NICOLAE TESTEMITANU
STATE UNIVERSITY
OF MEDICINE AND PHARMACY
OF THE REPUBLIC OF MOLDOVA

MJHS Moldovan Journal of Health Sciences

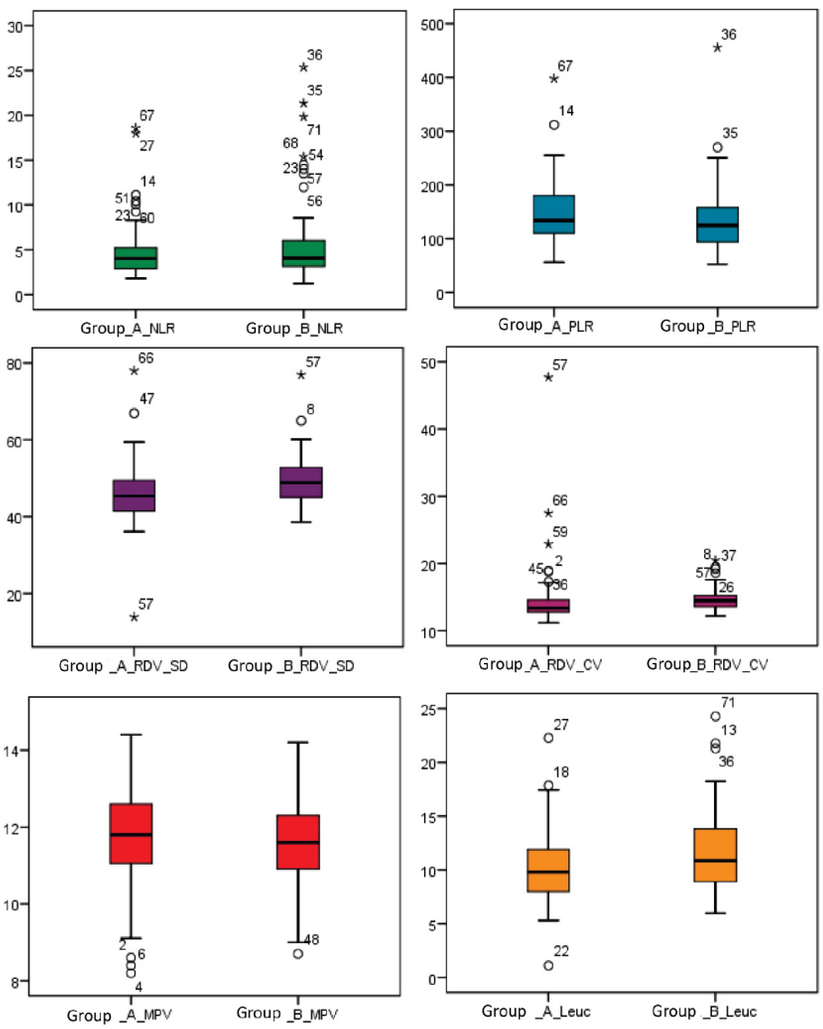
REVISTA DE ȘTIINȚE ALE SĂNĂTĂȚII DIN MOLDOVA

Vol. 28
2/2022

Categoria B

9 772345 146002

CONTENT HIGHLIGHTS:



ISSN 2345-1467

MJHS
Moldovan Journal of Health Sciences
Revista de Ştiinţe ale Sănătăţii din Moldova
28(2)/2022

CONTENT

Revista de Ştiinţe ale Sănătăţii din Moldova
Moldovan Journal of Health Sciences

Ediţie bilingvă: română, engleză

Fondator:
Instituţia Publică Universitatea de Stat de Medicină şi Farmacie „Nicolae Testemiţanu" din Republica Moldova

Redactor-şef:
Serghei Popa, dr. şt. med. conferenţiar universitar.

Colectivul redacţiei:
Dorian Sasu, redactor stilist
Sergiu Iacob, redactor stilist
Ana Orlic, redactor stilist

Adresa redacţiei:
biroul 407, blocul Administrativ, Universitatea de Stat de Medicină şi Farmacie „Nicolae Testemiţanu" bd. Ştefan cel Mare şi Sfânt, 165, Chişinău, Republica Moldova, MD-2004

Bilingual edition: Romanian, English

Founder:
Public Institution Nicolae Testemitanu State University of Medicine and Pharmacy from Republic of Moldova

Redactor-in-chief:
Serghei Popa, PhD.
university associate professor.

Editorial staff:
Dorian Sasu, redactor
Sergiu Iacob, redactor
Ana Orlic, redactor

Address of Editorial Office:
office 407; Administrative building, Nicolae Testemitanu State University of Medicine and Pharmacy
bd. Stefan cel Mare si Sfant, 165, Chisinau, Republic of Moldova, MD-2004

Editat: Editura „Lexon-Prim"
Tiraj: 200 ex.

Înregistrat la Ministerul Justiţiei cu nr. 250 din 01 august 2014 Categoria B (hotărârea comună a CSŞDT/CNAA nr. 169 din 21.12.2017) înregistrat IBN/IDSI la 16.11.2015

UDC: 616.62-008.222-089.819:615.217.5

RESEARCH ARTICLE

Variability of urodynamic parameters in patients with idiopathic overactive bladder before intradetrusor botulinum toxin injections

Mihaela Ivanov[1*†], Emil Ceban[1†]

[1]Department of Urology and Surgical Nephrology, „Nicolae Testemitanu" State University of Medicine and Pharmacy, Chisinau, Republic of Moldova.

Manuscript received on: 12.04.2022
Accepted for publication on: 17.05.2022

Corresponding author:
Mihaela Ivanov, PhD fellow
Department of Urology and Surgical Nephrology,
Nicolae Testemitanu State University of Medicine and Pharmacy
165 Stefan cel Mare si Sfant, Chisinau, Republic of Moldova, MD-2004
e-mail: mihaella.litovcenco@gmail.com

Short title: *The impact of urodynamics on management the overactive bladder*

What is not known yet, about the topic

No predictive parameters are established for urodynamic variables used in diagnosis of detrusor overactivity before surgical treatment with botulinum toxin A intradetrusor injections.

Research hypothesis

There are scientific controversies regarding the necessity of preoperative urodynamic investigation, which may allow the evaluation of effectiveness of botulinum toxin A injections.

The novelty brought to the scientific literature

Urodynamic tests have the potential for being used as a biomarker to provide a rapid assessment of effectiveness of botulinum toxin injections and being an early indicator of severity of idiopathic overactive bladder symptoms.

Abstract

Introduction. The aim of study was to establish if the treatment of overactive bladder in women and its result reported by patients were different depending on the findings of urodynamics, performed before botulinum toxin injection. We obtained clinical data based on the necessity and importance of performing urodynamic tests up to surgical treatment with botulinum toxin A injection in detrusor muscle, at patients diagnosed with idiopathic overactive bladder and detrusor overactivity, offering a guarantee of an effective and long-lasting treatment and assure with predictive parameters for some postoperative complications.

Material and methods. The research was carried out at the Department of Urology and Surgical Nephrology, during the years 2019 – 2022. After applying the inclusion and exclusion criteria, 36 women diagnosed with overactive bladder were enrolled in the prospective pilot study, aged between 18 and 70 years, refractory to drug treatment and investigated urodynamic by excluding/including the presence of detrusor overactivity and clinically using voiding diary/24h, bladder symptoms and Quality of Life questionnaires before and after botulinum toxin A injection. The primary data were analyzed and presented as a mean and standard deviation.

Results. All women involved in the study were diagnosed clinically and urodynamic with overactive bladder, of which 55.5% of cases were associated with detrusor overactivity and subsequently received BTX-A injectable surgical treatment with a dose of 100U. At urodynamics, the low values of the indices obtained at cystometry were established: first sensation of bladder filling (79.8 ± 56.3 ml), first desire to void (117.8 ± 103.2 ml), strong desire to void (162 ± 125 ml) and maximal cystometric capacity (183.4 ± 139.8 ml), which correlated in 100% cases with OAB symptoms (urinary urgency, frequency and nocturia) from the OABSS validated questionnaire. The capacity of the bladder at each sensation was lower, being inversely proportional to the detrusor overactivity present in women with OAB. Based on UDS data, the diagnosis of OABi with detrusor overactivity was confirmed by establishing the presence of phasic detrusor contractions (3.9 ± 1.1), increased values of detrusor pressure (45.9 ± 23.9 cmH2O) and the presence of bladder hypocompliance (10.6 ± 11.5 ml/cmH2O), these data in 100% of cases predicted an effective BTX-A injection. Daily activity and psychosocial behavior improved after intradetrusor botulinum injections toxin was influenced by reducing daytime urination from 28% to 40% and urinary urgency from 30 to 69%.

https://doi.org/10.52645/MJHS.2022.2.01

Conclusions. This study identified a number of statistically significant urodynamic variables associated with objective clinical data, which confirm the impact of severity of idiopathic overactive bladder symptoms and the subsequent effect of botulinum toxin type A injections in case of urodynamic confirmation of detrusor overactivity presence.

Key words: idiopathic overactive bladder, detrusor overactivity, botulinum toxin, intravesical injections, lower urinary tract.

Introduction

Urodynamics (UDS) is considered an important test in case of lower urinary tract symptoms (LUTS). The *International Continence Society* (ICS) states that the goal of urodynamic studies is to reproduce the patient's symptoms while performing measurements that aim to determine the underlying cause of LUTS and assess the associated pathophysiological processes [1].

UDS is the „gold standard" for assessing the bladder and sphincter function, used as an objective parameter to the administered treatment. A superior advantage of UDS over urinary diary used to evaluate the drug at an early stage is that UDS has the potential to capture a physiological response without waiting for the patient's behavior (urination habit) in response to physiological improvement [2].

Idiopathic overactive bladder (OABi) is a clinical syndrome characterized by urinary urgency, usually associated with frequency and nocturia, with or without urinary urge incontinence, in the absence of urinary tract infection (UTI) or other pathologies. Idiopathic overactive bladder symptoms affect approximately 17% of women, and its prevalence increases with patient's age, reaching up to 30.9% cases [3, 4].

OABi denotes a syndrome with unknown etiology, detrusor overactivity (DO) considered being a major cause of basic OAB pathophysiology. UDS is a sensitive investigation for detection DO in up to 70% of women with OAB [5, 6].

Idiopathic DO is an objective finding based on urodynamics, characterized by contractions of detrusor during filling phase and is associated with the urgency urinary sensation. Urgency, frequency and urinary urge incontinence have been shown to be the most sensitive factors for predicting DO (61.0%) in women. In a study by Khan *et al.* (2009) was established that urinary incontinence and nocturia were associated with DO in approximately 65.9% of patients with OAB, the authors also showed involuntary contraction of detrusor at urodynamics during cystometry at low volume of infusing the bladder [5, 7].

Some researchers believe that UDS is mandatory for diagnosis and treatment among women with symptoms of OAB, otherwise determining the diagnosis only on the basis of urinary symptoms would lead to underdiagnosis of detrusor overactivity. Some studies may be needed to diagnose female urinary disorders, which are represented by post-urinary symptoms that coexist with storage symptoms. Symptoms of OAB was present in 90% of women with infravesical obstruction [8]. Thus, the role of performing UDS at early stages in diagnosis the OAB in women is essential.

Many clinicians use UDS to diagnose DO before detrusor injection treatment. According to the recommendations of the NICE Guide (National Institute for Excellence in Health and Care) it is mandatory to investigate „urodynamics" to confirm the diagnosis of detrusor overactivity before performing minimally invasive treatment such as botulinum toxin type A (BTX-A) injections [1, 9, 10].

ICS defines detrusor overactivity as involuntary contractions of the detrusor during the filling phase, which can be spontaneous or induced, further classifying into two distinct subgroups: neurogenic and idiopathic. The clinical symptoms of idiopathic DO are associated with involuntary phasic contractions of the detrusor muscle, which prevent the storage of urine, manifested by urinary urgency and/or urge urinary incontinence. These phasic contractions can be diagnosed only based on urodynamic investigation at cystometry. Urodynamics is a diagnostic test that involves inserting a catheter into the bladder and one into the vagina or rectum, reproducing urinary symptoms and identifying the underlying pathology [10-12].

ICS states that „the clinical purpose of urodynamics is to reproduce symptoms while is performing accurate measurements to identify the underlying causes of symptoms and to quantify the link to pathophysiological processes". Studies about pathophysiological mechanisms underlying the occurrence of OAB are usually based on urodynamic findings [9].

It is widely accepted that urodynamics is not indicated to all women with bladder disorders, and practical guidelines recommend urodynamic testing when the diagnosis remains uncertain after clinical evaluation, when symptoms do not correlate with objective physical findings or in case that patient is refractory to drug treatment [13].

The clinical research done by Brubaker *et al.* established that in patients with refractory OAB diagnosed by urodynamics with DO and subsequently undergoing BTX-A injections surgery, approximately 60% of cases had a positive clinical response. However, there are many scientific studies that appears to be contradictory about urodynamic confirmation of detrusor overactivity before treatment, that may not be predictive in determining treatment success. Cystometry, most important part of urodynamic study, is an essential part in the diagnosis of pathology and the evaluation of treatment of OAB, acting as a diagnostic test to identify etiology, such as involuntary phasic contractions of detrusor or low bladder compliance [10, 14].

The NICE Institute UK, the American Association of Urology (AUA) and the European Association of Urology (EAU) recommend injecting BTX-A bladder detrusor muscle at women with refractory OABi and associated DO who are willing and able for self-catheterization [4].

BTX-A leads to selective paralysis of small-amplitude detrusor muscle contractions in time of strong contractions during urination, by influencing the afferent and efferent

pathways of detrusor control during filling and urination. At least two mechanisms of action of BTX-A are assumed: efferent modulation (reducing the acetylcholine release) and the ability to reduce the related urinary reflex transmission. In addition, this neurotoxin reduces the level of nerve growth factor [16, 17].

Multiple clinical trials have reported results about BTX-A injection therapy for patients with idiopathic DO, for evaluating and establishing a necessary dose for improving present urinary symptoms. An improvement in urodynamic parameters was established from a dose of 100U. At this dose, urinary urgency symptoms, frequency and urinary incontinence urge decreased sharply, and bladder capacity, evacuation pressure, and quality of life were improved significantly, with a successful rate of 73.3%. The side effects were less common at this dose than at 150 or 200U. According to these findings, 100U of BTX-A has become a standard dose for idiopathic DO and has shown a good outcome. Contradictory, Sahai *et al.* (2020), in an analysis of urodynamic data from their randomized clinical trial, reported that the maximum detrusor pressure was very high (>110 cmH_2O), which may predict a poor response to treatment with 200U of BTX-A, indicating that that higher doses may be required at these patients. The effectiveness of BTX-A injections in patients with low bladder compliance has a shorter duration of action (12-24 weeks) than in those with normal compliance. Preoperative bladder compliance was significantly lower in patients who did not respond to BTX-A injections [14, 15].

The AUA and EAU currently recommend a dose of 100U BTX-A as the starting dose in the treatment of refractory OAB. BTX-A detrusor muscle injection is a relatively minimally invasive procedure compared to alternative surgical treatment and is usually performed as outpatient surgery. The success rate of BTX-A injections varies between 60-80% [4, 16].

The clinical results of an individual treatment with BTX-A are expressed in 1-3 weeks and can last up to 9-12 months, patients usually requiring repeated treatments. Repeated BTX-A injections in the treatment of lower urinary tract disorders and pelvic floor dysfunction is recommended by the EAU Guideline. The reinjection period should not be less than 3 months and a range of 6 to 9 months is recommended to prevent the production of circulating antibodies, which could induce a decrease in post-injection therapeutic effect with BTX-A.

Side effects after BTX-A injections of the detrusor muscle at patients with OAB can lead to develop a high volume of post-void residual urine volume (PVR) in the first month after injection, acute urinary retention, ranging from 6-45% and requiring intermittent self-catheterization (11%), occurrence of UTI with a reported incidence rate between 0-45% and/or poor treatment efficacy (13%) [4, 15, 17].

Material and methods

A prospective clinical study was performed and a post-hoc analysis of data based on the results of urodynamic parameters before injection with botulinum toxin type A intradetrusor at patients with OABi. This study provided a unique opportunity to describe the variability of the results after botulinum toxin injections of detrusor muscle, performed after the urodynamic investigation per patient over a period of 3 years.

This research included 36 women diagnosed clinical and urodynamic with OAB, aged between 18 and 70 years from a single clinic, refractory to drug treatment and treated with botulinum toxin type A injections, during the years 2019 – 2022, at the Department of Urology and Surgical Nephrology, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova. In this study, fundamental ethical principles of research have been respected. All patients gave informed consent for study entry. The study protocol was endorsed positively by *Nicolae Testemitanu* SUMPh Research Ethics Committee (Minutes №. 24, 05.03.2021). Patients who underwent the surgical procedure, were asked to indicate that they understood the nature of the surgical procedure for being informed and after they gave the permission for operation by signing the informed consent.

Study procedure

This analysis was performed as part of a prospective pilot study to evaluate the clinical and urodynamic parameters of detrusor muscle contractility before botulinum toxin type A injections in patients with overactive bladder associated with idiopathic detrusor overactivity.

Inclusion criteria were women (>18 years age) diagnosed with idiopathic OAB, refractory to anticholinergic therapy for more than 6 weeks, due to ineffectiveness or tolerability, DO confirmed by urodynamic test with or without urge urinary incontinence and completed the voiding diary/24h and valid questionnaires before and after injections.

The exclusion criteria were – neurogenic urinary bladder, bladder pain syndrome/interstitial cystitis, infravesical obstruction diagnosed on urodynamics, UTI, lithiasis/bladder tumors.

Before and after botulinum toxin injection, all patients completed voiding diary/24h, the OAB validated urinary symptoms questionnaire (OABSS), and the health-related quality of life questionnaire (OABq-HRQoL). Patients underwent ultrasound investigation to establish PVR and preoperative UDS (uroflowmetry, cystometry and voiding pressure study).

Study equipment

The detailed medical history of the patients was analyzed (assessment of urinary symptoms, filling and incontinence, previous investigations and/or conservative, pharmacological and/or surgical treatments for OAB and relevant surgical medical history). Clinical examination was performed, including assessment of stress urinary incontinence, prolapse of pelvic organs, tumor masses or other pelvic pathologies.

The collected information included patient demographic factors, medical history (history of recurrent UTI, history of pelvic floor surgery, presence/absence of prolapse, menopause).

Voiding diary was completed for at least 24 hours as a valid journal prior to the urodynamic investigation. All patients were asked to complete the OABSS and OABq-HRQoL questionnaires before and after injections. LUTS/OAB symptoms assessed by validated OABSS developed by Blaivas *et al.* (2007) and Man *et al.* (2006), was completed by all women before the intravesical botulinum toxin type A injection and during a post-injection follow-up.

The minimally invasive (urodynamic) and noninvasive clinical evaluation of women with urinary OAB symptoms that were performed in the study included: cystometry, free uroflowmetry ± pressure flow study, bladder ultrasound for determine the presence/absence of PVR and urinalysis with urine culture to exclude the presence of UTI.

UDS investigation

Urodynamic studies were performed for the diagnosis of OAB and DO using urodynamic equipment Medica SpA Memphis Division (Medolla-Italy). Women with infravesical obstruction, detrusor underactivity, and detrusor overactivity with inadequate contractility were excluded from this study.

The evaluated urodynamic parameters were PVR assessed by ultrasound, maximum cystometric capacity (MCC), maximum detrusor pressure (MDP), maximum urinary flow pressure (PdetQmax), maximum urinary flow rate (Qmax) and bladder compliance (BC). BC was calculated using the ratio urine volume/detrusor pressure, being considered low when $\Delta V/\Delta Pdet$ was ≤ 30-40 ml/cmH_2O, despite not well-established and insufficient data regarding the normal values.

The actual procedure was explained to all patients in a clear manner by providing a scenario, instructions on how to report the 4 sensations during the cystometry. Patients signed the informed consent before the procedure.

Rectal urodynamic catheter was inserted ~10 cm, after that the urethra was catheterized using a 7Fr double lumen urodynamic catheter. The bladder was emptied after confirmation of the lack of residual urine based on the urodynamic investigation. Patients placed in a sitting position after the filler wires have been connected. The transducers were placed at reference heights according to ICS standards with the respective calibration of atmospheric pressures. The working of transducers was confirmed asking patients to cough and the filling of the bladder was performed with saline solution prepared at room temperature (filling speed 20 ml/min). The filling was stopped once the patient reached the maximum cystometric capacity, then the patient urinated.

BTX-A injection procedure

Surgical treatment was performed under intravenous anesthesia. All patients received antibiotic prophylaxis (Ciprofloxacin 500mg, KRKA, Slovenia). The used dose was 100U of BTX-A (Neuronox®, Medytox Inc., Korea). The dose was diluted with 10 ml of 0.9% saline solution. Under the guidance of the Karl Storz 19Fr rigid cystoscope, the bladder was dilated by infusing ~200 ml of 0.9% saline solution. Using a rigid injection needle with a diameter of 5Fr, 4mm long, inserted ~2-3mm into the detrusor supratrigonal muscle, BTX-A was injected, 10 units/mL in 20 separate places at a distance of ~1 cm, using 0.5 ml for each injection site. The trigone, the ventral wall and dome of the bladder were avoided due to its close relationship with the peritoneal cavity. The bladder was emptied after the injection.

Statistical analysis

Statistical data analysis was performed using unifactorial dispersion analysis designed in Microsoft Excel 2019 software and IBM SPSS Statistics 22, using the standard and paired t-tests, with a significance level of 0,05. The categorical data were presented as absolute and relative values and the continuous data – in the form of mean and standard error, or as a percentage of results, comparing results before and after procedure.

Results

There were analyzed data from 36 women, with clinical and urodynamic diagnosis of overactive bladder associated with detrusor overactivity in 55.5% of cases who received 100U of BTX-A injectable surgical treatment, between January 2019 and January 2022.

Table 1. *Demographic data of patients with idiopathic overactive bladder.*

Demographic data	(N = 36)
Age (years)	40.7 ± 13.64
Reproductive period (18 - 44 years)	21 (58.3%)
Pre-menopausal (45 - 55 years)	6 (16.6%)
Menopause (56 - 65 years)	8 (22.2%)
Post-menopausal (> 65 years)	1 (2.7%)
Disease outcome (years)	5.61 ± 3.9
Body mass index	24.5 ± 2.5
Symptoms	
Urinary frequency	36 (100%)
Urinary urgency	36 (100%)
Nocturia	36 (100%)
Urge urinary incontinence	2 (5.5%)
Natural births	20 (55.5%)
DOi	20 (55.5%)
Conservative previous treatment	36 (100%)
Anticholinergic preparations (Solifenacin, Trospium Chlorid, Tolterodine)	23 (63.8%)
Selective β3-adrenoceptor agonists (Mirabegron)	13 (36.1%)
Behavioral treatment	36 (100%)
Nr. of repeated injections	1 (5%)

Note: DOi - idiopathic detrusor overactivity.

The mean age of women included in this study was 41 years (18-67 years), which corresponds to the reproductive period; the duration of OAB symptoms was ~6 years (Table 1). Before the injection, the period of drug treatment was 3 months by administration of combined anticholinergic medicine (63.% of cases) and selective β3-adrenoceptor agonists (36.1% of cases), without improvement of LUTS/OAB. In 100% of cases, patients had urinary symptoms like urinary frequency, urgency, and nocturia.

Urodynamic data was obtained before injection in 36 patients, from which in 20 patients (55.5% of cases) were confirmed the presence of DO. The bladder contractility index (CI) was found to be within the normal range in 100% of cases in patients investigated urodynamic and diagnosed with DO (Table 2). In 100% of cases the PVR was measured before surgery, averaging 4.9 ml (between 0-10 ml).

Table 2. *Urodynamic parameters in patients with idiopathic overactive bladder before botulinum toxin type A injection treatment.*

Urodynamic parameters		BTX-A pre-injection (n = 36)	Normal values
Uroflowmetry	Maximum voided volume (ml)	132.7 ± 136.7	150-500
	Qmax (ml/s)	9.8 ± 4.1	17.04
	Qave (ml/s)	2.2 ± 1.6	13.2
Cystometry	FS (ml)	79.8 ± 56.3	170-250
	FDV (ml)	117.8 ± 103.2	250-330
	SDV (ml)	162 ± 125	350-560
	MCC (ml)	183.4 ± 139.8	450-550
	MDP (cmH_2O)	45.9 ± 23.9	25-60
	Nr. of contractions	3.9 ± 1.1	1 before void contraction
BC (ml/cm H_2O)		10.6 ± 11,5	>30-40
CI		124.6 ± 39,4	100-150
PVR (ml)		4.9 (0-10)	0-7

Note: Qmax - maximum flow rate; Qave - average flow rate; FS -first sensation of bladder filling; FDV - first desire to void; SDV - strong desire to void; MCC - maximum cytometric bladder capacity; MDP - maximum detrusor pressure; BC - bladder compliance; CI - index of detrusor contractility; PVR - post-void residual urine volume; BTX-A - botulinum toxin type A.

Based on UDS data, the diagnosis of OABi with DO was confirmed by establishing the presence of phasic contractions of the detrusor muscle (3.9±1.1), increased values of detrusor pressure (45.9 ± 23.9 cmH_2O) and the presence of bladder hypocompliance (10.6 ± 11.5 ml/cmH_2O), these data in 100% of cases predicted an effective BTX-A injection.

Urodynamic parameters, such as bladder capacity at each sensation and detrusor pressure were affected by the presence of DO in women with OAB. Low values of indices obtained at cystometry: first sensation of bladder filling (79.8±56.3 ml), first desire to void (117.8±103.2ml), strong desire to void (162±125 ml) and maximal cystometric capacity (183.4±139.8 ml) were 100% correlated with OAB symptoms (urgency, frequency and nocturia) from the OABSS validated questionnaire. We showed that the capacity of the bladder at each sensation was lower, being inversely proportional to the detrusor overactivity in women with OAB.

PVR values ($\bar{x}$ = 4.9 ml) did not correlate with the occurrence of acute urinary retention or the need for intermittent post-injection self-catheterization, as well as the low value of urinary flow rate (9.8 ± 4.1ml/s).

The urinary symptoms were evaluated before BTX-A injection – urinary frequency (100%), urinary urgency (100%), nocturia (100%) and urge urinary incontinence (10%). Our observations were the same as results of botulinum toxin type A therapy in OAB from other international studies. Which has shown that botulinum toxin influence on urination (daytime urination has been reduced from 28% to 40%), and the urgency urination being reduced from 30% to 69%.

Based on the voiding diary, we analyzed the following indices before and after injection – total voided volume, functional bladder capacity, nocturia index, and nocturia polyuria index, number of daytime voiding and total index of urgency and frequency urination (Table 3).

Table 3. *Primary post-injection efficacy according to voiding diary parameters.*

Voiding diary parameters	BTX-A pre-injection (n = 20)	BTX-A post-injection (after 6 weeks) (n = 20)
TVV / 24h (ml)	1314 ± 645	1565 ± 168
FBC (ml)	163.1 ± 123.9	338 ± 69
IN	2.86	0.7 ± 0.1
IPN (%)	28.7 ± 9.4	15.8 ± 5.1
DV	11.3 ± 1.68	5.1 ± 2
TUFS	31.7 ± 7.8	7.7 ± 3.8

Note: TVV - total voided volume; FBC - functional bladder capacity; IN - index of nocutia; IPN - index of nocturia polyuria; DV - daytime voiding; TUFS - total urgency and frequency score; BTX-A - botulinum toxin type A.

All validated self-report questionnaires quantifying OAB symptoms (daytime urinary frequency, nocturia, urinary urgency and urge urinary incontinence) were completed by all women prior to intravesical BTX-A injection and during a follow-up visit at first and third month after intravesical injections. A significant decrease of negative impact of LUTS/OAB on daily indoor and outdoor activity, physical and social activity was reported by patients following the completion of post-injection quality of life questionnaire (OABq-HRQoL).

From the study group, 19 patients received a single BTX-A injection. One patient (5% of cases) underwent repeated botulinum toxin type A injections, when an insufficient response or recurrence of urinary symptoms was observed after a minimum waiting time of 6 months. All patients were followed-up for at least 3 months after their first BTX-A injection.

Intravesical BTX-A injection has a positive effect on patients' emotions, with each follow-up visit (1st, 3rd and 6th month) an improvement in emotional state was observed. Sleeping difficulties and fatigue were reduced after the first month after the injection. Moreover, a statistically significant reduction in LUTS/OAB severity was observed using the OABSS questionnaire (Table 4).

The OABSS questionnaire indices and their improvements after BTX-A injection are shown in table 5. The results showed improvement of more than 2 points in 65% of treated patients, remained unchanged in 20% and worsened in 14%.

Table 4. *Injection efficacy according to the degree of impairment of symptoms from OABSS questionnaire.*

Severity of OABSS	BTX -A pre-injection (n = 20)	BTX-A post-injection (after 6 weeks) (n = 20)
Absence of symptoms	0	11 (55%)
Mild	8 (40%)	8 (40%)
Severe	12 (60%)	1 (5%)

Note: OABSS - overactive bladder symptoms questionnaire; BTX-A - botulinum toxin type A.

OABSS values improved on average by 35% after treatment, and in 55% of cases improved by 3 or more points. Scores for daytime urinary frequency, nocturia and urinary urgency improved significantly after BTX-A injection by 41.7%, 26.1% and 34.1%, respectively (Table 5).

Table 5. *Post-injection efficacy according to the symptoms from OABSS questionnaire.*

OABSS	BTX-A pre-injection (n = 20)	BTX-A post-injection (after 6 weeks) (n = 20)
Urinary frequency	100%	65.9%
Urinary urgency	100%	58.3%
Nocturia	100%	73.9%
Urge urinary incontinence	10%	0

Note: OABSS - overactive bladder symptoms questionnaire; BTX-A - botulinum toxin type A.

Our results showed that the impact of idiopathic refractory OAB on patients was improved. The overall impact of the bladder problem on quality of life increased slightly at the 6th and 9th month after treatment, compared with the 3rd month (Table 6).

Table 6. *The degree of impairment of quality of life in patients with idiopathic overactive bladder before and after the treatment with botulinum toxin type A injection, based on the OABq-HRQoL questionnaire.*

Degree of impairment on quality of life	BTX-A pre-injection (n = 20)	BTX-A post-injection (after 6 weeks) (n = 20)
OAB-QoL Subscale Symptom-Severity	75%	10%
HRQoL subscales Coping	70%	10%
HRQoL subscales Concern	70%	25%
HRQoL subscales Sleep	95%	25%
HRQoL subscales Social	55%	0
HRQoL total	70%	25%

Note: BTX-A - botulinum toxin type A; OAB-QoL - the quality-of-life questionnaire related to OAB symptoms; HRQoL - health-related quality of life.

Regarding the side effects of intravesical therapy with BTX-A, in the actual group of patients, 7 cases of urinary tract infection were detected (35% of cases), diagnosed by a positive urine culture. None of the patients required clean intermittent self-catheterization (CIC) due to acute urinary retention or increased post-void residual urine volume.

Discussion

Original definition of OAB is based on symptoms that highlight urinary urgency as a cardinal symptom. Although still controversial, it is believed that this urgency stems from an overactive bladder contraction (detrusor overactivity), which causes a "sudden, compelling urge to void" [18].

Urodynamic testing is a useful diagnostic tool and could be of great value in evaluating the efficacy before BTX-A injection. Urodynamic studies will not replace studies using voiding diary and OAB validated questionnaires to confirm symptoms, the outcome, and efficacy of treatment in OAB. However, clinical evidence from concept studies using voiding diary and OAB symptoms questionnaires may take approximately 9-16 months [2].

DO was considered one of the major features typical of OAB and OAB symptoms are thought to be indicative of a subsequent finding of DO on UDS. Digesu *et al.* found that only 54% of women with OAB had DO based on UDS, and 27% of women diagnosed with DO based on UDS had clinical symptoms of OAB. According to the findings, only the symptomatic diagnosis of OAB was not recommended for women with LUTS, especially for BTX-A injection [8].

A significant number of women with symptoms of overactive bladder showed LUTS, but the presence of OAB symptoms alone is not sufficient to predict the diagnosis of OAB with detrusor overactivity and the effectiveness of subsequent treatment.

Maximum cystometric capacity and bladder compliance were the variables with the most statistically significant associations in this study. Capacity is a measure of the potential for retention of bladder volume, smaller volumes being associated with more severe OAB symptoms. Bladder compliance, which is described as the complex interaction of volume and pressure during bladder filling, in the case of low compliance, was a bladder with reduced adaptation mechanisms and therefore associated with the severity of clinical manifestations.

Another important urodynamic parameter was the volume infused at the onset of the first sensation of bladder filling, that appears at smaller volumes were associated with more severe symptoms of urinary urgency. The first sensations of bladder at early stages of the filling phase are expected to be associated with several episodes of urgency.

These variables are also proposed by some authors as potential indicators between idiopathic and neurogenic DO. The value of these parameters has recently been supported by studies that evaluates the effect of OAB treatments based on urodynamic results. The effect of BTX-A in patients with idiopathic DO has been shown to improve bladder compliance and cystometric capacity, increased bladder filling volume, and decreased severity at first sensation of bladder filling [19]. The proven sensitivity of these urodynamic measurements offers good results in further research and in the treatment of patients with OAB.

We demonstrated the presence of a clinically relevant correlation between urodynamic findings and subsequent results of BTX-A injection therapy in patients with OAB.

Although both urodynamics and voiding diary with OABSS questionnaires may influence the clinical decisions of subsequent treatment, our study supports the role of UDS as predictors of outcome in patients with bladder overactivity.

Urodynamics seems to influence treatment decisions made by clinicians in determining treatment pathways in women with OAB. Patients that were diagnosed by UDS before treatment, appears to have greater reductions of symptoms than those who did not perform the investigation [1].

Based on our own study, we presented the short-term results of 36 patients treated with a dose of 100 and 200U with BTX-A in a single institution, urodynamic investigated before injection, with OAB and detrusor overactivity. There are some subjective and objective differences between the effect of BTX-A treatment on women who were diagnosed with DO by urodynamic tests before injection and those who were not, as evidenced by numerous worldwide clinical trials, suggesting that being OAB with DO as a serious disorder of bladder function among women.

International studies have suggested that maximal detrusor pressure, poor compliance, and maximal cystometric capacity were predictors of nonresponse and identified urinary retention and infection as potential side effects, but subsequent studies have refuted these data, but have identified that episodes of urge urinary incontinence and smoking status may be additional predictors of non-response to BTX-A injections in patients with refractory DO [20].

We found that lower Qmax values was predictive for urinary dysfunction requiring intravesical injection, this finding could be the result of weaker detrusor muscle contractility. However, low preoperative PVR values are not correlated with the occurrence of acute post-injection urinary retention or the need for clean intermittent self-catheterization.

In correlation with the results of an international study performed in two medical centers using 200U dose of BTX-A in patients with OABi, we can assume that low Qmax could be predictive of CIC use. The data in the literature contradict this, with some studies suggesting that preoperative PVR is associated with increased CIC and others not. However, in most preoperative studies PVR > 200 ml was an exclusion criterion. Poor contractility of the detrusor, leading to the presence of high post-injection PVR with BTX-A have been associated with the onset of ITU [16].

A significant improvement in LUTS/OAB symptoms was observed in the evaluation of own results. Daily activity and psychosocial behavior have improved due to reduced symptoms of urinary frequency, urge urinary urgency and nocturia, intradetrusor injections with botulinum toxin influence daytime urination, which have been reduced from 28% to 40%, reducing the urinary urgency from 30 to 69%.

A randomized clinical trial by Dmochowski *et al.* (2012) found that doses of 100U and higher provided better efficacy compared to 50U. Another study by Denys *et al.* (2007) showed no significant difference in urgency and incontinence episodes between groups of patients receiving BTX-A injections at doses from 100 and 150U after 3 months of follow-up. It was also shown that a dose of 50U had no benefit, as the results were not significantly different from a placebo injection [16].

In addition to the significantly improved number of daily voids, urgency episodes and nocturia, there was an improvement of quality of life observed in patients bothered especially by urinary urgency.

Surprisingly, in our study we did not report acute urinary retention that requires CIC. Ultrasound did not show any significant post-void residual urine volume. This is probably due to the small research group.

Rates of CIC requirement after BTX-A injection into the bladder are highly variable, ranging from 4% to 45% of cases. In other worldwide clinical trials, the mean CIC rate was approximately 23% of cases [21, 22].

The urodynamic test may be considered a predictive diagnostic method for some postoperative complications of botulinum toxin injection in patients with idiopathic overactive bladder and detrusor overactivity.

BTX-A is generally safe, effective treatment approved in many countries for overactive bladder symptoms. Barriers for using the toxin include meeting the clinical criteria for approved use, access to specialists, and the financial cost of treatment, which can be significant, although subsidized to varying degrees depending on use [23].

Conclusions

Clinical and urodynamic evaluation are essential in preparing for botulinum toxin type A injection in patients with OAB, suggesting a more severe bladder storage disorder when DO is present.

This study identified several urodynamic variables that have statistically significant correlations with objective clinical data, which report an impact on the severity of idiopathic overactive bladder symptoms and on the treatment efficiency with botulinum toxin type A injection in case of urodynamic confirmation of detrusor overactivity presence. Maximum cystometric capacity, bladder compliance, maximum voided volume and severity of first sensations of bladder filling could be considered predictive and useful values for being implemented in routine clinical practice for the diagnosis.

Urodynamic investigation reveals the particular characteristics of the results of the patients, despite the inherent variability of the parameters of urodynamic tests, women diagnosed with detrusor overactivity are significantly affected by their quality of life and have a severe degree of bladder dysfunction.

Declaration of conflicting interests

The author declares the absence of any conflict of interest in the elaboration of this article.

Authors' contribution

Both authors contributed equally to the development of the manuscript and approved its final version.

References

1. Verghese T.S., Middleton L.J., Daniels J.P., Deeks J.J., Latthe P.M. The impact of urodynamics on treatment and outcomes in women with an overactive bladder: a longitudinal prospective follow-up study. *International Urogynecology Journal,* 2018; 29(4): 513-519.
2. Frenkl T.L., Railkar R., Palcza J., Scott B.B., Alon A., Green S., Schaefer W. Variability of urodynamic parameters in patients with overactive bladder. *Neurourology and Urodynamics*, 2011; 30(8): 1565-1569.
3. Miotla P., Futyma K., Cartwright R., Bogusiewicz M., Skorupska K., Markut-Miotla E., Rechberger T. Effectiveness of botulinum toxin injection in the treatment of de novo OAB symptoms following midurethral sling surgery. *International Urogynecology Journal,* 2016; 27(3): 393-398.
4. Chohan N., Hilton P., Brown K., Dixon L. Efficacy and duration of response to botulinum neurotoxin A (onabotulinumA) as a treatment for detrusor overactivity in women. *International Urogynecology Journal and Pelvic Floor Dysfunction,* 2015; 26(11): 1605-1612.
5. Chen S.L., NG S.C., Huang Y.H., Chen G. Are patients with bladder oversensitivity different from those with urodynamically proven detrusor overactivity in female overactive bladder syndrome? *Journal of the Chinese Medical Association*, 2017; 80(10): 644-650.
6. Giarenis I., Mastoroudes H., Srikrishna S., Robinson D., Cardozo L. Is there a difference between women with or without detrusor overactivity complaining of symptoms of overactive bladder? *B.J.U. International*, 2013; 112(4): 501-507.
7. Diamond P., Hassonah S., Alarab M., Lovatsis D., Drutz H.P. The prevalence of detrusor overactivity amongst patients with symptoms of overactive bladder: A retrospective cohort study. *International Urogynecology Journal,* 2012; 23(11): 1577-1580.
8. Cho K.J., Kim H.S., Koh J.S., Kim J.C. Evaluation of female overactive bladder using urodynamics: Relationship with female voiding dysfunction. *International Braz. J. Urol.,* 2015; 41(4): 722-728.
9. Malone-Lee J.G., Al-Buheissi S. Does urodynamic verification of overactive bladder determine treatment success? Results from a randomized placebo-controlled study. *B.J.U. International*, 2009; 103(7): 931-937.
10. Abdel-Fattah M., Chapple C., Guerrero K., Dixon S., Cotterill N., Ward K., Hashim H., Monga A., Brown K., Drake M.J., Gammie A., Mostafa A., Bladder Health U.K., Breeman S., Cooper D., Maclennan G., Norrie J. Female Urgency, Trial of Urodynamics as Routine Evaluation (FUTURE study): a superiority randomised clinical trial to evaluate the effectiveness and cost-effectiveness of invasive urodynamic investigations in management of women with refractory overactive bladder symptoms. *Trials,* 2021; 22(1): 745.
11. Harris S., Rizzolo D. Botulinum toxin as a treatment for refractory overactive bladder. *JAAPA: official journal of the American Academy of Physician Assistants,* 2016*;* 29(2): 1-4.
12. Grishin A., Spaska A., Kayumova L. Correction of overactive bladder with botulinum toxin type A (BTX-A). *Toxicon*, 2021; 200: 96-101.
13. Kopp Kallner H., Elmér C., Altman D. Urodynamics as a Prognosticator of Mirabegron Treatment Outcomes. *Gynecologic and Obstetric Investigation*, 2019; 84(5): 472-476.
14. Przydacz M., Golabek T., Chlosta P. How to assess and predict success or failure of intra-detrusor injections with onabotulinumtoxinA. *Advances in clinical and experimental medicine: official organ Wroclaw Medical University*, 2019; 28(4): 555-567.
15. Chen J.L., Chorng H.K. Clinical application of intravesical botulinum toxin type a for overactive bladder and interstitial cystitis. *Investigative and clinical urology,* 2020; 61(1): 33-42.
16. Abrar M., Stroman L., Malde S., Solomon E., Sahai A. Predictors of Poor Response and Adverse Events Following Botulinum Toxin-A for Refractory Idiopathic Overactive Bladder. *Urology*, 2020; 135: 32-37.
17. Abeywickrama L., Arunkalaivanan A., Quinlan M. Repeated botulinum toxin type A (Dysport®) injections for women with intractable detrusor overactivity: A prospective outcome study. *International Urogynecology Journal and Pelvic Floor Dysfunction*, 2014; 25(5): 601-605.
18. Guralnick M.L., Grimsby G., Liss M., Szabo A., O'Connor R.C. Objective differences between overactive bladder patients with and without urodynamically proven detrusor overactivity. *International Urogynecology Journal*, 2010; 21(3): 325-329.
19. Giarenis I., Zacchè M., Robinson D., Cardozo L. Is there any association between urodynamic variables and severity of overactive bladder in women with idiopathic detrusor overactivity? *Neurourology and Urodynamics*, 2017; 36(3): 780-783.
20. Owen R.K., Abrams K.R., Mayne C., Slack M., Tincello D.G. Patient factors associated with onabotulinum toxin A treatment outcome in women with detrusor overactivity. *Neurourology and Urodynamics*, 2017; 36(2): 426-431.
21. Barba M., Lazar T., Cola A., Marino G., Manodoro S., Frigerio M. Learning Curve of Botulinum Toxin Bladder Injection for the Treatment of Refractory Overactive Bladder. *International Journal of Women's Health*, 2022; 14: 1-7.
22. Juszczak K., Adamczyk P., Maciukiewicz P., Drewa T. Clinical outcomes of intravesical injections of botulinum toxin type A in patients with refractory idiopathic overactive bladder. *Pharmacological Reports*, 2018; 70(6): 1133-1138.
23. Brennan A., Hickey M. Botulinum toxin in women's health: An update. *Maturitas,* 2019; 119: 21-24.

Authors's ORCID ID:
Mihaela Ivanov https://orcid.org/0000-0002-5990-320X
Emil Ceban https://orcid.org/0000-0002-1583-2884

UDC: 616.65-007.61-089.87+615.849.19

RESEARCH ARTICLE

Thulium: yttrium-aluminium-garnet laser transurethral vapoenucleation – a new standard in the surgical treatment of large benign prostatic hyperplasia

Alexei Plesacov[1,2*], Ivan Vladanov[1,2], Vitalii Ghicavii[1,2]

[1]Republican Clinical Hospital „Timofei Moșneaga", Chisinau, Republic of Moldova
[2]Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

Manuscript received on: 26.04.2022
Accepted for publication: 23.05.2022

Corresponding author
Alexei Plesacov, PhD student
Department of Urology and Surgical Nephrology
Nicolae Testemitanu State University of Medicine and Pharmacy
29 NicolaeTestemițanu str., Chisinau, Republic of Moldova, MD-2025
e-mail: alex_pleshacov@mail.ru

Short title: *Transurethral Thulium: yttrium-aluminium-garnet laser prostate vapoenucleation*

What is not yet known on the issue addressed in the submitted manuscript

The treatment of large benign prostate hyperplasia is a topic of great interest. The use of laser energy in prostate surgery opens up new possibilities for minimally invasive treatment.

Research hypothesis

Transurethral Thulium:YAG laser prostate vapoenucleation becomes a new standard for surgical treatment of large benign prostate hyperplasia**.**

The novelty added by manuscript to the already published scientific literature

The use of laser energy in the surgical treatment of large benign prostatic hyperplasia allows the improvement of functional postoperative results, along with rapid recovery and significantly lower surgical risks.

Abstract

Introduction. According to the current recommendations of the European Association of Urology and the International Society of Urology, open simple prostatectomy is the reference standard in the surgical treatment of benign prostatic hyperplasia of large size (>80 ml). Extended trauma and a high complication rate reduce the chances to use this method. In this context, several minimally invasive laser surgical treatment techniques have been proposed in order to provide an optimal treatment to patients. Transurethral endoscopic vapoenucleation of the prostate using laser energy is a new concept in endourological surgery.

Material and methods. Between September 2019 and December 2019, 93 patients with benign prostatic hyperplasia underwent surgical treatment. Two surgical methods were applied: transurethral Thulium:YAG laser prostate vapoenucleation (45 patients) and open simple prostatectomy (48 patients). All patients were evaluated preoperatively and postoperatively (3 and 6 months) using the International Prostate Symptom Score, Quality of Life Score and International Erectile Function Index, physical examination and digital rectal examination, prostate specific antigen assessment, transrectal prostate ultrasound examination and assessment of residual urine volume, uroflowmetry. Postoperative complications were recorded according to the Clavien-Dindo classification, 2004. The inclusion criteria were total prostate volume ≥80 cm3, age ≤80 years, residual urine volume ≥70 ml, Qmax ≤10 ml/s.

Results. Transurethral Thulium:YAG laser vaponucleation of prostate has proven a high surgical efficiency level. The baseline urodynamic and ultrasonographic indicators after transurethral vapoenucleation at 6 months postoperatively were similar to those in the control group (open simple prostatectomy). The duration of recovery of patients after classical surgery was significantly longer. At the same time, the rate of postoperative complications after prostate vapoenucleation was lower. Patients in the ThuVEP group did not require blood transfusions.

Conclusions. According to obtained results, we can assume that transurethral vapoenucleation of the prostate with laser energy will soon become a new „gold standard" in the surgical treatment of large benign prostate hyperplasia.

Keywords. Laser, benign prostatic hyperplasia

https://doi.org/10.52645/MJHS.2022.2.02

Introduction

Benign prostatic hyperplasia (BPH) is one of the most common pathological conditions contributing to the development of lower urinary tract symptoms (LUTS) in older men [1]. The prevalence of BPH increases progressively in men aged 40, reaching approximately 100% at age 90 [2]. The progressive evolution of lower urinary tract symptoms interfere significantly the quality of life in patients with BPH [3]. According to current guidelines, the surgical approach is the treatment of choice in patients with BPH. Transurethral resection of the prostate (TURP) and open prostatectomy (OP) have been considered the reference standard for the treatment of medium (<80 ml) and large (>80 ml) BPH [4, 5]. For decades, OP has been the only surgical treatment option for large BPH. However, the extensive and traumatic surgical approach in open surgery is commonly associated with significant perioperative complications, as well as an increased morbidity, catheterization time, and long-term hospital stay [6, 7]. To minimize surgical complications and enhance rapid postoperative recovery, several minimally invasive methods were suggested for the treatment of large BPH, such as laser surgery, aquablation, etc. Due to technical features, only laser surgery makes it possible to radically treat large BPH through a minimally invasive transurethral approach [8]. However, the safety and efficacy of these surgical procedures in the treatment of large BPH is still relevant. Transurethral endoscopic vapoenucleation of the prostate using laser energy is a novel concept in transurethral surgery. This surgical approach used in the treatment of BPH is showing promise and is becoming increasingly popular for the treatment of severe LUTS secondary to large BPH. The use of Holmium: YAG, KTP, diode and Thulium: YAG lasers is becoming more widespread, as data on their effectiveness and surgical safety has become more available [9, 10]. Features of the surgical technique using laser energy make it possible to carry out complete enucleation or vapoenucleation of hyperplastic prostate tissues with preservation of the surgical capsule only, which is similar to OP. In addition, these laser endoscopic techniques show an equivalent efficiency, and are sometimes even superior to OP [11, 12]. Thus, transurethral laser enucleation of the prostate is likely to completely replace OP in the surgical treatment of large BPH.

Material and methods

During September 2019 - December 2019, 93 patients with BPH underwent surgical treatment. Two surgical methods were applied: ThuVEP (45 patients) and OP (48 patients). All patients were assessed preoperatively and postoperatively (at 3 and 6 months of follow-up) by using the International Prostate Symptom Scale (IPSS), Quality of Life (QoL) score and International Erectile Function Index (IIEF-5), as well as by physical examination and digital rectal examination, assessment of prostate-specific antigen (PSA), estimation of prostate gland volume with transrectal ultrasound, measurement of postvoid residual urine volume (PVR), and Q_{mean} and Q_{max} measured by uroflowmetry. Postoperative complications were recorded according to the Clavien-Dindo (2004) classification. Inclusion criteria comprised total prostate volume ≥ 80 cm^3, age ≤80 years, postvoid residual urine volume (PVR) ≥ 70 ml, Q_{max} ≤10 ml/s. Exclusion criteria were the neurogenic bladder, confirmed prostate or bladder cancer. In ThuVEP, patients were placed in a lithotomy position. A Karl Storz 26Fr resectoscope with continuous flow and saline irrigation was used to perform ThuVEP in all cases. The 80 W settings of Thulium:YAG laser (Revolix Duo, Lisa Laser, Germany) were used to vaporize the tissue. The laser energy was delivered through RigiFib 550mc fiber optics with terminal emission.

First, a superficial circular incision is made in the mucous membrane of the prostatic urethra posterior to veru montanum using laser energy. After performing the dissection plane along the prostatic pseudocapsule, the prostate nodes were detached via vapoenucleation providing concomitant hemostasis. Vapoenucleated nodules were removed from the bladder lumen using a morcelator or via a devascularized tissue resection technique on the pedicle. At the end of the operation, a double-lumen autostatic urethral bladder Foley 20Fr was installed in all patients for postoperative bladder drainage. The removed tissues were sent for histological examination. In cases of severe hematuria in the early postoperative period, a continuous irrigation system was applied.

OP was performed with the patient positioned in supine position under spinal anesthesia. Enucleation of the hyperplastic prostate tissue was performed based on Fuller-Freyer procedure. Lower median laparotomy was used to access the bladder. Cystotomy allowed accessing the bladder neck and the nodes of the prostatic hyperplasia. An anatomical enucleation plane is created under digital transrectal control. Once created, the enucleation trajectory is accompanied by circular enucleation movements until the hyperplastic nodules of prostate are completely dissected. Installation of a Foley autostatic probe and cystostomy was mandatory in all patients. In all cases, a system of continuous irrigation was installed for a period of 12-24 hours in order to prevent bladder tamponade. The enucleated nodules were sent for histological examination.

The data were computer processed using Excel tables. Data is presented in absolute and relative values, or in mean values and standard deviation. Descriptive statistics.

Results

All the patients included in the study completed questionnaires throughout the entire study. During their visits, all parameters presented in the study were evaluated. At the end of the follow-up, all data were analysed by using the Student's t-test. There were no statistically significant differences in the study groups. Thus, the study groups were relatively homogeneous (Table 1).

Table 1. *Preoperative assessment (93 patients).*

Group characteristics	ThuVEP (n=45)	OP (n=48)
No. patients	45	48
Age, years	65±2	64±3
Q_{max}, ml/s	8.1±1.3	8.2±1.5
Q_{mean}, ml/s	7.4±1.1	7.5±1
IPSS	24±1	23±2
QoL	4±1	4±1
Prostate volume, ml	91±7	92±10
PVR, ml	85±14	89±11
PSA, ng/ml	3.3±0.6	3.1±0.4

***Note*:** ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation; OP – open prostatectomy; Q_{max} – maximum urinary flow rate; Q_{mean} – average urinary flow rate; IPSS - International Prostate Symptom Score; QoL – quality of life index; PVR – postvoiding residual urine volume; PSA – prostate specific antigen.

The operator indices were also recorded and analysed (Table 2). The removed tissue volume and the intervention timing were higher in the ThuVEP group due to complete enucleation of adenomatous tissue, which subsequently underwent fragmentation. The most significant blood loss was recorded in the OP group. The catheterization timing in patients who underwent OP was significantly higher (+400%) due to surgical trauma of the bladder and unfeasible definitive hemostasis. The hospitalization length was determined by the postoperative catheterization timing, which was also incomparably longer in patients following OP (+140%).

Table 2. *Operative data (93 patients).*

Group characteristics	ThuVEP	OP
Operating time, min	118±11	55±11
Blood loss volume, g/l	1.2±0,4	2.7±1.2
Catheterization time, days	2±1	10±1
Hospitalization length, days	5±1	12±2

***Note*:** ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation; OP – open prostatectomy.

After a 6-month follow-up, no statistically significant differences were found between the two study groups regarding IPSS, QoL, Q_{max} and PVR. However, at 3 months postoperatively, patients undergoing ThuVEP reported a more significant improvement in the IPSS score (15 points), QoL (2 points). PVR in the ThuVEP group showed a much faster positive trend. PVR volume decreased by 70.5% in the ThuVEP group and by 60.6% in the OP group 3 months after surgery. The trend towards greater improvement persisted 6 months postoperatively: 87% in the ThuVEP group and only 71% in the OP group). A faster improvement in reference values can easily be explained by the less traumatic features of ThuVEP (Table 3).

Table 3. *Postoperative dynamics (93 patients).*

Evaluation parameters	Preoperatively	Postoperatively	
		3 months	6 months
IPSS			
ThuVEP	24±1	9±1 (-62.5%)	8±2 (-66.6%)
OP	23±2	12±2 (-47.8%)	9±1 (-60.8%)
QoL			
ThuVEP	4±1	2±1 (-50%)	2±1 (-50%)
OP	4±1	3±1 (-25%)	2±1 (-50%)
PVR, ml			
ThuVEP	85±14	20±10 (-70.5%)	11±6 (-87%)
OP	89±11	35±11 (-60.6%)	25±7 (-71%)

Note: ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation; OP – open prostatectomy IPSS - International Prostate Symptom Score; QoL – quality of life index; PVR – postvoiding residual urine volume.

A significant improvement in Qmax was also reported in both groups, being faster in the ThuVEP group. At 6-month follow-up, the differences in Qmax were not so significant. Thus, the control values at 3 and 6 months were approximated. Changes in the urodynamic parameters during the study are shown in Table 4.

Table 4. *Changes in urodynamic values (93 patients).*

Intervention type	Preoperatively Qmax (ml/s)	Postoperatively	
		3 months, Qmax (ml/s)	6 months, Qmax (ml/s)
ThuVEP	8.1±1.3	17.1±1 (+111%)	18±1 (+122%)
OP	8.2±1.5	15±1 (+82%)	17.2±1 (+109%)

Note: ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation; OP – open prostatectomy; Qmax – maximum urinary flow rate.

In the preoperative period, 31% of patients in the ThuVEP group and 33% in the OP group reported satisfactory erectile function according to the IIEF-5 questionnaires. Erectile dysfunction in the postoperative period did not differ (Table 5). Thus, in patients who underwent OP, a significant decrease in erectile function was reported (on average -5 points (45%)). A slight improvement in erectile function in these patients was found only 6 months after surgery. At the same time, patients after ThuVEP reported a slight decrease in erectile function 3 months after surgery (on average -3 points (25%)). Repeated check-ups at 6 months of follow-up showed an improvement in erectile function reaching the preoperative values. Significant impairment of erectile function in patients undergoing open surgery is due to injury to vascular structures and peri-prostatic nerves during surgery.

Table 5. *Perioperative changes in erectile function (IIEF-5) (93 patients).*

Intervention type	Preoperatively	Postoperatively	
		3 months	6 months
ThuVEP	12±2	9±1	11±1
OP	11±2	6±1	9±1

Note: ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation; OP – open prostatectomy;IIEF-5 – International Index of Erectil Function.

The incidence of postoperative complications varied in the studied groups (Table 6) due to the extremely different surgical technique, various operative traumas and long-term catheterization in patients after OP. In sexually active patients, retrograde ejaculation in the postoperative period was reported in 35 patients out of 45 (77%) in the ThuVEP group and in 41 patients out of 48 (85%) in the OP group. Retrograde ejaculation has been shown to be stable over time and persisted in all patients, who reported this type of complication throughout the follow-up. During the follow-up period, a series of complications of a varying severity were identified, however not posing any threat on patient's life. No major bleeding events were reported in the ThuVEP group. At the same time, blood transfusions were required in 2 patients of the OP group, one of whom underwent a second operation with secondary hemostasis. Postoperatively, 5 patients (11%) in the ThuVEP group and 8 patients (16.6%) in the OP group reported transient urinary incontinence, which disappeared within 3 months of follow-up. One episode of acute urinary retention was recorded in both groups. A 48-hour re-catheterization with non-steroidal anti-inflammatory drugs was used for this type of complication.

Table 6. *Postoperative complications based on the 2004 Clavien-Dindo classification. (93 patients).*

Complication type	ThuVEP, no. patients (%)	OP, no. patients (%)	Complication severity
Transient urinary incontinence	5 (11%)	8 (16.6%)	Grade I
Repeated catheterization	1 (2.2%)	1 (2%)	
Blood Transfusion	-	2 (4%)	Grade II
Urinary tract infections	2 (4.5%)	5 (10.4%)	Grade III
Urethral stricture	1 (2.2%)	-	Grade IIIb
Bladder neck sclerosis	-	2 (4%)	
TURP syndrome	-	-	Grade IV
Total	9 (19.9%)	18 (35%)	

Note: ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation; OP – open prostatectomy; TURP syndrome – transurethral prostate resection syndrome.

During the 6-month follow-up, only one case of urethral stricture was reported in the ThuVEP group. At the same time, 2 patients with cervical sclerosis were identified in the OP group. These complications were corrected surgically by incision of the stricture with a cold blade and a bipolar incision of the bladder neck.

Urinary tract infections were reported in the early postoperative period in 2 patients (4.4%) in the ThuVEP group and in 5 patients (10.4%) in the OP group. An increase in the frequency of infectious and inflammatory complications after OP can probably be explained by a long-term bladder catheterization in the postoperative period. It is also worth mentioning that postoperative infectious complications were much more common among patients who underwent a prolonged bladder catheterization due to urinary retention (Table 7). Grade IV complications were not registered.

Table 7. *Incidence of infectious postoperative complications (93 patients).*

Complication type	ThuVEP		Open prostatectomy	
	Catheterized patients	Non-catheterized patients	Catheterized patients	Non-catheterized patients
Urethritis	1 (2,2%)	-	2 (4%)	1 (2%)
Orchoepididymitis	1 (2,2%)	-	2 (4%)	-

Note: ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation.

The role of the infectious-inflammatory factor in postoperative evolution is even more obvious when examining the urine cultures obtained from patients with preoperative bladder catheterization. Thus, the results of microbiological studies highlighted two main problems: the importation of nosocomial infections and an increasing antibacterial resistance. Among microorganisms found, Klebsiella pneumonia, Enterococus faecalis and Proteus mirabilis, well known for their antibiotic resistance, were increasingly being detected. At the same time, Escherichia coli, being one of the most common bacteria in urinary tract infections, has acquired a higher resistance to conventional antibacterial drugs (Table 8). The titer of pathogens detected in urine cultures varied, however no relationship between bacteriuria and the incidence of postoperative infectious complications was found.

Table 8. *Incidence of asymptomatic bacteriuria. (93 patients).*

Pathogenic agent	ThuVEP		Open prostatectomy	
	Catheterized patients	Non-catheterized patients	Catheterized patients	Non-catheterized patients
Klebsiella pneumonia	2	-	2	-
Proteus mirabilis	1	-	2	-
Enterococus faecalis	1	-	1	-
Escherichia coli	2	2	1	1
Other microorganisms	-	1	-	1
Sterile urine culture	4	32	5	35

Note: ThuVEP - transurethral Thulium:YAG laser prostate vapoenucleation.

Pre- and postoperative antibiotic therapy is becoming increasingly important, especially for the prevention of septic complications. Therefore, it seems very important to diagnose large BPH and timely establish indications for surgical treatment in order to avoid catheterization due to acute urinary retention leading to urinary tract infections.

Discussions

The study results confirmed the priority of the new minimally invasive method. Postoperative recovery and improvement in reference indices were much faster in patients treated with ThuVEP. This is due to massive surgical trauma in OP and the need for delayed bladder drainage by cystostomy with concomitant bladder catheterization in the postoperative period.

Surgical efficacy was assessed by changes in uroflowmetry and symptom scaling. Thus, in both groups, there was a significant improvement in Qmax. Patients who underwent ThuVEP resumed spontaneous urination much faster (on average 2 days after surgery) and reported a satisfactory and stable improvement in Qmax as early as 3 months after surgery. The Qmax values after OP reached similar values only 6 months after the intervention. The positive dynamics observed by the patients on the IPSS and QoL scale was faster in the ThuVEP group. Symptom assessment 6 months after surgery showed comparable results. Thus, it is worth mentioning that the ThuVEP efficacy is similar to the standard classical surgery, namely, open prostatectomy, showing faster functional outcomes. The data obtained during the study are also confirmed by a series of recent studies [13-15].

The overall incidence of postoperative complications was significantly higher in the OP group. Thus, patients after OP showed a significant decrease in the level of hemoglobin (2.7±1.2 g/l) due to the unfeasibility of definitive hemostasis intraoperatively. Subsequently, the frequency of blood transfusions after open surgery is relatively high - 4%. Due to the good hemostatic properties of the Thulium:YAG laser, the decrease in hemoglobin level was significantly lower - 1.2±0.4 g/l, and none of the patients included in the study required a blood transfusion. Similarly, there is a two-fold increase in the incidence of infectious complications after OP - 10.4% compared with 4.5% after ThuVEP. The data obtained during the study are similar to other researches found in specialized literature [4, 16]. This is due to the long-term urinary catheterization within the postoperative period. The presence of bacteriuria in patients was also assessed. The incidence of bacteriuria was reported significantly higher in patients with preoperative urinary catheterization, which directly affected the number of infectious complications in this category of patients. In the ThuVEP group, infectious complications occurred only in patients with preoperatively installed urinary catheters - 4.5% of patients, and in the OP group - 8% (compared to patients who urinate on their own - only 2%). Erectile dysfunction was more pronounced in patients who underwent OP, whereas full recovery occurred only in the ThuVEP group during the follow-up period. Postoperative sclerotic complications were also more common in patients after OP - 4% of cases developed bladder neck sclerosis during the follow-up period. Only one patient (2.2% of cases) experienced urethral stricture after ThuVEP. The results obtained in the study demonstrate the high efficacy of ThuVEP in combination with a significantly lower complication rate compared to OP, which have also been confirmed by other researchers [4, 17, 18].

Conclusions

Evaluation of patients under study during the postoperative period showed a significant progressive improvement in the patients' overall condition according to the IPSS scale and QoL in both groups. A positive evolution of Q_{max}, was also reported, characterized by a 122% improvement in maximum flow in the ThuVEP group and 109% in the OP group. Ultrasound examination showed a significant decrease in the total prostate volume and post-void residual urine volume. At the same time, a significantly lower complication rate was registered in the ThuVEP group (19.9%) compared with OP (35%). Postoperative recovery after ThuVEP was also significantly faster, being 5 days on average. Based on the obtained results, it can be assumed that laser transurethral vapoenucleation of the prostate will soon become the new "gold standard" of surgical treatment of large BPH.

Declaration of conflict of interests

Nothing to declare

Authors' contribution

All authors contributed equally to the research, data analysis, and writing of the manuscript. All authors read and approved the final article.

References

1. Lokeshwar S., Harper B., Webb E., *et al.* Epidemiology and treatment modalities for the management of benign prostatic hyperplasia. *Transl Androl Urol,* 2019; 8 (5): 529-539.
2. Vuichoud C., Loughlin K. Benign prostatic hyperplasia: epidemiology, economics and evaluation. *Can J Urol,* 2015; 22 Suppl 1: 1-6.
3. Egan K. The Epidemiology of Benign Prostatic Hyperplasia Associated with Lower Urinary Tract Symptoms: Prevalence and Incident Rates. *Urol Clin North Am,* 2016; 43 (3): 289-297.
4. Gunseren K., Akdemir S., Çiçek M., *et al*. Holmium Laser Enucleation, Laparoscopic Simple Prostatectomy, or Open

Prostatectomy: The Role of the Prostate Volume in terms of Operation Time. *Urol Int,* 2021; 105 (3-4): 285-290.
5. Long Z., Zhang Y., He L., Zhong K., Tang Y., Huang K. Comparison of transurethral plasmakinetic and transvesical prostatectomy in treatment of 100-149mL benign prostatic hyperplasia. *Asian J Surg,* 2014; 37 (2): 58-64.
6. Ofoha C., Raphael J., Dakum N., Shu'aibu S., Akhaine J., Yaki I. Surgical management of benign prostate hyperplasia in Nigeria: open prostatectomy versus transurethral resection of the prostate. *Pan Afr Med J,* 2021 May 1; 39.
7. Suer E., Gokce I., Yaman O., Anafarta K., Göğüş O. Open prostatectomy is still a valid option for large prostates: a high-volume, single-center experience. *Urology,* 2008; 72 (1): 90-94.
8. Ghicavii V., Plesacov A., Vladanov I. Transurethral Thulium laser vapoenucleation of prostate – a good alternative for open surgery. *International medical congress MedEspera 2020: The 8th International Medical Congress for Students and Young Doctors, 24-26 september, Chişinău, Rep Moldova: Abstract Book,* 2020; 436.
9. Herrmann T., Liatsikos E., Nagele U., Traxer O., Merseburger A. EAU Guidelines Panel on Lasers, Technologies. EAU guidelines on laser technologies. *Eur Urol,* 2012; 61 (4): 783-795.
10. Herrmann T. Enucleation is enucleation is enucleation is enucleation. *World J Urol,* 2016; 34 (10): 1353-1355.
11. Dellabella M., Castellani D. Anatomical Control of Adenoma Technique: An Accurate Surgical Approach to Thulium Laser Enucleation of the Prostate. *Urology,* 2018; 113:252.
12. Reichelt A., Suarez-Ibarrola R., Herrmann T., Miernik A., Schöb D. Laser procedures in the treatment of BPH: a bibliometric study. *World J Urol,* 2021; 39 (8): 2903–11.
13. Ghicavii V., Plesacov A., Vladanov I. Early outcomes of transurethral Thulium laser vapoenucleation of prostate. *International medical congress MedEspera 2020: The 8th International Medical Congress for Students and Young Doctors, 24-26 september, Chişinău, Rep Moldova: Abstract Book,* 2020; 436.
14. Becker B., Buttice S., Magno C., Gross A., Netsch C. Thulium Vaporesection of the Prostate and Thulium Vapoenucleation of the Prostate: A Retrospective Bicentric Matched-Paired Comparison with 24-Month Follow-Up. *Urol Int,* 2018; 100 (1): 105-111
15. Gross A., Orywal A., Becker B., Netsch C. Five-year outcomes of thulium vapoenucleation of the prostate for symptomatic benign prostatic obstruction. *World J Urol,* 2017; 35 (10): 1585-1593.
16. Nestler S., Bach T., Herrmann T., *et al.* Surgical treatment of large volume prostates: a matched pair analysis comparing the open, endoscopic (ThuVEP) and robotic approach. *World J Urol,* 2019; 37 (9): 1927-1931.
17. Leonardo C., Lombardo R., Cindolo L., *et al.* What is the standard surgical approach to large volume BPE? Systematic review of existing randomized clinical trials. *Minerva Urol Nefrol,* 2020; 72 (1): 22-29.
18. Lin Y., Wu X., Xu A., *et al*. Transurethral enucleation of the prostate versus transvesical open prostatectomy for large benign prostatic hyperplasia: a systematic review and meta-analysis of randomized controlled trials. *World J Urol,* 2016; 34 (9): 1207-1219.

Authors's ORCID ID:
Alexei Plesacov https://orcid.org/ 0000-0002-0139-4772
Ivan Vladanov https://orcid.org/ 0000-0002-9703-2775
Vitalii Ghicavii https://orcid.org/0000-0002-2130-9475

UDC: 616.36-008.811.6-074:618.2

RESEARCH ARTICLE

Assessment of the role of hematological inflammatory markers in the severity of intrahepatic cholestasis of pregnancy

Maria Cemortan[1†*], Olga Cernetchi[1†]

[1]Department of Obstetrics and Gynecology, Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova, Chisinau, Republic of Moldova

Manuscript received on: 15.04.2022
Accepted for publication on: 23.05.2022

Corresponding author:
Maria Cemortan, PhD student,
Department of Obstetrics and Gynecology
Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova
bd. Ştefan cel Mare şi Sfant 165, Chisinau, Republic of Moldova, MD-2004
e-mail: mariacemortan@yahoo.com

Short title: *Assessment of severity of ICP*

What is not known, yet about the topic: The role of hematological inflammatory markers in intrahepatic cholestasis of pregnancy.

Research hypothesis: The purpose of the study was to assess the role of hematological inflammatory markers in the severity of intrahepatic cholestasis of pregnancy.

Article's added novelty on this scientific topic: The study revealed the role of hematological inflammatory markers in intrahepatic cholestasis of pregnancy.

Abstract

Introduction. Hematological inflammatory markers may be promising diagnostic markers for assessing the severity of intrahepatic cholestasis of pregnancy. The aim of the study was to evaluate and compare the levels of hematological inflammatory markers in intrahepatic cholestasis of pregnancy.

Material and methods. A prospective cohort study of 142 clinical cases, divided into two groups depending on the presence of cholestasis gravidarum, was carried out. The research was conducted by assessing the level of bile acids and hematological inflammatory markers in the mother's blood, as well as by studying medical records. The IBM Statistics SPSS 21 program was used to process the statistical data. To compare categorical variables, the χ^2 without the Yates' continuity correction test was applied.

Results. There was an increase in platelet-to-lymphocyte ratio in pregnant women with intrahepatic cholestasis of pregnancy compared to the control group (146.0±6.8 versus 135.2±7.3, respectively). The values of neutrophil-to-lymphocyte ratio and the average volume of platelets were similar in both groups. At the same time, the study found a decrease in the values of erythrocyte distribution among women whose pregnancy was complicated by cholestasis gravidarum.

Conclusions. The study showed a significant increase in platelet-to-lymphocyte ratio in women whose pregnancy was complicated by cholestasis gravidarum, as well as an increase in this indicator with increasing levels of bile acids. Thus, this ratio may be a promising diagnostic marker in assessing the severity of intrahepatic cholestasis of pregnancy.

Keywords: pregnancy, childbirth, intrahepatic cholestasis of pregnancy, hematological inflammatory markers

Trial registration number

International Standard Randomised Controlled Trial Number ISRCTN21187408

Introduction

Intrahepatic cholestasis of pregnancy (ICP), also known as cholestasis gravidarum, is a liver disease with a global incidence of 0.5-1% [1]. The onset of cholestasis gravidarum is characterized by the appearance of cutaneous pruritus, typically on the palms and legs that cannot be explained by other factors [2].

The etiology and pathogenesis of cholestasis gravidarum are multifactorial, depending on environmental factors, hormonal changes, and genetic variations [3]. It should be

https://doi.org/10.52645/MJHS.2022.2.03

noted that the diagnosis of intrahepatic cholestasis of pregnancy is one of exclusion and should be differentiated from other pregnancy-related liver pathologies, which may have similar laboratory results, such as preeclampsia, acute fatty liver of pregnancy, and HELLP syndrome [4].

The standard diagnostic criterion in ICP remains the assessment of serum bile acid (BA) levels (BA ≥10 μmol/L) and liver function test (LFT) results [5]. The assessed serum BA levels are considered the definitive biochemical markers in the diagnosis of ICP, and they are also used to monitor the condition of patients [2]. Based on BA values, cholestasis gravidarum can be classified as being mild (BA 10-39 μmol/L) and severe (BA ≥40 μmol/L) [6]. In ICP, alanine aminotransferase values increase about 2-10 times, being a more sensitive marker of ICP compared to aspartate aminotransferase, the values of which do not significantly increase in women with this condition [7]. There is data in the literature suggesting increased total bilirubin levels in about 10% of cases complicated by ICP, although its values rarely exceed 85.5 μmol/L [8, 9]. Serum ɣ-glutamyltransferase levels decrease during pregnancy, while alkaline phosphatase activity increases due to placental isoenzyme production and increased bone isoenzyme activity. Nevertheless, simultaneous increases in ɣ-glutamyltransferase and alkaline phosphatase indicate liver pathology [10].

There is a discrepancy in the literature regarding the onset of ICP and whether it is signified by an increase in BA and LFT values or the onset of clinical symptoms. In some studies, the majority of patients were primarily diagnosed with increased BA levels before presenting clinical symptoms or other LFT changes [7]. Other studies have described cases of cholestasis gravidarum in which clinical symptoms have been observed at the onset of the pathology, with changes in BA and LFT values only occurring after 4-5 weeks [11]. Following delivery, clinical symptoms of cholestasis gravidarum resolve in most cases within 48 hours, with LFT values returning to normal over 2-8 weeks postpartum [3, 12].

Recent studies have demonstrated the prognostic role of hematological inflammatory markers in both cardiovascular diseases and malignancies [13, 14]. At the same time, there are few studies that have focused on studying hematological inflammatory markers in intrahepatic cholestasis of pregnancy [3]. Considering that bile acids are cytotoxic substances; their high values induce the release of proinflammatory mediators [15].

Some authors suggest that in ICP, women experience specific changes in hematological inflammatory markers, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), mean platelet volume (MPV), red cell distribution width – standard deviation (RDW-SD) and red cell distribution width – coefficient of variation (RDW-CV), although the results are inconclusive. Recently, NLR was found to be a prospective diagnostic marker in assessing the severity of ICP [16]. However, these data have not been refuted by other researchers who did not detect a difference between NLR values in women whose pregnancy was complicated by ICP compared to a control group [3]. Abide.Y. et al. discovered that MPV values increase significantly in cholestasis gravidarum, correlating with the pathology's severity [3]. Hence, studying hematological inflammatory markers may be a promising diagnostic method in assessing the severity of intrahepatic cholestasis of pregnancy.

Material and methods

A prospective study was conducted during 2020-2022 in three institutions, including the Department of Obstetrics and Gynecology of the Nicolae Testemitanu State University of Medicine and Pharmacy, the Institute of Mother and Child, and the Clinical Hospital „Gheorghe Paladi" in Chisinau, Republic of Moldova. The study protocol was approved by the Research Ethics Committee of the Nicolae Testemitanu State University of Medicine and Pharmacy on April 17, 2020.

The representative research sample was calculated using *EpiInfo 7.2.2.6 in the StatCalc Sample Size and Power* section based on the following parameters:

- Confidence interval for 95.0% significance of results;
- Statistical power - 80.0%;
- The difference in the course and outcome of pregnancy in pregnant women with intrahepatic cholestasis of pregnancy compared to pregnant women without ICP constitutes on average up to 20.0% [17];
- Ratio between the investigated groups = 1:1;
- Result: for the 95.0% CI the calculated value is 44 with 10.0% non-response rate, n=48;
- Therefore, there must be no less than 48 pregnant women with ICP in the research group;

Two groups were created for the prospective research:

- Group A - 71 pregnant women whose pregnancy was complicated by intrahepatic cholestasis of pregnancy (main group);
- Group B - 71 pregnant women whose pregnancies were not complicated by intrahepatic cholestasis of pregnancy (control group).

The research was carried out by assessing the BA levels and hematological inflammatory markers in the blood of participants, as well as by studying medical records (obstetric medical observation form no. 96/e). The diagnosis of ICP was established on the basis of anamnestic, clinical, and biochemical data. Biochemical analysis of blood was performed using an *Abbott Architect c8000* analyzer. The NLR and PLR values were calculated using standard formulas. NLR was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes [18, 19]. PLR was calculated by dividing the absolute number of platelets by the absolute number of lymphocytes [19]. The rest of the hematological markers were calculated automatically using an automated hematological analyzer - *SYSMEX XN-1000*.

The statistical data were processed using IBM Statistics SPSS 21 and the QuickCalcs section of GraphPad. Arithmetic mean and standard deviation (M (SD)) values were calculated to describe numerical indicators. To assess distributions of characteristics that differed from normal the median (Me) and the interquartile range (Q1; Q3) were cal-

culated. For comparison of categorical variables by groups, the χ^2 test without Yattes' continuity correction was used; a p-value<0.05 was considered statistically significant.

Results

The mean age was 29.5 (0.7) years (Me 30 (25; 34)) for women in group A and 27.3 (0.6) years (Me 27 (23; 31)) in group B. The age of the pregnant women included in the study ranged from 18-43 years, while 16/71 (22.5%) women in group A vs. 8/71 (11.3%) women in group B were aged over 35 years. All of the women in the study were included in their family doctor's register as having a current pregnancy, with the majority having seen their family doctor early in pregnancy. In group A, 41/71 (57.7%) women were multiparous, compared to 34/71 (47.8%) in group B. The study found that 15/142 (10.5%) pregnant women had developed cholestasis gravidarum in previous pregnancies, 14/71 (19.7%) of whom were in group A and 1/71 (1.4%) were in group B (χ^2 12.597, p=0.0004).

In pregnant women in group A, multiple pregnancies occurred in 8/71 (11.3%) cases, of which 6/8 were twin pregnancies and 2/8 were pregnancies with triplets. In the control group, multiple pregnancy occurred in 4/71 (5.6%) pregnant women (χ^2 1.456, p=0.2275), 2/4 cases being with twins and 2/4 with triplets. Of note is that 5/71 (7.0%) pregnancies in group A were achieved by in vitro fertilization (IVF) compared to 3/71 (4.2%) cases of IVF in group B (χ2 0.530, p=0.4667).

Cutaneous pruritus of varying location and intensity was experienced by all women in group A (Table 1), being the main clinical symptom of cholestasis gravidarum. At the same time, 32/71 (45.1%) women from group A reported skin pruritus simultaneously in several parts of the body. While some women from group B (7/71 (9.8%) experienced occasional skin pruritus, which in most cases was localized in the abdominal region, participants explained symptoms to have resulted from an increase in pregnancy term, topical application of different cosmetic substances, and discomfort caused by underwear.

***Table 1**. Location of cutaneous pruritis in women included in the study.*

	Location of cutaneous pruritis	Group A, n=71 (abs., %)	Group B, n=71 (abs., %)
1	Pruritus with localization on palms	25 (35.2%)	1 (1.4%)
2	Pruritus with localization on legs	23 (32.4%)	0
3	Pruritus with localization on abdomen	18 (25.4%)	5 (7.0%)
4	Generalized cutaneous pruritus	37 (52.1%)	1 (1.4%)

Note: the total percentage exceeds 100% as some women experienced more than one symptom at the same time.

In order to assess the severity of intrahepatic cholestasis of pregnancy, serum BA levels were assessed in women from both study groups. In group A, the BA levels ranged from 10 – 211.3 μmol/L, the mean value was 34.7 (4.3) μmol/L (Me 18.9 (11.1; 44.0)). Hence, mild ICP was found in 50/71 (70.4%) cases and severe ICP in 21/71 (29.6%) cases. The mean value of BA in group B was 3.3±0.1 μmol/L.

It was of interest to study the levels of hematological inflammatory markers in the women included in the study (Figure 1). Thus, mean NLR values were 4.7 (0.3) (Me 4.0 (2.9; 5.2)) in group A and 5.6 (0.5) (Me 4.0 (3.1; 6.1)) in group B. Mean PLR values were 146.0 (6.8) (Me 134.0 (110.2; 180.2)) in group A compared to 135.2 (7.3) (Me 124.7 (93.7; 158.2)) in group B. Mean values of MPV in group A were 11.6 (0.1) fl (Me 11.8 (11.0; 12.6)); in group B mean values of this marker were 13.3 (1.7) fl (Me 11.6 (10.9; 12.3)). The mean values of erythrocyte distribution indices were: RDW-SD 45.9 (0.9) fl (Me 45.4 (41.4; 49.5)) and RDW-CV 14.6 (0.5)% (Me 13.4 (12.8; 14.6)) in group A compared to RDW-SD 49.0 (0.7) fl (Me 48.8 (44.9; 53.1)) and RDW-CV 14.7 (0.1)% (Me 14.5 (13.6; 15.3)) in group B. Likewise, mean leukocyte (leuc.) values were 10.2 (3.3) $x10^3$/μL (Me 9.8 (7.9; 11.9)) in group A compared to group B, where they were 11.8 (3.8) $x10^3$/μL (Me 10.8 (8.7; 13.8)).

In order to assess the role of hematological inflammatory markers in the severity of intrahepatic cholestasis of pregnancy, women in group A were divided into two subgroups based on serum BA levels. The levels of hematological inflammatory markers in each subgroup were then analyzed (Table 2). A significant increase in PLR values was detected in severe ICP cases (159.3 (11.6)) compared to mild cases of the condition (140.4 (8.4)), correlating with the severity of the pathology.

Discussions

The findings are consistent with the literature, which shows that cholestasis gravidarum occurs more frequently in pregnant women with twin pregnancies than in singleton pregnancies (2.1% vs. 0.3%), also linked to an increased rate of ICP in IVF pregnancies (2.7%) [20, 21]. Some authors suggest that this pathology more often affects patients older than 35 years [22]. In the current study, 9.3% of women in the control group experienced cutaneous pruritus, whereas in the general population, according to literature data, up to 23% of pregnant women with physiological pregnancy experience cutaneous pruritus [11].

Assessment of BA levels is extremely important for patients with ICP, not only because of the maternal impact of the condition, but also in terms of perinatal outcomes related to cholestasis gravidarum. *Glantz A. et al.* reported an increased rate of adverse perinatal outcomes in pregnant women with serum BA levels >40 μmol/L [23]. The same study reported a 1-2% increase in the risk of spontaneous preterm birth, fetal asphyxia, or the presence of meconium in the amniotic fluid, with an increase for each additional μmol/L of BA above 40 μmol/L [9]. A recent study suggests that in cases of ICP with BA values >40 μmol/L, women give birth on average two weeks earlier than pregnant women in the control group [24]. In a recent meta-analysis of published studies, perinatal outcomes in pregnant women with ICP were evaluated and it was found that extremely high serum BA levels (>100 μmol/L) significantly increase the risk of intrauterine fetal death [25].

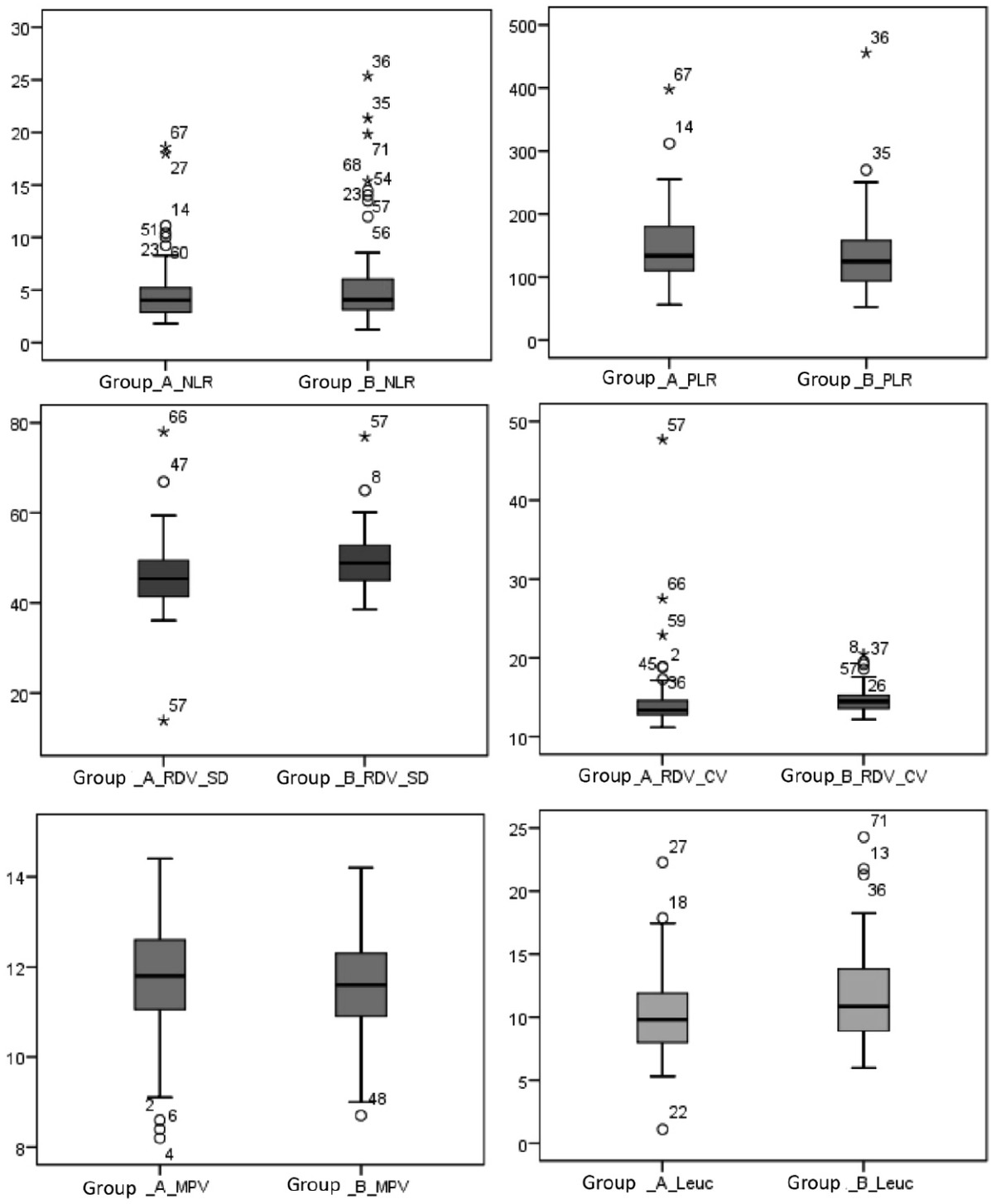


Fig. 1 *The level of hematological inflammatory markers in women included in the study.*

Note: NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MPV – mean platelet volume; RDV-SD – red cell distribution width – standard deviation; RDV-CV – red cell distribution width – coefficient of variation; leuc. – leucocyte.

Table 2. *The mean values of hematological inflammatory markers in women included in the study.*

	Hematological inflammatory markers	Group A n=71 Subgroup I Mild ICP n=50 M (SD) Me (Q1; Q2)	Subgroup II Severe ICP n=21 M (SD) Me (Q1; Q2)	Group B n=71 M (SD) Me (Q1; Q2)
1	NLR	4.8 (0.4) Me 3.9 (2.9; 5,4)	4.3 (0.5) Me 4.0 (2,3;5,0)	5.6 (0.5) Me 4.0 (3.1; 6.1)
2	PLR	140.4 (8,4) Me 126.4 (103.5; 170.7)	159.3 (11.6) Me 158.4 (119.,8; 184.3)	135.2 (7.3) Me 124.7 (93.7; 158.2)
3	MPV, fl	11.8 (0.1) Me 12.0 (11.1; 12.6)	11.3 (0.3) Me 11.5 (9.9; 12.7)	13.3 (1.7) Me 11.6 (10.9; 12.3)
4	RDW-SD, fl	46.2 (1.0) Me 44.9 (41.3; 49.4)	45.1 (1.9) Me 47.0 (41.7; 50.0)	49.0 (0.7) Me 48.8 (44.9; 53.1)
5	RDW-CV, %	14.2 (0.3) Me 13.4 (12.7; 14.6)	15.5 (1.6) Me 13.3 (12.8; 14.7)	14.7 (0.1) Me 14.5 (13.6; 15.3)

Note: NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MPV – mean platelet volume; RDV-SD – red cell distribution width – standard deviation; RDV-CV – red cell distribution width – coefficient of variation.

Despite the fact that the assessment of the NLR level is used to predict the severity of different pathologies, there is no conclusive opinion in the literature regarding NLR reference values, as they are dependent on several factors, such as age, gender, etc. However, there are studies in which authors have compared NLR and PLR levels in physiological pregnancies and those complicated by pre-eclampsia, reporting an increase in these parameters in cases of pre-eclampsia [26, 27]. However, the data presented is inconclusive, thus requiring further research.

The results of the study conducted by *Abide Ç. Y. et al.* showed that BA levels correlated positively and significantly with PLR (r=0.343, p=0.003) and women whose pregnancies were complicated by ICP showed significantly increased PLR values compared to the control group [3]. MPV, which is the most widely used measure of platelet size, is also an index of platelet activation. Platelets release thrombin, which plays a role in inflammation and angiogenesis. At the same time, a large platelet volume leads to increased coagulability and fibrinolysis [28, 29]. There are a limited number of studies that have focused on the correlation between MPV and ICP severity and on the correlation between MPV and perinatal outcomes, although an increase in MPV may be observed in women with ICP [3, 30]. Moreover, one study found that MPV correlates with ICP severity and could be a valuable marker for assessing the severity of the condition [3].

Conclusions

The study of hematological inflammatory markers showed a significant increase in platelet-to-lymphocyte ratio in women whose pregnancy was complicated by ICP. Moreover, the study data show an increase in PLR values in severe intrahepatic cholestasis of pregnancy. The values of NLR and MPV were similar in the group of women with ICP and in the control group. RDW-SD and RDW-CV values were lower in the group with cholestasis gravidarum compared to the control group. Thus, given these results, we can conclude that PLR can be a promising diagnostic marker in assessing the severity of ICP.

Declaration of conflict of interests

Nothing to declare

Authors' contributions

The authors have equally contributed to the manuscript drafting, design and editing. The final version of the manuscript was approved by all authors.

References

1. Ozkan S., Ceylan Y., Ozkan O.V., Yildirim S. Review of a challenging clinical issue: Intrahepatic cholestasis of pregnancy. In: *World J. Gastroenterol*. 2015; 21(23): 7134-7141. Disponibil pe: URL: http://www.wjgnet.com/1007-9327/full/v21/i23/7134.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i23.7134
2. Williamson C., Geenes V. Intrahepatic cholestasis of pregnancy. In: *Obstet. Gynecol.* 2014; 124: 120-133 [PMID: 24901263 DOI:10.1097/AOG.0000000000000346]
3. Abide Ç.Y., et al. Can we predict severity of intrahepatic cholestasis of pregnancy using inflammatory markers? In: *Turkish Journal of Obstetrics and Gynecology*. 2017; 14(3): 160.
4. Geenes V., Williamson C. Liver disease in pregnancy. In: *Best Pract. Res. Clin. Obstet. Gynecol*. 2015; 29: 612-624.
5. Kenyon A.P., Piercy C.N., Girling J., et al. Pruritus may precede abnormal liver function tests in pregnant women with obstetric cholestasis: a longitudinal analysis. In:

BJOG. 2001; 108: 1190-1192.

6. Smith D.D., Rood K.M. Intrahepatic Cholestasis of Pregnancy. In: *Clinical Obstetrics and Gynecology*. 2020; 63(1): 134-151.
7. Gabzdyl E.M., Schlaeger J.M. Intrahepatic cholestasis of pregnancy: a critical clinical review. In: *J. Perinat. Neonatal Nurs*. 2015; 29: 41-50. [PMID: 25633399 https://doi.org/10.1097/JPN.0000000000000077]
8. Wood Amber M., et al. Intrahepatic cholestasis of pregnancy: a review of diagnosis and management. In: *Obstetrical & Gynecological Survey*. 2018; 73(2): 103-109. Disponibil pe: https://doi.org/10.1097/ogx.0000000000000524
9. Geenes V., Williamson C., Chappell L.C. Intrahepatic cholestasis of pregnancy. In: *The Obstetrician & Gynaecologist.* 2016; 18(4): 273-281. Disponibil pe: https://doi.org/10.1111/tog.12308
10. Ammon F.J., Kohlhaas A., Elshaarawy O., et al. Liver stiffness reversibly increases during pregnancy and independently predicts preeclampsia. In: *World Journal of Gastroenterology.* 2018; 24(38): 4393. Disponibil pe: https://doi.org/10.3748/wjg.v24.i38.4393
11. Morton A., Laurie J. The biochemical diagnosis of intrahepatic cholestasis of pregnancy. In: *Obstetric Medicine*. 2019; 12(2): 76-78.
12. Brouwers L., Koster M.P., Page-Christiaens G.C., et al. Intrahepatic cholestasis of pregnancy: maternal and fetal outcomes associated with elevated bile acid levels. In: *Am. J. Obstet. Gynecol*. 2015; 212: 100.e1-e7.
13. Nunez J., Nunez E., Bodi V., et al. Usefulness of the neutrophil to lymphocyte ratio in predicting long-term mortality in ST segment elevation myocardial infarction. In: *Am. J. Cardiol*. 2008; 101: 747-752.
14. Zhang W.W., Liu K.J., Hu G.L., Liang W.J. Preoperative platelet/lymphocyte ratio is a superior prognostic factor compared to other systemic inflammatory response markers in ovarian cancer patients. In: *Tumour Biol*. 2015; 36: 8831-8837.
15. Cai S., Li M., Boyer J.L. The Role of Bile Acid-Mediated Inflammation in Cholestatic Liver Injury. In: *The Liver*. 2020; 728–736. Disponibil pe: doi:10.1002/9781119436812.ch56
16. Kirbas A., Biberoglu E., Daglar K., et al. Neutrophil-to-lymphocyte ratio as a diagnostic marker of intrahepatic cholestasis of pregnancy. In: *Eur. J. Obstet. Gynecol. Reprod. Biol*. 2014; 180: 12-15.
17. *Еремина* Е.Ю., *Машарова* А.А. *Внутрипеченочный холестаз у беременных*. В: *Экспериментальная и клиническая гастроэнтерология*. 2011; 6.
18. Forget P., Khalifa C., Defour JP., et al. What is the normal value of the neutrophil-to-lymphocyte ratio? In: *BMC Res. Notes*. 2017; 10: 12. Disponibil pe: https://doi.org/10.1186/s13104-016-2335-5
19. Dharmapuri S., et al. Predictive value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in advanced hepatocellular carcinoma patients treated with anti-PD-1 therapy. In: *Cancer Medicine*. 2020; 9(14): 4962-4970.
20. *Вороник Ю.Н., Мацюк Я.Р. Холестаз беременных: этиопатогенез*, лечение и прогноз (обзор). В: *Вестник Смоленской государственной медицинской академии*. 2018; 3.
21. Radu-Ionita F., Pyrsopoulos N.T., Jinga M., et al. (Eds.). *Liver Diseases*. 2020. Disponibil pe: doi:10.1007/978-3-030-24432-3
22. Manzotti C., et al. Total serum bile acids or serum bile acid profile, or both, for the diagnosis of intrahepatic cholestasis of pregnancy. In: *Cochrane Database of Systematic Reviews*. 2019; 7.
23. Glantz Anna, Hanns-Ulrich Marschall, Lars-*Åke* Mattsson. Intrahepatic cholestasis of pregnancy: relationships between bile acid levels and fetal complication rates. In: *Hepatology*. 2004; 40.2: 467-474.
24. Guszczynska-Losy M., et al. Evaluation of predictive value of biochemical markers for adverse obstetrics outcomes in pregnancies complicated by cholestasis. In: *Ginekologia Polska*. 2020; 91(5): 269-276.
25. Ovadia C., Seed P.T., Sklavounos A., et al. Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy with biochemical markers: results of aggregate and individual patient data meta-analyses. In: *Lancet*. 2019; 393: 899–909.
26. Kirbas A., Ersoy A.O., Daglar K., et al.. Prediction of preeclampsia by first trimester combined test and simple complete blood count parameters. In: *Journal of Clinical and Diagnostic Research*. 2015; 9: QC20–QC23.
27. Gezer C., Ekin A., Ertas I.E., et al. High first-trimester neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios are indicators for early diagnosis of preeclampsia. In: *Ginekologia Polska*. 2016; 87: 431–435.
28. Kisucka J., Butterfield C.E., Duda D.G., et al. Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage. In: *Proc. Natl. Acad. Sci. USA*. 2006; 103: 855-860.
29. Ross R. Atherosclerosis: an inflammatory disease. In: *N. Engl. J. Med*. 1999; 340: 115-126.
30. Oztas E., Erkenekli K., Ozler S., et al. Can routine laboratory parameters predict adverse pregnancy outcomes in intrahepatic cholestasis of pregnancy? In: *J. Perinat. Med.* 2015; 43: 667-674.

Authors's ORCID ID:

Maria Cemortan https://orcid.org/0000-0003-3137-7524

Olga Cerneţchi https://orcid.org/0000-0002-9229-8080

UDC: [616.98:578.834.1]-036.22(478)

RESEARCH ARTICLE

Clinical-epidemiological characteristics of adults hospitalized with Covid–19 in the Republic of Moldova

Galina Buta[1*†], Stela Cojocaru[2†], Raisa Puia[3†], Tudor Costru[4†], Maria Ciobanu[5†]

[1]Family medicine department, Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova
[2]Infectious diseases, tropical and parasitological department, Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova
[3]Nicolae Testemițanu Department of Social Medicine and Management, Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova
[4]Department of Therapeutic Dentistry, Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova
[5]Student of the Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova.

Date of the manuscript receipt: 11.04.2022
Date of publication acceptance: 12.05.2022

Corresponding author:
Galina Buta: PhD, assoc.prof.
Family medicine department
Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova, bd. Ștefan cel Mare și Sfânt, 165, Chișinău, Republic of Moldova, MD-2004
e-mail: galina.buta@usmf.md

Short title: *Peculiarities of Covid 19 infection in adults in Moldova*

What is not known yet, about topic

The impact of SARS CoV-2 infection on the health status of hospitalized patients with the COVID-19 in the Republic of Moldova; in particular: epidemiological data, clinical features, comorbid conditions, disease progression, mortality rate of the patients during the acute period of the disease.

Research hypothesis

COVID-19 has a nonspecific clinical manifestation with negative impact on the health of the infected patients, especially old people and those with chronic comorbidities.

Article's added novelty on this scientific topic

Old age, male sex, chronic cardiovascular diseases, diabetes mellitus, chronic kidneys diseases, and malignant tumors unfavorable influence the evolution of COVID-19. In providing a hospital-type medical care to the patients with COVID-19, the emphasis should be placed mainly on adults over the age of 50.

https://doi.org/10.52645/MJHS.2022.2.04

Summary

Introduction: The COVID-19 pandemic has a major negative impact on health and socio-economic well-being. Understanding the characteristics of COVID-19 disease and identifying the wide range of factors affecting health and quality of life can be the key to providing viable solutions to improve the management of patients and their physical and psycho-emotional rehabilitation. The purpose of the present study was to evaluate the influence of SARS CoV-2 infection on the health status of adults hospitalized with the diagnosis of COVID-19 in the Republic of Moldova.

Material and methods: The presented study is a retrospective, cohort, consisting of a sample of 7441 patients randomly selected, aged 18 y.o. and older, hospitalized in 10 public medical institutions in Chisinau, Moldova. Diagnosis of COVID-19 was confirmed by detection of CoV-2 SARS RNA. The data in the patients' medical records were processed and stored according to the unified, pre-established form, prepared in accordance with the requirements of the software „Electronic Patient Record COVID-19". The severity of COVID-19 disease was assessed using two principles: (1) according to the criteria of the National Clinical Protocol PCN-371; (2) according to the 7-point graduated scale developed by the WHO Special Committee (V.3.0, 3 March 2020) in randomized multicenter clinical trials.

Result: Only 30.07% patients mentioned the presence of a close contact with a COVID-19 positive person. The average age of the patients in the study was 52.83 years. Mild form was diagnosed in 5.00% of patients, medium - 66.15%, severe –20.67%, critical-8.18%. The main complaints of patients were fever, fatigue or physical asthenia, cough, and headache. More than 1/4 of those hospitalized have severe or critical forms of COVID-19; more than 1/3 - require oxygen therapy, and every 6-th patient needs non-invasive high-flow oxygen ventilation or mechanical ventilation. Old age, male sex, chronic comorbidities increase statistically significantly the probability of patients having an unfavorable prognosis in COVID-19. 7.93% of patients died, according to the age group: every 2-nd patient over 90 years, every 3-rd over 80 years, every 5-th over 70 years, and every 9-th over 60 years died.

Conclusions: (1) The uncertainty of the source of infection lead to delay specific prophylactic public health measures; (2) In COVID-19, in a hospital-type medical management, the emphasis should be placed mainly on patients over the age of 50; (3) There is no specific clinical manifestation in COVID-19, that would allow to distinguish the disease from other pathologies; (4) Age over 60 y.o., male sex, and chronic cardiovascular diseases, diabetes mellitus,

chronic kidneys diseases and malignant tumors unfavorable influence the evolution of COVID-19; (5) Antibiotic administration remains at a high level in hospitalized patients and is often unjustified and unnecessary.

Keywords: COVID-19, clinical, manifestations, comorbidities

Introduction

COVID-19 infection, that was declared by the World Health Organization (WHO) as pandemic in March 2020, cause a multitude of challenges in the public health system on national and international level. The consequences of the pandemic were felt by triggering an unprecedented crisis that adversely affects not only the physical health of population, but also mental welfare, and socioeconomic wellbeing [1, 2, 3]. Since the start of the pandemic, several variants of SARS-CoV-2 have been identified: alpha, beta, gamma, delta, omicron, each of them with different impact on health and healthcare system. SARS-CoV-2, like other RNA viruses, while adapting to new human hosts, is prone to genetic evolution, resulting in mutant variants that may have different characteristics than the original strains and threatens the progress made so far in limiting the spread of this disease [4]. Only understanding the characteristics of COVID-19 infection and identifying the wide range of factors affecting health and quality of life can be the key to providing viable solutions to improve the management of patients and their physical and psycho-emotional rehabilitation.

The evolution of COVID-19 varies from asymptomatic and mild infection to critical one and death, the most frequent clinical manifestations being fatigue, fever, cough, headache, dyspnea, anosmia, ageusia [5-9]. The presence of comorbidities such as diabetes, malignant tumors, immunosuppression, obesity, chronic renal failure, cardiovascular pathologies, as well as old age are often associated with a more severe course of the disease [10-17]. In aggressive clinical forms, the patients often develop such complications as acute respiratory distress, shock, myocardial damage, heart failure, acute kidney damage, etc. [18-23]. The risk of such complications as venous thromboembolism and pulmonary embolism may persist in some situations and in the post-acute period of COVID-19 [24-29].

The mortality rate during COVID-19 infection varies in different countries and regions, which may reflect differences in the age structure of the population, in the ability to test for suspected cases and seroprevalence, in the manner of reporting death cases, etc. [30-33]. The knowledge of the complexity of COVID-19 disease has evolved over time. Currently reaching the understanding that the risks that the infection presents do not end only at the stage of hospitalization. Acute disease survivors may have various long-term health problems, especially fatigue and cognitive dysfunction [34-40].

The purpose of the present study was to evaluate the impact of SARS CoV-2 infection on the health status of adults hospitalized with the diagnosis of COVID-19 in specialized clinics in the Republic of Moldova. The study examined epidemiological data, clinical features, comorbid conditions, disease progression, and mortality rate of the patients during the acute period of the disease.

Materials and methods

This study is a retrospective cohort, consisting of a sample of 7441 randomly selected patients aged 18 y/o and older, hospitalized with the diagnosis of COVID-19 in 10 public medical institutions in Chisinau municipality, Republic of Moldova, between March 1, 2020 and June 30, 2021. The diagnosis of COVID-19 was confirmed by the detection of SARS CoV-2 RNA by molecular biology tests. The data from the patients' medical records were processed and stored according to a unified, pre-established form, developed in accordance with the requirements of the „Covid-19 electronic patient record Register" software. The form included epidemiological, socio-demographic, clinical, paraclinical, laboratory data and information on therapeutic management.

The severity of COVID-19 disease was assessed based on two principles (1) according to the National Clinical Protocol and (2) according to the WHO special committee, that permitted to appreciate not only the need for additional oxygen supply, but also the type of oxygen therapy.

Assessment of the form of the disease according to the criteria of the National Clinical Protocol „New type coronavirus infection (COVID-19), PCN-371" [41]:

- mild form: patient with any signs and symptoms without shortness of breath, dyspnea or abnormal chest images (without pneumonia);
- moderate form: patient with fever and signs of non-severe pneumonia, no need for additional oxygen;
- severe form: patient with signs of severe pneumonia (significant dyspnea FR > 30 / min), or / and hypoxemia SpO2 < 94% at rest, or/and PaO2/FiO2 below 300 mmHg, or/and rapid negative evolution of lung imaging over the last 24-48 hours by more than 50%, or/and progressive decrease in peripheral lymphocyte count and rapid increase in lactate;
- critical form: patients with COVID-19 and one or more of the following: ARDS; shock; any organ failure, requiring care in the intensive care unit; other conditions with a major danger to the patient's life.

Assessment of the form of the disease according to the 7-point graded scale developed by the WHO special committee (V. 3.0; 3 March, 2020) in randomized multicenter clinical trials [42]:

- score 1: non-hospitalized patients without limitation in activity;
- score 2: non-hospitalized patients limited in activity, pre-COVID clinical status;
- score 3: hospitalized patients without need for oxygen therapy;
- score 4: hospitalized patients with oxygen therapy through the mask or nasal cannula;
- score 5: patients hospitalized with oxygen therapy with non-invasive high-flow oxygen ventilation;
- score 6: hospitalized patients with intubation and

mechanical ventilation oxygen therapy or extracorporeal membrane ECMO-oxygenation;
- score 7 (deceased patients).

In the present study, death from COVID-19 was defined as a death, that occurred in a patient with a confirmed case of COVID-19 except where there was another clear cause of death that cannot be related to COVID-19, and where there was no complete recovery period between the disease and the time of death, and where the death was not attributed to a pre-existing disease.

Results

The socio-demographic structure of the people in the study was as follows: from urban regions - 5730 (77.01%) patients, from rural regions - 1711 (22.99%) patients, employed - 4051 (54.44%) patients, retired - 1861 (25.01%) patients, unemployed - 1144 (15.37%) patients, disabled - 293 (3.94%) patients, pupils/students - 92 (1.24%) patients. At the time of hospitalization, 325 (4.37%) patients had no medical insurance.

The patients got referrals to the hospitalization by the Emergency Medical Assistance team in 2948 (39.61%) cases; by the medical specialists - 199 (2.67%) cases, by the family doctor - 171 (2.29%) cases. The patients were hospitalized by means of the transfer from the COVID center, or any other healthcare institutions in 3065 (41.19%) cases. On personal address to the inpatient wards were admitted 1058 (14.21%) patients.

For the last 14 days before the illness only 2234 (30.07%) patients mentioned the presence of a close contact with a COVID-19 positive person, and only 67 (0.9%) patients traveled outside the country. Thus, in the absence of the epidemiological clue, COVID-19 disease was the referral diagnosis only in 3360 (45.15%) cases.

The average age of the patients in the study was 52.83 years, with the prevalence of the 50-59 age group - 20.41% and 60-69 age group - 25.88%. The female gender predominated in the total group – 65.42% (4868 patients), with the male / female ratio below 1 in all age groups (Figure 1).

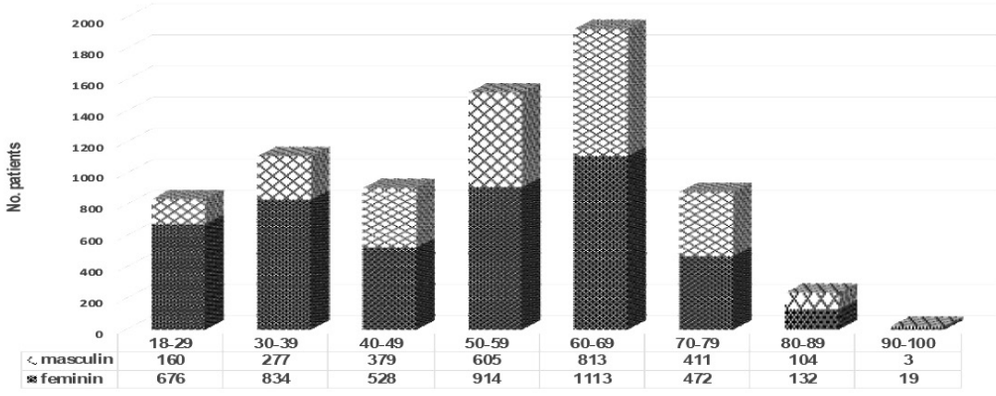


Fig. 1 *Distribution of adults in the study by age group and gender.*

The form of the disease ranged from mild to critical. Mild form was diagnosed in 372 (5,0%) patients, medium - in 4922 (66.15%), severe - 1538 (20.67%), critical - 609 (8.18%) patients (Figure 2). It is essential to specify that the dynamics and profile of hospitalized patients with COVID-19 in the Republic of Moldova has evolved over time according to the severity of the pandemic. Thus, in the first stage, all patients with the confirmed diagnosis of COVID-19 were hospitalized, regardless of age and severity of illnesses (PCN-371 Edition I). In the later stages, starting from mid-June 2020, hospitalization was not mandatory for adults aged 18-60 with asymptomatic and mild forms, without comorbidities and major risk of deterioration (PCN-371 editions II-V). The male/female ratio was under 1 for the mild (0.65), medium (0.51) and severe (0.67) clinical forms, but over 1 in the critical patients (1.08), as seen in Figure 2.

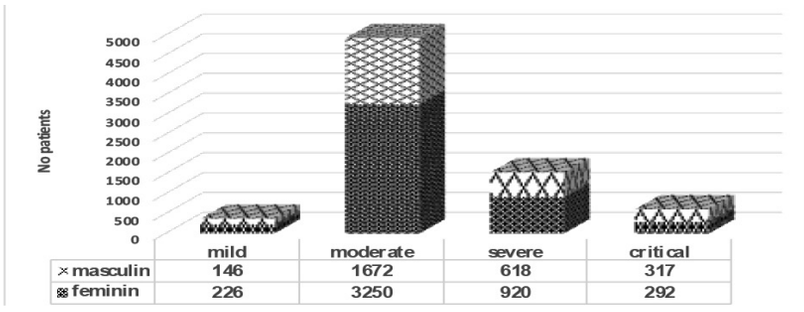


Fig. 2. *Distribution of patients by disease form, according to National Clinical Protocol „New type coronavirus infection (COVID-19), PCN-371" criteria, and gender.*

The health impairment of the patients were categorized also according to the graded scale developed by the WHO special committee in randomized multicenter clinical trials that allows assessing the severity of the disease focusing mainly on the need of patients for different types of oxygen therapy. Thus, a score of 3 (no oxygen therapy) had 4429 (59.52%) patients, male/female ratio – 0.50; a score of 4 (oxygen therapy via face mask or nasal cannula) had 1819 (24.45%) patients, male/female ratio – 0.63; a score of 5 (oxygen therapy in ventilation, non-invasive, with the oxygen flow rate increased) - 152 (2.04%) patients, male/female ratio – 0.81; a score of 6 (oxygen therapy by intubation and mechanical ventilation or ECMO) had 451 (6.06%) patients, male / female – 0.76; a score of 7 (deceased) - 590 (7.93%) patients, male/female ratio – 1.11 (Figure 3).

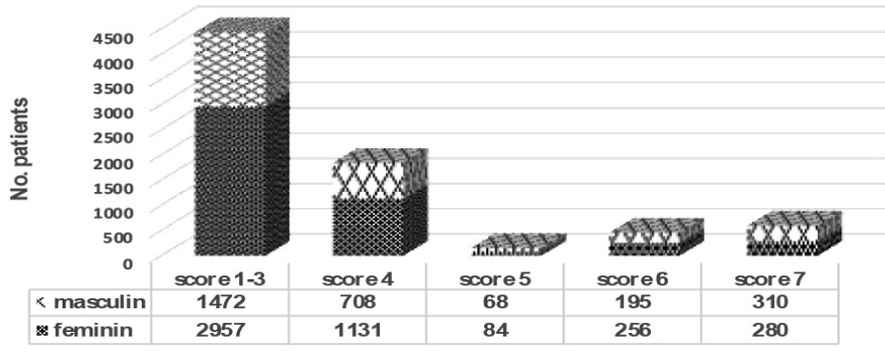


Fig. 3. *The degree of impairment of the health status of patients according to the graded scale developed by the WHO special committee in randomized multicenter clinical trials.*

The average age of patients with the mild form of the disease was 42.60 years, moderate form – 50.08 years, severe – 58.02 years, critical – 68.17 years. In general, patients who developed a milder form of the disease had a lower age compared to those who had a more severe course (Table 1).

Table 1. *Distribution of patients by age group and disease severity form according to National Clinical Protocol.*

	Age group 18-29 (n=836) n (%)	Age group 30-39 (n=1111) n (%)	Age group 40-49 (n=409) n (%)	Age group 50-59 (n=1519) n (%)	Age group 60-69 (n=1926) n (%)	Age group 70-79 (n=883) n (%)	Age group 80-89 (n=236) n (%)	Age group 90-99 (n=23) n (%)
Mild	93 (11.12%)	82 (7.38%)	64 (7.05%)	79 (5.20%)	40 (2.07%)	13 (1.47%)	1 (0.42%)	0
Moderate	675 (80.74%)	889 (80.01%)	639 (70.45%)	993 (65.37%)	1189 (61.73%)	453 (51.30%)	82 (34.74%)	2 (8.69%)
Severe	66 (7.89%)	130 (11.7%)	118 (20.72%)	363 (23.89%)	477 (24.76%)	234 (26.50%)	70 (29.66%)	10 (43.47%)
Critical	2 (0.23%)	10 (0.9%)	16 (1.76%)	84 (5.52%)	220 (11.42%)	183 (20.72%)	83 (35.16%)	11 (47.82%)

After completing the treatment in the hospital were discharged home 6575 (88.36%) patients, transferred to other public medical and health institutions - 276 (3.70%) patients, died of COVID-19 disease - 590 (7.92%) patients. Thus, each 8-th hospitalized person in the study lot died. The average age of the deceased was 68.37 years, the majority belonging to the age group 60-69 (35.59%) and 70-79 (30.67%). Analyzing the share of the deceased in each age group, the following profile was formed: 18-29 y/o - 2 patients out of 836 died; 30-39 y/o - 10 out of 1111; 40-49 y/o - 15 out of 907; 50-59 y/o - 79 out of 1519; 60-69 y/o - 210 out of 1926; 70-79 y/o - 181 out of 883; 80-89 y/o 82 - out of 236; 90-99 y/o - 11 out of 23 (Figure 4).

The male gender had a negative impact upon the cases of the deceased patients, male/female ratio – 1.1 (310/280), while among the survivors the female sex prevailed: male/female ratio in severe form – 0.67 (618/920), in the medium form – 0.51 (1672/3250), in the mild form – 0.64 (146/226). Similar data of disaggregation by gender with the case-fatality rate greater than 1 for the male sex have been reported by several researchers from different countries affected by COVID-19 [43-46].

During hospitalization the main complaints of patients were: fever - 5742 (77.16%) patients, fatigue or physical asthenia - 6613 (88.87%), cough - 5467 (73.47%), and headache - 4063 (54.60%) patients. Such clinical manifestations as pharyngitis, ageusia and hypo/anosmia were more often complained by young people in the age group 18-29 and 30-39, while such manifestations as dyspnea and vertigo - in people aged over 40, and the newly emerging behavioral changes - in those aged over 70 (Table 2).

Table 2. *The most common clinical manifestations during the course of the disease depending on the age group (%)*

Clinical manifestation	Age group 18-29 (n=836) n (%)	Age group 30-39 (n=1111) n (%)	Age group 40-49 (n=907) n (%)	Age group 50-59 (n=1519) n (%)	Age group 60-69 (n=1926) n (%)	Age group 70-79 (n=883) n (%)	Age group 80-89 (n=236) n (%)	Age group 90-99 (n=23) n (%)
Ageusia	173 (20.69%)	207 (18.63%)	92 (10.14%)	111 (7.31%)	127 (6.59%)	45 (5.10%)	6 (2.54%)	2 (8.70%)
Arthralgia	30 (3.59%)	55 (4.95%)	72 (7.94%)	126 (8.29%)	172 (8.93%)	84 (9.51%)	15 (6.36%)	2 (8.7%)
Behavior changes	3 (0.36%)	4 (0.36%)	14 (1.43%)	12 (0.79%)	31 (1.61%)	29 (3.28%)	9 (3.81%)	4 (17.39%)
Cough	533 (63.76%)	785 (70.66%)	675 (74.42%)	1169 (76.96%)	1459 (75.75%)	652 (73.84%)	181 (76.69%)	13 (56.52%)
Diarrhea	24 (2.87%)	39 (3.51%)	33 (3.64%)	49 (3.23%)	63 (3.27%)	21 (2.38)	90 (3.81%)	0.00
Dyspnea	168 (20.10%)	341 (30.69%)	487 (53.69%)	945 (62.21%)	1308 (67.91%)	604 (68.40%)	173 (73.31%)	13 (56.52%)
Fatigue/asthenia	726 (86.84%)	944 (84.97%)	80 (88.20%)	1385 (91.18%)	1749 (90.81%)	786 (89.01%)	204 (86.44%)	19 (82.1%)
Fever <38.0°C	515 (61.60%)	676 (60.85%)	512 (56.45%)	878 (57.80%)	1111 (57.68%)	528 (59.80%)	129 (58.90%)	3 (13.04%)
Fever >38.0°C	110 (13.16%)	196 (17.64%)	205 (22.60%)	304 (20.01%)	359 (18.64%)	156 (17.67%)	37 (15.68%)	13 (56.52%)
Headache	477 (57.06%)	653 (58.78%)	478 (52.70%)	815 (53.65%)	1025 (53.22%)	471 (53.34%)	133 (56.36%)	11 (47.83%)
Hypo/anosmia	286 (34.21%)	346 (31.14%)	135 (14.88%)	145 (9.87%)	171 (8.88%)	51 (5.78%)	8 (3.39%)	2 (8.70%)
Myalgia	132 (15.79%)	212 (19.08%)	237 (26.13%)	346 (22.78%)	4280 (22.22%)	178 (20.16%)	42 (17.80%)	3 (13.04%)
Pharyngitis	451 (53.95%)	501 (45.09%)	206 (22.71%)	236 (15.54%)	261 (13.55%)	92 (10.42%)	23 (9.75%)	2 (8.70%)
Vertigo	32 (3.83%)	53 (4.77%)	87 (9.59%)	142 (9.35%)	177 (9.19%)	101 (11.44%)	21 (8.90%)	2 (8.70%)

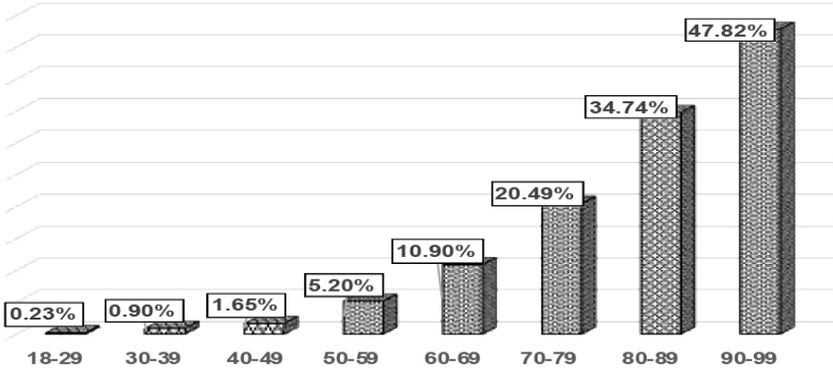


Fig. 4. *Share of the deceased patients in each age group.*

The patients who have died of COVID-19 (590 patients) suffered from various chronic diseases such as hypertension - 124 (21.01%) patients, chronic ischemic heart diseases - 164 (27.79%), disorders of the heart rate - 78 (13.22%), chronic heart failure - 165 (27.96%), diabetes mellitus - 229 (38.81%), chronic kidney diseases - 143 (24.23%); chronic liver diseases - 89 (15.08%), chronic anemia - 46 (7.79%), chronic lower respiratory tract diseases - 35 (5.93%), malignant tumors - 19 (3.22%), advanced degree of obesity - 18 (3.05%). Chronic comorbidities of survivors discharged at home (6575 patients) were as follows: hypertension diseases - 1374 (20.90%) patients; chronic ischemic heart diseases - 687 (10.44%), disorders of the heart rate - 261 (3.96%), chronic heart failure - 1293 (19.66%), diabetes mellitus

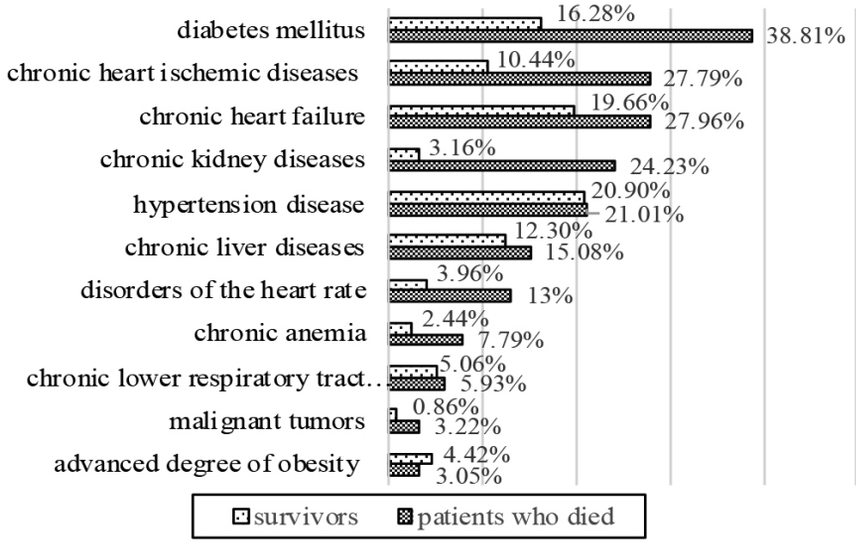


Fig. 5. *Structure of chronic comorbidities in survivors and in deceased patients*

- 1071 (16.28%), chronic liver diseases - 809 (12.30%), chronic kidney diseases - 208 (3.16%), chronic lower respiratory tract diseases 333 (5.06%), advanced degree of obesity - 291 (4,42%), chronic anemia - 161 (2.44%), malignant tumors - 57 (0.86%) patients (Figure 5). Thus, the presence of such chronic comorbidities as diabetes mellitus, chronic heart failure, chronic ischemic heart diseases, heart rate disorders, chronic kidney diseases, malignant tumors, chronic anemia – increase statistically significantly the probability of patients having an unfavorable prognosis in COVID-19 (P = 0.0001 in the Fisher test).

Antibiotic treatment was administered in 6819 (91.64%) cases, including: 359 (5.26%) patients with no lung imaging pathologies detected during the disease and 253 (3,71%) patients diagnosed with mild form of the disease.

The average duration of hospitalization varied depending on the form of the disease and the age of the patients. The fewest days of hospitalization - 13 days had patients aged under 40 years, while the duration of hospitalization in patients aged over 40 years was 15-16 days. The mean duration of hospitalization in deceased patients was 12 days. It should be mentioned that the duration of hospitalization, especially in mild and moderate severity cases, was influenced by the discharge criteria stipulated in the orders and recommendations in force that evolved over time according to the severity of the pandemic and the capacities of the health system (PCN-371 editions I-V).

In COVID-19 the quality of life is affected not only during the acute period of the disease, but also long after. People who were discharged at home continued to complained of different clinical manifestations such as fatigue, physical asthenia, cough, headache, dyspnea, myalgia, sub-febrility in 2646 (40.25%) cases. Out of 3929 patients who had no complaints at discharge in 2207 (56.17%) cases pulmonary imaging (Rx or CT) were abnormal.

Discussions

The source of SARS-CoV-2 infection remains unrevealed in most cases. Thus, out of the 7441 COVID-19 patients included in the study less than ⅓ mentioned the presence of contact with a COVID-19 positive. According to the WHO recommendations, the identification of the source of infection by case investigation „backward tracing" is essential to detect the transmission chains of SARS-CoV-2 [47]. Respectively, in more than ⅔ of the cases, neither the source of infection nor the risk factors that would favor the transmission of the infection to the patients were identified, leading to the delaying in applying of anti-epidemic prophylactic measures in general population. The similarity of clinical manifestations of COVID-19 with other respiratory infections in the absence of epidemiological connection with the source of infection also explains that in more than ½ of the cases the referral diagnosis was other than COVID-19 infection.

More than ¼ of those hospitalized have severe or critical forms of COVID-19 and more than ⅓ of patients require oxygen therapy, and every 6-th patient needs non-invasive high-flow oxygen ventilation or mechanical ventilation.

Patients aged over 50 years tend to have more severe clinical manifestations and a higher fatality rate, which means that they require more careful monitoring and more comprehensive medical interventions. Thus, every 9-th patient over 60 years, every 5-th patient over 70 years, every 3-rd patient over 80 years, 2-nd patient over 90 years died. According to our study, the proportion of patients who died (7.93%) was higher compared to some studies in other countries.

The non-specific complains of the patients with COVID-19 do not allow, without special laboratory investigations, their differentiation from other pathologies of the

respiratory tract. Most patients manifest physical fatigue or asthenia, fever, cough, and each 2-nd - headache. Such complaints as pharyngitis, ageusia, and hypo/anosmia are characteristic mainly of young people, and dyspnea and behavioral changes - of the elderly. Disorders of the gastrointestinal system caused by COVID-19 are insignificant in number and intensity in all age groups.

The presence of certain comorbidities such as diabetes mellitus, chronic heart failure, chronic ischemic heart diseases, heart rhythm disorders, chronic kidney diseases, malignant tumors, chronic anemia increase significantly the likelihood of patients having an unfavorable prognosis in COVID-19 (deceased vs survivors). Thus, the vaccination for COVID-19 should not be delayed in this category of patients. Surprisingly, in our study, chronic lower respiratory tract diseases do not influence dramatically the evolution of COVID-19, as was suggested previously.

Antibiotics were administered empirically, without laboratory validation, in majority of the hospitalized patients. In pandemic period, when there is a very high level of hospitalization, the excessive and unjustified administration of antibiotics can become dangerous, leading to different complication, including infection with *Clostridium difficile*, which can only aggravate the evolution of COVID-19 disease.

Discharge from the hospital does not mean recovery for majority of the patients, who continue to present different clinical manifestations, imaging and paraclinical pathologies. There should be additional investigations regarding clinical manifestation of Long-Covid and duration of the recovery period of the hospitalized patients. In COVID-19 the favorable prognosis and complete recuperation at discharge is most commonly associated with young age under 40 years, female sex and the lack of chronic comorbidities.

Conclusions

1. The uncertainty of the source of infection lead to delay specific anti-epidemic prophylactic public health measures, contributing to uncontrolled spread of COVID-19 and maintaining pandemic situation.
2. In providing a hospital-type medical care to the patients with COVID-19 the emphasis should be placed mainly on adults over the age of 50.
3. There is no specific clinical manifestation in COVID-19, characteristic for majority of the patients, that would allow to distinguish the disease from other pathologies, without special laboratory investigations.
4. Age over 60, male sex, chronic cardiovascular diseases, diabetes mellitus, chronic kidneys diseases, and malignant tumors unfavorable influence the evolution of COVID-19.
5. Antibiotic administration remains at a high level in hospitalized patients and is often unjustified and unnecessary.
6. The most of the discharged patients need supplementary period to complete recovery.

Declaration of conflicting interests

Authors certify the absence of conflict of interests.

Authors' contributions

All authors contributed equally to the research, data analysis, and writing of the manuscript. Final manuscript was read and approved by all authors

References

1. Haldane V., De Foo C., Abdalla S.M., *et al.* Health systems resilience in managing the COVID-19 pandemic: lessons from 28 countries. *Nature Medicine*, 2021; 27 (6): 964-980. doi: 10.1038/s41591-021-01381-y.
2. Akay A. The local and global mental health effects of the Covid-19 pandemic. *Econ Hum Biol.*; 2022; 45:101095. doi: 10.1016/j.ehb.2021.101095.
3. Hasan M.Z., Neill R., Das P., *et al.* Integrated health service delivery during COVID-19: a scoping review of published evidence from low-income and lower-middle-income countries. *BMJ Glob Health*, 2021; 6 (6): e005667. doi: 10.1136/bmjgh-2021-005667.
4. Long B., Carius B.M., Chavez S., *et al.* Clinical update on COVID-19 for the emergency clinician: Presentation and evaluation. *Am. J. Emerg. Med.;* 2022; 54: 46-57. doi: 10.1016/j.ajem.2022.01.028.
5. Tsai P.H., Lai W.Y., Lin Y.Y., *et al.* Clinical manifestation and disease progression in COVID-19 infection. *Journal of the Chinese Medical Association*; 2021; 84 (1): 3-8. doi: 10.1097/jcma.0000000000000463.
6. Koupaei M., Mohamadi M.H., Yashmi I., *et al.* Clinical manifestations, treatment options, and comorbidities in COVID-19 relapse patients: A systematic review. *J. Clin. Lab. Anal.;* 2022; Apr 8:e24402. doi: 10.1002/jcla.24402.
7. Blair P.W., Brown D.M., Jang M., *et al.* The Clinical Course of COVID-19 in the Outpatient Setting: A Prospective Cohort Study. *Open Forum Infectious Diseases;* 2021; 8 (2): ofab007. doi: 10.1093/ofid/ofab007.
8. Sung H.K., Kim J.Y., Heo J., *et al.* Clinical Course and Outcomes of 3,060 Patients with Coronavirus Disease 2019 in Korea, January-May 2020. *Journal of Korean Medical Science;* 2020; 35 (30): e280. doi: 10.3346/jkms.2020.35.e280.
9. Guan W.J., Ni Z.Y., Hu Y., *et al.* Clinical Characteristics of Coronavirus Disease 2019 in China. *New English Journal of Medicine;* 2020; 382 (18): 1708-1720. doi: 10.1056/NEJMoa2002032.

10. Sanyaolu A., Okorie C., Marinkovic A., *et al.* Comorbidity and its Impact on Patients with COVID-19. *SN Comprehensive Clinical Medicine*; 2020; 2 (8): 1069-1076. doi: 10.1007/s42399-020-00363-4.
11. Kaur H., Thakur J.S., Paika R., *et al.* Impact of Underlying Comorbidities on Mortality in SARS-COV-2 Infected Cancer Patients: A Systematic Review and Meta-Analysis. *Asian Pac. J. Cancer Prev.*; 2021; 22 (5): 1333-1349. doi: 10.31557/APJCP.2021.22.5.1333.
12. Singh A.K., Gupta R., Ghosh A., *et al.* Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. *Diabetes Metab. Syndr.*; 2020; 14 (4): 303-310. doi: 10.1016/j.dsx.2020.04.004.
13. Li J., Huang D.Q., Zou B., *et al.* Epidemiology of COVID-19: A systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes. *J. Med. Virol.;* 2021; 93 (3): 1449-1458. doi: 10.1002/jmv.26424.
14. Cheng Y., Luo R., Wang K., *et al.* Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.;* 2020; 97 (5): 829-838. doi: 10.1016/j.kint.2020.03.005.
15. Hessami A., Shamshirian A., Heydari K., *et al.* Cardiovascular diseases burden in COVID-19: Systematic review and meta-analysis. *Am. J. Emerg. Med.;* 2020; S0735-6757(20)30908-6. doi: 10.1016/j.ajem.2020.10.022.
16. Holban T., Cojocaru S., Iarovoi L., Băstrițchi I., Russu I., Potâng-Raşcov V. Manifestările clinice şi particularităţile evolutive în infecţia COVID-19 (review). *Moldovan Journal of Health Science*. Chişinău, vol 24 (2), 2020, 70-83. ISSN ISSN 2345-1467.
17. Ng W.H., Tipih T., Makoah N.A., *et al.* Comorbidities in SARS-CoV-2 Patients: a Systematic Review and Meta-Analysis. *mBio.*; 2021; 12 (1): e03647-20. doi: 10.1128/mBio.03647-20.
18. Wang Y., Lu X., Li Y., *et al.* Clinical Course and Outcomes of 344 Intensive Care Patients with COVID-19. *Respir. Crit. Care Med.;* 2020; 201 (11): 1430-1434. doi: 10.1164/rccm.202003-0736LE.
19. Yang X., Yu Y., Xu J., *et al.* Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir. Med.*; 2020; 8 (5): 475-481. doi: 10.1016/S2213-2600(20)30079-5.
20. Contou D., Cally R., Sarfati F., *et al.* Causes and timing of death in critically ill COVID-19 patients. *Crit. Care*; 2021; 25 (1): 79. doi: 10.1186/s13054-021-03492-x.
21. Roquetaillade C., Bredin S., Lascarrou J.B., *et al.* Timing and causes of death in severe COVID-19 patients. *Crit. Care.*; 2021; 25 (1): 224. doi: 10.1186/s13054-021-03639-w.
22. Elezkurtaj S., Greuel S., Ihlow J., *et al.* Causes of death and comorbidities in hospitalized patients with COVID-19. *Sci. Rep.;* 2021; 11 (1): 4263. doi: 10.1038/s41598-021-82862-5.
23. Zhou F., Yu T., Du R., *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*; 2020; 395 (10229): 1054-1062. doi: 10.1016/S0140-6736(20)30566-3.
24. Elsoukkary S.S., Mostyka M., Dillard A., *et al.* Autopsy Findings in 32 Patients with COVID-19: A Single-Institution Experience. *Pathobiology*; 2021; 88(1): 56-68. doi: 10.1159/000511325.
25. Satturwar S., Fowkes M., Farver C., *et al.* Postmortem Findings Associated With SARS-CoV-2: Systematic Review and Meta-analysis. *Am. J. Surg. Pathol.*; 2021; 45 (5): 587-603. doi: 10.1097/PAS.0000000000001650.
26. Suh Y.J., Hong H., Ohana M., *et al.* Pulmonary Embolism and Deep Vein Thrombosis in COVID-19: A Systematic Review and Meta-Analysis. *Radiology*; 2021; 298 (2): E70-E80. doi: 10.1148/radiol.2020203557.
27. Jevnikar M., Sanchez O., Humbert M., *et al.* Prevalence of pulmonary embolism in patients with COVID-19 at the time of hospital admission and role for pre-test probability scores and home treatment. *Eur. Respir. J.*; 2021; 2101033. doi: 10.1183/13993003.01033-2021.
28. Van Twist D.J.L., Luu I.H.Y., Kroon F.P.B., *et al.* Pulmonary Embolism in COVID-19: The Actual Prevalence Remains Unclear. *Radiology*; 2021; 299 (2): E254. doi: 10.1148/radiol.2021204671.
29. Malas M.B., Naazie I.N., Elsayed N., *et al.* Thromboembolism risk of COVID-19 is high and associated with a higher risk of mortality: A systematic review and meta-analysis. *E.Clinical Medicine*; 2020; 100639. doi: 10.1016/j.eclinm.2020.100639.
30. Ioannidis J. Infection fatality rate of COVID-19 inferred from seroprevalence data. *Bull. World Health Organ*; 2021; 99 (1): 19-33F. doi: 10.2471/BLT.20.265892.
31. Karlinsky A., Kobak D. The World Mortality Dataset: Tracking excess mortality across countries during the COVID-19 pandemic. *medRxiv;* 2021; 2021.01.27.21250604. doi: 10.1101/2021.01.27.21250604.
32. Islam N., Shkolnikov V.M., Acosta R.J., *et al.* Excess deaths associated with covid-19 pandemic in 2020: age and sex disaggregated time series analysis in 29 high income countries. *B.M.J.*; 2021; 373: n1137. doi: 10.1136/bmj.n1137.
33. Fountoulakis K.N., Fountoulakis N.K., Koupidis S.A., *et al.* Factors determining different death rates because of the COVID-19 outbreak among countries. *J. Public Health (Oxf.);* 2020; 42 (4): 681-687. doi:10.1093/pubmed/fdaa119.
34. Nalbandian A., Sehgal K., Gupta A., *et al.* Post-acute COVID-19 syndrome. *Nat. Med.;* 2021; 27 (4): 601-615. doi: 10.1038/s41591-021-01283-z.
35. Darcis G., Bouquegneau A., Maes N., *et al.* Long-term clinical follow up of patients suffering from moderate to severe COVID-19 infection: A monocentric prospective observational cohort study; *J. Infect. Dis.;* 2021; 109: 209–16. doi: 10.1016/j.ijid.2021.07.016.
36. Bourmistrova N.W., Solomon T., Braude P., *et al*. Long-term effects of COVID-19 on mental health: A systematic review. *J. Affect. Disord*.; 2022; Feb 15; 299: 118-125. doi: 10.1016/j.jad.2021.11.031.
37. Lopez-Leon S., Wegman-Ostrosky T., Perelman C., *et al.* More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. *Sci. Rep.*; 2021; 11 (1):16144. doi: 10.1038/s41598-021-95565-8.

38. Davis H.E., Assaf G.S., McCorkell L., *et al.* Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *E. Clinical Medicine*; 2021; Jul 15: 101019. doi: 10.1016/j.eclinm.2021.101019.
39. Beghi E, Helbok R, Ozturk S, *et al*. Short- and long-term outcome and predictors in an international cohort of patients with neuro-COVID-19. *Eur. J. Neurol.*; 2022; 29 (6): 1663-1684. doi: 10.1111/ene.15293.
40. Drake T.M., Riad A.M., Fairfield C.J., *et al.* Characterisation of in-hospital complications associated with COVID-19 using the ISARIC WHO Clinical Characterisation Protocol UK: a prospective, multicentre cohort study. *Lancet*; 2021; 398 (10296): 223-237. doi: 10.1016/S0140-6736(21)00799-6.
41. MSMPS, „Infecția cu coronavirus de tip nou (Covid-19), PCN 371," https://msmps.gov.md/.
42. Ader F., Discovery French Trial Management Team. Protocol for the DisCoVeRy trial: multicentre, adaptive, randomised trial of the safety and efficacy of treatments for COVID-19 in hospitalised adults. *B.M.J.*; 2020; 10 (9): e041437. doi: 10.1136/bmjopen-2020-041437.
43. Thompson K., Vassallo A., Finfer S., Woodward M. Renewed rationale for sex- and gender-disaggregated research: A COVID-19 commentary review. *Womens Health (Lond.)*; 2022;18:17455065221076738. doi:10.1177/17455065221076738.
44. Jin J.M., Bai P., He W., *et al.* Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. *Front Public Health*; 2020; Apr. 29; 8:152. doi: 10.3389/fpubh.2020.00152.
45. Gomez J.M.D., Du-Fay-de-Lavallaz J.M., Fugar S., *et al.* Sex Differences in COVID-19 Hospitalization and Mortality. *J. Womens Health (Larchmt)*; 2021; 30 (5): 646-653. doi: 10.1089/jwh.2020.8948.
46. Vahidy F.S., Pan A.P., Ahnstedt H., *et al.* Sex differences in susceptibility, severity, and outcomes of coronavirus disease 2019: Cross-sectional analysis from a diverse US metropolitan area. *PLoS One*; 2021;16 (1): e0245556. doi: 10.1371/journal.pone.0245556.

Authors's ORCID ID:
Galina Buta https://orcid.org/0000-0002-7376-1498
Stela Cojocaru https://orcid.org/0000-0002-1420-8772
Raisa Puia https://orcid.org/0000-0003-4988-7442
Tudor Costru https://orcid.org/0000-0002-9245-1399

UDC: 616.72-002:616.517-07

RESEARCH ARTICLE

Expressions and difficulty of clinical manifestations in the early diagnosis of psoriatic arthritis

Eugeniu Russu[1*], Liliana Groppa[1,2], Lia Chişlari[1], Lucia Dutca[1]

[1]Discipline of Internal Medicine, Department of rheumatology and nephrology, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova.
[2]Rheumatology laboratory, "Timofei Moşneaga" Republican Clinical Hospital.

Manuscript received on: 11.04.2022
Accepted for publication: 31.05.2022

Corresponding author:
Eugeniu Russu, PhD, associate professor
Discipline of Internal Medicine, Department of rheumatology and nephrology, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova.
29 N. Testemiţanu, MD-2025, Chisinau, Republic of Moldova
e-mail: eugeniu.russu@usmf.md

Short title: *Early manifestations of psoriatic arthritis.*

What is not known yet, about the topic

It is of particular interest to determine the early clinical manifestations of psoriatic arthritis (PsA), including variants of the onset of the disease, the relationship between its main syndromes and their specific expression.

Research hypothesis

The difficulties in the early diagnosis of PsA, the disability and the decrease in the quality of life in the first years of the disease are the reason for detailed research of the specificity of the first clinical expressions, which would allow in the future the recommendation of criteria for the classification of the early stage.

Article's added novelty on this scientific topic

In the diagnosis of PsA, the most frequently detected features were dactylitis and peripheral arthritis. These features allow the practicing dermatologist, the possibility of suspecting the presence of PsA in a patient with psoriasis. During the diagnosis of PsA, attention should be paid to the combination of psoriasis of the skin and of the nails, as well as comorbid conditions such as: diabetes mellitus, hypertension, ischemic disease and obesity of the first degree, since these patients are at an increased risk of developing PsA.

Abstract

Objectives. Study of clinical manifestations in psoriatic arthritis: enthesitis, dactylitis, peripheral arthritis, axial arthritis, skin manifestations, for early diagnosis, which would allow the establishment of an adjusted treatment and the elaboration of measures to prevent complications.

Materials and methods. The performed clinical study has an analytical-observational retrospective and included the patients who were hospitalized in the Rheumatology and Arthrology departments of the "Timofei Moşneaga" Republican Clinical Hospital during 2015-2021. The study included 103 patients with psoriasis (47 men and 56 women) with various clinical forms of psoriasis and different ages.

Results. The clinical examination of primary or referred patients with cutaneous manifestations, revealed peripheral arthritis in 15 patients (24.6%), dactylitis – in 37 (60.7%), heel pain was detected in 32 (52.5%), axial arthritis – in 30 (49.2%), enthesitis, distal interphalangeal arthritis and tendonitis were not detected. Those who primary or referred with joint manifestations, in 35 (57.4%) of patients was present peripheral arthritis, dactylitis in 40 (65.6%), enthesis of the elbow joints in 11 (18%), knee joints - in 8 (13.1%), the calcaneal region - to 25 (41%), arthritis of the distal interphalangeal joints was detected in 21 (34.4%), tendinitis - to 13 (21.3%).

Discussions. The obtained results allow us to state that one of the directions in improving the diagnosis of PsA consists in the use of ultrasound examination and MRI imaging of the joints of the hands and feet. This makes it possible to effectively identify the characteristic of enthesopathy, edema of tissues, as well as destructive changes in the joints of the hands and feet.

Conclusions. In the study group of patients with psoriasis, in 25 (24.2%) no joint damage was detected. The frequency of detection of psoriatic arthritis and other rheumatic diseases (rheumatoid arthritis, osteoarthritis, ankylosing spondylitis etc.) was 59.2% and 16.6%, respectively, 32 (52.5%) out of 61 (59.2%) patients with PsA were primarily diagnosed. The sensitivity and specificity of the mPEST (Psoriasis Epidemiology Screening Tool) questionnaire in relation to the CASPAR criteria were 77% and 69% respectively. The highest indicators of sensitivity, specificity and accuracy in the diagnosis of PsA has ultrasound and MRI: 88%, 92%, 89% and respectively, 89%, 94%, 91%

Keywords: psoriatic arthritis, early clinical manifestations.

https://doi.org/10.52645/MJHS.2022.2.05

Introduction

Rheumatic diseases currently present themselves as chronic diseases, imposing themselves as being quite important among diseases with immune component due to the complex problems that raise them in relation to their clinical-biological individuality [1, 2]. They have come to possess a profound socio-economic impact, being responsible for 50% of absenteeism in the workplace and about 60% of the rate of permanent incapacity for work, as they largely affect people in full functional capacity. In Europe, the treatment of these diseases accounts for a quarter of the total annual expenditure on health (240 billion EUR). Also, the economic losses caused by absenteeism in the workplace amount to 650 million EUR per year. Moreover, rheumatic diseases can be disabling and often lead to early retirement [3-5].

Psoriasis (Ps) is a chronic non-infectious pathology, a disease characterized by the deterioration of the skin, caused by determined immunopathological reactions. The disease can occur at any age, but in recent years, there is a tendency towards its „rejuvenation": Ps has begun to appear at a younger age, in particular, the onset of the disease is observed in 75% patients before the age of 40 years [6].

Psoriatic arthritis (PsA) is considered as a severe form of Ps, regardless of the area of skin lesions. PsA is a chronic, inflammatory disease from the seronegative spondyloarthritis group; the clinical picture presents mainly with inflammation of the peripheral joints, enthesis and inflammation of the tendons of the fingers, hands and feet, manifested by tenosynovitis and dactylitis, but the spine (spondylitis) and sacroiliac joints (sacroiliitis) can also be affected. PsA is a severe pathology because it decreases the quality of life and functional state of patients. The aforementioned clinical forms are often manifested by erosion and deformation, change the structure and lead to disability. Particularly severe cases of PsA are more often recorded in young men [7].

According to summary data, the incidence of Ps in the general population is 2-3%, of which 13.5 to 47% of patients are diagnosed with PsA. Most often the onset of PsA is between 20 and 50 years of age. The ratio between males and females is approximately the same. In latest studies, enough data have been accumulated that prove that in 40-60% of patients with PsA, joint lesions appear in the first years of the disease [8, 9].

Obviously, the issue of treating patients with PsA is extremely relevant and no less important. The possibility of early diagnosis of the disease and the earlier start of treatment, when the structural damage to the joints is less evident, provides the chances of improving the patient's quality of life. Early PsA (lasting less than 2 years) are conditionally distinguished in a special subgroup, since the first years in the course of PsA are decisive in the development and progression of the pathological process [10], even though until recently, the early diagnosis of PsA presented difficulties associated with a variety of clinical manifestations of the disease. In the space of a long time, the international medical community and, in particular, rheumatologists have made several attempts to systematize these manifestations [11]. At the same time, the most successful classification of PsA is currently CASPAR (The ClASsification for Psoriatic Arthritis), which was developed in 2006 by a group of 30 rheumatology centers (from 13 countries). It is based on five signs of PsA, most often detected clinically, instrumentally and laboratory. The CASPAR criteria have high specificity (98.7%) and sensitivity (91.4%), including in the early stages of PsA, are based on the analysis of data from patients with an average duration of the disease of 12.5 years. To date, the CASPAR classification is generally recognized and an important diagnostic tool in the clinical practice of the rheumatologist. However, patients with Ps, due to how PsA develops, are treated in most cases by the dermatologist, and are referred to the rheumatologist at an advanced stage, which is not appropriate, due to the lack of interdisciplinary collaboration between these specialists [11, 12].

Thus, despite the availability of modern diagnostic capabilities, an algorithm for the early detection of PsA in a cohort of patients with Ps has not yet been developed, in relation to the practice of a dermatologist. Furthermore, at the moment, there is no close interaction between a dermatologist and a rheumatologist, which is extremely necessary both in relation to the primary detection of patients and in the context of further management and monitoring [13, 14].

In addition to the CASPAR criteria, in the diagnosis of PsA, especially at the early stage, a great significance has the history of the disease, the nature of clinical manifestations, the use of instrumental research methods (scintigraphy, densitometry, conventional radiography, ultrasound, magnetic resonance imaging (MRI)); laboratory data such as C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor. Although it has been established that in PsA joint erosions on radiography are detected only in some patients, and at an early stage in just 20-30% of cases [15, 16].

Timely detection of PsA in patients with Ps involves early treatment and contributes to the prevention of the further development of functional disorders [17].

In this regard, it is important to optimize the methods of early diagnosis of PsA and in dermatological practice by timely detection of early clinical signs of the disease and the development of a diagnostic algorithm for early PsA [18].

The purpose of the study of our study was to study clinical manifestations in psoriatic arthritis: enthesitis, dactylitis, peripheral arthritis, axial arthritis, skin manifestations, for early diagnosis, which would allow the establishment of an adjusted treatment and the development of measures to prevent complications.

Our objectives were:

(1) Analysis of clinical aspects of patients with early manifestations in psoriatic arthritis;

(2) Highlighting of risk factors, incidence and clinical diversity of PsA;

(3) Preparation of recommendations and management plans in the early detection of clinical manifestations in psoriatic arthritis.

Material and methods

The clinical study performed has an analytical-observational retrospective character and included patients who were hospitalized in the Rheumatology and Arthrology departments at the Republican Clinical Hospital „Timofei Moşneaga", during 2015-2021. The study included 103 patients with Ps (47 men and 56 women) with various clinical forms of Ps and of different ages. The group of patients was analyzed multilaterally, according to different distribution groups that comply with the following criteria: sex, age, performing mPEST (Psoriasis Epidemiology Screening Tool) screening before establishing the diagnosis, smoking, residence environment. For all patients, the general and systemic clinical examination was performed, as well as analysis of the results of laboratory and instrumental investigations. Data presented in the study were collected from patients' medical records.

Criteria for inclusion in the study:

(1) age between 18 and 70 years;

(2) definite diagnosis of psoriatic arthritis according to CASPAR criteria (2006);

(3) assessment of the severity of the lesions and the area affected by Ps according to the PASI score (Psoriasis Area Severity Index);

(4) assessment of the total area of skin lesions according to BSA (Body Surface Area, 2007);

(5) evaluation of mPEST screening until the diagnosis has been established;

(6) highlighting and evaluation of early manifestations: enthesitis, dactylitis, peripheral arthritis, axial, cutaneous and nail manifestations in patients with PsA.

Criteria for exclusion from the study:

(1) the presence in patients of other autoimmune diseases, other skin diseases or decompensated chronic conditions such as diabetes mellitus, viral hepatitis, cirrhosis of the liver, hematological, nephrological, oncological, or cardiac diseases and other severe diseases.

(2) mismatch of diagnostic criteria for psoriatic arthritis.

Radiological examination of the hands, feet, pelvis, as well as other joints and spine was carried out in the department of radiology. The radiological stage was determined according to the Steinbroker stages. To quantify the severity of bone cartilage damage, the modified Sharp/van der Heijde method for PsA was used to calculate the total number of erosions (maximum 5 points in the hands and 10 points in the legs) and the narrowing of the intraarticular joint space of the hands and feet (maximum 4 points). The maximum score of erosion and narrowing of articular spaces in hands and feet in PsA was 518 points.

The statistical analysis of the data was performed at the statistical package Statistics 9.0 and EpiInfo, version 5 using simple descriptive statistics. For quantitative traits that have a normal distribution, the results are presented in the form of mean values and standard average deviations (M ± SD). For signs that do not correspond to the normal distribution, the median (Me) and the interquartile interval (25%; 75%) were used. To determine the reliability of differences in the average values of numerical parameters, the student's *t*-test was used, and in the absence of a normal distribution and the presence of a large data dispersion, the nonparametric method - the Mann-Whitney criterion. The analysis of the relationship between the two characteristics was carried out using non-parametric correlation analysis using the Spearman method. The statistical significance of disease risks (the evolutionary variant) was assessed using criterion X^2 and Fisher criteria. The results were considered reliable at a level of significance $p < 0,05$.

Results

The study included 103 patients with Ps (47 men and 56 women) with various clinical forms of Ps between 2019 and 2021. The age of the patients was on average 44.00 ± 13.69 years (from 18 to 70 years). The duration of Ps disease ranged from 6 months to 50 years. The average duration of Ps was 10.7 ± 1.2 years (table 1).

Table 1. *Distribution of patients according to clinical forms of Ps*

Clinical forms of Ps	Number of patients	Share (%)
Ps vulgaris	39	37.9
Ps of the scalp	24	23.3
Ps palmo-plantar	16	15.5
Ps on flexion surfaces	15	14.6
By type of erythroderma	4	3.9
Ps guttat	3	2.9
Ps pustulous	2	1.9

Note: Ps – psoriasis.

Almost a third of the patients – 39 (37.9%) had Ps vulgaris, 24 (23.3%) – Ps of the scalp, 16 (15.5%) – palmo-plantar Ps, 15 (14.6%) – Ps on the flexion surfaces, at 4 (3.9%) – the pathological process went after the type of erythroderma, 3 (2.9%) – Ps gutta, 2 (1.9%) – pustular form.

Evaluation of patients based on the mPEST questionnaire and according to CASPAR criteria

Based on the mPEST questionnaire, the diagnosis of PsA was suspected in 60 (58.2%) patients out of 103 patients. Diagnosis of PsA confirmed according to CASPAR criteria in 47 of them (45.6%). mPEST < 3 (indicating a low probability of having PsA) was observed in 43 out of 103 patients (41.7%). According to CASPAR criteria, PsA were absent at 29 (28.2%) of them.

Identification of early clinical manifestations in PsA at the first visit

Considering that the first visit of a patient with Ps can be with both cutaneous and joint manifestations, depending on what appears primarily, we evaluated the early clinical manifestations in accordance with the primary address of the patient and the detection of PsA at the initial clinical examination.

After the clinical examination, in primary or addressed patients with skin manifestations, peripheral arthritis in 15 patients (24.6%), dactylitis in 37 (60.7%), heel pain was detected in 32 (52.5%), axial arthritis in 30 (49.2%), enthesi-

tis, distal interphalangeal arthritis and tendinitis were not detected (table 2).

Table 2. Diagnosis of PsA based on the clinical manifestations of the disease

Early manifestations	Primary addressing with cutaneous manifestations	Primary address with joint manifestations
Peripheral arthritis	15 (24.6%)	35 (57.4 %)
Dactylitis	37 (60.7%)	40 (65.6%)
Axial arthritis	30 (49.2%)	-
Enthesitis of the elbow joints	-	11 (18%)
Enthesitis of the knee joints	-	8 (13.1%)
Enthesitis in the calcaneal region	32 (52.5%)	25 (41%)
Arthritis of DIP	-	21 (34.4%)
Tendinitis	-	13 (21.3%)

Note: PsA – psoriatic arthritis; DIP – distal interphalangeal joints.

Those who addressed primarily with joint manifestations, 35 (57.4%) of patients had peripheral arthritis, dactylitis – 40 (65.6%), entheses of the elbow joints – 11 (18%), knee joints – 8 (13.1%), calcaneal region – at 25 (41%), arthritis of the distal interphalangeal joints was detected in 21 (34.4%), tendinitis – 13 (21.3%).

The structure of the lesion of the osteoarticular apparatus in patients with psoriasis and the prevalence of psoriatic arthritis

Out of 103 patients with Ps, 61 had PsA (according to CASPAR criteria), which constituted 59.2%. In the remaining 42 cases (40.8%), other chronic inflammatory diseases of the joints were diagnosed. In 13 (12.6%) cases other rheumatic diseases (table 3): dermatomyositis, rheumatic polymyalgia, ankylosing spondylitis, rheumatoid arthritis, gout, etc., in 4 (4%) patients - a combination of two pathologies: PsA and gout, in 25 (24.2%) patients – no rheumatic diseases.

Table 3. The structure of the lesion of the osteoarticular apparatus in patients with Ps

Conditions	Number of patients	Share
Ps + PsA	61	59.2%
Ps without other rheumatic pathologies	25	24.2%
Ps + other rheumatic pathologies	13	12.6%
The combination of 2 rheumatic pathologies	4	4%

Note: Ps – psoriasis; PsA – psoriatic arthritis.

Identification of characteristic early clinical manifestations in psoriatic arthritis

In 61 patients of the study, the group with the presence of PsA, all the clinical features characteristic of this disease were noted: in 35 (57.4%) of patients peripheral arthritis was present, dactylitis in 40 (65.6%), enthesitis of the elbow joints in 11 (18%), knee joints – in 8 (13.1%), the calcaneal region – in 25 (41%), arthritis of the distal interphalangeal joints was detected in 21 (34.4%), tendinitis – 13 (21.3%).

Identification of patients according to the duration of PsA

It should be emphasized that from the 61 patients with PsA, in 32 (52.5%) of them the diagnosis was established for the first time at an early stage (up to 2 years). In 24 (39%) of 61 patients with PsA, the duration of the disease was less than 1 year, at 17 (28%) – from 1 to 2 years, in 11 (18%) – from 2 to 3 years, in 9 (15%) > 3 years.

Assessing the severity of Ps and PsA

BSA was determined during clinical examination in all patients and noted in the medical records, respectively. Based on the condition as a slap of the patient to the middle phalanges of the fingers corresponds to 1%.

According to the BSA index, patients were divided into 3 groups:

- BSA < 5% - Mild Ps (without affecting the patient's quality of life), in 43 patients (41.7%);
- BSA 5-20% - Moderate Ps (with impact on the patient's quality of life), in 53 patients (51.5%);
- BSA > 20% - Severe Ps, in 7 patients (6.8%).

Ps type I (the first cutaneous manifestation occurred before 25 years, in patients with a positive family history) – observed in 31 patients (30%), Ps type II (after the age of 25 years) – observed in 72 (70%) patients.

Relationship between nail Ps and PsA

Out of 103 patients, nail Ps was detected in 69 (67%) patients, and in the group of patients with PsA (n=61) – in 44 (63.7%), without PsA – 25 (36.2%). Nails Ps were not detected in 17 patients (50%) with PsA and in 17 (50%) without PsA.

When analyzing the period of manifestation of the nails in Ps, it was noticed that in most cases (47.8%), the nail lesions were detected after the cutaneous manifestations of Ps, but before PsA. This relationship may have prognostic value and may be a risk factor for the development of PsA. Therefore, in order to clarify its potential predictor, additional analysis may be necessary in some cases.

Effect of smoking on the development of PsA

We evaluated the impact of smoking as a risk factor for the development of PsA in patients with Ps. Significant difference in the frequency of detection of Ps with PsA and without PsA was not detected (26% to 37%) (Table 4).

Table 4. Effect of smoking on the development of PsA

Presence of PsA	Smokers	Nonsmokers	Total
Not	31 (73.81%)	11 (26.19%)	42
Yes	38 (62.30%)	23 (37.70%)	61
Total	69	34	103

Note: PsA – psoriatic arthritis.

Concomitant pathology in patients with Ps

Out of 103 patients examined, comorbidities were present in 77 (74.8%) of them. Among comorbidities: hypertension was observed in 23 (22.3%) patients with PsA and in 11 (10.7%) patients without PsA; diabetes mellitus – in 7 (6.8%) patients with PsA and in 4 (3.9%) without PsA; coronary heart disease – in 10 (9.7%) patients with PsA and 5 (4.9%) without PsA.

Table 5. Characteristic of patients depending on the concomitant pathology

Pathology	Patients with PsA	Patients without PsA	Total
Hypertension	23 (22.3%)	11 (10.7%)	34 (33%)
Type II DM	7 (6.8%)	4 (3.9%)	11 (10.7%)
Coronary artery disease	10 (9.7%)	5 (4.9%)	15 (14.6%)
Digestive disorders	7 (6.8%)	6 (5.8%)	13 (12.6%)
Chronic respiratory diseases	5 (4.9%)	6 (5.8%)	11 (10.7%)
Gynecological and urological diseases	7 (6.8%)	8 (7.8%)	14 (13.6%)

Note: PsA – psoriatic arthritis; DM – diabetes mellitus.

Diseases of the digestive system (gastritis, gastric and duodenal ulcers, gallstones, etc.) were observed in 13 (12.6%) patients. Chronic respiratory diseases (asthma, obstructive bronchitis, etc.) were observed in 11 (10.7%) patients. Gynecological and urological diseases (prostatitis, urolithiasis, chronic pyelonephritis, fibroids, etc.) were detected in 14 (13.6%) patients (Table 5).

Assessment of the sensitivity and specificity of instrumental diagnostic methods

One of the important components of the diagnosis of PsA is the use of instrumental methods.

In 94 patients out of 103, X-rays of the hands, feet and pelvis were performed, which totaled 91.3%. According to the results of the X-ray, the following changes were identified in patients: narrowing of the joint space in 60 patients out of 94 (63.8%), erosion of the articular surfaces in 14 (14,9%), bone proliferation (excessive growth of bone tissue at the edges of the joints) in 12 (12,8%) patients. However, in the early stages of PsA, radiological changes were not detected.

Ultrasound of the joints and entheses was performed in 53 patients out of 103 (51.5%). Following the ultrasound, the following changes were revealed: blurred contours of the articular surfaces in 38 patients out of 53 (71.7%), erosion of the ends of the bones in 18 (34%) patients, synovitis (inflammation of the synovial membrane of the joint) in 45 (85%), enthesopathy in 44 (83%), calcifications in 8 (15%).

MRI of the hands and feet was performed in 47 patients out of 103 (45.6%). MRI allows to identify numerous pathological changes in the study group of patients already at the early stage (in some cases, from 3 to 6 months): synovitis in 39 patients out of 47 (83%), tenosynovitis (inflammation of the synovial sheath of the tendon) in 40 (89.3%) patients, edema of bone tissue in 29 (61.7%) patients, erosions in 19 (40.4%) and soft tissue edema in 34 (72,3%) patients.

Discussions

This study represents a complex research on the latest discoveries in the field of detecting early manifestations of PsA. In a large group of patients with Ps, it has been shown that, in addition to PsA, this cohort of patients can develop any other rheumatic pathology: rheumatoid arthritis (RA), ankylosing spondylitis (AS), osteoarthritis (OA), reactive arthritis (ReA), gout, etc., which indicated the need for an interdisciplinary approach.

For the diagnosis of PsA there has not been identified any specific laboratory tests, however more informative for the diagnosis of inflammatory changes in the joints are radiography, USG and MRI, which have demonstrated an important significance [3-8].

In the diagnosis of PsA, the most frequently detected features were dactylitis and (less often) peripheral arthritis. These features allow the practicing dermatologist, the possibility of suspecting the presence of PsA in a patient with Ps. However, the identification of some clinical manifestations, such as enthesitis, spondylitis and tendinitis, requires additional investigations require close collaboration for the dermatologist with a rheumatologist [10, 11].

During the diagnosis of PsA, attention should be paid to the combination of Ps of the skin and Ps of the nails, as well as comorbid conditions such as: diabetes mellitus, hypertension, ischemic disease, and obesity of the first degree, since these patients are at an increased risk of developing PsA [12-15].

The results of the study provide fundamental support in the context of early diagnosis of systemic manifestations of the disease, which have an impact on the patient's quality of life. They allow diagnosing the disease in the early stages and developing treatment as quickly as possible, so that patients do not end up with destructive injuries.

The results obtained allow us to state that one of the directions in improving the diagnosis of PsA consists in the use of ultrasound examination and MRI imaging of the joints of hands and feet. This makes it possible to effectively identify the characteristic of enthesopathy, edema of tissues, as well as destructive changes in the joints of the hands and feet.

Conclusions

1. In the study group of patients with Ps, in 25 (24.2%) of them were not detected joint damage. The frequency of detection of PsA and other rheumatic diseases (RA, OA, AS, etc.) was 59.2% and 16.6%, respectively, 32 (52.5%) out of 61 (59.2%) patients with PsA were diagnosed for the first time.
2. The sensitivity and specificity of the mPEST questionnaire in relation to the CASPAR criteria were 77% and 69% respectively.
3. The highest indicators of sensitivity, specificity and accuracy in the diagnosis of PsA has ultrasound and MRI: 88%, 92%, 89% and, respectively, 89%, 94%, 91%.
4. The most frequently detected specific signs of PsA at the first address with cutaneous manifestations, it turned out to be peripheral arthritis and dactylitis - 24.6% and 60.7%. Enthesitis, spondylitis and tendinitis in people with severe skin manifestations were detected at a low rate.
5. For a better diagnosis of PsA in patients with Ps should be carried out mPEST screening. Depending on the score and the presence or absence of joint complaints, patients should be further examined. We should note the need for interdisciplinary interaction of cutaneous and joint manifestations, developing training programs for the early diagnosis of PsA, adequate therapy and improving the patient's quality of life.

Abbreviations

AS – Ankylosing Spondylitis; BSA – Body Surface Area; DIP – Distal Interphalangeal Joints, DM – Diabetes Mellitus; OA – Osteoarthritis; MRI – Magnetic Resonance Imaging, PsA – Psoriatic Arthritis; Ps – Psoriasis; RA – Rheumatoid Arthritis; USG – Ultrasonography.

Declaration of conflict of interest

Nothing to declare.

Authors' contribution

Study conception and design: ER, LG. Data acquisition: ER, LC, LD. Analysis and interpretation of data: ER, LC. Drafting of the manuscript: ER. Significant manuscript review with significant intellectual involvement: ER, LD. Approval of the „ready for print" version of the manuscript: ER, LG, LC, LD**.**

References

1. Collantes E., Zarco P., Munoz E., Juanola X., Mulero J., Fernandez-Sueiro J. L., J. C. Torre-Alonso, Gratacos J., Gonzalez C., "Disease Pattern of Spondyloarthropathies in Spain: Description of the Second National Registry Extended Report," *Rheumatology*, Vol. 46, No. 8, 2017, p.1309-1315.
2. Eder L., Haddad A., Rosen C.F., Lee K.A., Chandran V., Cook R., et al. The incidence and risk factors for psoriatic arthritis in patients with psoriasis - a prospective cohort study. *Arthritis Rheumatol*. 2015 Nov 10, p.78-85.
3. Alenius, G.M., Stegmayr, B.G., & Dahlqvist, S.R. (2001). Renal abnormalities in a population of patients with psoriatic arthritis. *Scand J Rheumatol*, 30(5), p.271-274.
4. Akassou A., Bakri Y. Does HLA-B27 Status Influence Ankylosing Spondylitis Phenotype?. Clinical medicine insights. *Arthritis and musculoskeletal disorders*. 2018; 11, p.117.
5. Alinaghi F., Calov M., Kristensen L.E., Gladman D.D., Coates L.C., Jullien D., et al. Prevalence of psoriatic arthritis in patients with psoriasis: A systematic review and meta-analysis of observational and clinical studies. *J Am Acad Dermatol*. 2018 Jun p.18.
6. Baker J.F., Krishnan E., Chen L. & Schumacher H.R. (2005). Serum uric acid and cardiovascular disease: recent developments, and where do they leave us? *Am J Med*, 118(8), p.816-826.
7. Carter J.D., Hudson A.P. Reactive arthritis: clinical aspects and medical management. *Rheum Dis Clin North Am*. 2009 Feb. 35(1), p.21-44.
8. Casals-Sanchez J.L., Garcia De Yebenes Prous M.J., Descalzo Gallego M.A., Barrio Olmos J.M., Carmona Ortells L., Hernandez Garcia C., & Grupo de Estudio em, A.R., II. (2012). Characteristics of patients with spondyloarthritis followed in rheumatology units in Spain. emAR II study. *Reumatol Clin*, 8(3), p.107-113.
9. Bakland G., Alsing R., Singh K., Nossent J.C. Assessment of SpondyloArthritis International Society criteria for axial spondyloarthritis in chronic back pain patients with a high prevalence of HLA-B27. *Arthritis Care Res* (Hoboken). 2013.
10. Bakland G., Nossent H.C. Epidemiology of spondyloarthritis: a review. *Curr Rheumatol Rep*. 2013 Sep;15(9, p.351.
11. Bennett JE, Dolin R, Blaser MJ. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. Elsevier Saunders; 2015.
12. Eliçabe R.J., Cargnelutti E., Serer M.I., Stege P.W., Valdez S.R., Toscano M.A., et al. Lack of TNFR p55 results in heightened expression of IFN-γ and IL-17 during the development of reactive arthritis. *J. Immunol*. 2010 Oct 1. 185(7), p.4485-4495.
13. Braun J.; Bollow M.; Neure L.; Seipelt E.; Seyrekbasan F.; Herbst H.; Eggens U.; Distler A.; Sieper J. Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. *Arthritis Rheum*. 1995, 38, p.499–505.
14. Bruce I.N., Schentag C.T. & Gladman D.D. (2000). Hyperuricemia in psoriatic arthritis: prevalence and associated features. *J Clin Rheumatol*, 6(1), p.6-9.
15. Chi C.C., Wang J., Chen Y.F., Wang S.H., Chen F.L. & Tung T.H. (2015). Risk of incident chronic kidney disease and end-stage renal disease in patients with psoriasis: A nationwide populationbased cohort study. *J Dermatol Sci*, 78(3), p.232- 238.
16. Colmegna I., Cuchacovich R., Espinoza L.R. HLA-B27-associated reactive arthritis: pathogenetic and clinical considerations. *Clin Microbiol Rev*. 2004, p.56-61.
17. Dougados M. Results from the International ASAS-COMOSPA Study The Prevalence of Renal Impairment in Patients with Spondyloarthritis. *The Journal of Rheumatology,* April, 2020, p.38-42.
18. Alvarez-Navarro C., Cragnolini J.J., Dos Santos H.G., Barnea E., Admon A., Morreale A., et al. Novel HLA-B27-restricted epitopes from Chlamydia trachomatis generated upon endogenous processing of bacterial proteins suggest a role of molecular mimicry in reactive arthritis. *J Biol Chem*. 2013 Sep 6. 288(36), p.25.
19. Baeten D., Breban M., Lories R., Schett G., Sieper J. Are spondylarthritides related but distinct conditions or a single disease with a heterogeneous phenotype? *Arthritis Rheum*. 2013 Jan;65(1), p.12-20.

Authors's ORCID ID:
Eugeniu Russu, https://orcid.org/ 0000-0001-8957-8471
Liliana Groppa, https://orcid.org/ 0000-0002-3097-6181
Lia Chişlari, https://orcid.org/ 0000-0002-7088-568X
Lucia Dutca, https://orcid.org/ 0000-0002-1815-2294

UDC: 616.721-002.77-07:616.34-022

RESEARCH ARTICLE

Improvement of early diagnosis of axial spondyloarthritis in intestinal infectious diseases

Lia Chișlari[1*], Liliana Groppa[1,2], Eugeniu Russu[1], Svetlana Agachi[1]

[1]Discipline of Internal Medicine, Department of rheumatology and nephrology, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova
[2]Rheumatology laboratory, Timofei Moșneaga Republican Clinical Hospital.

Manuscript received on: 13.04.2022
Accepted for publication: 31.05.2022

Corresponding author:
Lia Chișlari, PhD, associate professor
Discipline of Internal Medicine, Department of rheumatology and nephrology, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova.
29 N. Testemițanu St., MD-2025, Chisinau, Republic of Moldova
e-mail: lia.chislari@usmf.md

Short title: *Early diagnosis of axial spondyloarthritis in intestinal infectious diseases*

What is not known yet, about the topic

For the first time, were studied and determined in detail the features of early manifestations of axial arthropathies in infectious bowel diseases in order to improve their early diagnosis. Also, there was created an algorithm for the early detection of axial spondyloarthritis in intestinal infectious diseases.

Research hypothesis

Appreciation of the peculiarities of early manifestations of axial arthropathies in infectious bowel diseases to improve their early diagnosis.

Article's added novelty on this scientific topic

It has been identified the following clinical variants of arthropathies in patients with intestinal infectious diseases: axial lesion; arthralgia and arthritis. The 2009 ASAS classification criteria are applicable for patients with intestinal infectious diseases for the evaluation of spinal inflammatory pain with 75.9% sensitivity and 68.3% specificity. There was proposed an algorithm for the early detection of axial arthropathy in patients with intestinal infectious disease with a certain diagnostic value of the ASAS criteria and an odds ratio for spinal inflammatory pain, arthritis, arthralgia, uveitis and the diagnosis of *Salmonella enteritidis* or *Shigella flexneri*.

https://doi.org/10.52645/MJHS.2022.2.06

Abstract

Introduction. Despite numerous fundamental clinical studies on the frequency, pathogenesis, clinical features of spondyloarthritis (SpA) in intestinal infectious diseases (IID) and intestinal damage in SpA, there are currently a number of unresolved problems, one of which is the problem of early diagnosis of arthropathies, especially associated with IID, which makes it necessary to continue the research in the scientific problem.

Purpose of the study. Appreciation of the features of early manifestations of axial arthropathies in intestinal infectious diseases in order to improve their early diagnosis.

Objectives of the study. Identification of clinical variants of axial arthropathies in patients with infectious intestinal diseases and instrumental description of axial lesion by conducting comparative analysis of clinical and laboratory parameters in these patients depending on the presence of axial arthropathies. Development of an algorithm for the early detection of axial spondyloarthritis in intestinal infectious diseases.

Material and methods. During 2015-2021, 141 patients were analyzed retrospectively, out of which 50 patients with SpA from the Republican Clinical Hospital „Timofei Moșneaga" and 91 patients with infectious intestinal diseases from the gastro-enterology and hepatology departments of the Republican Clinical Hospital „Timofei Moșneaga". Depending on the etiology of IID, subjects were divided into 2 groups: the first group included patients with *Yersinia enterocolitica* or *Campylobacter jejuni* (*Y*±C), the second with *Salmonella enteritidis* or *Shigella flexneri* (*S*±*Sh*).

Results. In patients with IID, the following clinical variants of arthropathies have been identified: axial lesion (spinal inflammatory pain according to ASAS criteria (2009) in 42.9%, axial spondyloarthritis (axSpA) in 28.6%, AS in 15.4%), arthralgia - 38.5%, arthritis - 13.2%. Conventional radiography imaging and magnetic resonance imaging (MRI) of the sacroiliac joints (SI) increased the incidence of SpA from 6.6% (n = 6) to 28.6% (n = 26).

Conclusions. In patients with IID, the following clinical variants of arthropathies have been identified: axial lesion (spinal inflammatory pain according to ASAS criteria (2009) in 42.9%, axSpA in 28.6%, ankylosing spondylitis in 15.4%), arthralgia – 38.5%, arthritis – 13.2%. Imaging in the form of conventional radiography and MRI of SI joints increased the incidence of SpA from 6.6% (n = 6) to 28.6% (n = 26). Axial arthropathies, arthralgia, arthritis, and uveitis are encountered more frequently. At the same time, the possibility of detecting axSpA is greater if arthritis, arthralgia, inflammatory back pain, uveitis is present. Patients with *S*±*Sh* have

a higher chance of developing axSpA compared to patients with *Y*±C.

Keywords: *Yersinia enterocolitica*, *Salmonella enteritidis*, *Shigella flexneri*, axial spondyloarthritis.

Introduction

One of the difficult problems of internal medicine are intestinal infectious diseases (IID). The IID clinical picture is extremely diverse, therefore they should be considered as systemic diseases. The lesions, unfortunately, are not limited only to the gastrointestinal tract; some of the patients develop extraintestinal manifestations [1-3].

Extraintestinal manifestations are separated into two distinct pathogenetic groups: immune-mediated diseases due to a common pathogenetic link and non-immune-mediated diseases, its clinical picture due to metabolic processes that are secondary to intestinal infectious disease. The first group includes arthropathy, skin lesions (erythema nodosum, gangrenous pyoderma), eye damage (uveitis, iridocyclitis), primary sclerosing cholangitis; in the second group - cholelithiasis, urolithiasis, osteoporosis [4-6].

According to clinical studies, up to half of patients with IID suffer from at least one extraintestinal manifestation, and in some patients, it occurs before the onset of intestinal symptoms, which causes additional difficulties in the early diagnosis of the disease. The presence of one or more extraintestinal immune-mediated manifestations changes the prognosis, increases the severity the disease, and modifies the management [7-9].

Arthropathy, lesions of the skin, eyes, and mucous membranes are among the most common extraintestinal manifestations. Various cellular and humoral mechanisms have been identified that account for intestinal and joint inflammation. Damage to peripheral joints and spine is the most common manifestation of associated spondyloarthritis. Axial spondyloarthritis (axSpA) belongs to the group of spondyloarthritis, which includes ankylosing spondylitis (AS), psoriatic arthritis, reactive arthritis, and arthritis associated with IID [10-12]. According to studies in patients with IID, the incidence of SpA ranges from 17% to 39%.

Establishing the diagnosis of IID-associated SpA at an early stage is a very difficult task: the clinical manifestations are minor, but the radiological manifestations are reliable and are usually detected on average 7.1 years after the onset of symptoms [13]. A group of Italian researchers reported that the diagnosis of SpA in patients with IID is after around 5.2 years from the onset of dorsal pain. Currently, the incidence of undiagnosed cases remains to be quite high [14, 15].

Despite numerous fundamental clinical studies on the frequency, pathogenesis, clinical features of SpA in IID and intestinal damage in AS, there are currently several open issues, one of which is the problem of early diagnosis of arthropathies, especially associated with IID. Therefore, it was decided to initiate a study with the following purpose – *Determination of the peculiarities of early manifestations of axial arthropathies in intestinal infectious diseases in order to improve their early diagnosis*.

The objectives of the study are:

(1) identification of clinical variants of axial arthropathies in patients with infectious intestinal diseases and instrumental description of axial lesion;

(2) conducting comparative analysis of clinical and laboratory parameters in patients with intestinal infectious disease, depending on the presence of axial arthropathies;

(3) study of clinical features of gastrointestinal lesions in patients with ankylosing spondylitis;

(4) determination of diagnostic value of ASAS criteria, 2009 for dorsal inflammatory pain in infectious bowel disease;

(5) development of an algorithm for the early detection of axial spondyloarthritis in intestinal infectious diseases.

The scientific novelty of the study is – for the first time in patients with IID, the incidence of spinal inflammatory pain was determined (according to the ASAS criteria, 2009), which was 42.9%, which contributed to the early diagnosis of axSpA in 28.6% of patients. Furthermore, an attempt was made to determine the factors that are most commonly encountered in the presence of SpA in patients with IID. It has been determined that the presence of arthritis - odds ratio (OR) 10.77 [95% CI 2.26-44.2], p=0.005, arthralgia – OR 4.12 [95% CI 1.55-10.95], p = 0.005, inflammatory back pain - OR 8.07 [95% CI 2.8-23.23], p = 0.0001, uveitis - OR 19.2 [95% CI 2.18-169.13], p = 0.008 increases the risk of developing SpA in patients with IID. Patients with *S±Sh* have a higher risk of developing SpA compared to patients with *Y*±C – OR 2.92 [95% CI 1.14-7.48], p = 0.025.

We determined the diagnostic significance of the ASAS criteria of inflammatory pain in infectious bowel diseases. The sensitivity was 75.9%, the specificity – 68.3%, the positive predictive value – 0.51, the negative predictive value – 0.87; the probability ratio of the positive result – 2.3, the probability ratio of the negative result was 0.3. When using the DISQ questionnaire to identify intestinal symptoms in patients with AS, it was demonstrated for the first time that 92% of patients had remission of gastrointestinal symptoms.

Based on the obtained results, we proposed clinical predictors of the presence of axSpA in patients with IID. These include arthralgia, arthritis, inflammatory back pain, and uveitis. It is necessary to consider the early detection of SpA in patients with *S±Sh*. The diagnostic significance of 2009 ASAS classification criteria in patients with SpA and IID was determined, which allows them to be used in clinical practice. An algorithm is proposed for the early diagnosis of axSpA in patients with IID, which helps in identifying the opportune time for rheumatology referral. It should be taken into account that in patients with AS with a long course and severe activity, the manifestations on the part of the gastrointestinal tract are subclinical.

Material and methods

During 2015-2021, 141 patients were analyzed retrospectively. Of the 141 patients, 50 patients were with AS from the Republican Clinical Hospital „Timofei Moşneaga”

and 91 patients were with infectious intestinal diseases from the gastro-enterology and hepatology departments of the Republican Clinical Hospital „Timofei Moşneaga". Depending on the mediation of the inflammatory response, all patients with IID were divided into 2 groups: the first group included patients with *Yersinia enterocolitica* or *Campylobacter jejuni* (*Y*±C), the second group with *Salmonella enteritidis* or *Shigella flexneri* (*S*±*Sh*).

The criteria for inclusion in the study were:

- age over 18 years;
- the presence of an established diagnosis of inflammatory infectious disease;
- a diagnosis of AS;
- combination of IID with extraintestinal manifestations (including SpA);
- signed informed consent for inclusion in the study.

Exclusion criteria:

- pregnancy and lactation;
- the patient's refusal to participate in the study.

The study included 91 patients with IID: 52 (57.1%) with *Yersinia enterocolitica* or *Campylobacter jejuni* (*Y*±C), and 39 (42.9%) with *Salmonella enteritidis* or *Shigella flexneri* (S+-Sh). There were 47 men (51.6%) and 44 women (48.4%); as well as 50 patients with AS: men – 33 (66%), women – 17 (34%). Diagnoses of *Y*±C, *S*±*Sh* have been established in accordance with modern recommendations for the corresponding nosological form. Back pain was considered inflammatory if it met the 2009 ASAS criteria for inflammatory low back pain (if at least 4 out of 5 criteria were met).

The diagnosis of AS was established according to the criteria of the 1984 New York amended criteria. The diagnosis of axSpA was established by a joint consultation between a gastroenterologist and rheumatologist based on typical SpA complaints (in particular, spinal inflammatory pain and changes detected by imaging methods – MRI and pelvic X-ray). Imaging data were considered positive if the patient had significant sacroiliitis (SI) (Ro+) (Van der Linden *et al.*, 1984) or signs on MRI (MRI+). All patients diagnosed with IID-associated SpA met the ASAS classification criteria for axial SpA.

The examination of patients with IID and AS was based on the standard of examination of patients with *Y*±C, *S*±*Sh*, AS and recommendations for the corresponding nosological form. Additional methods of examination included the ASAS questionnaire for dorsal inflammatory disease (2009) in patients with IID, determination of fecal calprotectin and the DISQ questionnaire in patients with AS.

Statistical analysis

The nature of the distribution of the data obtained was evaluated using a graphical method using the Kolmogorov-Smirnov criterion. Descriptive statistical methods were used for the statistical processing of the research data. The description of signs that have a normal distribution is presented as M±δ, where M is the arithmetic mean, δ is the standard deviation. The statistical processing of 10 data obtained was carried out using the Mann-Whitney test, X^2 and the exact Fisher test. The differences were considered statistically significant at $p < 0.05$.

Sensitivity (Sn) and specificity (Sp) were calculated using the formulas: Sn = a/(a+c); Sp = d/(b+d), where *a* – true positive results (number of patients with spinal inflammatory pain and diagnosed with axSpA); *b* – false positives (number of patients with spinal inflammatory pain and not corresponding to the diagnosis of axSpA); *c* – false negative results (number of patients without dorsal inflammatory pain, but diagnosed with axSpA); *d* - true negative results (number of patients without dorsal inflammatory pain and without a diagnosis of axSpA).

We also calculated the positive predictive value (PPV) – the probability of having SpA if the patient has spinal inflammatory pain, and the negative predictive value (NPV) – the probability of not having axSpA if the patient does not have spinal inflammatory pain. The probability ratio of positive (LR+) and negative (LR-) results was determined. The higher the LR+, the higher the risk of being diagnosed with SpA if a patient with IID has spinal inflammatory pain. The higher the LR-value, the higher the likelihood of the absence of SpA in the absence of spinal inflammatory pain in a patient with IID.

To determine the odds ratio (OR), a binary logistics regression was built, a multivariate logistics regression model. The calculation was performed using the IBM SPSS 23 statistical program for Microsoft Windows, GraphPad Prism 8.

Results

In patients with IID the most common extraintestinal involvement was of the musculoskeletal system, which occurred in 45 (49.5%) patients. Arthralgia was one of the most common clinical manifestations, occurring in almost a third of patients with IID – 35 (38.5%) patients. Arthritis was detected in 12 (13.2%) patients with IID: in 8 (20.5%) with *S*±*Sh* and 4 (7.7%) with *Y*±C. SpA axial skeletal lesions were present in 26 (28.6%) patients and AS in 14 (15.4%) patients. The incidence of eye injuries was as follows: iridocyclitis – 6 (6.6%) patients, uveitis – 2 (2.2%) patients.

All patients with IID and spinal pain (n = 84) completed the 2009 ASAS questionnaire for spinal inflammatory pain. When evaluating each criterion for spinal inflammatory disease in patients with IID (n = 84), *S*±*Sh* (n = 36), *Y*±C (n = 48) and back pain, it was found that the onset of back pain before the age of 40 years was observed in 63 (75%) patients with IID – 32 (88.6%) patients with *S*±*Sh* and 31 (64.6%) with *Y*±C. The gradual occurrence of back pain was reported by 65 (77.4%) patients with IID – 25 (69.4%) with *S*±*Sh* and 40 (83.3%) with *Y*±C.

Pain relief after effort was present in 53 (63.1%) patients with IID – 24 (66.7%) had *S*±*Sh* and 29 (60.4%) had *Y*±C. Increased pain during rest periods was observed in 42 (50%) patients with IID: 17 (47.2%) with *S*±*Sh*, 25 (52.1%) with *Y*±C. The presence of nighttime back pain (with improvement after waking up) was reported by 52 (61.9%) patients with IID – 20 (55.6%) with *S*±*Sh* and 32 (66.7%) with *Y*±C.

Of all 91 patients with IID, 39 (42.9%) met the criteria for spinal inflammatory disease. Pelvic radiography was performed in 55 patients with IID: all patients with IID (n

= 39), as well as patients with IID with suspected spinal inflammatory disease (3 criteria out of 5, n = 16). Changes according to pelvic radiography were detected in 40 patients. Significant SI joints changes were detected in 14 patients.

MRI of the SI joints in T3 mode, was performed in 41 patients with IID and back pain. Bone marrow edema was detected in 6 patients (14.6%), and structural changes were detected in 20 patients with IID (48.7%). It should be noted that at the time of inclusion in the study, the number of patients with AS was 6 (6.6%). According to imaging, SpA was diagnosed in 26 (26.8%) patients – 14 with ankylosing spondylitis and 12 with non-radiological SpA.

It is important to note that in 12 patients with IID, the axial lesion was diagnosed at the early stage. According to the literature, the prevalence of IID-associated SpA varies in the range of 15-37%. The frequency of AS in our study was 15.4%, which is higher compared to the literature data (3-10%) (Table 1).

Table 1. *Axial spondyloarthritis in patients with IID*

Parameters	IID with SpA (n = 26)	IID without SpA (n = 65)
Male (n (%))	16 (61.5) 31 (47.7)	16 (61.5) 31 (47.7)
Age, years, (M ± δ)	40.35 ± 10.35	40.18 ± 12.36
Age at the onset of the disease, years, (M ± δ)	30.52 ± 11.36	32.61 ± 14.06
Duration of the disease, years, (n)	8.23 ± 7.14	7.47 ± 7.876
Arthralgia, (n (%))	16 (61.5)	19 (29.2)*
Arthritis, (n (%))	9 (34.6)	3 (4.6)**
Inflammatory spinal pain (ASAS), (n (%))	20 (76.9)	19 (29.2)**
Uveitis, (n (%))	6 (23.1)	1 (1.5)**
Hemoglobin, (M ± δ)	123.23 ± 23.7	124.63 ± 23.61
Platelets, (M ± δ)	349.09 ± 119.1	310.73 ± 133.49
ESR, (M ± δ)	25.95 ± 18.66	18.24 ± 12.07
C-reactive protein, (M ± δ)	11.78 ± 30.51	10.95 ± 25.197
y globulins, (M ± δ)	19.525 ± 4.76	18.44 ± 3.80
S±Sh, (n (%))	16 (61.5)	23 (35.4)*
Activity *Y±C*	5.33 ± 1.94	5.05 ± 2.45
Activity *S±Sh*	264.27 ± 159.18	225.17 ± 125.92

Note: *p < 0.05; **p < 0.01. M – arithmetic mean; δ – standard deviation; IID - intestinal infectious diseases; SpA – spondyloarthritis; ASAS – Assessment of SpondyloArthritis International Society; *Y±C - Yersinia enterocolitica* or *Campylobacter jejuni*; *S±Sh – Salmonella enteritidis* or *Shigella flexneri*.

The variability of prevalence rates is explained by the heterogeneity of the studies: the incidence of axial joint damage in IID was influenced by the used classification criteria.

Thus, in patients with a combination of IID and SpA, clinical signs of joint damage such as spinal inflammatory pain (p < 0.01), arthralgia (p < 0.05), arthritis (p < 0.01) as well as uveitis (p < 0.01) were encountered significantly more frequently. An analysis of the SpA frequency was made according to the nosology. It has been proven that in patients with *S±Sh*, axSpA was detected significantly more often (p<0.05) than in patients with *Y*±C. When building a logistic regression, it was found that in patients with IID, the risk of detecting SpA was higher in the presence of extraintestinal manifestations - OR 8.07 [95% CI 2.80-23.23], p = 0.0001, arthritis - OR 10.77 [95% 2.26-44.2], p= 0.005, arthralgia - OR 4.12 [95% CI 1.55-10.95], p = 0.005, uveitis - OR 19.2 [95% CI 2.18-169.13].

The activity, duration of the disease and the extent of IID did not increase the risk of axSpA. It was also determined that the risk of having axSpA is higher in patients with *S±Sh* compared to *Y*±C - OR 2.92 [95% CI 1.14-7.48], p = 0.025.

Taking into account the findings, in order to verify the reliability of the association of the confusion factor (influence factor) on the detection of axSpA in patients with IID, a multivariate logistics regression model was developed. It turned out that the third model, which included arthritis, *S±Sh* and spinal inflammatory pain (R2 = 0.486), set the highest rate of correct predictions. Given this, each parameter – spinal inflammatory pain, arthritis, arthralgia and the diagnosis of *S±Sh* are associated with an increased risk of developing SpA independently of each other, which allows us to state about the applicability of each parameter for the early diagnosis of axSpA in patients with IID.

Lesions of the gastrointestinal tract in ankylosing spondylitis

The analysis of the results of the questionnaire DISQ for the identification of symptoms of gastrointestinal lesions in patients with AS (n = 50) revealed a very heterogeneous frequency of symptoms. The most common symptoms were fatigability – 42 (84%), bloating – 32 (64%) patients, abdominal (or stomach) pain – 33 (66%) patients, and subfebrile condition – 29 (58%) patients.

In addition, the questions were grouped into four blocks depending on the severity of each symptom: the first block included – relation with the frequency of defecation; the second reflected the painful syndrome; the third - dyspepsia; the fourth – the syndrome of asthenia and fever. When calculating the average severity of each symptom, it was found that the complaints predominated in asthenia (2.12 ± 1.29), bloating (1.31 ± 1.19), abdominal pain (1.25 ± 1.17), as well as the subfebrile state.

The average score for the final value of the questionnaire was 9.76 ± 7.73. According to our study, in 46 patients (92%), the score of the results of the questionnaire was less than 19, which was considered remission. Subsequently, patients with a combination of AS and IID (n = 14) were compared with patients with IID-free AS (n = 50).

Thus, patients with a combination of IID and AS were significantly more likely to have signs of eye damage – uveitis and iridocyclitis compared to patients with IID-free AS (p < 0.05). HLA-B27 was detected significantly more frequently in patients with AS than in patients with a combination of IID and AS (p < 0.05).

The diagnostic value of 2009 ASAS classification criteria for spinal inflammatory pain and the possibility of their application to patients with a combination of IID and axSpA has not been determined. In this study, the diagnostic signif-

icance of ASAS classification criteria for spinal inflammatory pain was calculated in patients with IID and a concomitant diagnosis of axSpA.

Patients without dorsal pain were excluded from the study (n = 7). Therefore, the number of patients for calculating the diagnostic significance was 84. Patients were separated into two groups according to the presence of IID according to the criteria for spinal inflammatory pain 2009 ASAS classification criteria. The first group consisted of patients with IID and spinal inflammatory pain (n = 39); the second – patients with IID without spinal inflammatory pain (n = 45). Then, in each group, patients were divided into 4 subgroups according to the presence/absence of changes according to imaging data.

Mathematical calculation of the diagnostic significance

The Sn of the spinal inflammatory pain criteria was 75.9%, Sp – 68.3%, PPV – 0.51, NPV – 0.87, LR+ = 2.3, LR - = 0.3. Noteworthy is the high value of NPV – 0.87, that is, in the absence of spinal inflammatory pain, the probability of absence of SpA is 87%.

Therefore, 2009 ASAS classification criteria in IID shows a sensitivity of 75.9% for IID with dorsal inflammatory pain and 77.8% for dorsal inflammatory pain without IID and a specificity of 68.3% for IID with dorsal inflammatory pain, and 74.8% for dorsal inflammatory pain without IID.

Thus, our study allowed us to establish the following parameters arthritis, arthralgia, spinal inflammatory pain as well as the diagnosis of *S±Sh*, is raises the suspicion of SpA. It was possible to determine the sensitivity and specificity of the 2009 ASAS classification criteria for spinal inflammatory pain, which demonstrated significantly superior sensitivity and specificity compared to the criteria studied in a cohort of patients with chronic back pain. An algorithm for the early diagnosis of axial arthropathy in patients with IID has been proposed (fig. 1).

Conclusions

1. In patients with IID, the following clinical variants of arthropathies have been identified: axial lesion (spinal inflammatory pain according to 2009 ASAS classification criteria in 42.9%, axSpA in 28.6%, AS in 15.4%); arthralgia – 38.5%, arthritis – 13.2%. Conventional radiography imaging and MRI of SI joints increased the incidence of SpA from 6.6% (n = 6) to 28.6% (n = 26).
2. In patients with IID and axial arthropathies, arthralgia ($p < 0.01$), arthritis ($p < 0.01$), and uveitis ($p < 0.01$) are more common. At the same time, the possibility of detecting axSpA is higher in the presence of arthritis - OR 10.77 [95% CI 2.26-44.2], p = 0.005, arthralgia - OR 4.12 [95% CI 1.55-10.95], p = 0.005, inflammatory back pain - OR 8.07 [95% CI 2.80-23.23], p= 0.0001, uveitis - OR 19.2 [95% CI 2.18-169.13], p = 0.008. Patients with *S±Sh* have a higher chance of developing axSpA compared to patients with *Y*±C - OR 2.92 [95% CI 1.14–7.48], p = 0.025.
3. In patients with AS, according to the results of the questionnaire for the identification of gastrointesti-

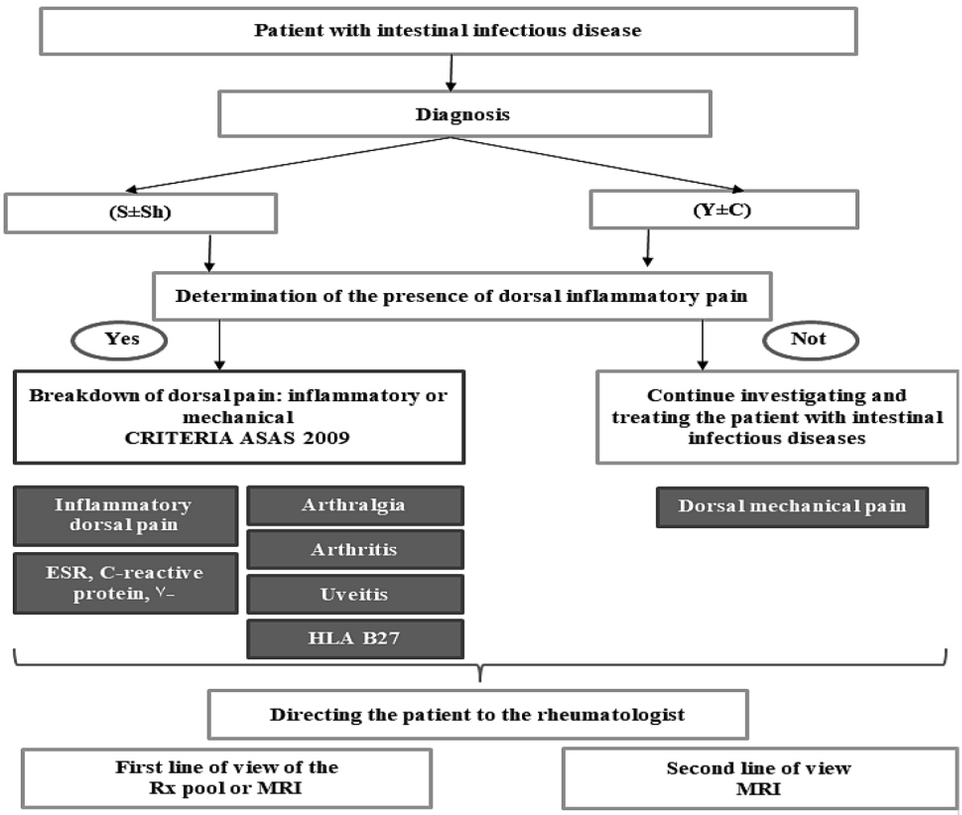


Fig. 1 Algorithm for diagnosing axSpA in patients with IID

ASAS – Assessment of SpondyloArthritis International Society; Y±C – *Yersinia enterocolitica* or *Campylobacter jejuni*; *S±Sh* – *Salmonella enteritidis* or *Shigella flexneri*; MRI – Magnetic Resonance Imaging; Rx – Radiography; HLA – Human Leukocyte Antigens; ESR – Erythrocyte Sedimentation Rate.

nal symptoms DISQ, complaints of asthenia prevailed, an average score of 2.12 (moderately pronounced), bloating and abdominal pain (an average score of 1.31 and 1.25, respectively), subfebrile condition (average score 1.08). At the same time, the lesion of the gastrointestinal tract was subclinical, since, according to the total score, 92% of the patients corresponded to the remission criteria.

4. The 2009 ASAS classification criteria for the evaluation of spinal inflammatory pain are applicable for patients with IID: sensitivity – 75.9%, specificity – 68.3%, positive predictive value – 0.51, negative predictive value – 0.87, the ratio of the probability of a positive result - 2.3, the probability ratio of a negative result - 0.3; for *S+Sh*: Sn = 71.4%, Sp = 72.7%, PPV = 0.63, NPV = 0.8, LR+ = 2.62, LR- = 0.39; for *Y*±C : Sn = 83.3%, Sp = 63.9%, PPV = 0.44, NPV = 0.92, LR+ = 2.31, LR- = 0.26.
5. An algorithm was proposed for the early detection of axial arthropathy in patients with IID with a certain diagnostic value of the ASAS classification criteria and an OR for spinal inflammatory pain, arthritis, arthralgia, uveitis, and the diagnosis of *S*±*Sh*.

Abbreviations:

ASAS – Assessment of SpondyloArthritis International Society; IID – Intestinal Infectious Diseases; MRI – Magnetic resonance imaging; Rx – X-ray; *S*±*Sh* – *Salmonella enteritidis* or *Shigella flexneri*; SpA – Spondyloarthritis; Y±C – *Yersinia enterocolitica* or *Campylobacter jejuni*.

Declaration of conflict of interest

Nothing to declare

Authors' contribution:

Study conception and design: LC, LG. Data acquisition: LC, ER, AS. Analysis and interpretation of data: LC, ER. Drafting of the manuscript: LC. Significant manuscript review with significant intellectual involvement: LC, ER. Approval of the "ready for print" version of the manuscript: LC, ER, LG, AS.

References:

1. Pedersen S., Maksymowych W. The Pathogenesis of Ankylosing Spondylitis: an Update. *Curr Rheumatol Rep* 21, 58 (2019).
2. Harkins P., Burke E., Swales C., Silman A. 'All disease begins in the gut'—the role of the intestinal microbiome in ankylosing spondylitis, *Rheumatology Advances in Practice*, Volume 5, Issue 3, 2021.
3. Breban M., Beaufrère M., Glatigny. The microbiome in spondyloarthritis. *Best Practice & Research Clinical Rheumatology* 33.6 (2019): p.101495.
4. Fragoulis C., George E., et al. Intestinal infectious diseases and spondyloarthropathies: from pathogenesis to treatment. *World journal of gastroenterology* 25.18 (2019): p.2162.
5. Belousova E., Abdulganieva D., Odintsova A. Frequency of Inflammatory Back Pain and Structural Changes of Axial Skeleton on Inflammatory Bowel Disease. *Book of abstracts. 2nd Prague European Days of Internal Medicine*. Prague, 2016, № 1-2., p. 8.
6. Belousova E., Abdulganieva D., Odintsova A. Performance of ASAS criteria for inflammatory back pain in patients with inflammatory bowel disease. *Journal of Crohn's &*Colitis. 2017. Vol. 11 (February), p. 56.
7. Ahsan T., Erum U., Jabeen R., Khowaja D. Ankylosing Spondylitis: A rheumatology clinic experience. *Pak J Med Sci*. 2016;32(2), p.365-368.
8. Young A., Lianjun A W., Liping A W., Xin A X., Cory J. A L., Hai D A. Possible Role of Intestinal Microbiota in the Pathogenesis of Ankylosing Spondylitis. 2016 *J International Journal of Molecular Sciences*, V 17 N 12, p.2126.
9. Rocha F., Castro A., et al. Microbes, helminths, and rheumatic diseases. *Best Practice & Research Clinical Rheumatology* 34.4 (2020): p.101528.
10. Tingting W., Shuhui M., Ping C., Laiyou W., Cuilian L., Donge T., Dongzhou L., Zhenyou J., Xiaoping H. Comprehensive analysis of differentially expressed mRNA and circRNA in Ankylosing spondylitis patients' platelets. *Experimental Cell Research*, (2021).
11. Antoniou A., Lenart I., Kriston-Vizi J., et al. Salmonella exploits HLA-B27 and host unfolded protein responses to promote intracellular replication. *Annals of the Rheumatic Diseases* 2019;78, p.74-82.
12. Kurtz J., Goggins J., McLachlan J. Salmonella infection: Interplay between the bacteria and host immune system. *Immunol Lett*. 2017;190: p.42-50.
13. Ashrafi M., Kuhn K., Weisman M. The arthritis connection to inflammatory bowel disease (IID): why has it taken so long to understand it?. *RMD Open* 2021; p.7.
14. Cardoneanu A., et al. Characteristics of the intestinal microbiome in ankylosing spondylitis. *Experimental and therapeutic medicine* 22.1 (2021): p.1-14.
15. Zhang X., Sun Z., Zhou A., et al. Association Between Infections and Risk of Ankylosing Spondylitis: A Systematic Review and Meta-Analysis. *Front Immunol*. 2021;12:768741. Published 2021 Oct 22.

Authors's ORCID ID:
Lia Chișlari, https://orcid.org/ 0000-0002-7088-568X
Liliana Groppa, https://orcid.org/ 0000-0002-3097-6181
Eugeniu Russu, https://orcid.org/ 0000-0001-8957-8471
Svetlana Agachi, https://orcid.org/ 0000-0002-2569-7188

UDC: 616.742.7-073.7:616.314

RESEARCH ARTICLE

Electromyographic values of masticatory muscles in middle-aged dentate patients

Mihail Mostovei[1*], Oleg Solomon[1†], Andrei Mostovei[2†], Nicolae Chele[2†]

[1]Department of prosthetic dentistry „I. Postolachi", Nicolae Testemitanu State University of Medicine and Pharmacy
[2]Department of OMF surgery and oral implantology „A. Guțan", Nicolae Testemitanu State University of Medicine and Pharmacy.

Manuscript received on: 16.04.2022
Accepted for publication: 30.05.2022

Corresponding author:
Mihail Mostovei, PhD fellow
Department of prosthetic dentistry „I. Postolachi",
Nicolae Testemitanu State University of Medicine and Pharmacy,
42, Toma Ciorbă street, Chișinau, Republic of Moldova, MD-2004
Email: mihail.mostovei@usmf.md

Short title: *Electromyography of dentate patients*

What is not known yet about the topic

Surface electromyography of masticatory muscles is highly dependent on different factors including age, sex, ethnic group. There is no available data about the surface electromyographic activity of masticatory muscles in middle-aged Moldavian people.

Research hypothesis

Middle-aged people that have most of the teeth healthy should be in the normal range of the muscular activity according to the mean values of the device.

The novelty added by the manuscript to already published scientific literature

The obtained data can be used further as a reference for comparison of masticatory muscle activity in healthy individuals vs patients of the same ethnical group and age with different treatment options to assess the differences or the quality of the provided treatment. The values in healthy subjects differ with 20.5% from the average values provided by the manufacturer for the percentage overlapping coefficients.

Abstract

Introduction. Surface electromyography has proven to be a useful instrument for the assessment of success rate for different dental treatments. However, due to numerous variables that may influence the data like age, gender, fat tissue thickness etc. it is necessary to adjust the obtained values to a reference group with the same parameters.

Material and methods. A number of 33 patients were included in the study (21 women and 12 men) aged between 43-67 years old (mean 54± SD1.26). Surface electromyography of masticatory muscles was used in these patients to assess the average value in a time span and overlapping coefficients. Totally 10 parameters from each patient were recorded: TAL, TAR, MML, MMR, PocTA, PocMM, BAR, Asym, TORS, Impact. Data were stored in an Excel spreadsheet and then analyzed statistically using RStudio software.

Results. Based on the results of our study, the main EMG activity indices in middle-aged Moldavian individuals had the following values: for the left temporalis (TAL) was 42 μV, median 18.8 with a minimum of 3.8 and a maximum of 190 μV. For the right temporalis (TAR) the mean was 51.4 μV, median – 32.9, a minimal value of 7.9 and a maximum of 248 μV. The left masseter had a mean of 48.7 μV, median 12.3, a minimal value of 1.5 and a maximum value of 439 μV. The right masseter had a mean of 42.1 μV, a median of 16.3, minimum value 11.4 and maximum 243 μV. The overlapping coefficients deviated with 20.5% from the normal range provided by the manufacturer.

Conclusions. Electromyographic activity of masticatory muscles in healthy patients can be used for comparison with patients that had various dental treatment but have the same age, gender, ethnicity, etc. The overlapping coefficients did not perfectly match in the normal range provided by manufacturer even if these subjects had previously minimum dental procedures.

Key words: surface electromyography, middle-aged individuals, overlapping coefficients.

Introduction

After the first record of muscle contraction in 1890 by Marey, electromyography has known a huge development being constantly upgraded and having its applications in different medical pathologies like temporomandibular disorders, dystonia, lesions of cranial nerves, sports medicine etc. [1-3]. Surface electromyography (sEMG) is often used due to the lack of tissue damage, ease of use and it provides often similar data to the needle EMG [4, 5]. Despite its extensive use, recording is influenced by many factors such

https://doi.org/10.52645/MJHS.2022.2.07

as electrode type and positioning, fat tissue thickness, sex, age etc. [6, 7]. Different researches have shown that elder patients have less muscle activity than younger ones, which can be explained by the possible decrease of motor units [8]. Dentists often use investigations like x-ray, MRI, functional analysis with standard parameters to assess the skeletal or functional symmetry in patients. In this perspective, asymmetrical work of masticatory muscles can lead to different complications both in dental structures, temporomandibular joint (TMJ) or even muscles themselves. In most cases, the electromyographic activity is assessed as mean values of muscle activity in a time span. However, this data is hard to compare between different groups due to numerous variables that influences the data [2, 5]. EMG activity of masticatory muscles in dentistry is mainly influenced by the occlusal changes that dentists perform. There are many articles that analyze the change in EMG activity in numerous dental procedures like implant-supported dentures, complete dentures, orthodontic treatments etc. [9-12]. In order to be able to use this data for comparison, a group of healthy subjects is required that would have also similar age, ethnicity, gender as the study group.

Material and methods

The study was based on analysis of electromyographic activity of masticatory muscles in 33 dentate subjects (21 women and 12 men) aged between 43-67 years old (mean/SD – 54±1.26 years). Patients were included in the study from three dental clinics based on the following criteria:

1. Lack of TMJ or masticatory muscle pathology.
2. Lack of fix dental or implant prostheses that exceed more than 2 teeth per arch.
3. Edentulous spans that don't exceed 1 tooth on the same side of dental arch.
4. Lack of heart pacemakers or any electronic device that may interfere with the muscle signal.
5. Patients that have signed the informed consent.

The study was conducted according to the criteria of Helsinki Declaration and the protocol was approved by the Ethic Committee of N. Testemiţanu State University of Medicine and Pharmacy on 16th of March 2018, no. 43. Taking into account the age of patients there were no patients completely without dental procedures performed in the past. From overall number of subjects 12 had single unit edentulous spans on one or both arches, 9 had implant supported restorations, 12 had fixed tooth supported restorations, 25 patients had associated tooth attrition with the dentin isles exposed in molars and premolars. Only patients with class I malocclusion were accepted and those with removable prostheses were not accepted in the study. After routine dental examination, patients were sited upright in the chair with the Frankfort line parallel to the ground. Muscles were identified through manual palpation, and the electrodes were positioned on the most prominent area of masseter and anterior temporal muscles (Figure 1a). Placement of bipolar electrodes may influence the amplitude of EMG records [13]. In this research, we have used the 4 channel electromyograph (ForEMG, Quatrotii, Italy) with concentric electrodes which are not such susceptible to positioning. The acquired data are available both as raw and as mean values in a time span (Figure 1 b, c). Patients were instructed to stay relaxed for first 3 seconds of registration then, to clench on their teeth for another 3 seconds. The values were related as percentage of initial values that were obtained during 3s clenching on cotton rolls as described previously by Ferrario [5]. Having the same time span, only the average values were compared. Besides the 4 basic values (TAL, TAR, MML, MMR) another 6 parameters were evaluated (Figure 1c): Percentage overlapping for temporalis (PocTA) and masseter (PocMM), Percentage overlapping for both masseter and temporalis (BAR), torque coefficient (Tors), percentage overlapping of right and left side muscles (Asym), muscular work related to vertical dimension of occlusion (IMPACT). The acquired data were introduced into RStudio software for descriptive statistical analysis. Shapiro-wilk test was applied for normality distribution, mean and median values were calculated.

Results

Statistical analysis of electromyographic indices has shown a wide distribution of values that can be explained by the small number of subjects included in the study. However, a wide range of parameters has been also reported in other studies [14]. Statistical data have been shown in Table 1. The average value for temporalis left (TAL) was 42 μV, median 18.8 with minimum of 3.8 and maximum of 190 μV. For temporalis right (TAR) the mean was 51.4 μV, median 32.9, minimal value 7.9 and maximum of 248 μV. Masseter left had a mean of 48.7 μV, median 12.3, minimal value of 1.5 and maximum 439 μV. Masseter right 42.1 μV, median 16.3, minimum value 11.4 and maximum 243 μV.

The obtained value can be also analyzed depending on their relation toward the normal range provided by the manufacturer for assessment of muscle symmetrical work. For this, another 6 parameters previously emphasized are available. They are formed depending on the interaction of first 4 main parameters of muscle activity. The result of statistical analysis is given in table 2.

The percentage overlapping coefficients have a range in which the value indicates a symmetrical function. For PocTa and PocMM it is between 83 and 100%, BAR and Tors coefficients have a normal range between 90 and 100% and Asym from -10 to 10% range.

Even though, the subjects in this study had minimal dental procedures, they did not fit perfectly in the normal range provided by the manufacturer. The value with highest deviation from the normal range was IMPACT. This might be due to wider range that this coefficient has (from 85 to 115%) or instability of vertical dimension of occlusion that is changed after full mouth rehabilitation procedures. The overall mean deviation percentage was 20.5% from normal parameters of the device.

Table 1. *Descriptive analysis of EMG activity indices in healthy subjects*

		LC (N=33)
TAL, µV	Mean (SD)	42.0 (48.5)
	Median (IQR)	18.8 (36.3)
	[Min, Max]	[3.80, 190]
	Shapiro-Wilk normality test	W = 0.72061, p = 1.361e-06
TAR, µV	Mean (SD)	51.4 (56.8)
	Median (IQR)	32.9 (35.2)
	[Min, Max]	[7.90, 248]
	Shapiro-Wilk normality test	W = 0.67751, p = 3.031e-07
MML, µV	Mean (SD)	48.7 (107)
	Median (IQR)	12.3 (24.8)
	[Min, Max]	[1.50, 439]
	Shapiro-Wilk normality test	W = 0.45479, p = 5.957e-10
MMR, µV	Mean (SD)	42.1 (64.4)
	Median (IQR)	16.3 (16.7)
	[Min, Max]	[11.4, 243]
	Shapiro-Wilk normality test	W = 0.51507, p = 2.646e-09

Note: SD – standard deviation; IQR – interquartile deviation; Min – minimal value; Max – maximal value; TAL – temporalis left; TAR – temporalis right; MML – masseter left; MMR – masseter right.

Discussions

The obtained data were a part of broader study conducted in a PhD thesis that require healthy patients of middle age to be assessed and compared to the ones that had full mouth implant-supported rehabilitation. The electromyographic signal can vary during the same registration due to different factors that influences the muscle contraction. In order to provide reproducibility of acquired signals there are different protocols that aim to position the electrodes depending on different landmarks [13, 15]. In this case, the concentric electrodes allow their positioning without taking into account the interelectrode distance that can in some cases lead to „crosstalk" signals from other muscles. Analysis of percentage overlapping coefficients allows a better understanding of muscle interaction during dental rehabilitation procedures with the modification of occlusal surfaces [5].

The acquired data has shown that even in cases were dental procedures were minimum; there is a deviation from the normal range in the percentage overlapping coefficients of 20.5%.

The wide range of value distribution inside the sample group shows that surface electromyography is hardly comparable due to high individuality of evaluated parameters. It cannot be said that these parameters are high or low in accordance with the data from the literature because there are various devices available in the market with different electrode types that can provide non-comparable data and different reference values. The registration method is technique sensitive and depends also on the positioning of the electrodes, tissue thickness, age, sex, anatomical peculiarities etc.

Table 2. *Statistical analysis of percentage overlapping coefficients*

		LC (N=33)
POCTA	Mean (SD)	74.7 (17.2)
	Median (IQR)	81.0 (18.9)
	[Min, Max]	[29.0, 90.9]
POCMM	Mean (SD)	73.6 (17.2)
	Median (IQR)	76.2 (18.1)
	[Min, Max]	[14.5, 98.7]
BAR	Mean (SD)	76.4 (14.7)
	Median (IQR)	80.1 (21.4)
	[Min, Max]	[23.4, 93.1]
TORS	Mean (SD)	80.5 (12.4)
	Median (IQR)	83.5 (15.8)
	[Min, Max]	[40.2, 93.9]
ASYM	Mean (SD)	12.7 (13.5)
	Median (IQR)	6.60 (11.3)
	[Min, Max]	[0, 56.4]
IMPACT	Mean (SD)	95.0 (28.2)
	Median (IQR)	95.0 (31.0)
	[Min, Max]	[0, 150]

Note: SD – standard deviation; IQR – interquartile deviation; Min – minimal value; Max – maximal value; PocTA – temporalis muscle overlapping; PocMM – masseter muscles overlapping, BAR – masseters and temporalis muscles overlapping, TORS – torque coefficient, Asym – right and left muscles overlapping, IMPACT – vertical dimension correlated index.

The middle-aged patients are hard to be found with most of the teeth sound. Most of the patients already have dental treatment of different type and extent that can disturb the acquired data. Additional to that, the low awareness of Moldovan people towards the oral health leads to early tooth loss, which minimize the number of healthy subjects for establishing normal reference values.

Conclusions

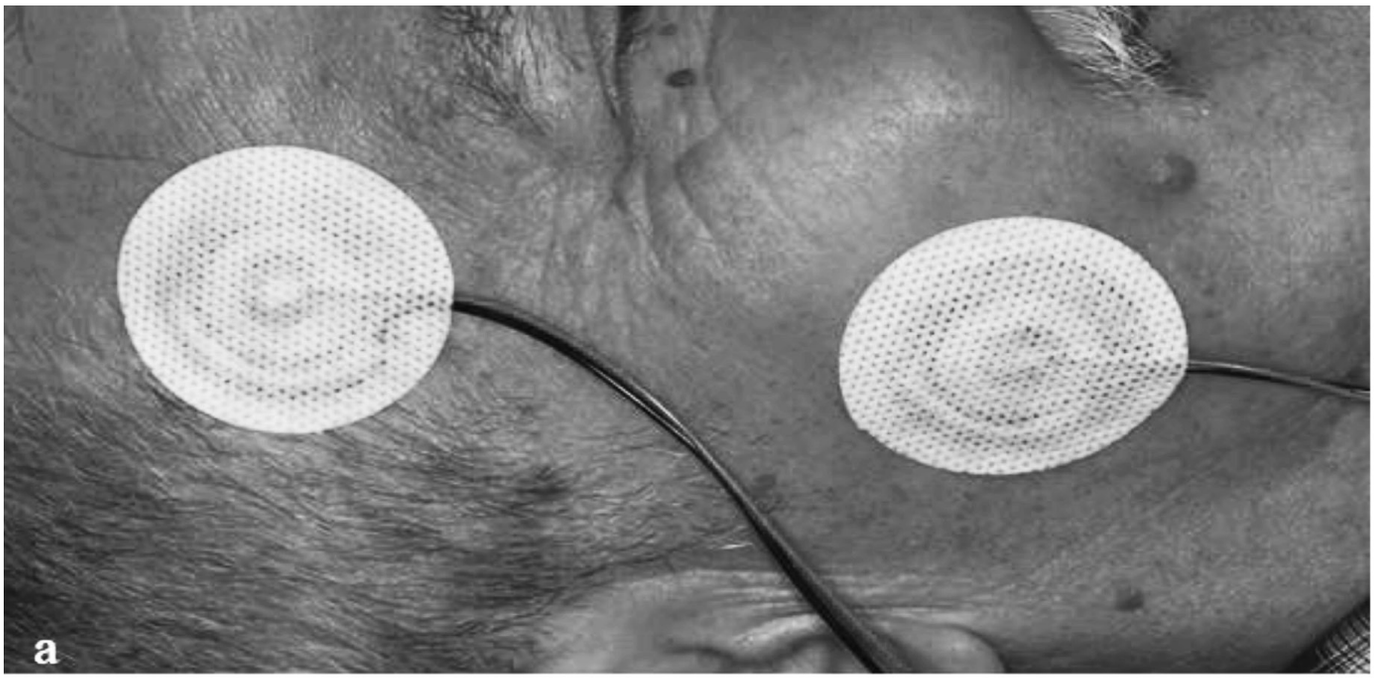
Surface electromyography has been widely used in dentistry to validate the dental treatment and try to create the most suitable restorations that would integrate into stomatognathic system. However, there is a big number of variables that can influence the acquired data among different population. Thus, in order to say if a treatment is valid or interfering with the muscular system, a reference group of the same age, sex and ethnicity is required.

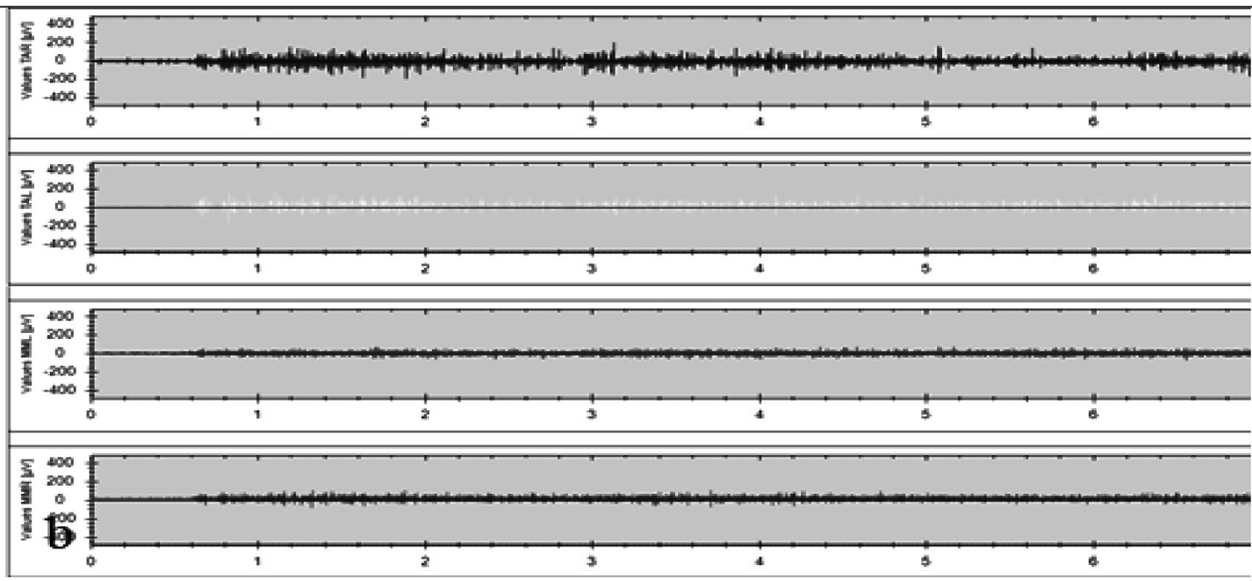
Declaration of conflict of interests:

Nothing to declare.

Authors' contributions:

AM – Conception and design of study; MM – Acquisition of data and manuscript drafting; NC – Analysis and interpretation of data; OS – Revising the manuscript critically for important intellectual content.





	Value
TA L	15.9uV
TA R	22.2uV
MM L	10.2uV
MM R	16.6uV

	Value	Range
POC TA	87.6R (51)	83≤(%)≤100
POC MM	88.9R (70.9)	83≤(%)≤100
BAR	91.5A (68.5)	90≤(%)≤100

	Value	Range
IMPACT	94	85≤(%)≤115
TORS	92.7L (64.4)	90≤(%)≤100
ASYM	2.4	-10≤(%)≤10

c

Fig. 1. *Evaluation of masticatory muscle EMG activity.*

a – placement of concentric electrodes, b – raw data of EMG activity, c – average values in a time span and percentage overlapping coefficients.

References

1. Nishi S. E., Basri R., Alam M. K. Uses of electromyography in dentistry: An overview with meta-analysis. *European Journal of Dentistry*, 2016; 10(03): 419–425.
2. Klasser G. D., Okeson J. P. The clinical usefulness of surface electromyography in the diagnosis and treatment of temporomandibular disorders. *The Journal of the American Dental Association,* 2006; 137(6): 763–771.
3. Raez, M. B., Hussain, M. S., Mohd-Yasin, F. Techniques of EMG signal analysis: detection, processing, classification and applications. *Biological procedures online*, 2006; *8*, 11–35.
4. Belser U. C., Hannam A. G. The contribution of the deep fibers of the masseter muscle to selected tooth-clenching and chewing tasks. *The Journal of Prosthetic Dentistry*, 1986; 56(5): 629–635.
5. Ferrario V. F., Sforza C., Colombo A., Ciusa, V. An electromyographic investigation of masticatory muscles symmetry in normo-occlusion subjects. *Journal of Oral Rehabilitation,* 2000; 27(1): 33–40.
6. Suvinen T.I., Kemppainen P. Review of clinical EMG studies related to muscle and occlusal factors in healthy and TMD subjects. *Journal of Oral Rehabilitation*. 2007; 34(9):631-44.
7. Suvinen TI, Malmberg J, Forster C, Kemppainen P. Postural and dynamic masseter and anterior temporalis muscle repeatability in serial assessments. *Journal of Oral Rehabilitation*, 2009; 36(11):814-20.
8. Jensen R., Fuglsang-Frederiksen A. Quantitative surface EMG of pericranial muscles. Relation to age and sex in a general population. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 1994; 93(3): 175–183.
9. Nishi S. E., Rahman N. A., Basri R., Alam M. K., Noor N., Zainal S. A., Husein A. Surface Electromyography (sEMG) Activity of Masticatory Muscle (Masseter and Temporalis) with Three Different Types of Orthodontic Bracket. *BioMed research international*, *2021*; 6642254.
10. Hamada T., Kotani H., Kawazoe Y., Yamada S. Effect of occlusal splints on the EMG activity of masseter and temporal muscles in bruxism with clinical symptoms. *Journal of Oral Rehabilitation*, 1982; 9: 119-23.
11. Szyszka-Sommerfeld L., Machoy M., Lipski M., Woźniak K., The Diagnostic Value of Electromyography in Identifying Patients With Pain-Related Temporomandibular Disorders. *Frontiers in Neurology,* 2019; 10, 180p.
12. Sonego M., Goiato M., Santos D. Electromyography evaluation of masseter and temporalis, bite force, and quality of life in elderly patients during the adaptation of mandibular implant-supported overdentures. *Clinical Oral Implants Research*, 2017; 28(10):e169-e174.
13. Sabaneeff, A., Caldas L. D., *et al.* Proposal of surface electromyography signal acquisition protocols for masseter and temporalis muscles. *Research on Biomedical Engineering*, 2017; 33(4): 324–330.
14. Gracht I., Derks A., Haselhuhn K., Wolfart S. EMG correlations of edentulous patients with implant overdentures and fixed dental prostheses compared to conventional complete dentures and dentates: A systematic review and meta-analysis. *Clinical Oral Implants Research,* 2016; 28(7): 765-773.
15. Hermens HJ., Freriks B., Disselhorst-Klug C., Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of Electromyography and Kinesiology*, 2000; 10(5): 361-74.

Authors's ORCID ID:
Mihail Mostovei https://orcid.org/0000-0002-8112-4798
Oleg Solomon https://orcid.org/0000-0002-7341-1711

UDC: 616.314-007.24-089.23:616.313-006.314

RESEARCH ARTICLE

3D volumetric analysis of the tongue in patients with skeletal class III malocclusion

Stanislav Strîșca[1]*

[1]Department of oro-maxillofacial surgery and oral implantology „Arsenie Guțan", Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova

Manuscript received on: 14.04.2022
Accepted for publication on: 23.05.2022

Corresponding author:
Stanislav Strîșca, PhD fellow, assistant professor
Department of oro-maxillofacial surgery and oral implantology „Arsenie Guțan",
Nicolae Testemitanu State University of Medicine and Pharmacy
1, Toma Ciorbă str., Chisinau, Republic of Moldova, MD-2004
e-mail: stanislav.strisca@gmail.com

Short title: *3D analysis of the tongue in skeletal class III*

What is not yet known on the issue addressed in the submitted manuscript

Currently in the scientific literature it is debated whether the tongue actively shapes the surrounding tissues or adapts to existing conditions. Also, the clinical studies started by Tamari et al. and Yoo et al., concluded that the volume of the tongue is correlated with the size of the lower dental arch, face height and chin position.

The research hypothesis

The null hypothesis assumes that there is no correlation between the volume of the tongue and the maxillo-mandibular relations in the third skeletal class patients.

The novelty added by manuscript to the already published scientific literature

Based on the obtained results, we will have a better understanding on how the volume of the oral cavity changes after performing orthognathic surgeries as well as its influence in the development of sleep apnea.

Abstract

Introduction. Mandibular prognathism is a facial disharmony characterized by an anterior relationship of the mandible to the upper jaw. Some authors associate the increased volume of the tongue with the development of this anomaly. Currently in the scientific literature it is debated whether the tongue actively shapes the surrounding tissues or adapts to existing conditions. The aim of this study is to evaluate the volume of the tongue at the patients with class III malocclusion compared to class I patients using three-dimensional volumetric analysis based on computed tomography.

Material and methods. The three-dimensional calculation of the tongue volume was performed using the technology of virtual surgical planning on 15 subjects with class III compared to class I, based on DICOM data of multidetector computed tomography.

Results. As a result of a detailed analysis, the volume of the language was correlated with the values of the angles SNA, SNB as well as the Witts values.

Conclusion. In conclusion, we attest to a higher volume of tongue at patients with class III compared to class I.

Keywords: dento-maxillary anomalies, orthodontics, OMF surgery, class III, virtual surgical planning, macroglossia.

Introduction

According to Moss's functional matrix theory, bone growth occurs in response to function, thus, tongue posture, nasal resistance and the presence of adenoids are thought to affect maxillofacial form [1, 2]. Class III dento-skeletal anomalies or mandibular prognathism is a facial disharmony characterized by an anterior relationship of the mandible to the upper jaw [3]. These changes in the sagittal plane of the upper jaw compared to the lower one translates into facial analysis through a face with a concave profile, sunken cheeks, the upper lip usually being placed in the opposite direction to the lower lip (Figure 1). These features lead to significant physiological and functional disorders with impaired quality of life [4]. The etiology of this anomaly has not yet been fully elucidated, but most authors assume that the occurrence of this disease may be conditioned by specific genetic factors, environment, and their relations [5].

The specific factors are characterized by the presence of disorders during embryological development, discrepancies in skeletal growth and / or dental arches, pituitary dwarfism, acromegaly but also early tooth loss. Genetic factors refer to the transmission of family tendencies, such as the disproportion between the size of the teeth and jaw bones which could generate crowding or spacing, as well as

https://doi.org/10.52645/MJHS.2022.2.08

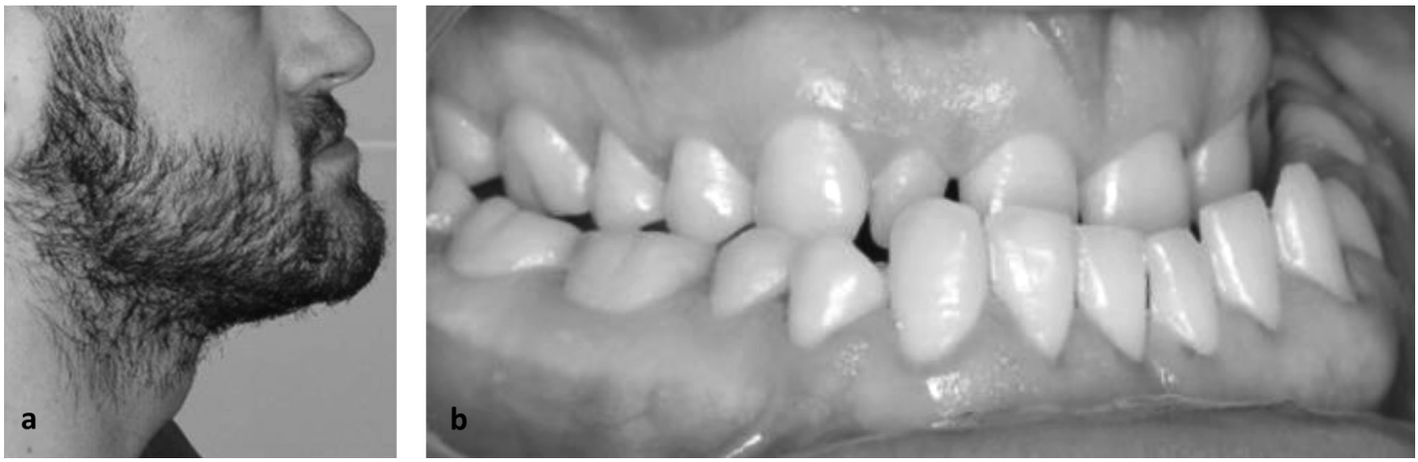


Fig. 1. *Patient with a Class III dento-skeletal malocclusion pattern.*

(a) – extra-oral lateral view, (b) – intra-oral presentation

the disproportion between the size and shape of the upper jaw to the lower [6]. Environmental factors are the disruption of the balance of harmonious development due to the influence of external pressure [6]. Such pressures can be caused by the interposition of objects between the dental arches, such as the lower or upper lip, fingers, extended use of the pacifier, infant swallowing, mouth breathing, etc. Similarly, authors such as Brodie et al., have assumed that tongue volume (VL), in addition to posture and function, is of notorious importance in the development of dento-facial anomalies [7].

Currently in the scientific literature, it is debated whether the tongue actively shapes the surrounding tissues or adapts to existing conditions. Also, the clinical studies started by Tamari et al. and Yoo et al., concluded that the volume of the tongue is correlated with the size of the lower dental arch [8], face height and chin position [9]. Previous research has also shown a correlation between the lower position of the tongue and the narrowing of the upper jaw in association with the posterior cross-bite, while infant swallowing is associated with open bite. Thus, lately, more and more authors show the importance of determining the volume of the tongue, because an increased volume will lead to a decrease in the total volume of the oral cavity. Consequently, by performing only mandibular setbacks, relapse may occur, therefore authors such as Clauser and Tieghi, in case of macroglossia, consider the surgical reduction of the tongue [10]. Studies on the surgical reduction of tongue at patients with Beckwith-Wiedmann syndrome in preadolescence have shown a change in skeletal class from III to I [10], but it was also associated with a lingual collapse of the dental arches [11]. For this reason, to better understand the influence of tongue on occlusion stability after orthognathic surgery, it is important to evaluate the oral cavity volume (OCV) and tongue volume (TV) to determine the volume ratio between TV and OCV.

The aim of this study is to evaluate the volume of the tongue in patients with class III malocclusion compared to class I patients, using three-dimensional volumetric analysis based on computed tomography. Thus, the null hypothesis assumes that there is no correlation between the volume of the tongue and the maxillo-mandibular relations in the third skeletal class patients.

Materials and methods

The current retrospective study involved 15 patients who came for dental treatment during 2019-2021. Patients were divided into 2 groups depending on their malocclusion type, thus the study group included 10 patients with class III, who had a mesialized molar ratio and a negative overjet, of which 3 women and 7 men (mean age – 29.8 years ± 8.25); and the control group included 5 patients with orthognathic occlusion, the relationship of class I molars with an overjet and overbite within the normal range, of which 2 women and 3 men (mean age – 36, 6 years ± 13.59).

The inclusion criteria were the presence of computed tomography (to minimize radiation exposure, the study also included class I patients who underwent radiological examination for purposes other than orthodontic examination, such as various traumas in the middle part region and upper face, tumors of the soft tissues of the face, etc.).

Exclusion criteria included: the presence of orthodontic treatment in the anamnesis, craniofacial developmental abnormalities, traumas in the region of the lower third of the face as well as disorders of the temporomandibular joint.

The study was approved by the Ethics Committee of the N. Testemițanu State University of Medicine and Pharmacy, no.23 from 12.04.2019 and informed consents were obtained from all subjects involved in the study.

During the radiological examination, the patient was placed in a supine position in the Siemens Somatotom Definition Edge tomography device, with the occlusal plane ori-

ented perpendicular to the tube (gantry), slice thickness 0.5 mm, field of view 218 mm, gantry tilt 00, at 120kV and 209 mA, for 12.4 seconds. Each radiological study included 523-612 native slices exported in DICOM format, the size of a voxel being 0.5 mm.

Applying the three-dimensional cephalometric analysis protocol, the maxilla-mandibular ratio in the sagittal plan was evaluated, using the angles: sella-nasion-A (SNA), SNB as well as FMA (Frankfort plan angle to the mandibular plan). The anatomical points were drawn manually in each patient.

The evaluation of the tongue volume was performed in the open-source software Slicer 3D 4.10.1 (https://www.slicer.org/), using the manual virtual segmentation procedure, based on the density range specific to muscle tissue, from -200 to 200 HU (Hounsfield units). In the segmented volume (Figure 2) were included 3 intrinsic muscles (longitudinal, transverse, vertical), 3 extrinsic muscles (genioglossus, palatoglossal, styloglossus). Hyoglossus muscle was not included in the segmentation.

To reduce segmentation errors, upper airway segmentation was additionally performed, and the Boolean subtraction procedure was carried out (Figure 3).

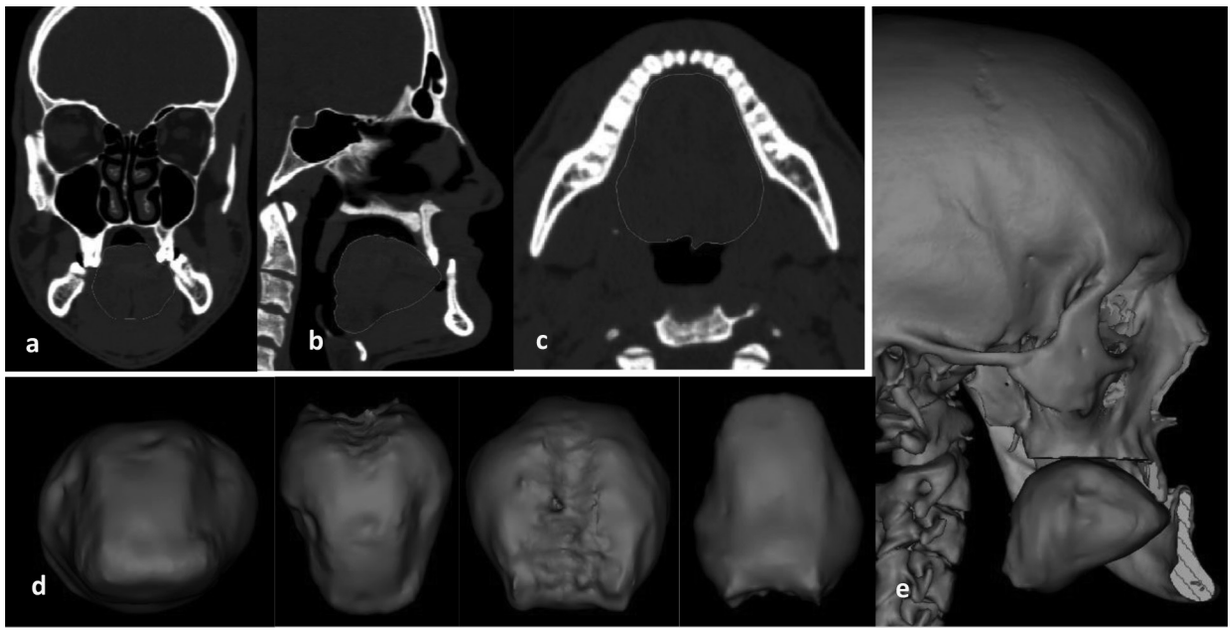


Fig. 2. *Manual virtual tongue segmentation.*

(a) – coronal view, (b) – sagittal view, (c) – axial view, (d) – the three-dimensional image of the tongue after surface smoothing procedure, I – volumetric reconstruction of the craniofacial skeleton with virtual segmental removal of the upper and lower jaw for a better visualization of the position of the tongue and the inverse maxilla-mandibular ratio.

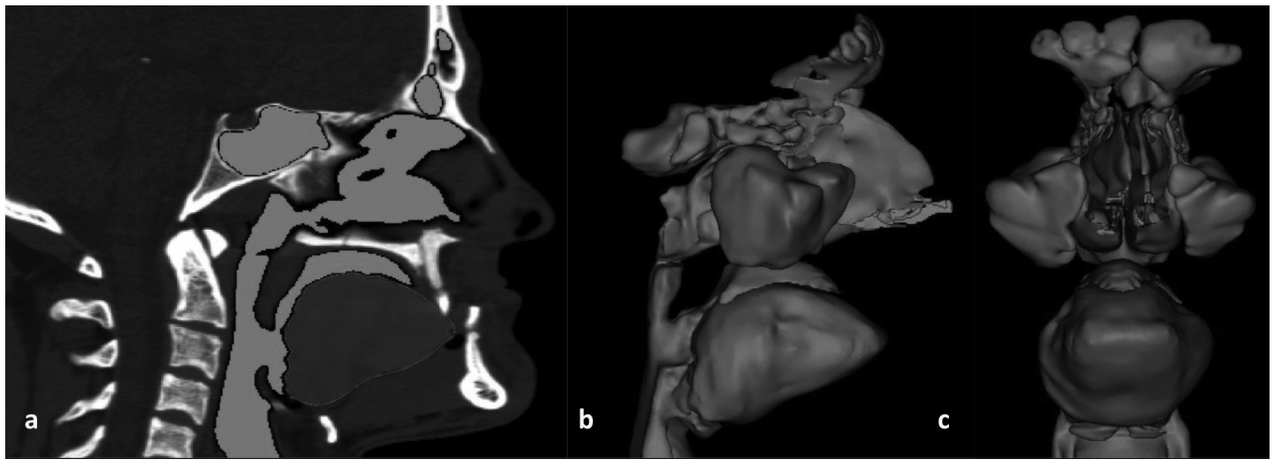


Fig. 3. *Segmentation of the upper airway*

A) Sagittal section. B) Three-dimensional reconstruction of the airway, lateral view. C) Three-dimensional reconstruction of the airways, frontal view

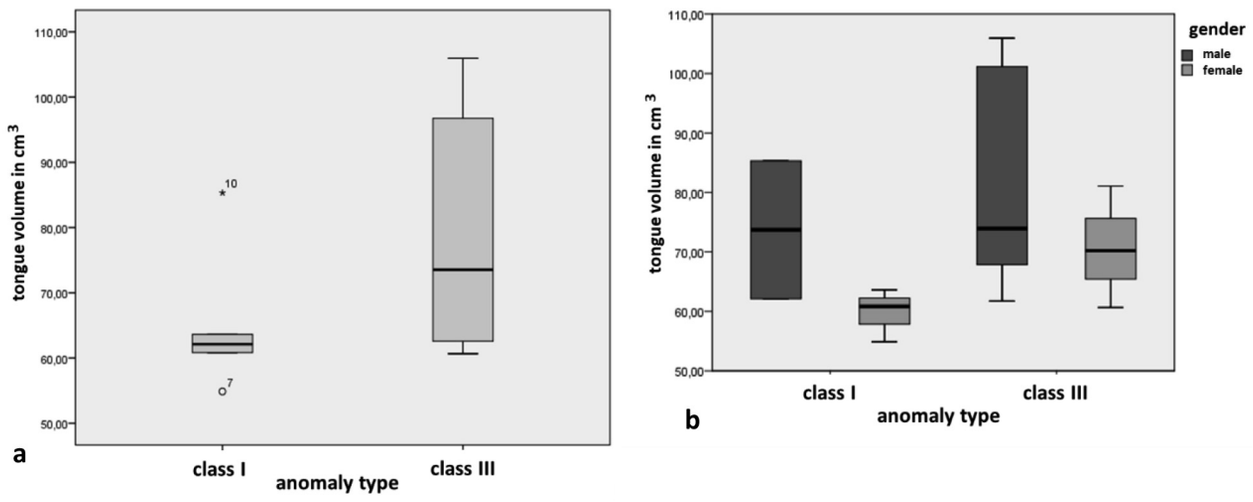


Fig. 4. *Boxplot type of tongue volume.*
(a) depending on the class of the anomaly, (b) depending on the class of the anomaly and gender.

In this study, the t-Student test was performed to assess the volume of tongue in males and females, but also to compare the study groups with each other, in the IBM SPSS statistical program. To evaluate the relationship between the volume of the tongue and the prognathism of the mandible the Pearson correlation was calculated (Class III Angle). Results are considered to be statistically significant at a level lower than 5% ($p < 0.05$). Data distribution assumption of normality was respected.

Results

The average volume of the tongue in this study was 74.55 cm^3, in class I – 65.35 cm^3 and in class III – 79.15 cm^3 (Figure 4). The data (mean and standard deviation) are represented in Table 1.

Table 1. *Statistical comparison of study groups*

	Class I		Class III	
	average	**stand.dev.**	**average**	**stand.dev.**
Age	36.60	13.594	29.89	8.257
SNA	82.20	2.588	81.20	3.364
SNB	80.80	3.962	87.75	5.195
ANB	2.20	0.836	-7.23	5.137
FMA	23.02	2.331	22.75	3.955
Witts	1.38	0.704	-12.19	6.469
Tongue volume in cm^3	65.89	11.645	79.15	17.612
Airway volume in cm^3	96.48	33.964	114.15	30.117

Note: SNA-the angle between the sella/nasion plane and the nasion/A plane; SNB-the angle between the sella/nasion plane and the nasion/B plane; ANB-difference between SNA and SNB angles; FMA-the angle formed by the intersection of the Frankfort horizontal plane with the mandibular plane; Witts-appraisal entails projecting points A and B in two perpendicular lines, along the functional occlusal plane.

Given the obvious sexual dimorphism, there was also conducted a comparison of the tongue volume, depending on gender (Figure 4).

The Pearson correlation revealed a positive and statistically significant correlation between the tongue volume and the SNB angle value, P = .034 (if the tongue volume is larger, the SNB angle increases) and negatively correlated with the Wits value P = .005 (if the tongue volume increases, Wit value decreases). The volume of the airways was 108.26 cm3.

Discussions

In 1965, Köle assumed that macroglossia could be the cause of the development of mandibular prognathism but failed to elucidate the role of language in the development of dento-maxillary anomalies [12]. This is due to the lack at that stage of the possibilities of objective quantitative and qualitative calculation of the volume of the language. The present study showed that the volume of the tongue was higher in patients with Class III compared to Class I (Figure 5).

Similar results were noticed in the study conducted by Ihan et al., in 2016, where the volume of the tongue in males was 100.8 ± 6.3 cm^3 in class III compared to 92.4 ± 9.8 cm^3 in class I, and in females – 77.4 ± 10.2 cm^3 in class III compared to 67.2 ± 5.6 cm^3 in the control group [12]. In another study conducted by Shigeta et al., in Japan on a group of 40 patients, the mean volume of the tongue was 79.00 ± 1.06 cm^3, the age of the subjects was between 25 and 77 years, however, in this study, no segmentation limits were defined and an automatic and/or semi-automatic segmentation protocol was used [13]. Similar results have been described by other authors, the results varying between 30 to 132 cm^3, these discrepancies are represented by the measurement method (direct measurements, use of different impression techniques), acquisition technique (multidetector computed tomography, conical beam computed tomography, magnetic resonance, ultrasonography) but also by the boundaries that the authors used in esti-

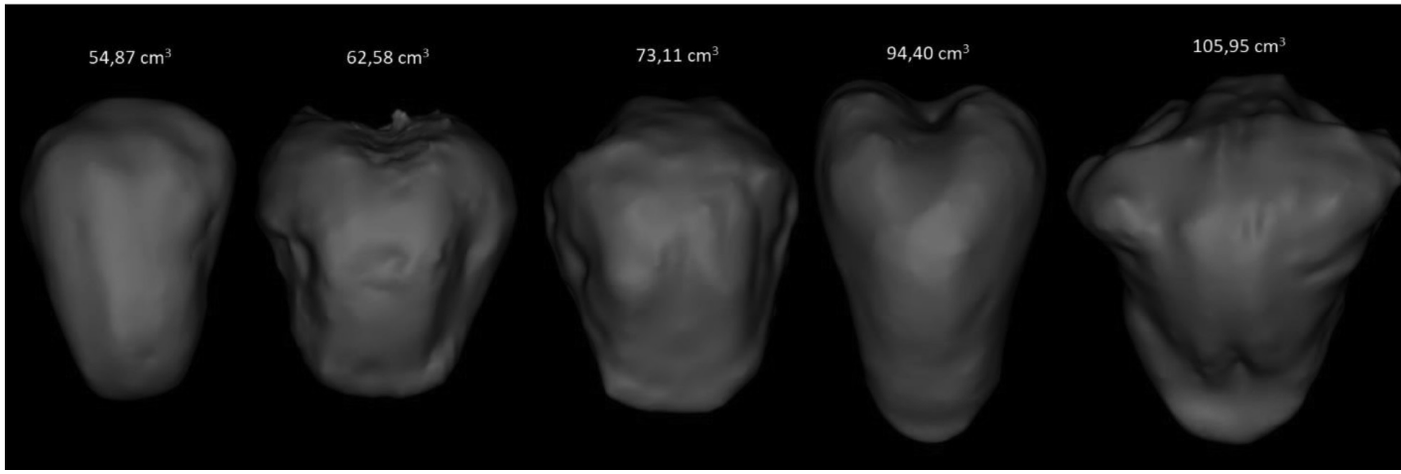


Fig. 5. *Tongue volume differences arranged in ascending order*

mating the volume of the tongue. Some studies also included the pharyngeal muscles, while in other publications the volume of the tongue was partially calculated, where the posterior nasal spine was used as a posterior landmark and the lower border was the enamel-cement junction of the first lower molars. For this reason, in some research there is a correlation between the volume of the tongue and the SNB angle values as well as the narrowing of the upper jaw, similar results being obtained by Iwasaki et al., in 2019. Based on the data presented, treatment planning should be performed with caution, considering the volume of the tongue, because in certain clinical situations, some authors recommend performing dental extractions to align and camouflage the dento-facial anomaly. However, due to the decrease in the perimeter of the dental arches, the pressure exerted by the tongue could favor the development of relapse. At the same time, the planning of orthognathic surgery must also be performed depending on the volume of the tongue and the oral cavity, to achieve a volumetric balance between them. Currently we cannot correlate the volume of the tongue with the pressure it exerts, because we do not know much about the self-adaptive abilities of the tongue and its changes in tonicity. Interestingly, in a study conducted by Frohlich et al., the pressure of the tongue on the teeth after surgical reduction did not change significantly at 12 months postoperatively, but the resting pressure was lower than before surgery [14]. For these reasons, in cases of increased tongue pressure following the posterior movement of the mandible, Wickwire et al., recommended reducing the tongue to achieve better stability of the intervention [15]. Still, such an approach is quite traumatic not only in our opinion but also in other authors' as well, because it can involve several postoperative complications. In such cases, we consider the bimaxillary approach to be rational, because the advancement of the upper jaw reduces the posterior movement of the mandible, so we do not adversely affect the volume of the airways.

In this study, the volume of the tongue was not correlated with the position of the hyoid bone; this remains to be elucidated in future research. Based on the obtained results, we are also going to study how the volume of the oral cavity changes after performing orthognathic surgery as well as its influence in the development of sleep apnea.

Conclusions

Based on the limitations of the current study, we can conclude that the increased volume of the tongue is correlated with mandibular prognathism. This phenomenon was encountered in both sexes, as well as the increased volume was correlated with the pronounced negative Witts values. The clinical significance of the present study is that the posterior movement of the mandible in orthognathic surgery should be carefully planned to minimize the risk of relapse as well as narrowing of the airways.

Declaration of conflict of interests

The authors declare that they have no conflicts of interest.

Financial disclosure statement

The research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Iwasaki T., Sato H. *et al*. Relationships among nasal resistance, adenoids, tonsils, and tongue posture and maxillofacial form in Class II and Class III children. *Am J Orthod Dentofacial Orthop.* 2017;151(5):929-940.
2. Iwasaki T., Suga H, *et al*. Relationships among tongue volume, hyoid position, airway volume and maxillofacial form in paediatric patients with Class-I, Class-II and Class-III malocclusions. *Orthod Craniofac Res*. 2019;22(1):9-15.
3. Trifan V., Lupan I. *et al*. Morbiditatea prin anomaliile dento-maxilare în Republica Moldova. *Medicina Stomatologică*. 2015;1(34):47.
4. Mendes de P. G., Adas S.G. *et al*. Dentofacial Deformities and Implications on Quality of Life: A Presurgical Multifactorial Analysis in Patients Seeking Orthognathic Surgical Treatment. *J Oral Maxillofac Surg*. 2019; 77(2):409.e1-409.
5. Mi H. *et al*. Influence of sociocultural factors on the selection of orthognathic surgery in patients with dental and maxillofacial deformities. *Shanghai Kou Qiang Yi Xue*. 2018;27(5):495-500.
6. Gabardo M., Zielak J. *et al*. Impact of orthognathic surgery on quality of life: Predisposing clinical and genetic factors. *J Craniomaxillofac Surg*. 2019;47(8):1285-1291.
7. Brodie A.G. Muscular factors in the diagnosis and treatment of malocclusions. *Angle Orthod*. 1953;23(2):71–7.
8. Tamari K., Shimizu K. *et al*. Relationship between tongue volume and lower dental arch sizes. *Am J Orthod Dentofacial Orthop.* 1991;100(5):453-8.
9. Yoo E., Murakami S., Takada K. *et al*. Tongue volume in human female adults with mandibular prognathism. *J Dent Res.* 1996;75(12):1957-62.
10. Miyawaki S. *et al*. Long-term changes in dentoskeletal pattern in a case with Beckwith-Wiedemann syndrome following tongue reduction and orthodontic treatment. *Angle Orthod*. 2000;70(4):326-31.
11. Liu Z.J., Shcherbatyy V. *et al.* Effects of tongue volume reduction on craniofacial growth: A longitudinal study on orofacial skeletons and dental arches. *Arch Oral Biol*. 2008;53(10):991-1001.
12. Ihan H. N., Barbič U. Tongue volume in adults with skeletal Class III dentofacial deformities. *Head Face Med*. 2016;22;12:12.
13. Shigeta Y., Ogawa T. *et al*. Influence of tongue/mandible volume ratio on oropharyngeal airway in Japanese male patients with obstructive sleep apnea. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;111(2):239-43.
14. Frohlich K., Ingervall B., Schmoker R. Influence of surgical tongue reduction on pressure from the tongue on the teeth. *Angle Orthod*. 1993;63(3):1918.
15. Wickwire N.A., Proffit W.R. Changes in tongue position and activity following mandibular osteotomy. *Am J Orthod*. 1972;62(1):94–5.).

Authors's ORCID ID:
Stanislav Strîșca https://orcid.org/0000-0002-6215-9434

UDC: 616-004.1-074/-078-037

REVIEW ARTICLE

Novel biomarkers in systemic sclerosis

Svetlana Agachi[1*†], Liliana Groppa[1,2†], Larisa Rotaru[1,2†], Elena Deseatnicova[1†], Lia Chişlari[1†], Eugeniu Russu[1,2†]

[1]Discipline of Internal Medicine, Department of rheumatology and nephrology, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova
[2]Rheumatology laboratory, Timofei Moşneaga Republican Clinical Hospital.

Manuscript received on: 13.04.2022
Accepted for publication: 31.05.2022

Corresponding author:
Svetlana Agachi, PhD, associate professor
Discipline of Internal Medicine, Department of rheumatology and nephrology, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova.
29 N. Testemiţanu, MD-2025, Chişinău, Republic of Moldova
e-mail: svetlana.agachi@usmf.md

Short title: *Novel biomarkers in systemic sclerosis*

What is not yet known on the issue addressed in the submitted manuscript

Given the limited therapeutic options and poor prognosis of many systemic sclerosis patients, a better understanding of the immune-pathophysiological profiles might aid to an adjusted therapeutic approach.

Research hypothesis

The qualitative, analytical study was performed in order to identify the possible biomarkers for diagnosis, disease progression, and complications in systemic sclerosis patients.

The novelty added by manuscript to the already published scientific literature

Recently described biomarkers for diagnosis, disease progression and complications in systemic sclerosis patients are summarized and systematized.

Abstract

Introduction. Due to the heterogeneous nature of systemic sclerosis, it is difficult to predict disease progression and complications. Despite the discovery of novel autoantibodies associated with systemic sclerosis (SSc), there is an unmet need for biomarkers for diagnosis, disease progression, and response to treatment.

Materials and methods. An analytical, qualitative study was performed with a narrative review of literature in the form of a synthesis article. Relevant primary sources published in 2020-2022 were identified and selected, using data extraction and analysis.

Results. Anti-citrullinated protein/peptide antibody could be useful in identifying patients with a more prominent joint disease. Of most interest, the anti-carbamylated protein antibodies (anti-CarP) could be a relevant biomarker related to fibrotic skin and lung disease. Positive anti-RNA (Ribonucleic acid) polymerase III antibody and antinuclear antibodies (ANA) negativity were significantly associated with GAVE (gastral antral vascular ectasia). Autoantibodies against telomeres may help identify systemic sclerosis with lung disease. Osteopontin links myeloid activation and disease progression in systemic sclerosis. CTRP (C1q tumor necrosis factor-related proteins) 9 protein levels may be biomarker of lung disease severity. CD (cluster differentiation) 21-low B cells are linked to vascular damage**.** L-tyrosine, L-tryptophan, and 1-methyl-adenosine distinguished healthy controls from SSc patients. L-leucine, L-isoleucine, xanthosine, and adenosine monophosphate differentiated between progressing and stable SSc-ILD. CECs (circulating endothelial cells) are a direct indicator of systemic vascular damage. Levels of the protein, galectin-3, are associated with heart involvement in people with systemic sclerosis. Low levels of the galectin-10 protein (Gal-10) in scleroderma patients associate with inflammation and vascular changes in the lungs, leading to pulmonary arterial hypertension (PAH). High levels of the CD146 protein may be a potential biomarker in identifying people with systemic sclerosis. Blood levels of the protein endocan increased in scleroderma patients who are at risk for pulmonary arterial hypertension. FLCs (free light chain) could be employed as useful potential biomarker of early diagnosis and to follow disease activity.

Conclusions. Novel discovered biomarkers could predict disease development, activity, and severity of diverse organ involvement, predict risk of complications of systemic sclerosis.

Keywords: systemic sclerosis, biomarkers, diagnosis, prediction of disease progression, complication.

https://doi.org/10.52645/MJHS.2022.2.09

Introduction

Systemic sclerosis (SSc) is an autoimmune disease, which is characterized by vasculopathy, tissue fibrosis, and activation of the innate and adaptive immune system. The disease course and severity have various clinical characteristics ranging from a relatively benign condition to a rapidly progressive disease with high mortality. Clinical features of the disease are variable and consist of skin thickening, vasculopathy and internal organ involvement. Due to the heterogeneous nature of the disease, it is difficult to predict disease progression and complications. Given the limited therapeutic options and poor prognosis of many SSc patients, a better understanding of the immune-pathophysiological profiles might aid to an adjusted therapeutic approach.

Despite the discovery of novel autoantibodies associated with SSc, there is an unmet need for biomarkers for diagnosis, disease progression, and response to treatment.

The aim of the study was to investigate recently described biomarkers for diagnosis, disease progression and complications in systemic sclerosis patients.

Materials and methods

A qualitative and analytical study was performed focused on primary studies published in 2020-2022 and dedicated to the identification of biomarkers for diagnosis, disease progression, and complications in systemic sclerosis patients.

In order to realize the proposed aim, scientific publications were searched over the PubMed, NCIB, Google Search, Medscape using the key words systemic sclerosis, biomarkers, diagnosis, prediction of disease progression, complication. More than 80 reference sources have been identified and 20 were selected for analysis.

Results and discussions

Systemic sclerosis (SSc) is an autoimmune disease that is characterized by vasculopathy, tissue fibrosis, and activation of the innate and adaptive immune system. The disease course and severity have various clinical characteristics ranging from a relatively benign condition to a rapidly progressive disease with high mortality. Clinical features of the disease are variable and consist of skin thickening, vasculopathy and internal organ involvement. Due to the heterogeneous nature of the disease, it is difficult to predict disease progression and complications. Given the limited therapeutic options and poor prognosis of many SSc patients, a better understanding of the immune-pathophysiological profiles might aid to an adjusted therapeutic approach.

Despite the discovery of novel autoantibodies associated with SSc, there is an unmet need for biomarkers for diagnosis, disease progression, and response to treatment.

SSc-specific autoantibodies are currently used for diagnosis and prediction of clinical features, as other biomarkers have not yet been fully vetted. Krebs von den Lungen-6 (KL-6), surfactant protein-D (SP-D), and CCL (C Chemokine Ligand) 18 have been considered as serum biomarkers of SSc-related interstitial lung disease. Moreover, levels of circulating brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) can provide diagnostic information and indicate the severity of pulmonary arterial hypertension. Assessment of several serum/plasma cytokines, chemokines, growth factors, adhesion molecules, and other molecules may also reflect the activity or progression of fibrosis and vascular involvement in affected organs. Recently, microRNAs have also been implicated as possible circulating indicators of SSc. In table 1 are presented some biomarkers that have been shown to possess predictive potential for various manifestations of systemic sclerosis.

In this review, we focus on several new potential SSc biomarkers that have been described in 2020-2022 and discuss their clinical utility.

Similarly, to what is seen in other connective tissue diseases, most SSc patients are seropositive for antinuclear antibodies and have disease-specific autoantibodies, which can be detected prior to the development of clinical symptoms. Disease-specific autoantibody profiles support not only conclusive and phenotypic diagnoses but can also be associated with clinical manifestations and disease progression of SSc. In fact, autoantibodies such as anticentromere antibody, anti-topoisomerase I antibody, and anti-RNA polymerase III antibody are specific and included in 2013 criteria for classification.

There are also several other autoantibodies that are considered as potential predictors of course and outcomes of systemic sclerosis.

Anti-endothelial cell antibodies are found in a substantial number of SSc patients (22–86%) [2]. These antibodies have been shown to target various vascular antigens, such as ICAM-1 [3], lamin A/C, tubulinβ-chain, and vinculin [4], which are responsible for endothelial cell activation via increased oxidative stress and proinflammatory responses. Some authors found anti-endothelial cell antibodies more frequently present in SSc patients with ILD than in those without ILD [5].

Based on two large SSc French cohorts (448 SSc patients) [6] the prevalence and associated factors with the autoantibodies linked to erosive arthritis were studied. Enzyme-linked immunosorbent assay (ELISA) for IgM rheumatoid factor (RF), IgG anti-citrullinated proteins (ACPA) and IgG anticarbamylated proteins antibodies (anti-CarP) were determined. The prevalence and clinical associations of the different antibodies were investigated. RF positivity was observed in 113 patients (25%) compared to 39 (9%) for ACPA and 63 (14%) for anti-CarP antibodies. Through multivariate regression analysis, both RF and ACPA positivity resulted to be associated with RA overlap disease (OR 5.7, 95% CI 2.3–13.8 and OR 44.1, 95% CI 15.4–126.3, respectively). Additionally, ACPA was found to be significantly related to synovitis/tenosynovitis (OR 1.7, 95% CI 1.0–2.6). RF positivity was associated to a „vascular subset" (i.e., any major vascular complication) (OR 2.1, 95% CI 1.3–3.4). Moreover, anti-CarP antibodies were associated with a fibrotic subset and with digital ulcers (OR 2.0, 95% CI 1.1–3.6 and OR 1.9, 95% CI 1.1–3.4). Authors have concluded that

Table 1. *Potential Biomarkers in Systemic Sclerosis [1].*

Biomarker	Clinical association
TGF-β+	Digital ulcers, dcSSc
TGF-β-	dcSSc, mRSS (in dcSSc)
VEGF+	Systemic organ involvement, PAH, shorter disease duration, skin sclerosis, reduced capillary density of nailfold
VEGF-	Digital ulcers
CTGF+	mRSS, ILD
GDF-15+	Skin sclerosis, PAH, ILD, respiratory dysfunction (FVC, DLCO)
IL-6+	mRSS, early progressive skin sclerosis, poor prognosis, DLCO decline in SSc-ILD
BAFF+	Skin sclerosis
APRIL+	Pulmonary fibrosis
CCL2+	ILD (lung dysfunction, CT scores), mRSS
CXCL4+	mRSS, lung fibrosis, PAH, disease progression
CXCL8+	Predictive of physical dysfunction
CXCL10+	Preclinical/early SSc
CX3CL1+	dcSSc, ILD, digital ulcer
ICAM-1+	Rapidly progressive disease, digital ulcers, dcSSc, ILD, joint involvement, renal crisis, predictive of respiratory dysfunction
VCAM-1+	Systemic organ involvement, renal crisis, disease activity
E-selectin+	Systemic organ involvement, renal crisis, disease activity
P-selectin+	Disease activity, predictive of physical disability
Endostatin+	PAH
Endoglin+	lcSSc, anticentromere Ab, cutaneous ulcer, telangiectasia, PAH.
Von Willebrand factor+	Raynaud's phenomenon, disease severity, ILD, predictive of PAH
KL-6+	Severity of ILD, maximum fibrosis scores on HRCT
SP-D+	Severity of ILD, maximum fibrosis scores on HRCT
CCL18+	Activity and severity of ILD, predictive worsening of ILD and mortality
BNP/NT pro-BNP+	Severity, stability, and prognosis of PAH
Endothelin-1+	PAH, systemic organ involvement, microangiopathy defined by capillaroscopy
Type I collagen (C-terminal telopeptide)+	Skin fibrosis, mRSS, pulmonary dysfunction, CRP
Type III collagen (N-terminal peptide)+	Disease activity, mRSS, HRCT score, prognosis
MMP-7+	ILD, disease severity
MMP-9+	mRSS, dcSSc
MMP-12+	Skin sclerosis, dcSSc, ILD, nailfold bleeding, lower FVC
CRP+	Skin sclerosis, PAH, renal dysfunction, risk of progressive early ILD, worse pulmonary function
sCD163+	ILD, PAH, skin sclerosis
YKL-40+	Pulmonary involvement, higher mortality rate

Note: + – upregulated; - – downregulated; TGF-β – transforming growth factor; GDF-15 – growth differentiation factor 15; BAFF – B-cell-activating factor belonging to the tumor necrosis factor family; APRIL – a proliferation-inducing ligand; MMP – matrix metalloproteinases; BNP – brain natriuretic peptide; NT-proBNP – N-terminal-pro hormone BNP; CTGF –connective tissue growth factor; mRSS – modified Rodnan total skin thickness score; ILD – interstitial lung disease; IL-6 – interleukin 6; DLCO – diffusing capacity of carbon monoxide; CT – computed tomography; PAH – pulmonary arterial hypertension; ICAM-1 – intercellular adhesion molecule 1; dcSSc – diffuse cutaneous systemic sclerosis; VEGF – vascular endothelial growth factor; lcSSc – limited cutaneous systemic sclerosis; KL-6 – krebs von den Lungen-6; HRCT – high resolution CT; SP-D – surfactant protein-D.

ACPA could be useful in identifying patients with a more prominent joint disease and RA overlap disease. Of the most interest was found that anti-CarP antibodies could be a relevant biomarker related to fibrotic skin and lung disease.

Based on a multicenter international cohort, the clinical associations of anti-PM/Scl (polymyositis/scleroderma) antibodies in patients with SSc was evaluated, with a particular focus on unresolved issues, including scleroderma renal crisis (RC), malignancies, and functional outcome of interstitial lung disease (ILD) [7]. Using the The European Scleroderma Trials and Research group (EUSTAR) database, SSc patient were analyzed with the following outcomes: anti-PM/Scl+ without SSc-specific autoantibodies were compared with 7202 anti-PM/Scl-, and then to 155 anti-Pm/Scl+ with SSc-specific antibodies. Additional data were collected for 165 anti-PM/Scl+ SSc patients (85 from the EUSTAR registry) and compared with 257 anti-PM/Scl- SSc controls, matched for sex, cutaneous subset, disease duration and age at SSc onset.

As a result, it appears that patients with isolated anti-PM/Scl+, as compared with anti-Pm/Scl-, had higher frequency of muscle involvement, ILD, calcinosis and cutaneous signs of dermatomyositis (DM), but similar frequency of scleroderma renal crisis (SRC) and malignancies (either synchronous with SSc onset or not). The presence of muscle involvement was associated with a more severe disease phenotype. Although very frequent, ILD had a better functional outcome in cases than in controls. In patients with both anti-PM/Scl and SSc-specific antibodies, a higher frequency of typical SSc features than in those with isolated anti-PM/Scl was observed.

Authors concluded that analysis of the largest series of anti-PM/Scl+ SSc patients so far reported helps to delineate a specific clinical subset with muscle involvement, cutaneous DM, calcinosis and ILD characterized by a good functional outcome. SRC and malignancies do not seem to be part of this syndrome.

Using a cohort study of SSc patients who were seen at Stanford between 2004 and 2018 and had undergone esophagogastroduodenoscopy (EGD), the clinical features between those with and without GAVE were compared, based on a multivariable logistic regression to identify clinical correlates with GAVE [8]. A total of 225 patients with SSc who underwent EGD were included in this study and 19 (8.4%) had GAVE. Those with GAVE were more likely to have scleroderma renal crisis (SRC) (21% vs 3%; $p<0.01$), positive anti-RNA polymerase III antibody (71% vs 19%; $p<0.01$), nucleolar pattern of anti-nuclear antibody (ANA) (33% vs 11%; $p=0.04$), and negative ANA (<1:80 by immunofluorescence) (33% vs 11%; $p=0.02$). On multivariate analysis with multiple imputation, anti-RNA polymerase III positivity (OR 4.57; 95% CI (1.57 – 13.23), $p<0.01$) and ANA negativity (OR 3.75; 95% CI (1.21 – 11.62), $p=0.02$) remained significantly associated with GAVE.

In conclusion was mentioned that positive anti-RNA polymerase III antibody and ANA negativity were significantly associated with GAVE. Further studies are necessary to de-

termine whether patients with these autoantibody profiles should undergo screening endoscopies for GAVE.

Autoantibodies against proteins of the telomeres – the protective caps of chromosomes and a marker of lifespan - were found in scleroderma patients with lung disease and shorter telomeres, a new study reveals [9]. The findings suggest these autoantibodies could serve as a novel biomarker for scleroderma with lung disease. Telomeres are coverings on the tips of chromosomes that, as cells age, become shorter and work as a kind of „molecular clock".

Previous studies have reported that people with scleroderma, or systemic sclerosis (SSc), who have shorter telomeres in white blood cells (lymphocytes) are at higher risk for interstitial lung disease (ILD). ILD is an umbrella term for a group of lung disorders characterized by inflammation and scarring (fibrosis) of the lungs. It is a frequent complication of scleroderma.

Researchers at the Johns Hopkins University in Baltimore, Maryland, Cedars-Sinai Medical Center in Los Angeles, California, and the University of California in San Francisco (UCSF), tested whether scleroderma patients with shorter telomeres carry autoantibodies against the telomerase and shelterin proteins of telomeres.

Two groups of patients, one from Johns Hopkins and the other from UCSF Scleroderma Centers, were included in the study.

To test for autoantibodies against telomerase, the researchers analyzed blood samples from 200 patients of the Johns Hopkins group, and 30 healthy individuals who served as controls. The analysis revealed that six patients (3%) were positive for these autoantibodies, while all the controls were negative. In addition, seven patients had autoantibodies against telomerase or one of the six shelterin proteins. Again, no autoantibodies were found in the healthy individuals. TERF1 was the most targeted shelterin, with 22 patients (11%) testing positive for such autoantibodies. In the UCSF group, 18 of 242 patients (7.4%) also had autoantibodies against TERF1.

Combining the two groups, a total of 40 patients (9%) tested positive for anti-TERF1 antibodies, in contrast to only 1.3% in 78 controls. To assess whether anti-TERF1 autoantibodies were exclusive of scleroderma, they measured their levels in 60 patients with rheumatoid arthritis and 60 with myositis (inflammation of the muscles). The analysis showed that only one patient with rheumatoid arthritis and one with myositis (1.7% each) were positive for anti-TERF1 autoantibodies, a rate similar to that seen in healthy individuals.

To assess whether the presence of the autoantibodies was linked with shorter telomeres, the researchers then measured the telomeres' length in white blood cells from the UCSF group.

Results showed that compared to patients without autoantibodies, the telomeres were shorter than expected for their age range in significantly more patients with anti-TERF1 autoantibodies – 78% vs. 43%.

Another method for quantifying telomere length (Flow-FISH), which is more accurate and sensitive, according to the researchers, confirmed the link between anti-TERF1 autoantibodies and shorter telomeres.

Patients with anti-TERF1 autoantibodies were generally younger than those negative for these antibodies (mean age 52.6 vs. 56.4). The presence of autoantibodies was linked with a history of severe lung disease and worse lung function, as shown by the lower percent predicted diffusion capacity (DLCO), 58.0 vs. 67.9. DLCO measures how much oxygen is transferred from the lungs into the bloodstream.

Also, anti-TERF1 autoantibodies were associated with a greater risk for severe muscle disease (three times higher) and inflammatory arthritis (about two times higher).

Finally, the researchers screened 152 patients with idiopathic pulmonary fibrosis (IPF), an inflammatory lung disease also linked with shorter telomeres, for anti-TERF1 autoantibodies. They found that 11 patients were positive (7.2%), which suggests that telomere-targeting antibodies might underlie lung disease.

"We describe a novel subgroup of patients with SSc and IPF with autoantibodies targeting the telomerase/shelterin complex that in SSc is associated with short telomeres in peripheral lymphocytes and the presence of lung disease," the researchers wrote. "These autoantibodies could serve as novel biomarkers for systemic sclerosis and specifically for systemic sclerosis lung disease," they concluded.

One of the important questions around SSc is whether there exists a dependency of clinical features of systemic sclerosis (SSc) patients negative for SSc related autoantibodies (autoAbs).

In a single-center retrospective study [10] of 546 SSc patients, 4.8% were negative for ANA and 5.3% were ANA positive but negative for SSc related autoAbs. Regarding clinical features, patients negative for ANA/SSc related autoAbs (n=55) had a significantly shorter disease duration, higher proportion of the diffuse type, contracture of phalanges, diffuse pigmentation, higher modified Rodnan total skin thickness score (mRSS), and lower incidence of telangiectasia than those with ACA (n=224). On the other hand, younger disease onset, lower mRSS, and lower incidence of scleroderma renal crisis were observed in patients negative for ANA/SSc related autoAbs than in those with antiRNAP Abs (n=52). Although pitting scars were less common in patients negative for ANA/SSc related autoAbs than in those with anti-topo I Abs (n=144), their clinical features were similar. Probably, patients negative for ANA/SSc related autoAbs form a clinically distinct subset among SSc patients.

Other study have shown that osteopontin links myeloid activation and disease progression in systemic sclerosis [11]. This study highlights that osteopontin is increased in lung tissue from patients with SSc and that lung macrophages are the main source of osteopontin in SSc-associated interstitial lung disease (SSc-ILD). The authors demonstrate that serum osteopontin is increased in SSc and that higher levels of osteopontin are associated with disease progression. From a pathogenic viewpoint, this study demonstrates that osteopontin secretion by monocytes/macrophages is induced by immune complexes in an IL-6- and M-CSF-de-

pendent manner. In patients with SSc, blockade of the IL-6 receptor by tocilizumab reduces the levels of circulating osteopontin. These results suggest that macrophagic osteopontin could be a key biomarker participating in the progression of SSc ILD.

Higher-than-normal blood levels of squamous cell carcinoma antigen 1 (SCCA1), a pro-fibrotic protein, are associated with a greater risk of interstitial lung disease (ILD) among people with systemic sclerosis (SSc-ILD), a study suggests [12]. This finding points to SCCA1 blood levels as a potential biomarker for early detection of SSc-ILD, and in helping to identify scleroderma patients with a low risk of ILD. In addition, SSCA1 could be a potential therapeutic target in SSc-ILD.

ILD is a group of disorders characterized by inflammation and scarring in tissue in and around the pulmonary air sacs, hampering the lungs' ability to transfer oxygen to the bloodstream. While it is well established that SSc patients are at high risk of developing ILDs, its diagnosis remains challenging due to the potential lack of symptoms in early stages of the disease.

Increasing efforts are focused on identifying potential biomarkers of early lung involvement in SSc patients, but few have been clinically confirmed so far.SCCA1, also known as SerpinB3, is involved in inflammatory diseases and cancer. It can be detected in the blood bound to another protein called IgM (SCCA-IgM).

Higher-than-normal levels of SCCA1 are found in the lungs of people with idiopathic pulmonary fibrosis (IPF), the most common ILD, and SCCA1 levels have been associated with the extent of fibrosis and fibrotic-associated processes in IPF patients and in people with chronic liver disease. However, whether SSCA1 plays a role in lung involvement among those with SSc remains unclear.

Researchers in Italy set out to evaluate a potential link between SCCA-IgM blood levels and clinical features of SSc patients. They analyzed demographic and clinical data on 97 SSc patients (82 women and 15 men) followed at a single tertiary center. Patients had a mean age of 55.4 and had lived with the disease for a median of 12 years. Most (63.9%) were classified as having limited scleroderma, and 36.1% had diffuse scleroderma. Pulmonary involvement was measured by high resolution computed tomography (HRCT) scans and lung function tests. Heart, gastrointestinal, and skin changes were also assessed. A cut-off value for blood levels of SCCA-IgM (higher than 200 AU/ml) was determined, based on measures made using 100 healthy people.

Results showed that 41 patients (42.3%) had ILD, which was significantly linked to diffuse SSc, severe skin involvement, poorer lung function, and the presence of autoantibodies specific for scleroderma – antibodies that wrongly attack healthy cells. Median levels of SCCA-IgM were significantly higher in patients with ILD than those without (218 vs. 87.5 AU/ml).

When comparing patients under and above the cut-off value for SCCA-IgM, the team found that significantly more patients with higher SCCA-IgM levels had ILD and a lower total lung capacity – the volume of air in the lungs after a maximum inhalation. No differences in any other clinical features were found between the two groups.

Further analyses significantly associated higher SCCA-IgM blood levels with an ILD diagnosis, increasing by 10 times the risk of developing ILD.

Interestingly, patients with a more recent ILD diagnosis (less than three years ago) had significantly higher levels of SCCA-IgM than those living with the disease for a longer period. Researchers suggested this associated with the transition from an inflammatory to a fibrotic phase in the lungs of SSc patients, in which SSCA1 may have a role.

The presence of low SCCA-IgM levels in SSc patients with normal values of total lung capacity also helped to identify those with a very low risk of ILD.

„SSCA-IgM is associated with interstitial lung disease in scleroderma patients and might be used in the assessment of SSc-ILD," the researchers wrote.

High levels of a protein known as CTRP9 appear to indicate worsening lung function in patients with systemic scleroderma (SSc), according to a recent study. The discovery suggests that CTRP9 may be a useful biomarker for the loss of lung function in SSc [13].

Such biomarkers are sorely needed, researchers said, given that diseases that cause scarring of lung tissue – known as interstitial lung diseases or ILD – are linked to poor outcomes for people with SSc.

While interstitial lung disease (ILD) is the main cause of morbidity and mortality in systemic sclerosis (SSc), there is still a lack of predictive markers to assess disease progression, the findings suggesting that CTRP9 may be a potential biomarker in SSc-associated ILD.

Past research had demonstrated that fat metabolism is disrupted over the course of SSc. Moreover, the cell-signaling molecule CTRP9, made within fat cells, is associated with pulmonary complications in that disorder.

In a recent work, researchers from across the United States teamed up to investigate this relationship further, looking at how changes in CTRP9 levels correlated with changes in pulmonary function in SSc patients.

Overall, they found high levels of CTRP9 associated with declining pulmonary function. Meanwhile, low CTRP9 protein levels corresponded to a more stable disease course over time.

These results make the protein a potential disease biomarker, the team said. To arrive at this conclusion, they reviewed the records of 110 individuals included in the Northwestern Scleroderma Patient Registry and Biorepository, led by Northwestern University, in Illinois. Among the cases reviewed, 61 involved patients with limited cutaneous SSc, and 49 pertained to those with diffuse cutaneous SSc. Most patients (70) had four years' worth (48 months) of pulmonary function tests, taken at 12-month intervals. Mean disease duration was 9.7 years.

Patients with more CTRP9 in their bloodstream (over 81.1 nanograms/ml) generally had significantly worse lung function at the study's start, and at 48 months, as measured

by forced vital capacity (FVC) and DLCO – standard measures of how much air a person can exhale after a forced breath, and of predicting the lungs' capacity to transfer oxygen to blood cells.

Additionally, high CTRP9 levels were associated with greater numbers of monocytes, cells known to contribute to lung fibrosis, or scarring. Low CTRP9 was associated with a decrease in monocytes, even after accounting for disease duration and treatment status. Notably, the data showed that patients with stable disease – those whose FVC changed by less than 3% over the study period – tended to have low levels of CTRP9.

Although CTRP9 levels did not appear to predict SSc progression so much as changes in lung function, the researchers suggested that the possibility cannot yet be ruled out.

"Development and progression of ILD in SSc is often early with a steep progression early on in disease", they wrote, adding that because the patients in this study had an average disease duration of about nine years, they may have already reached the „plateau" phase of their lung disease.

Other factors, such as the study's sample size and variability regarding disease duration and treatment status cannot be excluded, the investigators added. Future studies, they mentioned, should examine CTRP9's prognostic value in detail.

The group, involving researchers from the University of California San Francisco, the Yale School of Medicine, in Connecticut, the University of Michigan, and the University of Rochester Medical Center, in New York, in addition to Northwestern, now plans to study *CTRP9* gene variants, to determine how the gene's expression, or activity, might be altered in SSc with interstitial lung disease.

Their current findings, they concluded, „support a novel role for CTRP9 as a prognostic biomarker, and potentially a therapeutic target for SSc-associated lung disease."

Levels of a subtype of immune B-cells in the blood are increased in some people with scleroderma and associated with lower respiratory function, higher blood pressure in the lungs, and more kidney damage, a study reports [14].

Both inflammation and vascular complications in systemic sclerosis patients are influenced by B-cells, a part of the body's adaptive immune system. B-cells play a critical role in autoimmune disorders by producing both antibodies that target the body's own tissues, called autoantibodies, and signaling molecules called cytokines, which promote inflammation and fibrosis, or scarring.

A recent study found that B-cells with lower levels of a cell surface protein called CD21 were more prevalent in those with scleroderma. These cells, known as CD21-low cells, also described in people with systemic lupus erythematosus and other disorders, appear unreactive (anergic) to substances that would normally induce an immune response and are found in inflamed tissues.

This prompted researchers at Sapienza University in Rome to see whether the cells could be used to predict vascular complications in scleroderma. The team analyzed 74 scleroderma patients – 40 with limited scleroderma and 34 with diffuse disease, mean age 54.5 – and 20 healthy people (controls). Overall, the percentage of B-cells with reduced levels of CD21 was higher in people with scleroderma than in the healthy controls. Yet only one third of the patients showed increased percentages of such CD21-low B cells, suggesting that these may represent a particular subtype of scleroderma cases, the researchers said. These cells were more prone to a process called apoptosis, which refers to „programmed" cell death – as opposed to death caused by injury. Apoptosis, which involves the genetically determined elimination of cells, is a factor in many neurodegenerative diseases.

Greater proportions of CD21-low cells in people with scleroderma were associated with higher systolic pulmonary arterial pressure (sPAP), lower carbon monoxide diffusing capacity (DLCO) – a measure of respiratory function – and lower levels of vascular endothelial growth factor, which is essential for the formation of blood vessels.

Those with more CD21-low cells had stiffer renal arteries, compared with the other participants. Renal arterial stiffness is predictive of kidney damage, which is known to occur in scleroderma cases. Next to the to the alteration of B cell subpopulations, the results point towards a potential role of CD21-low B cells in the pathogenesis (disease progression) of some vascular manifestations of the disease.

No difference was noted between patients who developed digital ulcers and those who did not, suggesting that CD21-low B-cells might only operate on deeper, or visceral, tissue. It can be assumed that CD21-low have a selective homing for internal organs, however additional research is required to evaluate these assumptions.

Herein, were investigated how levels of serum metabolites correlated with different stages of SSc and SSc-ILD [15]. Serum samples of patients with SSc without ILD, stable and progressive SSc-ILD as well as of healthy controls (HC) were analyzed using liquid targeted tandem mass spectrometry. The best discriminating profile consisted of 4 amino acids (AA) and 3 purine metabolites. L-tyrosine, L-tryptophan, and 1-methyl-adenosine distinguished HC from SSc patients. L-leucine, L-isoleucine, xanthosine, and adenosine monophosphate differentiated between progressing and stable SSc-ILD. In SSc-ILD, both, L-leucine and xanthosine negatively correlated with changes in FVC% predicted.

Additionally, xanthosine was negatively correlated with changes in DLCO% predicted and positively with the prognostic GAP index. Validation of L-leucine and L-isoleucine by an enzymatic assay confirmed both the sub-stratification of SSc-ILD patients and correlation with lung function and prognosis score.

Serum metabolites may have potential as biomarkers for discriminating SSc patients based on the presence and severity of ILD. Confirmation in larger cohorts will be needed to appreciate their value for routine clinical care.

Skin biopsy samples from patients with systemic sclerosis (SSc) may help to identify potential biomarkers and pathways implicated in the disease pathogenesis, according

to study results published in *Arthritis Research and Therapy* [16].

In this study, the researchers used a high-throughput mass spectrometry technique to analyze samples from affected and unaffected areas of the skin from 7 patients (5 women). Proteins were extracted from the cryopulverized samples and analyzed to reveal differentially expressed proteins, which led to the identification of 2149 proteins. The samples were compared, revealing that 169 of the proteins were significantly differentially expressed in the affected vs unaffected tissues. Further analysis performed on these proteins identified many involved pathways that were associated with SSc pathogenesis including platelet activation and extracellular matrix (ECM)-receptor interaction. After these analyses, 15 proteins were selected for validation through affected/unaffected comparison, of which 5 were confirmed to be significantly differentially expressed in SSc-affected vs -unaffected skin biopsies.

Based on the results, the researchers confirmed that ECM proteins are a key part of SSc development. Three proteins that interacted with ECM receptors were overexpressed in the comparison analysis, which were previously suspected to be part of SSc pathogenesis, could serve as potential biomarkers for the disease.

Additional studies could include data from new patients with SSc to confirm the use of these proteins as biomarkers; these molecules isolated from easily accessible tissue may also provide an easier way for clinicians to diagnose patients and prevent painful procedures.

Endothelial damage and fibro-proliferative vasculopathy of small vessels are pathological hallmarks of systemic sclerosis (SSc). The consequence is the detachment of resident elements that become circulating endothelial cells (CECs). The aim of a study dedicated to this topic was to evaluate the potential of CECs as biomarker in SSc [17].

The study enrolled 50 patients with limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) subset of SSc, who underwent clinical evaluation to establish the organ involvement. CECs were measured by flow-cytometry utilizing a polychromatic panel.

An evident difference was observed in CEC counts comparing controls to SSc patients (median 10.5 vs. 152 cells/ml, $p < 0.0001$) and for the first time, between the two subsets of disease (median lcSSc 132 vs. dcSSc 716 CEC/ml, $p < 0.0001$). A significant correlation was established between CECs and some SSc clinical parameters, such as digital ulcers, skin, and pulmonary involvement, presence of Scl-70 antibodies, nailfold videocapillaroscopy patterns and EUSTAR activity index. After 12 months, CECs correlated with clinical worsening of patients, showing that a number higher than 414 CEC/ml is a strong negative prognostic factor (RR5.70).

The results indicate that CECs are a direct indicator of systemic vascular damage. Therefore, they can be used as a reliable marker of disease severity.

Levels of the protein, galectin-3, are associated with heart involvement in people with systemic sclerosis (SSc), according to a recent pilot study [18]. The protein could be used as a biomarker to detect and address cardiac problems more quickly. Another protein, called soluble suppression of tumorigenicity-2 (sST2), was also evaluated, but did not show any relationship to heart involvement. The study results imply that galectin-3 may be a useful and simple biomarker for the screening and early identification of SSc patients with cardiac involvement, while the usage of sST2 for the same purpose was not supported by the data.

SSc is marked by too much collagen, a protein found in connective tissue that helps to repair wounds, being produced, resulting in excessive scarring, or fibrosis, of the skin and internal organs.

Cardiac fibrosis can lead to heart dysfunction and poorer outcomes, or prognosis, so it is important to identify patients at risk for heart complications.

"Cardiac involvement implies poor prognosis in SSc, thus its early, biomarker-based screening would be crucial", the team wrote.

To determine usefulness of such biomarkers, the researchers evaluated 36 women and 4 men with SSc being treated at a University of Pécs center in Hungary. The patients' median age was 57.3. Levels of the two fibrosis-related proteins were measured before an echocardiogram and lung function tests were performed. Galectin-3 promotes fibrosis and mediates collagen production, while sST2 blocks interleukin-33, a protein that protects against fibrosis.

These proteins were chosen because previous studies showed they correlated with heart involvement and could predict mortality in heart failure patients. Galectin-3 has also been proposed as a blood biomarker of scleroderma.

Results from the lung tests showed that higher galectin-3 levels correlated with worse lung function.

Specifically, people with more galectin-3 had lower measures in forced expiratory volume (how much air can be exhaled in one breath), and diffusing capacity of carbon monoxide (how much oxygen travels from the lungs to the bloodstream).

An echocardiogram revealed that galectin-3 was also associated with several indicators of heart dysfunction.

Higher galectin-3 levels correlated with both left ventricle systolic dysfunction, or the heart's inability to contract appropriately during heartbeats, and diastolic dysfunction, the heart's inability to relax after a heartbeat.

High levels of the protein were also seen with mitral valve regurgitation, which occurs when the heart's mitral valve, responsible for keeping blood flowing in the right direction, doesn't fully close, allowing blood to travel backwards. Galectin-3 levels also correlated with disease duration, results showed.

In contrast, sST2 was not correlated with any clinical, lung, or echocardiographic measurements, a contrast to previous studies in heart failure patients.

Based on the results, the team concluded that Galectin-3 may be a useful biomarker for the screening and early diagnosis of SSc patients with cardiac involvement. However, compared to earlier research, the team was unable to show

any relationship between sST2 levels and the clinical characteristics of the disease or the echocardiographic markers of the [heart] mechanics in SSc patients.

Researchers noted that the study lacked a healthy control group, meaning they couldn't define normal galectin-3 or sST2 blood levels. Another study limitation was that, while galectin-3 and sST2 indirectly suggest the presence of cardiac fibrosis, they did not prove that fibrosis was present in these patients. "Circulating biomarker levels require careful interpretation in relation to [cardiac] involvement", the researchers wrote.

High levels of a form of the CD146 protein, normally found on endothelial cells – those that line blood vessel walls – may be a potential biomarker in identifying people with systemic sclerosis (SSc), a study suggests [19].Certain forms of the protein are implicated in lung scarring (pulmonary fibrosis) and in controlling skin thickness, having potential as therapeutic targets.

CD146 is involved in the formation of new blood vessels – a process called angiogenesis – inflammation, and controlling vascular permeability, or the capacity of certain cells and molecules to cross the vessel's wall. While the two major forms of CD146 are located in the cell's membrane, the protein also can exist in a soluble form in the blood.

Recent research suggests that measuring the levels of this soluble form of CD146, known as sCD146, could serve as a biomarker of disease activity in SSc, also known as scleroderma. Moreover, data from an animal model of SSc showed that sCD146 injections reduced disease severity. However, little is known about the mechanisms regulating sCD146 formation and whether different variants of the protein exist and play a role in SSc.

To answer this, an international team led by researchers at Aix-Marseille University, in France, conducted lab (*in vitro*) tests as well experiments in animal models of SSc. The lab tests revealed that two forms of CD146 – a short and a longer form, both present at the membrane of endothelial cells – can generate a shed form of the soluble protein. Enzymes called metalloproteinases cleaved the membrane proteins. This process represented about 75% of the sCD146 production. The remaining 25% of the soluble protein was not dependent on the action of metalloproteinases. The researchers went on to identify two new forms of sCD146 – called I5-13-sCD146 and I10-sCD146 – that arose as a result of alternative RNA splicing, a process that allows a single gene to produce a number of different messenger RNA (mRNA) sequences and as a consequence, different proteins. Of note, mRNAs are the molecules that carry genetic information to the sites where proteins are produced. The levels of sCD146 variants in blood samples from 117 SSc patients were then measured and compared with those of 81 participants serving as controls. Results showed that the sCD146 form that resulted from cleavage of the membrane protein and the splice variant I5-13-sCD146 had significantly higher levels in the blood of patients with SSc. The splice variant was particularly high in people with pulmonary fibrosis when compared with patients without lung disease. In contrast, levels of I10-sCD146 were decreased in SSc samples, as were those of cleaved sCD146 in participants with pulmonary hypertension, a disease associated with high blood pressure in the blood vessels that supply the lungs. The scientists then tested the effects of the different sCD146 forms when injected under the skin (subcutaneously) in a mouse model of SSc.

Compared with control animals, sCD146 and I10-sCD146 reduced the thickness of the dermis, the thicker layer of the skin. Later experiments linked this effect with CD146's role as a potentiator of blood vessels formation. No such effects were seen with I5-13-sCD146. Instead, lab tests suggest that it plays a role in promoting fibrosis, which is in agreement with its levels being particularly high among SSc patients with pulmonary fibrosis.

Overall, these findings suggest that "variants of sCD146, and in particular the novel I5-13-sCD146 splice variant, could thus constitute novel biomarkers and/or molecular targets for the diagnosis and treatment of SSc, but also of other angiogenesis – or fibrosis-related pathologies [diseases]", the investigators concluded.

Blood levels of the protein endocan may be a potential biomarker in identifying people with scleroderma who are at risk for pulmonary arterial hypertension (PAH), a study reported [20].

Scleroderma, or systemic sclerosis (SSc), can lead to fibrosis (scarring) in multiple organs, inflammation, and damage to blood vessels. In some patients, these changes can cause a rare disorder known as PAH, in which the blood pressure in the arteries of the lungs is higher than normal.

Although many studies have investigated PAH in scleroderma patients, biomarkers helping to establish a PAH risk are lacking. Proangiogenic hematopoietic cells (PHCs) that form in the bone marrow are a type of stem cell that circulates in the bloodstream. These cells can become heart muscle cells and smooth muscle cells, as well as endothelial cells - those that line blood vessel walls and endothelial precursor cells. However, their role in scleroderma remains unclear.

Endocan, a protein expressed by endothelial cells, has been suggested as a marker of blood vessel (vascular) stress. Additionally, vitamin D and several pro-inflammatory molecules are known to modulate the interaction between bone marrow-derived cells and changes in endothelial cell function.

„Taken together, it may be conceived that PHCs and endocan could be involved in the development of PAH, but their interrelationships have not been investigated so far in SSc," the researchers wrote. A team from Italy investigated the association among PHC cells, endocan, vitamin D levels, inflammatory markers, and clinical parameters in 36 women with scleroderma and PAH. Enrolled patients had a mean age of 64.1, and a mean disease duration of 78.3 months (about six and a half years). Twelve participants had limited SSc (33.3%), while 24 (66.6%) had diffuse SSc, a more severe disease form. Also included in the study were 36 age-matched healthy women as a control group. Blood samples

were collected, and each patient underwent a complete clinical assessment, including an echocardiogram to establish cardiac health.

No differences in the numbers of progenitor cells were evident between patients and controls, but endocan levels were significantly higher in those with scleroderma, 365.6 nanograms per mL (ng/mL), relative to controls, 280.4 ng/mL.

Vitamin D levels were also higher in patients compared with controls (27.34 ng/mL vs. 22.47 ng/mL). Among the patients, 12 had vitamin D levels under 20 ng/mL, nine had levels between 20 and 30 ng/mL, and 16 had normal levels (greater than 30 ng/mL).

Pro-inflammatory markers like C-reactive protein, erythrocyte (red blood cell) sedimentation rate, and fibrinogen were also all higher in women with scleroderma than in those without this disease.

No difference was seen regarding endocan, vitamin D levels, and progenitor cell numbers between women with diffuse SSc and those with limited SSc.

In patients, results showed a significant correlation between low numbers of progenitor cells and high endocan protein levels. Elevated endocan levels also correlated with higher pulmonary arterial blood pressure (sPAP). A low progenitor cell count correlated with higher sPAP and fibrinogen, as well as low vitamin D. Higher sPAP linked with elevated fibrinogen.

A first statistical analysis evaluated each variable with progenitor cell numbers. Here, a low cell count was associated with higher endocan, fibrinogen, and sPAP. A subsequent analysis found that endocan and fibrinogen were potential predictors of progenitor cell numbers.

A second analysis also assessed parameters such as skin thickness. It ultimately showed that endocan level was the only potential predictor of elevated pulmonary arterial blood pressure.

„Our study seems to enforce the findings on the potential role of endocan as a biomarker of vascular health in SSc," the scientists wrote. „The relationships between endocan and other angiogenesis [blood vessel formation] biomarkers should be evaluated by future prospective studies in order to investigate its ability in predicting vascular involvement, including PAH."

The amount of *free light chain* (FLC) molecules in blood and urine correspond to the severity of scleroderma, adding to the evidence supporting these molecules as biomarkers for early diagnosis and disease activity.

Free light chain molecules are made during the production of antibodies and are considered to be biomarkers for B-cell activity. B-cells, a part of the immune system, tend to become self-reactive in scleroderma, or systemic sclerosis (SSc), meaning that they target the body's own tissues, causing inflammation.

Activated B-cells produce antibodies – molecules that „flag" cells and other biological molecules for the immune response – called immunoglobulins. Immunoglobulins are made of light and heavy chains (two of each). The two light chains on each immunoglobulin will both be of a certain variant, called kappa and lambda.

Free light chains are the excess light chains made by activated B-cells and released into the blood. Here, they tend to be rapidly removed from the body by the kidneys, potentially making them direct biomarkers of B-cell activity.

Despite their association with B-cells and reports that they associate with scleroderma activity and severity, as well as with scleroderma-related interstitial lung disease, few studies have evaluated their levels in this disease, and none has examined their presence in urine.

To address this knowledge gap, researchers in Italy analyzed FLC levels in the blood and urine of 72 adult SSc patients (median age, 55) and 30 healthy controls, seeking to correlate these levels with disease severity and activity [21].

People with scleroderma showed significantly higher amounts of both kappa and lambda FLCs in their blood than did the control participants, with kappa FLC levels generally higher than those of lambda.

Likewise, urine levels of kappa FLCs were significantly higher in patients than in healthy controls, but no such significant difference between the two groups was seen in lambda FLCs. Greater levels of both FLCs tended to correspond to higher measures of inflammation, as assayed by C-reactive protein levels, and a faster erythrocyte sedimentation rate – a test that measures how fast red blood cells settle at the bottom of a test tube. They also correlated with more disease activity and severity, as measured using the disease activity index (DAI) and the disease severity scale (DSS).

DAI scores rose with urinary kappa FLCs, as SSc patients with more of these had statistically higher DAI scores than those whose kappa FLC levels remained below 15.1 mg/L.

The authors suggest the usage of FLCs as a reliable and useful potential biomarker of early diagnosis and to follow disease activity, allowing for the improvement of SSC patients clinical management. Study limitations mentioned by the investigators included its relatively small sample size and the fact that enrolled patients had a long disease duration (median of 11 years).

Low levels of the *galectin-10 protein* (Gal-10) in scleroderma patients associate with inflammation and vascular changes in the lungs, leading to pulmonary arterial hypertension (PAH).

This finding, from a recent study, provides the first evidence that GAL-10 may play a role in scleroderma [21].

Galectins are a subfamily of proteins involved in regulating numerous biological processes, including inflammation and blood vessel remodeling, both of which occur in scleroderma (also known as systemic sclerosis, SSc). Four galectins – galectin-1, galectin-3, galectin-7, and galectin-9 – have been investigated in scleroderma [22, 23, 24].

Gal-10 is mainly found in eosinophils, basophils, and regulatory T-cells (Tregs) – three types of immune cells involved in balancing the inflammatory response. Tregs, for instance, regulate the workings of T-effector cells and suppress the production of inflammatory molecules. A subtype

of eosinophils helps to provide that check, limiting T-effector cells' ability to proliferate by transferring Gal-10.

Based on these and other observations, researchers with the University of Tokyo's Graduate School of Medicine explored relationships between Gal-10 levels in blood serum and scleroderma [25].

The investigators recruited 38 patients with diffuse cutaneous SSc (dcSSc), 30 with limited cutaneous SSc (lcSSc), and 20 healthy people as controls to their study. Gal-10 levels in the blood were significantly lower in all SSc patients than in controls – a median of 20.3 nanograms (ng)/mL vs. 31.2 ng/mL. The effect was greatest in those with dcSSc. These patients averaged 19.3 ng/mL of Gal-10, compared with 23.9 ng/mL among people with lcSSc.

Because of the different degrees of skin fibrosis (scarring) involved in these two SSc subtypes, this suggests that lower levels of Gal-10 might associate with more severe skin sclerosis and the development of scleroderma. The researchers identified a moderate, but significant correlation between serum Gal-10 levels and modified Rodnan total skin thickness score (mRSS) values in total SSc patients. (mRSS scores measure skin thickness in scleroderma as a way of determining disease severity).

They also found that low Gal-10 levels are significantly associated with a lower percent diffusion lung capacity for carbon monoxide, which measures how well the lungs can transfer oxygen from their air sacs to the blood, and with higher right ventricular systolic pressure (RVSP), a measure of the pressure inside the artery that supplies blood to the lungs.

These findings suggested that lower Gal-10 levels are linked to the changes in pulmonary blood vessels that can lead to PAH. Decreasing Gal-10 also corresponded to higher overall white blood cell counts – a measure of immune response – C-reactive protein (CRP) levels, and a faster erythrocyte sedimentation rate, the last two being measures of inflammation. To the researchers, this indicated that lower Gal-10 levels correspond to an impaired ability to regulate inflammation.

In support of this potential association, SSc patients with joint pain, a symptom of active inflammation, had significantly lower Gal-10 levels than those without such pain. Statistical analysis also independently linked CRP and RVS with low Gal-10, reinforcing the idea of a relationship to systemic inflammation and pulmonary vascular changes leading to PAH.

A limitation for this study is related to missing data on the sources of Gal-10, however this still allows to examine the association and role of Gal-10 and SSc. This report being the first investigating the role of Gal-10in SSc, further research can potentially explore the connection further.

Conclusions

1. Specific autoantibodies for some autoimmune diseases could be a relevant biomarker related to fibrotic skin, lung disease, joint and muscle involvement, digestive complication in the systemic sclerosis patients.
2. Appearance and severity of interstitial lung disease in the systemic sclerosis patients could be predicted using the level of CTRP9 protein, osteopontin, serum metabolites such L-tyrosine, L-tryptophan, 1-methyl-adenosine, L-leucine, L-isoleucine, xanthosine, and adenosine monophosphate, presence of antibodies against telomers.
3. Indicators of systemic vascular damage in the systemic sclerosis patients could be considered circulating endothelial cells, subset of immune B-cells, galectin-10 protein and endocan, with the latter two found to be associated with increased risk for pulmonary arterial hypertension.
4. Levels of the protein, galectin-3, are associated with heart involvement in people with systemic sclerosis.
5. Related to early diagnosis of systemic sclerosis, high levels of a form of the CD146 protein may be a potential biomarker in identifying people with systemic sclerosis and FLCs could be employed as a reliable and useful potential biomarker of early diagnosis and for following disease activity.

Declaration of conflict of interest

Nothing to declare

Authors' contribution

All authors contributed equally to the research, data analysis, and writing of the manuscript. Final manuscript was read and approved by all authors

References

1. Utsunomiya A., Oyama N. and Hasegawa M. Potential Biomarkers in Systemic Sclerosis: A Literature Review and Update. *J. Clin. Med*., 2020; 9: 3388.
2. Mihai C., Tervaert J.W. Anti-endothelial cell antibodies in systemic sclerosis. *Ann. Rheum. Dis*., 2010; 69: 319-324.
3. Wolf S.I., Howat S., Abraham, D.J., Pearson, J.D., Lawson, C. Agonistic anti-ICAM-1 antibodies in scleroderma: Activation of endothelial pro-inflammatory cascades. *Vascul. Pharmacol*., 2013; 59: 19-26.
4. Dib H., Tamby M.C., Bussone G. *et al*. Targets of anti-endothelial cell antibodies in pulmonary hypertension and scleroderma. *Eur. Respir. J.,* 2012; 39: 1405-1414.
5. Ihn H., Sato S., Fujimoto M., Igarashi A., Yazawa N., Kubo M., Kikuchi K., Takehara K., Tamaki. Characterization of autoantibodies to endothelial cells in systemic sclerosis (SSc): Association with pulmonary fibrosis. *Clin. Exp. Immunol.,* 2000; 119: 203–209.
6. Riccardi A., Martinroche G., Contin-Bordes C. *et al*. Erosive arthritis autoantibodies in systemic sclerosis. *Seminars in Arthritis and Rheumatism*, Volume 52, February 2022.
7. Lazzaroni M. G., Marasco E., Campochiaro C. *et al*. The clinical phenotype of systemic sclerosis patients with anti-PM/Scl antibodies: results from the EUSTAR cohort. *Rheumatology* (Oxford), 2021; Nov 3; 60 (11): 5028-5041.
8. Serling-Boyd N., Pei-Shien Chung M., Li S. *et al*. Gastric Antral Vascular Ectasia in Systemic Sclerosis: Association with Anti-RNA Polymerase III and Negative Anti-Nuclear Antibodies. *Seminars in Arthritis and Rheumatism*, Available online 6 July 2020. https://doi.org/10.1016/j.semarthrit.2020.06.016.
9. Adler B. L., Boin F., Wolters P. J. *et al*. Autoantibodies targeting telomere-associated proteins in systemic sclerosis. http://dx.doi.org/10.1136/annrheumdis-2020-218918.
10. Miyake M., Matsushita T., Takehara K., Hamaguchi Y. Clinical features of Japanese systemic sclerosis (SSc) patients negative for SSc related autoantibodies: A single center retrospective study. https://doi.org/10.1111/1756-185X.13908.
11. Gao X., Jia G., Guttman A. *et al*. Osteopontin Links Myeloid Activation and Disease Progression in Systemic Sclerosis. https://doi.org/10.1016/j.xcrm.2020.100140.
12. Zanatta E., Martini A., Scarpieri E. *et al*. Squamous cell carcinoma antigen-IgM (SCCA-IgM) is associated with interstitial lung disease in systemic sclerosis. *Joint Bone Spine*, Volume 87, Issue 4, July 2020; p. 331-335.
13. Yang M. M., Balmert L. C., Marangoni R. G. *et al*. Circulating CTRP9 is Associated with Severity of Systemic Sclerosis-associated Interstitial Lung Disease. *Arthritis Care Res.* (Hoboken), 2021 Jul 12. doi: 10.1002/acr.24749.
14. Marrapodi R., Pellicano C., Radicchio G. *et al*. CD21-low B cells in systemic sclerosis: A possible marker of vascular complications. *Clinical Immunology*, Volume 213, April 2020; 108364. https://doi.org/10.1016/j.clim.2020.108364.
15. Meier C., Freiburghaus K., Bovet C. *et al*. Serum metabolites as biomarkers in systemic sclerosis-associated interstitial lung disease. *Scientific Reports*, Volume 10, 21912 (2020). https://doi.org/10.1038/s41598-020-78951-6.
16. Chairta P., Nicolaou P., Sokratous K., Galant K., Houssiau F., Oulas A. *et al*. Comparative analysis of affected and unaffected areas of systemic sclerosis skin biopsies by high-throughput proteomic approaches. *Arthritis Res. Ther.*. 2020; 22 (107). doi:10.1186/s13075-020-02196-x.
17. Di Martino M. L., Frau A., Losa F. *et al*. Role of circulating endothelial cells in assessing the severity of systemic sclerosis and predicting its clinical worsening. *Scientific Reports*, (2021) 11:2681. https://doi.org/10.1038/s41598-020-80604-7.
18. Vértes V., Porpáczy A., Nógrádi A. *et al*. Galectin-3 and sST2: associations to the echocardiographic markers of the myocardial mechanics in systemic sclerosis. *Cardiovascular Ultrasound*, 2022; 20: 1. doi:10.1186/s12947-022-00272-7.
19. Nolle M., Bachelier R., Joshkon A. *et al*. Multiple variants of soluble CD146 are involved in Systemic Sclerosis: identification of a novel pro-fibrotic factor. *Arthritis & Rheumatology.* First published: 09 January 2022. https://doi.org/10.1002/art.42063.
20. Lo Gullo A., Mandraffino G., Rodríguez-Carrio J. *et al*. Endocan and Circulating Progenitor Cells in Women with Systemic Sclerosis: Association with Inflammation and Pulmonary Hypertension. *Biomedicines*, 2021 May; 9 (5): 533. doi:10.3390/biomedicines9050533.
21. Gigante A., Pellicano C., Leodori G. *et al*. Serum and urine free light chains measurements in patients with systemic sclerosis: novel biomarkers for disease activity. *Clinical and Experimental Immunology*, Volume 205, Issue 2, August 2021; Pages 135-141. https://doi.org/10.1111/cei.13611.
22. Sundblad V., Gomez R. A., Stupirski J. C. *et al*. Circulating Galectin-1 and Galectin-3 in Sera From Patients With Systemic Sclerosis: Associations With Clinical Features and Treatment. *Front. Pharmacol*., 20 April 2021. https://doi.org/10.3389/fphar.2021.650605.
23. Chihara M., Kurita M., Yoshihara Y. *et al*. Clinical Significance of Serum Galectin-9 and Soluble CD155 Levels in Patients with Systemic Sclerosis. *Journal of Immunology Research* Volume 2018; Article ID 9473243, https://doi.org/10.1155/2018/9473243.
24. Sewgobind N. V., Albers S. and Roland J. Pieters R. J. Functions and Inhibition of Galectin-7, an Emerging Target in Cellular Pathophysiology. *Biomolecules* 2021; 11, 1720. https://doi.org/10.3390/biom11111720.
25. Awaji K., Miyagawa T., Fukui Y. *et al*. A potential contribution of decreased serum galectin-10 levels to systemic inflammation and pulmonary vascular involvement in systemic sclerosis. *Exp. Dermatol.* 2021 Jul; 30 (7): 959-965. doi: 10.1111/exd.14320.

Authors's ORCID ID:
Svetlana Agachi, https://orcid.org/ 0000-0002-2569-7188
Liliana Groppa, https://orcid.org/ 0000-0002-3097-6181
Larisa Rotaru, https://orcid.org/ 0000-0002-3260-3426
Elena Deseatnicova: https://orcid.org/0000-0001-5029-2994
Lia Chișlari, https://orcid.org/ 0000-0002-7088-568X
Eugeniu Russu, https://orcid.org/ 0000-0001-8957-8471

UDC: 616.53-002-08(478)

REVIEW ARTICLE

Evaluation of topical remedies in the treatment of acne available in the Republic of Moldova

Adrian Virlan[1*†], Diana Guranda[1†], Cristina Ciobanu[1†]

[1]Department of Drug Technology, Nicolae Testemitanu State University of Medicine and Pharmacy Chisinau, Republic of Moldova

Date of receipt of the manuscript: 14.04.2022
Date of acceptance for publication: 30.05.2022

Corresponding author:
Adrian Virlan, PhD student,
Department of Drug Technology,
Nicolae Testemitanu State University of Medicine and Pharmacy,
22 N. Testemitanu str., Chisinau, Republic of Moldova, MD-2025
e-mail: virlanadrian57@gmail.com

Titlu scurt: *Topical remedies in the treatment of acne*

What is not yet known on the issue addressed in the submitted manuscript

Pharmaceutical manufacture is in a continuously developing and improving state, including the formulations customized for dermato-cosmetics use in order to identify the most effective topical treatment for acne, which would improve the symptoms and diminished the negative impact on the quality of life of patients.

The research hypothesis

The systematization of data from the literature and evaluation of local pharmaceutical market on the topical treatment of acne would allow the unification of national and international therapeutic patterns and formulation of practical recommendations.

The novelty added by manuscript to the already published scientific literature

Current status and trends of the contemporary and effective pharmacotherapeutic approach in the treatment of acne and its availability for patients. Collation between topical industrial pharmaceutical forms and magistral individual compounded drugs, prescribed by dermatologists from the Republic of Moldova based on the data from Production department of the *Vasile Procopisin* University Pharmaceutical Center.

https://doi.org/10.52645/MJHS.2022.2.10

Summary

Introduction. A lot of research and studies are currently being carried out in the field of elaboration and optimization of topical pharmaceutical forms used in the treatment of various types of acne. *Acne vulgaris* is a disease with a complex etiopathogenesis with potential negative psychosocial effects on the quality of life of patients, and its prevention is based on the successful management of risk factors. There are currently many topical and systemic treatment options available, so the choice of therapy depends on the form and severity of the condition. The advantage of topical pharmaceutical forms is the ability to target early stages of acne lesions.

Material and methods. Statistical, systemic, informational and sociological analysis were used as methods of analysis. Documentation with the State Drug Nomenclature and Classification, evaluation of topical prescription for acne treatment, dispensed in the *Vasile Procopisin* University Pharmaceutical Center (UPhC), the study of active pharmaceutical ingredients (API) and technological characteristics of topical pharmaceutical forms.

Results. Topical treatment for acne should be based on its type and severity. The basic therapy for the treatment of mild acne refers to external applications, due to their action to prevent lesions. Treatment should be maintained over time to prevent recurrence. For domestic patients are accessible drugs with keratolytic, bacteriostatic and anti-inflammatory properties for topical treatments as benzoyl peroxide, salicylic acid, sulfur, azelaic acid, zinc oxide and antibiotic. The analysis of the use of anti-acne medications, in treating mild to moderate acne, by the age group, identified that for adolescents treatment is being focused on combating the causative agent of sebum production using salicylic acid in 32.5% cases and with age increase, the aim is to suppress inflammatory processes requiring retinoids, and in case of absence, can be substituted by benzoyl peroxide.

Conclusions. The therapeutic approach in clinical practice must be done individually, considering the severity and shape of acne lesions.

Keywords: acne, industrial, magistral, topical pharmaceutical forms.

Introduction

Currently, guidelines for the treatment of acne, as well as safety, tolerability and patient preferences are needed to determine the appropriate treatment steps. The literature may support evidence-based physician recommendations based on these factors. Thus, the bibliographic references,

are focused on the efficacy and tolerability of the combined topical therapies used in the treatment of acne. Proceeding from the multifactorial pathogenesis of acne and the obstacles to adherence to treatment, scientists opt for optimization of topical anti-acne pharmaceutical forms which shall lead to greater confidence in the future of patients [1].

Acne vulgaris is a chronic dermatological condition that affects about 80% of adolescents globally, of which 20% have a mild and severe form. The age of onset is between 10 and 17 years for females and between 14 and 19 years for males, but it can also occur at an older age [2, 3]. Acne is an inflammatory condition of the pilosebaceous unit of the skin, which occurs through excessive production of sebum, which leads to the formation of comedones, by blocking the pilosebaceous unit, by hyperkeratinization hair, blocking the elimination of sebum, with the formation of blackheads, and by added bacterial infections [4]. Anaerobic *Propionibacterium acnes* develops in acne lesions, maintaining inflammation and forming whitehead lesions characteristic of acne: papules, pustules, nodules and cysts, as shown in figure 1. Acne is often associated with seborrhea, so it affects especially the areas where the sebaceous glands are more abundant: face, head, neck, upper part (chest and arms) [5].

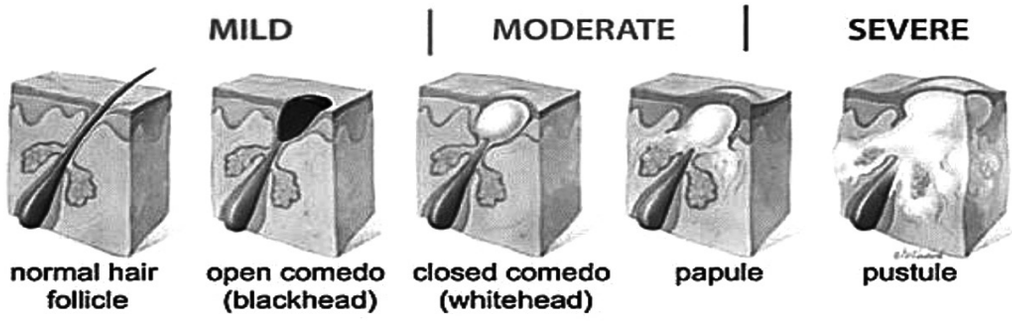


Fig. 1. *The stages of acne [6].*

Topical therapy in acne vulgaris includes multiple options: retinoids, antibiotics, substances with antibacterial, keratolytic, antimicrobial action (salicylic acid, sulfur, chloramphenicol, etc.). The choice of topical therapy is influenced by the patient's age, the location of the lesions, their extent and severity, as well as the patient's preference [6, 7].

According to international specialized literature, the most commonly used medications for the treatment of various forms of acne are *topical retinoids* (vitamin A derivatives), known for 4 generations to bind the specific retinoid receptors at the keratinocyte level, they reduce keratinization and follicular adhesion, leading to inhibition of comedogenesis and increased skin penetration for other topical treatments [8]. In international dermatological practice, retinoids are recommended for topical use due to their anti-comedogenic and anti-inflammatory effects as initiation or maintenance therapy and also for relapse prevention [9-11]. The combination of a topical retinoid with an antimicrobial agent is recommended as a first-line treatment for most patients, as it targets the multiple pathological factors that underlie the appearance of inflammatory and non-inflammatory acne lesions [12]. Retinoids stimulate the turnover of epithelial cells, with the release of the cutaneous pilosebaceous unit, and have anti-inflammatory properties by inhibiting the chemotaxis of neutrophils and monocytes [3]. Their impact varies with vehicle formation, skin type, frequency and mode of application, use of moisturizers, and environmental factors such as sun exposure or temperature [13, 14].

Current research, in accordance with published guidelines and protocols, considering the effectiveness, safety and tolerability of anti-acne treatment, as well as patient allegations can help the physician to achieve an effective evidence-based effect on all factors that cause acne.

The aim of this study is to get an overview of the current and future direction of topical pharmacotherapy for acne and evaluate industrial drugs and magistral formulations available in the Republic of Moldova, also to identify the most prescribed API (active pharmaceutical ingredients) by domestic dermatologists.

Material and methods

In order to achieve the purpose of the paper, the materials used were in the form of scientific publications, national and international guides on the methods of treatment of various forms of acne. As methods of analysis were applied statistical, systemic, informational, and sociological analysis. Analytical evaluation of State Drug Nomenclature and Classification of the administrative authority Agency for Medicines and Medical Devices by pharmaceutical active ingredients and manufacturing countries, also evaluation of the magistral prescriptions of the *Vasile Procopisin* UPhC (University Pharmaceutical Center) regarding topical pharmaceutical forms used in the treatment of acne by age groups (11-16 y.o., 17-21 y.o., 21-28 y.o., 29 y.o. and more), by pharmaceutical form (ointments, pastes, creams, liniments) and by pharmaceutical active ingredients.

Results and discussions

There is currently a wide variety of anti-acne treatment options available in the R. of Moldova, offering integrative therapeutic approaches so that they are tailored to the patient's needs. The choice of treatment depends on the etiopathogenic cause of the acne, the personal or family history, the treatment used previously and the response obtained to these therapies, in association with a thorough clinical examination of the patient. Due to the fact that acne is a condition that can lead to psychosocial impairment of patients, it is important to start treatment as early as possible [15, 16]. The goals of anti-acne therapy is to reduce the number and severity of existing lesions, to prevent the formation of new lesions and the formation of permanent scars, leading to disfigurement. Thus, for anti-acne therapy, specialists from the R. of Moldova approach a complex treatment, in which topical therapy is associated with systemic therapy. For instance, in the Republic of Moldova at the moment is registered the 1st generation retinoid - *isotretinoin* - for oral administration in the form of capsules.

Topical therapy is the first intention in mild and moderate cases of acne, and the combination of several topical preparations for various aspects of acne pathogenesis may be useful. Most often dermatologists from different curative-prophylactic institutions opt for industrial pharmaceutical forms and mainly, in the form of ointment, cream, gel, suspension and local applications. In patients with sensitive skin prone to acne, doctors often prescribe such pharmaceutical forms as ointments-emulsions, liquid creams, hydrogels, which have permeable properties (moisturizing), but do not make skin oily, are easily washable, and are more effective from a biopharmaceutical point of view [17]. The penetration of topical drugs into and through the skin can be achieved in two ways: (1) through the pores - the walls of the hair follicles (transfollicular) and less through the sweat glands; (2) transepidermal - passing through the epidermis.

Thus, the skin absorbs the following categories of substances: substances soluble in skin lipoids (nonpolar organic solvents); substances soluble in both oil and water; fat-soluble volatile substances, due to a wide variety of ointment bases [18]. The factors that influence the penetration of topically administered drugs and implicitly the bioavailability and the action of the pharmaceutical form are the factors related to the structure and conditions of the skin, characteristics of the active and auxiliary substances, the composition of the applied topical formula, a special role being played by the vehicle - the excipient or the ointment base.

Nowadays, there is a wide assortment of dermato-products with singular or multicomponent medicines. Pharmaceutical industry has entered a new era, as there is a growing interest in increasing the standards of quality of dosage forms, through the implementation of more structured development and manufacturing technologies [19]. For topical formulation, as a semi-solid dosage form, that has suitable consistency for the treatment of skin diseases caused by bacteria, a key challenge relay to the properties of the active substance in association with excipient bases [20] and the objective in development includes physical-chemical studies of API proprieties and excipients and their compatibility, release rate, stability, *in vitro* and *in vivo* drug permeability studies, etc. Industrial topical anti-acne dosage forms are available in various pharmaceutical forms, majority with conventional, unmodified API release, such as: gels, ointments, creams, lotions, etc. On domestic pharmaceutical market are present dermato-products containing benzoyl peroxide, azelaic acid, sulfur and other substances, as displayed in table 1.

Table 1. *Topical industrial preparations used in the treatment of acne available in the Republic of Moldova*

International common name of the API	**Pharmaceutical form, concentration**
Benzoyl peroxide	Cream, 5%
Azelaic acid	Cream 20%, gel 15%
Erythromycin	Gel 25 mg/g
Clindamycin	Gel 10 mg/g
Sulfur	Paste, 10%
Zinc oxide	Paste, 10%, ointment, 100 mg/g
Salicylic acid	Ointment 30 mg/g

Evaluation of registered anti-acne industrial pharmaceutical forms available in our country revealed that the drugs are from different chemical groups starting with:

- benzoyl peroxide is used as an alternative to topical retinoids, associated with antibiotics in the treatment of acne. Benzoyl peroxide has comedolytic effects by increasing the renewal rate of epithelial cells and by favoring the opening of pores blocked by excess sebum and cell debris [21, 22]. Benzoyl peroxide side effects occur especially if high doses are used at the beginning of treatment. These side effects are excessive dry skin, flaking, erythema, sensitization and contact dermatitis [23, 24], if the allergic event is severe, the treatment is interrupted; benzoyl peroxide produces photosensitization; like retinoids, it is contraindicated in pregnancy [25]. Benzoyl peroxide used alone can relieve inflammatory acne, with antibacterial, anti-inflammatory and keratolytic action [26]. The decomposition of benzoyl peroxide releases oxygen as a by-product. The oxygen suppresses the growth of anaerobic bacteria *P. acnes* [27]. By hindering bacterial colonization in sebaceous ducts, oxygen markedly reduces bacterial count (even over 95% during 2 weeks).
- azelaic acid, as a dicarboxylic acid, has antibacterial, comedolytic and anti-inflammatory properties. It is considered an effective topical treatment for adult patients with acne and for maintenance therapy. A potential adverse effect of azelaic acid is the appearance of hypopigmented lesions, so it can be used in the treatment of post-inflammatory hyperpigmentation [28, 29].
- topical antibiotics, erythromycin and clindamycin are most commonly used in the treatment of acne. Erythromycin is used both in reducing inflammatory lesions and

in reducing non-inflammatory lesions. After its use for 6 to 12 weeks, a reduction in lesions was observed in 42% to 74% for inflammatory lesions and between 25% and 74% for non-inflammatory lesions [23]. Combined with zinc, erythromycin is much more effective in reducing pustules and papules [30]. Clindamycin is a lincosamide derivative, with antimicrobial action is effective in fighting gram-positive and gram-negative microorganisms, has the following effects – destroys bacteria, disinfects, regenerates cells, cleanses, dries, whitens [31].

- the sulfur commonly prescribed by dermatologists in pharmaceutical suspensions is known to be a very good agent in eliminating acne, it helps to absorb chemicals from the skin that are the main cause of acne. Sulfur acne treatment is done by drying excess fat on the skin and controlling sebum [32]. It makes the surface of the skin to peel off. This exfoliation helps to produce new cells, which leads to firmer and smoother skin.
- zinc oxide has the ability to act like a mild astringent. It will keep *P. acnes* from causing infections and acts as a natural skin-drying agent. Thus, zinc oxide on acne-prone skin treatment may help clear acne-causing bacteria and reduce oil production [33].
- salicylic acid is a topical medication and is found in a variety of anti-acne, one-component liquid and semi-solid forms and in combined formulations of alpha and beta hydroxy acids [14].

Doctors recommend starting the treatment with low doses of benzoyl peroxide (2.5%) as they have a lower irritant effect compared to higher concentration preparations [34]. Because of the risk of developing bacterial resistance when erythromycin and clindamycin are used as monotherapy, if prescribed, they should be combined with benzoyl peroxide [25].

After evaluation of State Drug Nomenclature of medicines [35] that are registered in the R. of Moldova, we had established that the major share of topical drugs used in acne belongs to domestic manufacturer of pharmaceuticals and that salicylic acid is the most utilized component in anti-acne products registered from different countries. Local pharmaceutical manufacture *Farmaprim LTD* produce drugs containing chloramphenicol, salicylic acid, sulfur and benzoyl peroxide, as shown in figure 2.

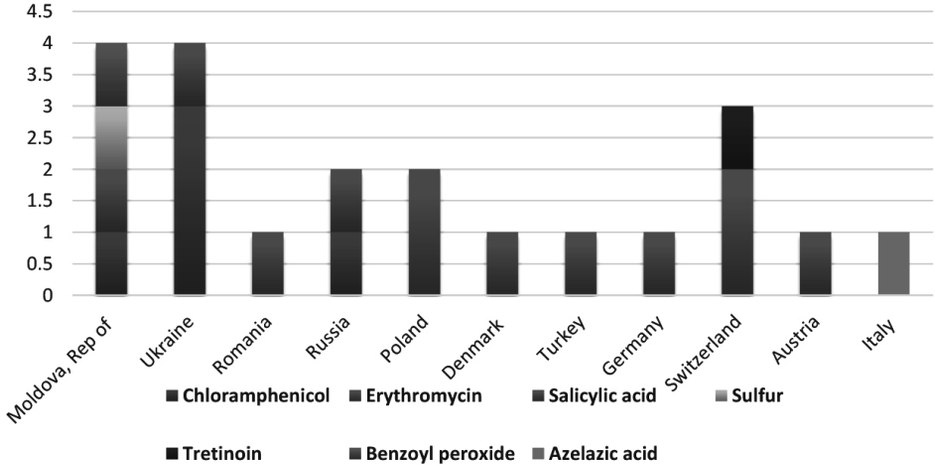


Fig. 2. *Anti-acne drugs registered in the Republic of Moldova, by the countries of manufacture, in units.*

An important direction in anti-acne treatment is emphasized by preparation by compounding pharmaceutical formulations according to medical prescriptions. Dermatologists from various medical institutions from our country prescribe in the treatment of acne magistral or extemporaneous prescriptions for drug preparation in pharmacies as topical formulations containing: salicylic acid, sulfur, chloramphenicol, and others. The evidence-based medication of individual medication maintains the trend for compounding. Pharmacies are still individually responsible for assessing the quality, stability, and effectiveness of every compounded preparation they dispense.

Compounding is an extemporaneous process and a compounded topical anti-acne product, formulated based on individual unique prescription by pharmacists, is more susceptible to physicochemical modifications than manufactured drug products that are made usually by a single drug. Thus, a proper combination of more than one active and inactive ingredients, in consideration of the quality, stability, and effectiveness of compounded preparations, rely on pharmacy technicians. Following the analysis of the prescription from *Vasile Procopisin* UPhC it was determined that the pharmaceutical forms for topical use (ointments, pastes, gels, ointments-emulsions, ointments-suspensions, creams, etc.) represent 4-6% of the total preparations prescribed by doctors and prepared by pharmacists. Among topical forms that are prepared and released, pastes occupy the largest share – 25.6% (figure 3), followed by ointments with water-oil base – 21.4%, that are considered to have advantages as dermato-cosmetics because after been applied on skin, evaporation occurs and increases the release concentration of the drug that was previously dissolved in water, thus enhancing the API effect and absorption.

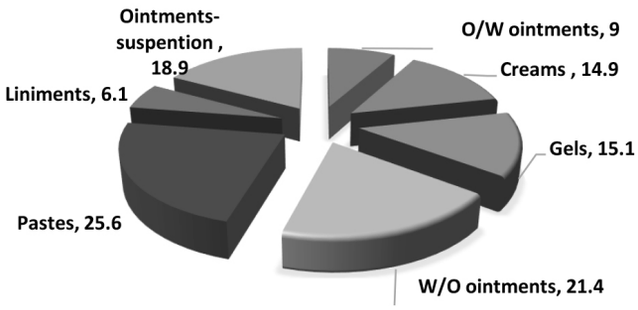


Fig. 3. *The share of topical anti-acne pharmaceutical forms in Vasile Procopisin UPhC, %.*

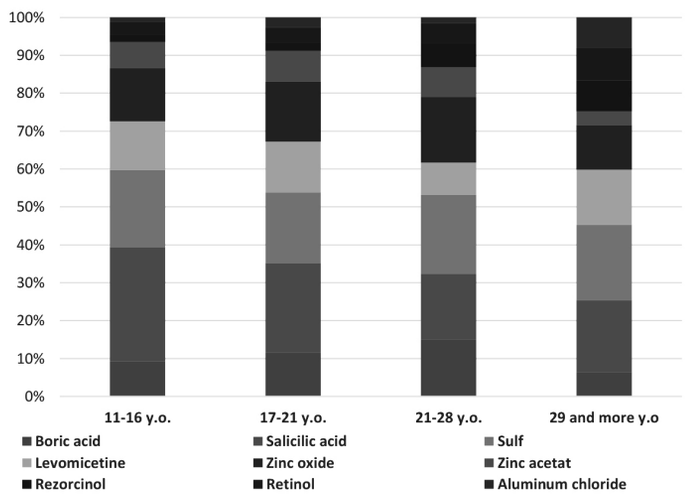


Fig. 4. *Evaluation of compounded magistral-prescribed active substances by age group, %.*

Evaluation of prescribed anti-acne API by dermatologists from the R. of Moldova was carried out. Following analysis of the prescriptions, based on the age of the patients, it was found that the most common API indicated in the age groups of 11-16 and 17-21 years (figure 4) is salicylic acid; sulfur is prescribed for both the juvenile segment and the adult group over 29 years of age, including the most common retinol and resorcinol.

Salicylic acid ointment is prescribed by doctors in a concentration of 1% for magistral formulations. It has a comedolytic effect, but with a lower efficiency than topical retinoids. Sulfur, commonly prescribed by dermatologists in pharmaceutical ointments-suspension, is known to be a very effective agent in eliminating acne because it helps to absorb chemicals from the skin that are the main cause of acne. Sulfur acts on acne on account of keratolytic properties and helps to quickly get rid of dead skin cells that block pores, which causes acne. It also clears comedones and prevents them from forming again. This exfoliation helps to produce new cells, which leads to firmer and smoother skin. The analysis revealed that the treatment of acne in adolescents focuses on combating the causative agent of sebum production, and with increasing age, the aim is to suppress inflammatory processes.

Conclusions

Acne vulgaris is considered a minor clinical problem but has a significant psychosocial impact on the patient's quality of life, leading to psychological stress or depression. Specialists pay attention not only to the physical manifestations of acne, but also to the emotional burdens resulting from the condition.

Currently on the pharmaceutical market there is a wide range of topical industrial forms, favored by salicylic acid. In the production department of *Vasile Procopisin* UPhC there is a large assortment of anti-acne remedies, with different therapeutically groups of medicinal substances that are prescribed in magistral prescription by dermatologists.

Pharmaceutical compounding of topical anti-acne remedies is opportune for continuous research in pharmaceutical optimization regarding dermato-products perusing an efficient and safety anti-acne pharmacotherapy.

Declaration of conflict of interest

The author declares the absence of any conflict of interest in the elaboration of this article.

Authors' contribution

All authors participated equally in the elaboration and writing of the paper. All authors read and approved the final version of the manuscript.

References

1. Yang Z., Zhang Y., Lazic Mosler E., Li H., Hu J., Zhang Y., Liu J., Zhang Q. Topical benzoyl peroxide for acne. *Cochrane Database of Systematic Reviews* 2014, Issue 6. Art. No.: CD011154. DOI: 10.1002/14651858.CD011154.
2. Bagatin, Edileia *et al.* Adult female acne: a guide to clinical practice. *Anais brasileiros de dermatologia* vol. 94,1 (2019): 62-75. doi:10.1590/abd1806-4841.20198203.
3. Benner N., Sammons D. Osteopathic Family Physician. Overview of the treatment of acne vulgaris. *Osteopathic Family Physician.* 2013, 5 (5): 185–90. doi:10.1016/j.osfp.2013.03.003.
4. Simonart T. Immunotherapy for Acne Vulgaris: Current Status and Future Directions. *Am J Clin Dermatol.* 2013 Dec 1;14(6):429–35.

5. Titus S., Hodge J. Diagnosis and treatment of acne. *Am Fam Physician.* 2012 Oct 15;86(8):734–40.
6. Eichenfield L.F., Draelos Z., Lucky A.W., *et al.* Preadolescent moderate acne vulgaris: a randomized trial of the efficacy and safety of topical apalenebenzoyl peroxides. *J Drugs Dermatol*. 2013;12:611-18.
7. Gonzalez P., Vila R., Cirigliano M. The tolerability profile of clindamycin 1%/ benzoyl peroxide 5% gel vs. adapalene 0.1%/benzoyl peroxide 2.5% gel for facial acne: results of a randomized, single-blind, split-face study. *J Cosmet Dermatol* 2012;11:251-60. DOI: 10.1111/jocd.12013.
8. Fox L, Csongradi C, Aucamp M, du Plessis J, Gerber M. Treatment Modalities for Acne. *Molecules.* 2016 Aug 13;21(8):1063.
9. Knutsen-Larson S., Dawson A.L., Dunnick C.A., Dellavalle R.P. Acne vulgaris: pathogenesis, treatment, and needs assessment. *Dermatologic Clinics.* 2012, 30 (1): 99–106. doi:10.1016/j.det.2011.09.001.
10. Practice Guidelines. Acne Vulgaris: Treatment Guidelines from the AAD. *J Am Acad Dermatol.* May 2016;74(5):945-973.e33. [accessed March 2022].
11. Thielitz A., Gollnick H. Topical retinoids in acne vulgaris: update on efficacy and safety. *Am J Clin Dermatol*. 2008;9(6):369-81. doi: 10.2165/0128071-200809060-00003.
12. See J.A., Goh C.L., Hayashi N., Suh D.H., Casintahan F.A. Optimizing the use of topical retinoids in Asian acne patients. *J Dermatol*. 2018 May;45(5):522-528. doi: 10.1111/1346-8138.14314. Epub 2018 Apr 3. PMID: 29611225; PMCID: PMC5969268.
13. Thielitz A., Abdel-Naser M.B., Fluhr J.W., Zouboulis C.C., Gollnick H. Topical retinoids in acne--an evidence-based overview. *J Dtsch Dermatol Ges*. 2008 Dec;6(12):1023-31. English, German. doi: 10.1111/j.1610-0387.2008.06741.x. Epub 2008 May 13. PMID: 18479477.
14. Nedelciuc B., Beţiu M., Guţu A. Acne vulgaris: retrospectives and perspectives. *Curierul Medical*, nr. 4(56) / 2013 / ISSN 1875-0666, p. 62-65.
15. Csongradi C., Aucamp M., du Plessis J., Gerber M. Treatment Modalities for Acne. *Molecules.* 2016 Aug 13;21(8):1063.
16. Keri J., Shiman M. An update on the management of acne vulgaris. Clinical, Cosmetic and Investigational Dermatology. 2009 Jun;105-10. doi: 10.2147/ccid.s3630.
17. Diug E., Guranda D., Ciobanu C. Biofarmacie şi farmacocinetică. Compendiu (ediţia a II-a). Chişinău: Print Caro, 2019, 156 p.
18. Guranda D., Ciobanu C. Rolul substanţelor auxiliare în formularea şi dezvoltarea unguentelor magistrale. *Revista Farmaceutică a Moldovei*, 2021, 47(3), pp. 37-41.
19. Guranda D., Solonari R., Ciobanu C., Diug E., Ciobanu N., Vîrlan A. Utilizarea nanotehnologiilor în formularea dermatocosmeticelor. *Revista Farmaceutică a Moldovei*. 2021, 47(3), pp. 32-36.
20. Guranda D., Ciobanu C. Rolul substanţelor auxiliare în formularea şi dezvoltarea unguentelor magistrale. *Revista Farmaceutică a Moldovei*, 2021, 47(3), pp. 37-41.
21. Practice Guidelines. Acne Vulgaris: Treatment Guidelines from the AAD. J Am Acad Dermatol. May 2016;74(5):945-973.e33. [accesat March 2022].
22. Primary Care Dermatology Society. Acne – Practical Advice and Maintenance. [accessed April 2022] http://www.pcds.org.uk/ee/images/uploads/general/Acne_Treatment_2015-web.pdf.
23. Management of acne. Clinical practice guidelines. Ministry of Health Malaysia. [accessed March 2022] https://www.moh.gov.my/moh/attachments/7190.pdf.
24. Rallis E., Verros C., Katoulis A., Katsarou A. Topical 5% Benzoyl Peroxide and 3% Erythromycin Gel: Experience with 191 Patients with Papulopustular Acne. *Acta Dermatovenerol Croat.* 2013;21(3):160-167.
25. Pastuszka M., Kaszuba A. Role of topical combination drug containing clindamycin and benzoyl peroxide in the treatment of common acne. *Postep Derm Alergol* 2012; XXIX, 4: 279–285 DOI: 10.5114/pdia.2012.30468
26. Titus S., Hodge J. Diagnosis and treatment of acne. *Am Fam Physician.* 2012 Oct 15;86(8):734–40.
27. Ningsih D. R. et al. Synthesis of Anti-Acne Ointment of Ethanol Extract of White Plumeria Leaves (Plumeria Alba L.). *IOP Conf. Ser.: Mater. Sci. Eng.,* 2017, 172 012013. doi:10.1088/1757-899X/172/1/012013.
28. Clive B. Archer. Atopic dermatitis. *Medicine*, Volume 45, Issue 6, 2017, pp. 379-382, ISSN 1357-3039. doi. org/10.1016/j.mpmed. 2017.03.001.
29. James W.D. Acne. *New England Journal of Medicine*. 2005, 352 (14): 1463–2. doi:10.1056/NEJMcp033487.
30. Feucht C.L., Allen B.S., Chalker D.K., Smith J.G. Jr. Topical erythromycin with zinc in acne. A double-blind controlled study. *J Am Acad Dermatol.* 1980 Nov;3(5):483-91. doi: 10.1016/s0190-9622(80)80114-9. PMID: 6452464.
31. Guay D.R. Topical clindamycin in the management of acne vulgaris. *Expert Opin Pharmacother.* 2007 Oct;8(15):2625-64. doi: 10.1517/14656566.8.15.2625.
32. Csongradi C., Aucamp M., du Plessis J., Gerber M.. Treatment Modalities for Acne. *Molecules.* 2016 Aug 13;21(8):1063.
33. Andronachi A., Guranda D., Ciobanu C., Diug E., Solonari R. Study of magistral topical pharmaceutical forms used in the treatment of psoriasis. *Materialele Congresului "Congresul consacrat aniversării a 75-a de la fondarea Universităţii de Stat de Medicină şi Farmacie „Nicolae Testemiţanu""*, Chişinău, Moldova, 21-23 octombrie 2020 Pag. 644-644.
34. Mills O.H. Jr, Kligman A.M., Pochi P., Comite H. Comparing 2.5%, 5%, and 10% benzoyl peroxide on inflammatory acne vulgaris. *Int J Dermatol.* 1986 Dec;25(10):664-7. doi: 10.1111/j.1365-4362.1986.tb04534.x. PMID: 2948929.
35. State Nomenclature of Drugs of the Republic of Moldova. Online database. [Accessed March 2022]. http://nomenclator.amdm.gov.md/.

Authors's ORCID ID:
Adrian Virlan https://orcid.org/0000-0001-5668-6265
Diana Guranda https://orcid.org/0000-0001-6296-9114
Cristina Ciobanu https://orcid.org/0000-0001-6550-6932

UDC: [616.98:578.834.1+616.36]-053.2

CASE REPORT

COVID-19 infection and liver damage in children. Clinical case study.

Svetlana Liubarscaia[1,2*†], Tatiana Raba[1†], Lucia Ciobanu[2†], Lilia Chiosea[2†], Olga Tihai[1,2†]

[1]Department of Pediatrics, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova.
[2]The Municipal Clinical Hospital for Children „Valentin Ignatenco", Chisinau, Republic of Moldova

Date of the manuscript receipt: 21.02.2022
Date of publication acceptance: 17.05.2022

Corresponding authors:
Liubarscaia Svetlana, Ph.D student
Department of Pediatrics
Nicolae Testemitanu State University of Medicine and Pharmacy
The Municipal Clinical Hospital for Children „Valentin Ignatenco"
165 Ştefan cel Mare şi Sfânt Bd., Chisinau, Republic of Moldova, MD-2004
e-mail: liubarscaia@mail.ru

***Short title**: COVID-19 infection and liver damage*

What is not yet known on the issue addressed in the submitted manuscript?

Currently, the infection with the new coronavirus SARS-CoV-2 is known as a systemic disease that primarily affects the lungs, followed by the heart, kidneys, central nervous system and digestive involvement. Severe forms of COVID-19 infection in children have been rarely described, whereas the damage degree of the digestive organs and, particularly, of the liver in infected children, as well as the clinical signs features of digestive disorders in children have not been sufficiently highlighted.

The research hypothesis

This present article aims to present the clinical manifestations of liver damage in children infected with COVID-19, as well as the problems that might interfere with the management of these patients.

The novelty added by manuscript to the already published scientific literature

This study proves the presence of digestive symptoms, particularly of the liver damage in children infected with the new coronavirus, which shows a diagnostic and therapeutic relevance.

Abstract

Introduction. COVID-19 is currently considered a systemic disease affecting the immune system, primarily, lungs, heart, central nervous system, kidneys, intestines, liver and spleen. Impaired liver function and the presence of biochemical changes in liver can be found in approximately 14-53% of adults infected with SARS-CoV-2. Impaired liver function in patients infected with COVID-19 may occur due to a direct effect of the virus on hepatocytes, as well as being secondary to factors such as a systemic inflammatory response of the infected host, the onset of hypoxia (associated with lung damage), multiple organ failure, or due to abusive treatment using overlapping and hepatotoxic drugs.

The purpose of this article is to describe a clinical case study regarding the clinical and paraclinical manifestations of liver damage in a 12-year-old child infected with SARS-CoV-2, hospitalized at the Municipal Children's Clinical Hospital „Valentin Ignatenco", Republic of Moldova.

Material and methods. The epidemiological, clinical and paraclinical data were used to highlight this study, followed by the conclusions of multidisciplinary specialists, retrieved from the inpatient medical records of the 12-year-old child with moderate COVID-19 infection, who was admitted for emergency treatment.

Results. A 12-year-old patient F. was admitted to the „Covid-19" subunit, complaining of severe general malaise, fever up to 39°C, cough, rhinorrhea. The objective clinical examination revealed hepatomegaly and lack of splenomegaly. Laboratory findings determined leukocytosis 15.88 $x10^9$/l, neutrophilia 72.2%, lymphopenia 26.1%, increased ESR (Erythrocyte Sedimentation rate) – 20 mm/h, increased CRP (C-reactive protein) >12.0 mg/l, increased ALT (alanine aminotransferase) by 16 (50.9-487-764 U/l) values compared to the normal reference and a 3-fold increase in AST (aspartate aminotransferase) that is higher than the normal range (55.8 - 113 - 181 U/l), an increased fibrinogen - 5.3 g/l, increased ferritin - 2834 pmol/l and D-dimer levels - 762 ng/ml. Hepatomegaly was detected on abdominal ultrasound. Covid-19 infection was confirmed by a rapid test of nasopharyngeal exudate for SARS-CoV-2 antigens.

Conclusions. Patients with the novel coronavirus (COVID-19) show varying degrees of liver dysfunction, especially those with increased levels of AST and ALT. A question arises within the clinical practice, as whether the liver damage occurred due to direct viral hepatotoxicity or due to the drugs used in COVID-19 treatment.

Keywords: COVID-19, liver damage, children

https://doi.org/10.52645/MJHS.2022.2.11

Introduction

COVID-19 continues to pose a global public health threat. Epidemiological data indicate that patients suffering from metabolic disorders and chronic diseases are the most susceptible to SARS-CoV-2 (severe acute respiratory syndrome coronavirus). COVID-19 is currently considered a systemic disease with impaired immune system function that primarily affects the lungs, as well as the heart, kidneys, intestines, liver, and spleen. The particular effect of COVID-19 on the liver remains unclear, changes in liver biochemistry are common and occur in approximately 14 to 53% [1] of people infected with SARS-CoV-2. Liver biochemistry disorders are typically characterized by a moderate (1-2 times higher than normal) increase in serum levels of alanine aminotransferases (ALT) and aspartate aminotransferases (AST) [2, 3, 4]. In a study conducted in China, AST/ALT levels were elevated in 18.2/19.8% of patients with mild disease and in 39.4/28.1% of patients with severe disease [5]. Another small study in China showed similar results: elevated AST levels in 62% of patients admitted to the Intensive Care Unit (ICU) compared to 25%, who did not undergo treatment in the ICU [6]. In patients with a subclinical course of the disease, the AST and ALT levels were increased to 8.7 and 8.9% of patients, respectively [7]. The mechanisms of liver damage that occur during SARS-CoV-2 infection have not been clearly defined yet. The main pathogenetic consequences of liver damage include the following:

- direct liver damage due to active replication of the virus in liver cells with the participation of ACE2 as receptors for introduction into the cell [8];
- induction of severe hypoxia, which is considered one of the main causes of ischemic / hypoxic liver damage in COVID-19, followed by acute lung failure and / or shock. The liver damage is associated with metabolic acidosis, calcium overload, and changes in mitochondrial membrane permeability, being commonly manifested by cytolysis [9, 10];
- immune activation and inflammation caused by circulating cytokines , followed by the cytokine „storm" onset and multiple organ failure [11, 12, 13];
- direct hepatotoxicity of the administered drugs [5, 14];
- reactivation of pre-existing chronic liver disease (such as chronic viral hepatitis B, C and E), as well as progression to decompensation of liver cirrhosis [1, 15].

The most common cause of liver damage in COVID-19 is associated with drug-induced toxicity resulting from the use of etiotropic treatment of SARS-CoV-2 infection according to approved clinical protocols and pathogenetic therapy for COVID-19 [5, 14]. Drug-induced toxic liver damage occurs in 10% of all drug-related adverse reactions [16]. There are many drugs with cumulative and hepatotoxic effects that can damage the liver. Some of them can cause an asymptomatic increase in liver enzymes, whereas in other cases, clinical signs may be characteristic of acute hepatitis. Liver damage may also be caused by the dose of the drug used (e.g. paracetamol overdose) or may not be dose-related. Liver toxicity can be induced by some drugs that can cause liver damage, such as antibiotics, anti-inflammatory drugs, and antivirals [17].

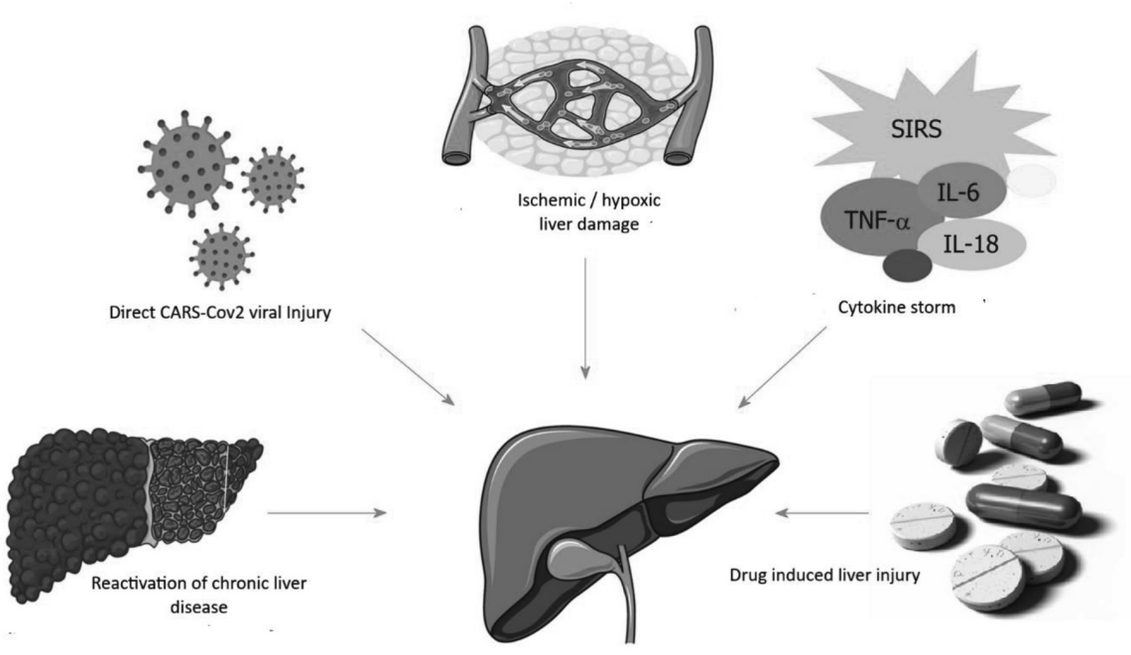


Fig. 1. *Etiology of liver lesions in patients with COVID-19.*

(Source: Rui-Xu Y., Rui-Dan Z., Jian-Gao F. Etiology and management of liver injury in patients with COVID-19. In: J Gastroenterol 2020 Aug 28;26(32): 4753-4762, modified by the author).

Paracetamol (acetaminophen). Paracetamol is the most common drug used as an antipyretic and anti-inflammatory drug, being used in medical practice by prescription and often as self-medication. Paracetamol is considered one of the most common hepatotoxic drugs with dose-dependent hepatotoxicity. The drug is safe if administered according to the dose, starting with body weight at one time or within 24 hours. It is necessary to consider the possibility of the synergistic effect when combining this drug with other ones, resulting in cumulative and hepatotoxicity effects. If the administered dose exceeds, the probability of developing drug-induced hepatitis sometimes is fulminant, followed by acute liver failure in 50% of pediatric patients [18].

It is the first antipyretic drug of choice in the treatment of coronavirus infection [19]. Undesirable side effects of paracetamol primarily include hepato- and nephrotoxicity. Toxicity is induced by the formation of metabolites derived from cysteine and mercapturic acid, which covalently bind to hepatocyte macromolecules, causing hepatocellular necrosis. When administering therapeutic paracetamol doses (10-15 mg/kg/ intake, not more than 60 mg/kg per day) the metabolite is inactivated by endogenous glutathione and excreted by the kidneys [20, 21]. Possibly, apart from the direct drug-induced hepatotoxicity, the occurrence of an idiosyncratic liver damage (immune- mediated) should be considered and identified separately, the most common cause being the antibiotic therapy [22].

Antimicrobial drugs Macrolides are widely used as antimicrobial drugs in children worldwide. Particular attention should be paid to monitoring their safety and harmfulness. According to the pharmacological properties of these drugs, almost all macrolides are hepatotoxic with varying degrees of severity. However, the doses used in clinical practice are insufficient for describing a direct hepatotoxic effect of macrolides. The adverse reactions commonly occur in presence of the following risk factors: high drug doses, genetic predisposition to hepatotoxicity, concomitant use of several drugs that foster the adverse effect of macrolides, as well as a present underlying liver disease. Special care should be given when prescribing potentially hepatotoxic drugs at highest doses, especially in pediatric patients. Cases of hepatotoxicity caused by azithromycin and clarithromycin have been reported in the scientific literature [23].

Anticoagulants may be one of the known causes of potential risk of liver damage [24]. Since the approval of sodium heparin by the Food and Drug Administration (FDA) in 1939, it is still widely used in medical practice for prevention and treatment of thrombosis [25]. The use of heparin has been associated with several well-documented side effects. The heparin-induced hepatotoxicity is less commonly recognized in clinical practice. Increases in AST and ALT levels have been frequently reported in the administration of both sodium heparin and low molecular weight heparins [26]. Previous clinical trials have shown that transaminase elevations occur in up to 90% of patients treated with heparin [27, 28]. An increase in the level of transaminases by more than 3 times from normal values indicates that it occurs in about 5% of patients receiving unfractionated heparin, and between 5% - 10% of patients administering low molecular weight heparins [29]. This increase in transaminase levels is usually temporary and self-limiting with no long-term effects.

Therefore, all patients receiving etiotropic and pathogenetic therapy for COVID-19 need to monitor liver parameters prior to following a treatment, throughout the treatment and in the post-COVID follow-up period to prevent severe drug-induced hepatitis in both active and post-COVID treatment.

Materials and methods

This article reports a clinical case study of a 12-year-old child, who was admitted to the Municipal Clinical Hospital for Children „Valentin Ignatenco" within the Covid-19 unit. The clinical and paraclinical data were retrieved from the inpatient medical records (lab findings included CBC, ALT, AST, total bilirubin and fractions, total protein, prothrombin index, fibrinogen, creatinine, urea, blood glucose, blood count, CRP, ferritin, D-dimers). According to medical records, the patient was confirmed with Covid-19 infection by performing a SARS-CoV-2 Rapid Antigen Test in the nasopharynx. Chest X-ray, abdomen ultrasound and check-ups at the neurologist, gastroenterologist-hepatologist, as well as at the infectious disease specialist were carried out.

Results and discussions

According to the medical history of the 12-years-old patient F., who was in contact with the mother infected with SARS-CoV-2, the child fell acutely ill on November 10 2021, experiencing severe malaise, an increase in body temperature up to 39°C, dry cough, sneezing, and rhinorrhoea. The mother self-administered antipyretics (ibuprofen combined with paracetamol) and antibiotics (Ceftriaxone intramuscular solution), which did not cause a clinical effect. Due to an increase in the malaise severity and the persistence of the febrile syndrome, the mother called an ambulance followed by urgent hospitalization on 10/20/21.

The patient has been monitored with severe psycho-verbal retardation and overweight since the age of one year. The objective clinical examination findings showed medium severity of the patient's overall condition, including T - 38.5°C, RF - 20 r/min, RHR (resting heart rate) - 116 bpm, SpO_2 - 97%, (SpO_2 95% - 94% at 5-6 days, when acute bilateral pneumonia was confirmed) and BP - 120/80 mm Hg. Body weight - 67 kg, height - 147 cm, Percentile >97, BMI - 31 kg/m^2 (+ 3DS). Pale and clean skin with no rash. No pathological picture of the mucous membranes and sclera, as well as a lacking peripheral lymphadenopathy was registered. Hyperemic pharyngeal isthmus. Free nasal breathing was observed. On auscultation, severe bilateral pulmonary respiration, with no rales was revealed. The heart sounds were clear and rhythmic. The abdomen was soft and painless to the touch; hepatomegaly +2.5-3 cm below the edge of the right costal arch, with a rounded edge, insensitive and painless, no ascites and splenomegaly. Physiological transit.

Laboratory examination revealed the following data (table 1): leukocytosis - 15.88 x10^9/l (on the underlying bilateral pneumonia), neutrophilia - 72.2%, lymphopenia - 24%, increased ESR - 20 mm/h, elevated CRP >12.0 mg/l (on the underlying acute pneumonia), increase in ALT by 16 (50.9-487-764 U/l) compared to the normal references and AST 3 times higher than the norm (55.8 - 113 - 181 U/l), total bilirubin was normal, elevated fibrinogen - 5.3 g/l, ferritin - 2834 pmol/l and D-dimer levels - 762 ng/ml..

Table 1. *Laboratory examination data in dynamics of patient F, a 12-year-old boy.*

Lab indices	**Data**					
	21.10.21 (Day 2)	**26.10.21 (Day 7)**	**31.10.21 (Day 12)**	**03.11.21 (Day 15)**	**08.11.21 (Day 20)**	**29.11.21 (after a 20-day follow up)**
Haemoglobin (N 110 - 120 g/l)	131	127	146	148	136	138
Erythrocyte count (N 4.4 -5.0 x10^{12}/l)	4.96	4.91	5.57	5.62	5.15	4.12
Leucocyte count (N 4.0 – 9.0 x 10^9/l)	6.10	7.35	15.88	14.94	7.04	7.15
Platelet count (180 - 400 x 10^9/l)	226	211	397	351	217	382
Neutrophil count (N 43.0 – 60.0%)	68.9	72.2	53.7	58.4	59.1	50.4
Lymphocyte count (N 30 – 46.0%)	26.1	24.0	32.5	34.1	29.8	42.1
ESR (N 0 - 10 mm/h)	19	20	14	16	32	23
CRP, mg/l	negative	>12,0	negative	negative	>21,0	negative
Total protein (N36 - 60 g/l)	60,1	61,6	-	-	69,9	-
Total bilirubin (8.5 – 20.5 mmol/l)	4.45	4.72	6.87	6.08	12.72	6.4
AlAT (N 0 - 45 U/l)	43.3	50.9	487.3	764.9	193.7	23
AST (N 0 - 45 U/l)	43.2	55.8	113.,8	181.,6	61.7	21
Glucose (3.5 – 5.5 mmol/l)	3.76	6.06	4.37	4.57	4.71	4.84
Urea (3.2 – 7.3 mmol/l)	5.52	3.86	6.71	5.49	5.67	-
Creatinine (34 - 65 mmol/l)	59,3	81,2	70,6	57,7	64,8	-
Prothrombin (95 - 105 %)	97	99	95	-	82	-
Fibrinogen (1.25 - 4 g/l)	4.0	5.3	3.1	-	.8	-
INR	1.03	1.01	1.05	-	1.22	-
Ferritin, pmol/l (N 61.6 - 803)	-	2834	-	-	853	
D-Dimer, ng/ml (N 100 - 500)	-	762	-	-		0.15

Note: N - normal reference value; ESR - Erythrocyte Sedimentation rate; CRP - C-reactive protein; AlAT - Alanine aminotransferase; AST - Aspartate aminotransferase; INR - International Normalized Ratio.

The chest X-ray revealed signs characteristic of right pneumonia, on day 5 of hospital stay, and negative radiological dynamics resulted in a bilateral pneumonia, 6-score Brexia, and cardiomegaly on day 11 of hospitalization. The abdominal ultrasound findings were suggestive for an inflammatory process in the liver, pancreas and kidneys; the liver was enlarged, RL = 134 mm, LS = 90 mm, portal vein = 7 mm with regular, clear contour, homogeneous parenchymal structure, increased echogenicity; gallbladder: atypical shape, fundal bend, dimensions 82 x 36 mm, perennials not thickened, no calculi; choledochus was not thickened; pancreas: increased echogenicity, homogeneous parenchymal echostructure, dimensions 17 x 16 x 22 mm, with regular contour, thickened; enlarged spleen with normal echogenic-

ity, homogeneous parenchymal echostructure, dimensions 127 x 46 mm, regular contour; kidneys showed regular, clear contour, pelvises were not dilated, the calyx system was bilaterally deformed; bladder with little content, the walls were not thickened. An accurate diagnosis was carried out, including blood tests negative for HBsAg, negative total anti-HBcor, and negative anti-HCV. Covid-19 infection was confirmed by the SARS-CoV-2 Rapid Antigen Test of the nasopharyngeal exudate. The patient was consulted multidisciplinary for infectious diseases, by the gastroenterologist - hepatologist, pulmonologist and neurologist. Based on the objective examination data, as well as on the laboratory and instrumental findings, the following clinical diagnosis was established: moderate-form COVID-19 infection with bilateral pneumonia, acute evolution, severe-to-moderate form. Type 1 acute respiratory failure. Third-degree toxic hepatitis (based on RUCAM score - 8 points). Severe psycho-verbal retardation. Autism? First-degree acquired obesity (BMI - 31 kg/m^2). The child received treatment according to the recommendations provided by the national and international pediatric medical protocols and standards. Antiviral treatment (Pranogir tab.); intensive symptomatic treatment: antipyretics (Paracetamol tab. 500 mg x 3 times/daily, per oral, at temperature ≥38.5°C); on first 4 days of hospitalization, bronchodilators (Salbutamol aerosol), probiotics (Subtil caps.), proton pump inhibitors (omeprazole caps., vitamin therapy). Considering the persistence of fever, the elevated CRP levels and the negative dynamics of the chest X-ray (acute pneumonia), a glucocorticoid therapy was administered to reduce the inflammatory response, namely low-dose Dexamethasone 0.2 mg/kg/day for 5 days and antibacterial treatment with macrolides in combination with third-generation cephalosporin (Ceftriaxon intravenous, Clarithromycin per oral). For the prevention of venous thromboembolism, basic anticoagulant therapy was carried out viz. Heparin 100 IU/kg/day subcutaneously for 9 days under coagulation testing. During treatment, intoxication syndrome, febrile syndrome, hypercoagulability, acute pneumonia and respiratory failure (SpO_2 98%) regressed. Another significant challenge arising on the underlying treatment was the development of cytolysis syndrome (table 1), most likely associated with the use of the following drugs: paracetamol, heparin, and clarithromycin. Moreover, the unfavorable underlying conditions should also be considered, such as the first-degree obesity and long-term steatosis (steatohepatitis), thus referring the patient F. to a possible risk group for drug hepatotoxicity. Hepatoprotectors like Silymarin tab. per oral, then Essentials caps. per oral, and Aminoplasmal Hepa intravenously were administered. The child was discharged with improved clinical and paraclinical features, being indicated to follow the oral therapy with hepatoprotectors, probiotics, enzyme and vitamin therapy under the control of hepatic transaminases, coagulation and abdominal ultrasound.

Conclusions

The upper digestive system in children is the „gateway" for many disease-casing agents, including the coronavirus. The SARS-VOC-2 virus enters the body through the faecal-oral route, attaching to the epithelial cells of the mucous membrane of the upper respiratory and digestive tract, and subsequently spreading to internal tissues and organs. The interaction of coronavirus with the digestive tract of a child may lead to consequences, sometimes by a long-term elimination of SARS-CoV-2 through feces. In some patients, nausea, vomiting, abdominal pain, diarrhea, and liver damage may be associated with respiratory manifestations. If chronic diseases of the digestive system (cholecystitis, pancreatitis) are recorded in a pediatric patient, they can be reactivated, clinically showing both respiratory signs and digestive manifestations characteristic of a flare-up period of a chronic disease. Apart from the toxic effect of coronavirus on the liver and gastrointestinal tract, patients with COVID-19 also might have frequent and sometimes toxic side effects due to the complex treatment, as well as synergism due to drug overlapping. Antibacterial, antiviral, anticoagulant and drugs, especially those with hepatotoxic effects, can cause toxic side effects due to an overlapping mechanism, inducing toxic hepatitis, colitis, toxic biliary sludge the phenomenon. Therapeutic approach for Covid-19 infection in children should be individually-customized based on clinical indications and dynamic monitoring of the clinical disease evolution. Although liver damage may be associated with the viral direct cytopathic effects, the mechanism of liver damage during SARS-CoV-2 infection requires further in-depth scientific study.

Declaration of conflicting interests

Nothing to declare.

Authors' contributions

The authors contributed equally to the search of scientific literature, the selection of bibliography, the reading and analysis of biographical references, the writing of the manuscript and its peer review. All authors read and approved the final version of the article.

References

1. Zhang C., Shi L., Wang F. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterojgy & Hepatology,* 2020; 5 (5): 428-430.
2. Sultan S., Altayar O., M Siddigue S. *et al*. AGA institute rapid review of the gastrointestinal and liver manifestations of COVID-19, meta-analysis of international data, and recommendations for the consultative management of patients with COVID-19. *Gastroenterology,* 2020; 159 (27): 320-334.
3. Richardson S., Jamie S., Narasimhan M. *et al.* Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York city area. *JAMA*, 2020; 323(20): 2052-2059.
4. Goyal P., Choi J., Pinheiro L. *et al.* Clinical characteristics of Covid-19 in New York city. *N. Engl. J. Med.* 2020*;* 382: 2372-2374.
5. Guan W-J, Ni Z-Y, Hu Y., *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020; 382:1708-1720.
6. Huang C., Wang Y., Li X. *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China*. Lancet*. 2020; 395: 497-506.
7. Shi H., Han X., Jiang N. *et al*. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan China: a descriptive study. *Lancet Infect Dis*. 2020; 20: 425 - 434.
8. Hamming I., Timens W., Bulthuis M.L. *et al*. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 2004; 203(2): 631 – 637.
9. Xu L., Liu J., Lu M. *et al*. Liver injury during highly pathogenic human coronavirus infections*. Liver Int.* 2020; 40(5): 998 - 1004.
10. Gupta A., Madhavan M.V., Sehgal K. *et al*. Extrapulmonary manifestations of COVID-19. *Nat Med.* 2020; 26:1017–1032.
11. Mehta P., McAuley D. F., Brown M. *et al*. COVID-19: consider cytokine storm syndromes and immunosuppression*. Lancet.* 2020; 395(10229): 1033 – 1034.
12. Wong CK., Lam CW., Wu AK. *et al*. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol*. 2004; 136(1): 95-103.
13. Wang D., Hu B., Hu C., *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* 2020; 323(11): 1061-1069.
14. Li J., Fan J.G. Characteristics and mechanism of liver injury in 2019 coronavirus disease. *J. Clin. Transl. Hepatol.* 2020; 8(1): 13-17.
15. Zippi M., Fiorino S., Occhigrossi G., *et al*. Hypertransaminasemia in the course of infection with SARS-CoV-2: incidence and pathogenetic hypothesis*. World J. Clin. Cases.* 2020; 8.(8): 1385 – 1390.
16. Ивашкин В.Т. (ред.). Болезни печени и желчевыводящих путей. М.: Издательский дом «М-Вести». 2002. 416 с. [Ivashkin V.T. (ed.) Diseases of the liver and biliary tract. *M.: Publishing house "M-Vesti",* 2002. 416 p. (In Rus.)].
17. Cascella M., Rajnik M., Cuomo A. *et al.* Features, evaluation, and treatment of coronavirus. [Updated 2020 Oct 4]. In: *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.* 2020.
18. EASL 2019. Clinical Practice Guidelines: Drug-induced liver injury. In: *J Hepatol*, 2019; 70(6): 1222 - 1261.
19. Временные методические рекомендации. Профилактика, диагностика и лечение новой коронавирусной инфекции (COVID-19). Версия 5 (08.04.2020). http://nasci.ru/?id=10642&download=1.
20. Маркова И.В., Афанасьев В.В., Цыбулькин Э.К. Клиническая токсикология детей и подростков. В 2-х томах. Т. 1. СПб.: Интермедика. 1998. 302 с. [Markova I.V., Afanasyev V.V., Tsybulkin E.K., *et al.* Clinical toxicology of children and adolescents: in 2 volumes. T. 1. *SPb .Intermedika*. 1998; p. 302].
21. Bessems J., Vermeulen N. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and prote. *Crit Rev Toxicol*.2001; 31(1): 55 - 138.
22. Звягинцева Т.Д., Чернобай А.И. Лекарственные поражения печени. НПВП-ассоциированные гепатопатии: актуальность проблемы и современные терапевтические подходы. Український медичний часопис. 2014; 1: 80-85. http://nbuv.gov.ua/UJRN/UMCh_2014_1_16.
23. Белоусов Ю., Лекарственные поражения печени, ассоциируемые с макролидами. Очевидна ли связь? РМЖ; 2011; 18: 1118 – 1121.
24. Mahamid M., Mader R., Safadi R. Hepatotoxicity of tocilizumab and anakinra in rheumatoid arthritis: management decisions*. Clin Pharmacol*. 2011; 3: 39 - 43.
25. Center for drug evaluation and research. Summary review. Heparin Sodium. Data of submission 11.04.2011. Verified 11.11.2018. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/201370Orig1s000SumR.pdf.
26. Bounameaux H. Unfractionated versus low-molecular-weight heparin in the treatment of venous thromboembolism. *Vasc Med.* 1998; 3(1): 41 - 46.
27. Bosco M., Kish T. Hepatotoxicity With Elevated Bilirubin Secondary to Prophylactic Doses of Unfractionated Heparin: A Case Report and Review of Heparin-Induced Hepatotoxicity. *J Pharm Technol.* 2019; 35(1): 36–40.
28. Harrill AH., Roach J., Fier I. *et al.* The effects of heparins on the liver: application of mechanistic serum biomarkers in a randomized study in healthy volunteers. *Clin Pharmacol Ther.* 2012; 92:214-220.
29. Arora N., Goldhaber S., Anticoagulants and transaminase elevation. *Circulation*. 2006; 113 (15): 698-702.

Authors's ORCID ID:
Tatiana Raba https://orcid.org/0000-0002-3970-6495
Lucia Ciobanu https://orcid.org/0000-0002-3583-4051

MJHS Moldovan Journal of Health Sciences
REVISTA DE ŞTIINŢE ALE SĂNĂTĂŢII DIN MOLDOVA

DECLARAŢIA AUTORILOR

Titlul manuscrisului: _____
_____
_____

Toate persoanele care îndeplinesc criteriile de autor sunt menţionate drept autori. Toţi autorii certifică faptul că au participat suficient elaborarea lucrării, încât să îşi asume responsabilitatea publică pentru conţinutul remis, inclusiv pentru concept, design, analiză, scris sau revizuire a manuscrisului. Mai mult decât atât, fiecare autor certifică faptul că acest material sau un material similar nu a fost şi nu va fi propus spre publicare sau publicat în orice altă ediţie periodică, înainte de apariţia lui în *Revista de Ştiinţe ale Sănătăţii din Moldova*.

Contribuţia autorilor:

Vă rugăm să indicaţi contribuţiile specifice efectuate de fiecare autor (înscrieţi iniţialele autorilor, urmate de numele lor, de exemplu: *A. Belîi, Gh. Rojnoveanu*). Numele fiecărui autor trebuie să apară cel puţin o dată în fiecare dintre cele trei categorii, menţionate mai jos.

Categoria 1

Concepţia şi design-ul studiului: _____, _____, _____, _____;

Achiziţia de date: _____, _____, _____, _____;

Analiza şi/sau interpretarea datelor: _____, _____, _____, _____.

Categoria 2

Elaborarea (*drafting*-ul) manuscrisului:_____, _____, _____, _____;
Revizuirea semnificativă a manuscrisului, cu implicare intelectuală semnificativă: _____, _____.

Categoria 3

Aprobarea versiunii „gata pentru tipar" a manuscrisului (trebuie menţionate numele tuturor autorilor):

_____, _____, _____, _____, _____,
_____, _____, _____, _____, _____.

Mulţumiri:

Toate persoanele care au adus contribuţii importante la lucrul raportat în manuscris (de exemplu, ajutor tehnic, scris şi asistenţă la editare, suport general), dar care nu îndeplinesc criteriile de autor, sunt menţionate în secţiunea *„Mulţumiri",* iar acestea şi-au dat acordul în scris ca să fie menţionate. Dacă secţiunea *„Mulţumiri"* lipseşte din manuscris, atunci acest fapt semnifică că nu au existat contribuţii substanţiale din partea non-autorilor.

_____, _____, _____, _____, _____,

Prezenta declaraţie este semnată de către toţi autorii:

(puteţi utiliza o fotocopie a formularului dat în cazul existenţei mai mult de 6 autori)

Numele autorului (tipărit)	Semnătura autorului	Data
_____	_____	_____
_____	_____	_____
_____	_____	_____

Vă rugăm să transmiteţi acest formular completat şi scanat pe adresa: editor.mjhs@usmf.md

AUTHORSHIP STATEMENT

Manuscript title: _____
_____
_____

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Moldovan Journal of Health Sciences.*

Authorship contributions:

Please indicate the specific contributions made by each author (list the authors' initials followed by their surname(s), e.g., *A. Belîi, Gh. Rojnoveanu*). The name of each author must appear at least once in each of the three categories below.

Category 1

Conception and design of study: _____,_____,_____,_____;
Acquisition of data:_____,_____,_____,_____;
Analysis and/or interpretation of data:_____,_____,_____,_____.

Category 2

Drafting the manuscript: _____, _____, _____, _____;
Revising the manuscript critically for important intellectual content:_____,_____.

Category 3

Approval of the version of the manuscript to be published (the names of all authors must be listed):

_____,_____,_____,_____,_____,
_____,_____,_____,_____,_____,

Acknowledgements:

All persons who have made substantial contributions to the work reported in the manuscript (e.g., technical help, writing and edit- ing assistance, general support), but who do not meet the criteria for authorship, are named in the „*Acknowledgements*" and have given us their written permission to be named. If we have not included an „*Acknowledgements*", then that indicates that we have not received substantial contributions from non-authors:

_____,_____,_____,_____,_____,

This statement is signed by all the authors:

(*a photocopy of this form may be used if there are more than 6 authors*):

Author's name (typed)	Author's signature	Date
_____	_____	_____
_____	_____	_____
_____	_____	_____

Please, send this filled and scanned form to: editor.mjhs@usmf.md

MJHS Moldovan Journal of Health Sciences
REVISTA DE ŞTIINŢE ALE SĂNĂTĂŢII DIN MOLDOVA

SCRISOARE DE ÎNSOŢIRE

Titlul manuscrisului: _____
_____

Relevanţa manuscrisului pentru scopul promovat de Revistă: _____
_____
_____
_____

Ce aduce nou manuscrisul domeniului (specialităţii) în care este publicat: _____
_____
_____
_____

Modul în care manuscrisul adaugă valoare la literatura științifică de specialitate: _____
_____
_____
_____

Noi, autorii subsemnaţi ai manuscrisului, declarăm că (bifaţi):

- ☐ lucrarea menţionată este originală;
- ☐ lucrarea menţionată nu a fost publicată anterior;
- ☐ lucrarea menţionată nu este depusă pentru publicare în altă revistă;
- ☐ toţi autorii subsemnaţi au contribuit la elaborarea manuscrisului;
- ☐ de la subiecţii incluşi în studiu a fost obţinut consimţământul informat;
- ☐ toţi autorii subsemnaţi au aprobat versiunea finală a manuscrisului;
- ☐ suntem de acord cu verificarea antiplagiat a manuscrisului;
- ☐ au fost declarate orice potenţiale conflicte de interes.

Prin prezenta, autorii sunt de acord să transfere drepturile de proprietate (copyright) Revistei de Ştiinţe ale Sănătăţii din Moldova – Moldovan Journal of Health Sciences, în caz că manuscrisul va fi publicat.

Autorii (nume, prenume complet, semnătură):

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

Locul şi data: _____

Datele de contact ale autorului corespondent:

Instituţia: _____
_____

Adresa: _____
_____
_____

e-mail: _____
tel./fax.: _____

Vă rugăm să transmiteţi acest formular completat şi scanat pe adresa: editor.mjhs@usmf.md

MJHS Moldovan Journal of Health Sciences
REVISTA DE ȘTIINȚE ALE SĂNĂTĂȚII DIN MOLDOVA

COVER LETTER

Manuscript title: _____
_____

Relevance of the manuscript for the Journal purposes: _____
_____
_____
_____

Contributions of the manuscript for to the research field: _____
_____
_____
_____

What is the added value of the manuscript to the already published scientific literature: _____
_____
_____
_____

We, the undersigned authors of the manuscript, declare that (please, tick):

- ☐ the paper contain original data;
- ☐ the paper has not been published before;
- ☐ the manuscript is not submitted for publication to another journal;
- ☐ all authors have contributed to the manuscript;
- ☐ the informed consent were obtained from all study subjects;
- ☐ all coauthors approved the final version of the manuscript;
- ☐ we agree for checking of the manuscript for plagiarism;
- ☐ any potential conflicts of interest were disclosed.

With this, the authors agree to transfer property rights (copyright) to the *Moldovan Journal of Health Sciences – Revista de Stiinte ale Sanatatii din Moldova*, in the event that the manuscript will be published.

Authors (name, surname, signature):

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

Place and date: _____

Corresponding author data:

Institution: _____
_____

Address: _____
_____
_____

e-mail: _____
tel./fax: _____

Please, send this filled and scanned form to: editor.mjhs@usmf.md

Revista de Ştiinţe ale Sănătăţii din Moldova

Moldovan Journal of Health Sciences

Ghidul autorului

Criterii pentru publicare

Articolele originale trebuie să conţină cercetări noi (originale), rezultatele cărora contribuie la acumularea de noi cunoştinţe în domeniul publicat şi cu condiţia că rezultatele prezentate nu au mai fost publicate înainte sau nu sunt depuse, în paralel, la o altă revistă, în vederea publicării.

Manuscrisele prezentate trebuie să corespundă standardelor STROBE (http://www.strobe-statement.org).

Prezentarea manuscrisului

Manuscrisele trebuie să fie prezentate doar în formă electronică, în limba română sau engleză. Dacă manuscrisul a fost depus doar în limba română, odată ce a trecut procedura de recenzare internă, acesta va fi tradus integral de către autori în limba engleză, pentru a putea trece procedura de recenzare externă. În acelaşi manuscris se permite utilizarea *US English* sau *British English*, dar nu mixt. Varianta tipărită (hârtie) nu va fi acceptată. Doar autorul corespondent va putea depune manuscrisul la redacţie; tot el va deţine responsabilitatea completă de procesul de depunere, de corespondenţa cu redacţia pe durata procesului de publicare.

Procesul de publicare poate fi amânat, întrerupt sau anulat, la discreţia autorului corespondent. Odată manuscrisul depus, autorul corespondent va primi un cod electronic de identificare a manuscrisului, pe care îl va folosi în corespondenţa ulterioară cu redacţia.

În scopul menţinerii integrităţii editoriale şi a standardelor internaţionale de calitate, Redacţia *Moldovan Journal of Health Sciences – Revista de Ştiinţe ale Sănătăţii din Moldova* utilizează un sistem de detectare a plagiatului şi va supune manuscrisul unei verificări antiplagiat. Depunerea manuscrisului pentru publicare înseamnă, implicit, acordul tuturor autorilor cu verificarea lui antiplagiat. În cazul suspectării că manuscrisul depus a încălcat politicile de publicare, acesta poate fi suspendat sau respins, indiferent de etapa procesului de publicare.

Scrisoarea de însoţire

La depunere, autorul corespondent va anexa la manuscris o scrisoare de însoţire. Formularul tipizat al Scrisorii de însoţire este oferit de către Redacţie. Scrisoarea de însoţire include: (1) titlul manuscrisului; (2) o scurtă descriere despre relevanţa manuscrisului pentru scopul promovat de Revistă; (3) contribuţiile aduse de manuscris pentru domeniul său; (4) modul în care manuscrisul adaugă valoare la literatura ştiinţifică de specialitate; (5) numele şi semnăturile tuturor coautorilor; (5) datele complete de contact ale Autorului corespondent, cu menţionarea instituţiei şi adresei instituţionale, nr. de telefon, nr. de fax şi adresa e-mail.

Instructions for Authors

Criteria for publication

Original articles should contain new (original) results, which bring new knowledge in the field. The submitted manuscripts should contain data unpublished before and not submitted in parallel for publication to another journal.

Manuscripts submitted must meet STROBE standards (http://www.strobe-statement.org).

Manuscript submission

Manuscripts must be submitted only in electronic form in Romanian or English. Once past the internal reviewing procedure, the manuscript was submitted only in Romanian will be fully translated by the authors in English to pass the external reviewing procedure. In the manuscript are allowed to use U.S. English or British English, but not mixed. Printed version (paper) will not be accepted. Only the corresponding author may submit the manuscript. The corresponding author holds full responsibility of the submission and correspondence with the editor during reviewing and publication process.

The publication of the manuscript may be postponed, stopped or canceled at the request of the corresponding author. Once the manuscript is submitted, the corresponding author will receive an electronic identification code of the manuscript, which should be used for subsequent correspondence with the editor.

In order to maintain editorial integrity and international quality standards, editor of the Moldovan Journal of Health Sciences reserves the right to use a plagiarism detection system. Thus the submitted manuscript will be checked for plagiarism. Manuscript submission involves agreement of all coauthors for checking for plagiarism. If the submitted manuscript violates copyright policies; it can be suspended or dismissed, regardless of the stage of the publishing process.

Cover letter

A submitted manuscript should be accompanied by a Cover letter. A template of Cover letter is provided by editor. Cover letter should include: (1) the title of the manuscript; (2) a short statement regarding the relevance of the manuscript for the journal proposes; (3) contributions of the manuscript for to field; (4) what is the added value of the manuscript to the already published scientific literature; (5) the names and signatures of all coauthors; (5) the full contact details of corresponding author, indicating the institution and institutional address, no. telephone, no. fax and e-mail.

In the Cover letter, the corresponding author should clearly indicate that: (1) the paper contain original data; (2) the paper has

În scrisoarea de intenţie, Autorul corespondent trebuie să indice în mod clar că: (1) lucrarea menţionată este originală; (2) lucrarea menţionată nu a fost publicată anterior; (3) lucrarea menţionată nu este depusă pentru publicaţie în altă revistă; (4) toţi autorii subsemnaţi au contribuit la elaborarea manuscrisului; (5) de la subiecţii incluși în studiu a fost obţinut consimţământul informat; (6) toţi autorii subsemnaţi au aprobat versiunea finală a manuscrisului; (7) acordul implicit de verificare antiplagiat al manuscrisului; (8) au fost declarate orice potenţiale conflicte de interes. De asemenea, Autorul corespondent poate include orice informaţie suplimentară în Scrisoarea de intenţie, dacă consideră că aceasta poate fi utilă pentru Redacţie.

Consimţământul informat

Orice manuscris care comunică rezultate experimentale, obţinute de la subiecţi umani, trebuie să fie bazat pe studii, în care a fost obţinut consimţământul informat de la subiect (ţi) şi/sau tutore (i). În scrisoarea de intenţie, autorul corespondent trebuie să indice în mod clar obţinerea consimţământului informat. În caz de necesitate, Redacţia este în drept să solicite probe suplimentare, care atestă obţinerea consimţământului informat.

Comitetul de Etică

Pentru orice studiu experimental, efectuat pe oameni sau animale, este necesar de a menţiona evaluarea etică a proiectului de cercetare. În acest sens, în articol vor fi menţionate numărul procesului verbal şi data şedinţei Comitetului de Etică, când a fost aprobat proiectul de cercetare.

Permisiuni

În conformitate cu ghidurile Comitetului Internaţional al Editorilor Revistelor Medicale (*ICMJE Guidelines*), în cazul când în manuscrisul prezentat este folosită sau reprodusă o informaţie publicată anterior, sau un material cu drepturi de autor, este de responsabilitatea Autorului corespondent să obţină permisiunea în scris a deţinătorului de drepturi (*Copyright*) şi să citeze corect sursa originală. Cu scopul de a menţine transparenţa, se recomandă ca această permisiune, sub formă de copie, să fie depusă împreună cu manuscrisul.

Fotografii cu pacienţi identificabili

În conformitate cu ghidurile internaţionale ale Comitetului de Etică a Publicaţiilor (*COPE Guidelines*), în cazul când în imaginile prezente în manuscris (fotografii, radiograme, rezultate de laborator, rezultatele investigaţiilor paraclinice, înregistrări video sau sonore ş. a.) o persoană este identificabilă fizic, de la aceasta trebuie obţinută o permisiune în scris de utilizare a imaginii date. Se recomandă ca permisiunea dată să fie depusă împreună cu manuscrisul, iar în manuscris să fie stipulat în mod clar, că această permisiune a fost obţinută.

Specificarea medicamentelor şi dispozitivelor

În manuscris se vor utiliza nume generice de medicamente, urmate, dacă este cazul, de denumirea lor comercială între paranteze. Pentru medicamente şi dispozitive, includeţi numele producătorului şi localizarea acestuia (ţara de origine).

Formatul fişierelor

Se acceptă următoarele formate de text pentru manuscrisul principal: Microsoft Word (97, 2003, 2007, 2010) şi formatele „.rtf“, „.doc“, „.docx“. Se acceptă următoarele formate pentru imag-

not been published before; (3) the manuscript is not submitted for publication to another journal; (4) all authors have contributed to the manuscript; (5) the informed consent were obtained from all study subjects (6) all coauthors approved the final version of the manuscript; (7) agreement for checking of the manuscript for plagiarism; (8) any potential conflicts of interest were disclosed. Corresponding author may include in the Cover letter any other additional information which could be useful for the editor.

Informed consent

Manuscripts that report experimental results obtained on human subjects must be based on studies in which informed consent was obtained from study subjects and/or their legal representative. The corresponding author should clearly indicate in his letter of intention about the obtaining of the informed. Editor reserved the right to request additional evidence attesting the obtaining of the informed consent.

Ethic Committee

For any experimental study conducted on humans or animals, it is necessary to mention in the article the ethical evaluation of the research project (such as date of evaluation and reference number of approval).

Permissions

In accordance with the guidelines of the International Committee of Medical Journals Editors (ICMJE Guidelines) if the submitted manuscript used or reproduced information/material previously published or copyrighted is the responsibility of the corresponding author to obtain a written permission from the owner of the copyright and properly cite the original source. In order to maintain transparency, it is recommended to submit the permission, as a copy, along with the manuscript.

Pictures

In accordance with international guidelines of the Publications Committee of Ethics (COPE Guidelines), if the manuscript contains pictures (photographs, radiograms, laboratory results, results of laboratory investigations, videos or sound etc.) which allows physical identification of the person, it must be obtained a written permission for the use of the image data. It is recommended to submit the permission along with the manuscript. Also in the manuscript text should be clearly stated that permission was obtained.

Drugs and devices specifications

In manuscript generic names of drugs, followed by their trade name in parentheses (if appropriate) should be used. For drugs and devices, manufacturer's name and location (country of origin) should be mentioned.

Files format

The following file formats for manuscript text are accepted: Microsoft Word (97, 2003, 2007, 2010) ".rtf", ".doc", ".docx". Pictures should be submitted in one of the following formats: ".jpeg", ". tiff", ".eps ", ".ppt", , ".pptx". The images could be transmitted also, in a format item ".ppt" or ".pptx" (one image one slide). Scanning

ini: „.jpeg“, „.tiff“, „.eps“, „.ppt“. „.pptx“. Este posibil ca imaginile articolului să fie transmise în format „.ppt“ sau „.pptx“ (o imagine – un slide). Calitatea imaginilor, indiferent de format, trebuie să fie, minim: pentru desene – 800 dpi, pentru imagini cu detalii fine – 1000 dpi, pentru imagini alb-negru – de 300 dpi.

Structura manuscrisului

Publicaţia Periodică *Moldovan Journal of Health Sciences – Revista de Ştiinţe ale Sănătăţii din Moldova* respectă recomandările STROBE de raportare a cercetărilor observaţionale biomedicale. Pentru a vă uşura procesul de elaborare şi structurare a manuscrisului, vă recomandăm să consultaţi informaţia respectivă, disponibilă online, pe site-ul www. strobe-statement.org.

Volumul textului unui manuscris nu trebuie să depăşească 6000 de cuvinte. Cu toate că numărul figurilor şi tabelelor în manuscris rămâne la discreţia autorilor, se recomandă ca numărul lor să fie limitate la 5, pentru a nu reduce din lizibilitatea articolului pe paginile Revistei.

Structura unui articol original trebuie să respecte următoarea consecutivitate:

- Titlul lung (formulat în conformitate cu ghidurile STROBE)
- Numele şi prenumele complete ale autorului (autorilor)
- Afilierile autorului (autorilor)
- Datele de contact ale autorului corespondent
- Titlul scurt (va fi utilizat în calitate de colontitlu pe paginile Revistei)
- Elementele scoase în evidenţă din articol:
  - Ce nu este, deocamdată, cunoscut la subiectul abordat (descris în 1-3 fraze)
  - Ipoteza de cercetare (formulată în 1-2 fraze)
  - Noutatea adusă de articol literaturii ştiinţifice din domeniu (limitată la 1-3 fraze).
- Rezumatul articolului (compus din: introducere, materiale şi metode, rezultate, concluzii), limitat la maximum 350 de cuvinte.
- Cuvinte cheie
- Introducere
- Materiale şi metode
- Rezultate
- Discuţii
- Concluzii
- Lista abrevierilor utilizate (dacă este cazul)
- Declaraţia de conflict de interese
- Contribuţiile autorilor
- Mulţumiri şi finanţare (dacă este cazul)
- Referinţe bibliografice
- Tabele şi legende la tabele (dacă este cazul)
- Ilustraţii şi figuri (dacă este cazul)
- Legendele figurilor (dacă este cazul)
- Descrierea datelor suplimentare, anexe (dacă este cazul)

Pe pagina de titlu a manuscrisului trebuie să fie prezente următoarele elemente:

- **Titlul manuscrisului:** formulat în conformitate cu ghidurile STROBE, trebuie să fie laconic, relevant pentru conţinutul manuscrisului, să reflecte tipul (*design*-ul) studiului şi să nu depăşească 25 de cuvinte. Nu se admit prezenţa abrevierilor în titlu.
- **Titlul scurt** (ce va fi utilizat drept colontitlu pe paginile Revistei) reprezintă o versiune scurtă, de esenţă, a titlului complet.

resolution should be as follows: drawings – at least 800 dpi, fine line images – 1000 dpi and greyscale images – at least 300 dpi.

Structure of the manuscript

Moldovan Journal of Health Sciences follows STROBE recommendations for reporting observational biomedical research studies. To facilitate the development of the manuscript, please consult this information available online at www.strobe-statement.org.

The volume of the manuscript text should not exceed 6000 words.

Although, the number of figures and tables in the manuscript is at the discretion of the authors, in order to not reduce article legibility it is recommended to limit their number to five.

Structure of original article must comply with the following sequence:

- Full title (according to the STROBE guidelines)
- Full authors' name
- Authors' affiliations
- Contact details of corresponding author
- Short title (to be used as a running head on the journal)
- Article highlights:
  - What is not yet known on the issue addressed in the submitted manuscript (described in 1-3 sentences)
  - The research hypothesis (described in 1-2 sentences)
  - The novelty added by manuscript to the already publishedscientific literature (limited to 1-3 sentences).
- Abstract (consisting of background, materials and methods, results and conclusions), to not exceed 350 words.
- Keywords
- Introduction
- Materials and methods
- Results
- Discussions
- Conclusions
- List of abbreviations used (if applicable)
- Declaration of conflict of interests
- Authors' contributions
- Acknowledgements and funding (if applicable)
- References
- Tables and tables' captions (if applicable)
- Pictures and figures (if applicable)
- Figures' legends (if applicable)
- Description of additional data, appendices (if applicable)

The cover page of the manuscript should include:

- **Title of the manuscript:** written according to the STROBE guidelines, should be concise, relevant to the content of the manuscript, and reflect the study design. The title length should not exceed 25 words. It is not allowed the presence of abbreviations in the title.

- **Short title:** (to be used as a running title) is a short version of the essential of the full title. Short title will be limited to 40 characters, including spaces.

- **Author(s) name:** Authors list must include only those persons who had a substantial contribution to the work. Exam-

Va fi limitat la 40 de caractere, inclusiv spaţiile.

• **Numele autorului (autorilor).** Autori sunt numiţi doar acele persoane, care au avut o contribuţie substanţială la lucrare. Exemple de contribuţie esenţială la lucrare sunt: elaborarea *design*-ului studiului, recrutarea pacienţilor, participarea în colectarea datelor, analiza datelor, interpretarea rezultatelor, scrierea propriu-zisă a articolului, realizarea tehnică a testelor, investigaţiilor, realizarea imaginilor, formularea concluziilor. Pot fi citaţi până la 10 autori individuali. În cazul când grupul de lucru depăşeşte 10 autori individuali, vor fi citaţi în secţiunea „Numele şi prenumele autorilor" doar primii doi, iar restul vor fi menţionaţi la sfârşitul articolului, la secţiunea „Mulţumiri şi finanţare".

Membrii grupului de lucru, care nu îndeplinesc criteriile formale de autor enumerate, dar au avut o oarecare contribuţie la lucrare, pot fi menţionaţi în secţiunea „Mulţumiri şi finanţare".

Notă: Pentru a diferenţia autorul corespondent şi autorii care au contribuit în aceeaşi măsură la lucrare, folosiţi caractere speciale, ca exponenţi, la sfârşitul numelor lor:

(*) – pentru Autorul corespondent;

(†) – pentru Autorii care au avut o contribuţie egală. (De exemplu: Adrian Belîi*, Adrian Belîi†)

Nu se vor menţiona gradele şi titlurile ştiinţifice şi cele ştiinţifico-didactice.

• **Afilieri.** Afilierea autorilor se va scrie după secţiunea „Numele autorului (autorilor)". În acest sens, se va menţiona numele complet al instituţiei de afiliere a autorului (autorilor), localitatea şi ţara.

Afilierea se marchează cu cifre arabe, în superscript (de exemplu: Adrian Belîi[1])

• Elementele scoase în evidenţă din articol:
  - Ce nu este, deocamdată, cunoscut la subiectul abordat (descris în 1-3 fraze)
  - Ipoteza de cercetare (formulată în 1-2 fraze)
  - Noutatea adusă de articol literaturii ştiinţifice din domeniu (limitată la 1-3 fraze).

Din pagină nouă:

Rezumatul

Rezumatul trebuie să fie scris la timpul trecut, persoana a treia. Acesta trebuie să ofere un sumar concis al scopului, obiectivelor, rezultatelor semnificative şi concluziilor studiului, în limitele la 350 de cuvinte, organizate în următoarele secţiuni:

- **Introducere** – unde se va reflecta, pe scurt, contextul şi scopul principal al studiului;
- **Materiale şi metode –** cum a fost realizat studiul şi ce teste statistice au fost aplicate;
- **Rezultate** – prezintă rezultatele principale ale studiului;
- **Concluzii** – o scurtă trecere în revistă a constatărilor făcute, cu posibile implicări pentru studii ulterioare.

Nu utilizaţi abrevieri şi citaţii în rezumatul articolului.

Cuvintele cheie

Enumeraţi 4-10 cuvinte cheie, care sunt reprezentative pentru conţinutul articolului. Pentru a uşura găsirea articolului Dvs. de către motoarele de căutare ale bazelor de date, folosiţi termeni recomandaţi din lista de titluri cu subiect medical de pe http://nlm.nih.gov/ mesh.

Înregistrarea trialului clinic

În caz dacă articolul Dvs. comunică rezultatele unui trial clinic,

ples of essential contribution to the work are: developing of the study design, patients recruitment, participation in data collection, data analysis, interpretation of results, writing of the manuscript, performing of the tests, pictures taking, drawing conclusions. The authors list should not exceed 10 persons. If the research group exceed 10 individual authors, in the "Authors name" section first two will be cited, all others should be mentioned at the end of the article, in the "Acknowledgements and funding" section.

Members of the research group who do not meet the formal criteria of the authorship, but have had some contribution to the paper, may be mentioned in the "Acknowledgements and funding" section.

Note: To differentiate the corresponding author, as well as authors who have an equal contribution to the work, using special characters as a superscript index at the end of their names is recommended:

(*) – Corresponding author;

(†) – Authors with equal contribution. (e.g. Adrian Belii*, Adrian Belii†)

• **Affiliation:** Please state the full name of institution, city and country to which the author(s) is affiliated. Affiliation should be marked with Arabic numerals in superscript after the author(s) name (e.g. Adrian Belii[1])

• **Article highlights:**
  - What is not yet known on the issue addressed in the submitted manuscript (described in 1-3 sentences)
  - The research hypothesis (described in 1-2 sentences)
  - The novelty added by manuscript to the already published scientific literature (limited to 1-3 sentences).

From new page:

Abstract

The abstract should be written using the past tense, third person. It should provide a concise summary of the purpose, objectives, significant results and conclusions of the study. The summary text should not exceed 350 words organized into the following sections:

- **Introduction** – reflect in short the context and purpose of the study;
- **Materials and methods** – describe how the study was conducted and specify the applied statistics;
- **Results** – present the key results of the study;
- **Conclusions** – a brief overview of the findings, with possible implications for further studies.

Do not use abbreviations or citations in the abstract of the article.

Key words

List 4-10 keywords that are representative for the contents of the article. To facilitate finding of your article by search engines of electronic databases, use MESH keywords list (available on http:// nlm. nih.gov/mesh).

Registered clinical trial

In case if your article reported the results of a clinical trial, please indicate Trial Register and the unique registration number of the trial.

vă rugăm să indicaţi Registrul trialului şi numărul unic de înregistrare a trialului.

Exemplu: *„Current Controlled Trials ISRCTN61362816”*. Atenţie! Nu trebuie să existe niciun spaţiu între literele şi cifrele numărului unic de înregistrare a trialului. Pentru mai multe informaţii, va rugam să accesaţi http://www.isrctn.org (*International Standard Randomised Controlled Trial Number*) şi http://www.icmje.org (*International Committee of Medical Journal Editors*).

Din pagină nouă:

Introducerea

Introducerea, scrisă la timpul trecut, persoana a treia, trebuie:

- să ofere informaţii care ar permite cititorilor din afara domeniului să intre în contextul studiului, să-i înţeleagă semnificaţia;
- să definească problema abordată şi să explice de ce aceasta este importantă;
- să includă o scurtă trecere în revistă a literaturii recente din domeniu;
- să menţioneze orice controverse sau dezacorduri relevante în domeniu;
- să formuleze ipoteza de cercetare şi să prezinte parametrul principal şi cei secundari de rezultat;
- să concludă cu scopul lucrării şi cu un comentariu care să ateste dacă scopul propus a fost atins.

Materiale şi metode

În secţiunea „Materiale şi metode” trebuie să fie descrise cu detalii suficiente procedurile efectuate. Aici se vor menţiona protocoalele detaliate privind metodele utilizate precum şi informaţii justificative. Se vor include: *design*-ul studiului, descrierea participanţilor şi materialelor implicate, descrierea clară a tuturor intervenţiilor şi comparaţiilor efectuate, precum şi testele statistice aplicate. Se vor specifica denumirile generice de medicamente. Atunci când în cercetare sunt folosite branduri, se indică în paranteze denumirea lor comercială. În cazul studiilor pe subiecţi umani sau pe animale, trebuie să fie menţionată aprobarea etică (data şi nr. procesului verbal al şedinţei Comitetului de Etică, preşedintele CE şi denumirea instituţiei, în cadrul căreia activează CE), precum şi consimţământul informat al persoanelor.

Rezultate

Rezultate şi discuţiile vor fi prezentate în secţiuni separate.

Autorii trebuie să prezinte rezultate clare şi exacte. Rezultatele prezentate trebuie explicate (nu justificate sau comparate, în această secţiune) cu constatări fundamentale, evident, referitoare la ipoteza care a stat la baza studiului. Rezultatele trebuie redate concis şi logic, cu accentuarea celor noi.

Discuţii

Se va descrie impactul, relevanţa şi semnificaţia rezultatelor obţinute în domeniul respectiv. Rezultatele obţinute se vor compara cu cele provenite din studiile anterioare din domeniu şi se vor trasa potenţiale direcţii viitoare de cercetare. Discuţiile trebuie să conţină interpretări importante ale constatărilor şi rezultatelor, în comparaţie cu studiile anterioare. De asemenea, se vor menţiona limitele studiului şi factorii potenţiali de *bias*.

Concluzii

Această secţiune trebuie să concludă laconic întregul studiu şi

E.g.: “Current Controlled Trials ISRCTN61362816”

Attention! There should be no space between letters and numbers of the unique record number of the trial. For more information, please visit http://www.isrctn.org (International Standard Randomized Controlled Trial Number) and http://www.icmje.org (International Committee of Medical Journal Editors).

From new page: Introduction

The Introduction section should be written using past tense, third person, and should:

- provide information that would allow readers outside of the field to enter the context of the study, to understand its meaning;
- define the problem addressed and explain why it is important;
- include a brief review of recent literature in the field;
- mention any controversy or disagreement existing in the field;
- formulate research hypothesis and present the main and secondary assessed outcomes;
- conclude with the research’ propose and a short comment whether the purpose has been achieved.

Materials and methods

“Materials and methods” section should present in sufficient details all carried out procedures. Here should be described protocols and supporting information on the used methods. It will include study design, subjects’ recruitment procedure, clear description of all interventions and comparisons and applied statistics. In the manuscript text the generic names of drugs should be used. When drug brands are used their trade name will be shown in parentheses. For studies on humans or animals a statement about ethical approval and informed consent of study subjects should be include. Please specify date and number of Ethics Committee (EC) decision, chair of the EC as well as institution within EC is organized.

Results

Results and discussion should be presented in separate sections. Authors must present results in a clear and accurate manner. Results should be explained (not justified or compared in this section) and include fundamental statements related to hypothesis behind the study. The results should be presented concisely and logically, emphasizing on new original data.

Discussions

Describe the impact, relevance and significance of the obtained results for the field. The results are compared with those from previous publications and draw potential future research directions. Discussions should include important interpretations of the findings and results compared with previous studies. Also, study limitations and potential bias should be mentioned.

Conclusions

This section should conclude laconically entire study, and highlight the added-value brought on the studied issue. The conclusions should not provide new information or double (repeat) those presented in the “Results” section.

să specifice, care este plus-valoarea adusă la informaţiile disponibile despre subiectul abordat. În concluzii nu se vor oferi informaţii noi şi nu se vor dubla (repeta) cele prezentate în secţiunea „Rezultate".

Abrevieri

Folosiţi numai abrevieri standard. De asemenea, pot fi formulate şi alte abrevieri, cu condiţia că acestea vor fi descifrate în text atunci când sunt utilizate pentru prima dată. Abrevierile din figuri şi tabele vor fi descifrate în legendă. Abrevierile trebuie folosite cât mai rar posibil.

Declaraţia de conflict de interese

După publicare, persoanele sau organizaţiile implicate în studiu vor deveni publice şi astfel poate fi influenţată reputaţia lor. Prin urmare, autorii trebuie să dezvăluie relaţia financiară sau non-financiară cu persoane sau organizaţii şi să declare conflictele de interese pentru datele şi informaţiile prezentate în manuscris. În conformitate cu ghidurile ICMJE, Autorul (autorii) trebuie să completeze o declaraţie privind Conflictele de interese, care va fi prezentată la sfârşitul articolului publicat.

Completând declaraţia referitoare la Conflictele de interes, se vor lua în consideraţie:

Pentru Conflicte de interese financiare

- specificaţi dacă vreo organizaţie are relaţie financiară cu lucrarea ştiinţifică reflectată în manuscris, inclusiv de finanţare, salariu, rambursări;
- menţionaţi, dacă articolul are un impact asupra organizaţiei date, ce ar genera pierderi sau profituri după publicare, în prezent sau în viitor;
- autorul (autorii) trebuie să precizeze dacă deţin cote de proprietate în orice organizaţie care ar putea să suporte pierderi sau să aibă profituri după publicare, în prezent sau în viitor. De asemenea, se recomandă să se specifice dacă autorul (autorii) deţin(e) sau aplică pentru orice drepturi de proprietate (brevet) în legătură cu conţinutul utilizat în manuscris;
- precizaţi dacă există oricare alte conflicte de interese.

Pentru Conflicte de interese non-financiare

- Vă rugăm să specificaţi oricare conflicte de interese non-financiare legate de politică, individuale, religioase, ideologice, educaţionale, raţionale, comerciale etc., care au legătură cu manuscrisul.

Contribuţia autorilor

Această secţiune a manuscrisului are rolul de a specifica contribuţia şi gradul de implicare a fiecărui autor. În acest sens, vă rugăm să respectaţi formatul exemplului propus: *„HW a conceput studiul, a participat la design-ul studiului şi a ajutat la redactarea manuscrisului. MG a efectuat procesarea exemplarelor, a metodelor de cultură ale ţesutului şi a elaborat manuscrisul. TK a efectuat testele de imunofluorescenţă. PN a participat la colorarea probelor şi la analiza citometrică prin flux. AR a participat la elaborarea design-ului studiului şi a efectuat analiza statistică. Manuscrisul final a fost citit şi aprobat de către toţi autorii".*

Fiecare Autor trebuie să aibă o contribuţie individuală în desfăşurarea cercetării, pregătirii manuscrisului şi publicării lucrării. Un Autor trebuie să contribuie semnificativ la conceptul şi *design*-ul lucrării, la efectuarea procedurilor experimentale, la colectarea datelor, la compilarea, analiza, interpretarea şi validarea rezultatelor.

Conform recomandărilor Comitetului Internaţional al Editorilor Revistelor Medicale, ICMJE, (www.icmje.org), drept autor poate fi considerată persoana care se încadrează în toate cele 4 criterii:

Abbreviations

Use only standard abbreviations. Other abbreviations may be defined and provided when are used for the first time in the manuscript. Abbreviations in the figures and tables will be explained in legend. Abbreviations should be used as rare as possible.

Declaration of conflict of interests

Following publication, persons or organizations involved in the study become public and thus their reputation may be influenced. Therefore, authors must disclose financial and non-financial relationship with people or organizations and to declare conflicts of interest related to the data presented in the manuscript. In accordance with the ICMJE guidelines, authors must fulfill a statement of conflicts of interest, which will be published at the end of the article.

Complementing the declaration of conflicts of interest the following will be taken into consideration

For financial conflicts of interest

- specify whether any organization has financial relationship with research presented in the manuscript, including funding, salary, reimbursements;
- mentioned, if the article has any impact on the eventually involved organization and could generate losses or profits after publication, now or in the future;
- authors must indicate if they have shares ownership in any organization that may incur losses or take profits after publication, now or in the future. Also, you should specify whether the author (s) own (s) or apply to any property rights (patent) on the content used in the manuscript;
- indicate if there are any other conflicts of interest.

For non-financial conflicts of interest

- Please specify any non-financial conflicts of interest: political individual, religious, ideological, educational, rational, commercial etc. related to manuscript.

Authors' contributions

This section of the manuscript is to specify the input and involvement of each author. In this regard, please follow the suggested format: *"HW conceived the study and participated in study design and helped drafting the manuscript. MG performed the processing of specimens and tissue culture methods and drafted the manuscript. TK performed immunofluorescence tests. PN participated in staining and flow-cytometry. AR participated in the study design and performed the statistical analysis. Final manuscript was read and approved by all authors".*

Each author must have an individual contribution to the research, manuscript preparation and work publication. An author should contribute substantially to one of the following: the concept and design of the work, performing of the experimental procedures, data collection, compilation, analysis, interpretation and validation of results.

According to the International Committee of Medical Journals Editors, ICMJE (www.icmje.org), as author may be a person who fit all four of following criteria:

o has made a substantial personal contribution in desi-gning,

- o a adus o contribuţie individuală substanţială conceperii, elaborării design-ului cercetării, sau a colectat, analizat sau interpretat datele;
- o a elaborat manuscrisul sau l-a revăzut în mod critic, aducând o contribuţie intelectuală importantă;
- o a aprobat versiunea finală a manuscrisului, gata pentru publicare;
- o este de acord să fie responsabilă pentru toate aspectele legate de cercetarea efectuată şi de manuscrisul depus pentru publicare şi să dea asigurare, că toate întrebările referitoare la acurateţea sau integritatea lucrării vor investigate şi rezolvate în mod corespunzător.

Notă: Persoanele, care au contribuit la realizarea lucrării, însă nu se încadrează în toate cele 4 criterii enunţate mai sus, nu pot fi considerate drept autori; contribuţia acestora va fi menţionată în secţiunea „mulţumiri şi finanţare" a manuscrisului. De asemenea, persoanele care au fost implicate doar în colectarea datelor, supraveghere, asistenţă tehnică şi finanţare, nu deţin drept de Autor, dar ei pot fi menţionaţi în secţiunea „mulţumiri şi finanţare". Simpla deţinere a funcţiei de şef de unitate, departament sau instituţie, în cadrul căreia s-a efectuat cercetarea, fără îndeplinirea tuturor celor 4 recomandări ale ICMJE, nu oferă dreptul de a fi (co)autor al lucrării.

Mulţumiri şi finanţare

Persoanele care au contribuit la elaborarea design-ul studiului, colectarea datelor, analiza şi interpretarea acestora, la pregătirea manuscrisului şi la redactarea lui critică, au oferit suport general sau tehnic, au contribuit cu materiale esenţiale pentru studiu, dar care nu îndeplinesc criteriile ICMJE de Autor, nu vor fi considerate drept Autori, dar contribuţia lor va fi menţionată în secţiunea „mulţumiri şi finanţare". Tot în această secţiune se vor menţiona sursele de finanţare ale lucrării. Menţionarea persoanelor fizice sau juridice, care au contribuit la realizarea lucrării şi manuscrisului, poate fi făcută doar după obţinerea unei permisiuni de la fiecare dintre ele.

Tabelele

Fiecare tabel va fi creat cu dublu-spaţiere şi amplasat pe o pagină separată, după textul manuscrisului. Enumerarea tabelelor va fi consecutivă, cu cifre arabe, în ordinea primei lor citări în text, scris cu caractere grase **(bold)**, alinierea – pe stânga, deasupra tabelului. Fiecare tabel va avea un titlu laconic, care va fi scris cu caractere normale (regular) sub numărul tabelului. Nu utilizaţi caractere bold în interiorul tabelului. Urmaţi exemplul prezentat:

Tabelul 1
Evenimente adverse intra-anestezice şi imediat post-extubare

	Lot experimental (n=100)	Lot control (n=100)	p
Disritmii	6,0%	3,0%	0,49
Instabilitate hemodinamică	7,0%	1,0%	0,034
Trezire prelungită*	11,0%	4,0%	0,19
GVPO† post-extubare	8,0%	27,0%	0,007
Durere intensă la trezire	17,0%	19,0%	1,0

Notă: * – trezire neobişnuit de lentă, după ce concentraţia cerebrală a reziduurilor de anestezice a trecut sub pragul de inducere a hipnozei; † – greaţă şi vomă postoperatorie. Analiza statistică utilizată: testul Fisher.

- o developed or reviewed critically the manuscript bringing a significant intellectual contribution;
- o approved the final version of the manuscript ready for publication;
- o agrees to be responsible for all aspects of the conducted research and submitted manuscript and to assure that all questions relating to accuracy or completeness of the work was adequately assessed and resolved.

Note: Persons who have contributed to the work, but not fit the four criteria mentioned above cannot be considered as authors. Their contribution will be mentioned in the "Acknowledgment and funding section" of the manuscript. Also, people who have only been involved in data collection, monitoring, technical assistance and funding, are not eligible as coauthors, but they may be mentioned in the "Acknowledgements and funding" section. Mere position of head of unit, department or institution, on which the research was conducted, without fulfilling all four ICMJE criteria, doesn't provide the right to be a coauthor of the work.

Acknowledgements and funding

People who contributed to the study design, data collection, analysis and interpretation, manuscript preparation and editing, offered general or technical support, contributed with essential materials to the study, but do not meet ICMJE authorship criteria will not be considered as authors, but their contribution will be mentioned in section "Acknowledgements and funding". Also in this section must be specified the sources of work funding. Mention of persons or institutions who have contributed to the work and manuscript can be made only after obtaining permission from each of them.

Tables

Content of each table should be double-spaced and placed on a separate page after the text of the manuscript. Tables numbering will be done using consecutive Arabic numerals in the order of their first citation in the text; it is should be written in **bold**, align to left and place above the table. Each table should have a concise title that will be written in bold (regular) under table number. Do not use bold within the table. Please follow the example:

Tabelul 1
Intra-anesthetic and immediately post-extubation adverse events

	Experimental Cohort (n=100)	Control Cohort (n=100)	p
Dysrhythmia	6,0%	3,0%	0,49
Hemodynamic instability	7,0%	1,0%	0,034
Prolonged awakening*	11,0%	4,0%	0,19
PONV† post-intubation	8,0%	27,0%	0,007
Strong pain on awakening	17,0%	19,0%	1,0

Note: * – Unusually slow awaking, after that cerebral concentration of the anesthetic reach the under hypnotic level; † – postoperative nausea and vomiting. Used statistical analysis: Fisher's exact test.

Legendele şi notele explicative vor fi făcute sub tabel. Toate abrevierile non-standard se vor explica în notele de subsol, folosind următoarele simboluri, în următoarea ordine: * , † , ‡ , § , | | , , ** , † † , ‡ ‡ , § § , | | | | , ¶ ¶ etc.

Menţionaţi, de asemenea, testele statistice aplicate şi tipul de date prezentate. Asiguraţi-vă că fiecare tabel este citat în text. Dacă utilizaţi date din altă sursă publicată sau nepublicată, trebuie să obţineţi permisiunea şi să declaraţi pe deplin sursa sub tabel.

Figurile

Figurile vor fi prezentate atât în manuscris, cât şi pe fişiere separate. În manuscris, figurile vor fi prezentate după textul lucrării, fiecare pe pagină separată şi vor fi numerotate consecutiv, cu cifre arabe, în ordinea citării lor în text. Numerotarea va fi scrisă abreviat (**Fig. 1**), cu caractere grase (**bold**), alinierea – pe stânga, sub figură. Fiecare figură va avea un titlu laconic, care va fi scris cu caractere normale (regular) în dreptul numerotării.

Figurile trebuie să fie calitative, vizibile în detaliu. Fotografiile cu persoane potenţial identificabile trebuie să fie însoţite de permisiunea scrisă de a utiliza fotografia. În caz contrar, faţa persoanelor trebuie acoperită cu o bandă neagră. În cazul în care o figură a fost publicată anterior, faceţi referinţă la sursa originală şi prezentaţi permisiunea scrisă de la deţinătorul drepturilor de autor pentru a reproduce figura. Permisiunea poate fi luată atât de la autorul figurii, cât şi de la editor, cu excepţia documentelor din domeniul public.

Pentru figuri, sunt acceptate următoarele formate de fişiere:

- o TIFF
- o JPEG
- o EPS (format preferat pentru diagrame)
- o PowerPoint (figurile trebuie să fie de mărimea unui singur diapozitiv)

Titlul fişierului va consta din numărul figurii şi un titlu scurt, identificabil.

Legendele figurilor

Legenda figurii va fi scrisă în continuare, imediat după titlul figurii. Descrierea figurii nu trebuie să repete descrierea din textul manuscrisului. Când sunt folosite simboluri, săgeţi, numere sau litere pentru a identifica, descrie părţi ale ilustraţiilor, identificaţi-le şi explicaţi-le pe fiecare în mod clar în legendă. Explicaţi scala internă şi identificaţi metoda de colorare în microfotografii.

Vă rugăm să reţineţi că este de responsabilitatea autorului (autorilor) de a obţine permisiunea de la deţinătorul drepturilor de autor pentru a reproduce figuri sau tabele care au fost publicate anterior în altă parte. Imaginile color vor fi tipărite din contul autorilor.

Referinţele bibliografice

Toate referinţele bibliografice trebuie să fie numerotate consecutiv, între paranteze pătrate [ ], în ordinea în care sunt citate în text. Citatele de referinţă nu trebuie să apară în titluri sau subtitluri. Fiecare referinţă trebuie să aibă un număr individual. Citările multiple din cadrul unui singur set de paranteze trebuie să fie separate prin virgulă şi spaţiu. În cazul în care există trei sau mai multe citări secvenţiale, acestea ar trebui să fie indicate sub formă de serie. Exemplu: [1, 5-7, 28].

Vă rugăm să evitaţi folosirea excesivă a referinţelor. În cazul în care se folosesc sisteme automate de numerotare, numerele de

Legends and notes will be place under the table. All non-standard abbreviations should be explained in footnotes, using the following symbols, in the following order: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡, § §, | | | |, ¶ ¶ etc.

Applied statistical tests and the type of presented data should be also mentioned. Make sure that each table is cited in the text. If you use data from another published or unpublished source, you must obtain permission and cited the source below the table.

Figures

Figures will be included in the main manuscript, and also submitted as separate files. The manuscript figures should be presented, each one on a separate page and should be numbered consecutively with Arabic numerals in the order of their citation in the text. Figure numbering will be written abbreviated (Fig. 1), using bold fonts, left alignment, and placed under the figure. Each figure should have a laconic title that will be written using regular font and place in the right of the figure's number. Figures' quality should assure the visibility of details. Pictures of persons potentially identified must be accompanied by written permission to use it. If a figure has been previously published, please cite the original source and submit the written permission to reproduce the figure from the copyright owner. Permission can be taken from both the author and the publisher, except the documents of public domain.

For figures, the following file formats are accepted:

- o TIFF
- o JPEG
- o EPS (preferred format for diagrams)
- o PowerPoint (figures should be of the size of a single slide)

The file title should include the figure number and an identifiable short title.

Figures' legends

Figure's legend should be written immediately after the figure's title. Figure's description should not repeat the description in the text of the manuscript. When used symbols, arrows, numbers or letters to describe parts of the figure, explain clearly each one of them in the legend. Explain the internal scale and identify the staining method of the photomicrographs.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures or tables that have been published previously elsewhere. Color images will be printed at the expense of the manuscript authors.

References

All references must be numbered consecutively, in square brackets [ ], in the order they are cited in the text. Reference citations should not appear in titles or subtitles. Each reference should have an individual number. Multiple citations within a single set of brackets must be separated by commas and spaces. If there is a sequence of three or more citations, they have to be given as a range (e.g. [1, 5-7, 28]).

Please avoid excessive use of references. If an automatic system of citation is used, reference numbers must be finalized and the bibliography must be fully formatted before submission. Reference list should include all authors. Journals' abbreviation must be in accordance with Index Medicus/MEDLINE. It may be cited only

referinţă trebuie să fie finalizate, iar bibliografia trebuie formatată complet înainte de depunere. Lista de referinţă trebuie să conţină toţi autorii. Abrevierea revistelor trebuie să fie în conformitate cu Index Medicus / MEDLINE. Pot fi citate doar articolele sau rezumatele care au fost publicate şi care sunt disponibile, accesibile prin intermediul serverelor publice. Orice rezumate sau articole nepublicate sau cu caracter personal nu trebuie să fie incluse în lista de referinţă, dar pot fi incluse în text şi citate în mod corespunzător, indicând cercetătorii implicaţi. Obţinerea permisiunii printr-o scrisoare de la autori pentru a le cita comunicările sau datele nepublicate sunt în responsabilitatea autorului corespondent al articolului.

Formatul referinţelor

Autorii sunt rugaţi să furnizeze cel puţin un link pentru fiecare referinţă bibliografică (preferabil PubMed).

o *Referinţă la revistă*

Numele şi iniţialele autorului sau al autorilor, separate prin virgulă (regular). Titlul articolului (regular). Forma abreviată a denumirii revistei (italice), urmat de anul, numărul volumului: numărul paginilor (regular). Articolele în curs de publicare citate vor fi menţionate cu „***In press***" (italic, bold), după numărul paginilor. Se vor menţiona toţi autorii articolului.

Ex: „1. Belîi A., Cobâleţchi S., Casian V., Belîi N., Severin G., Chesov I., Bubulici E. Les aspects pharmaco-economiques dans la gestion de la douleur perioperatoire. Mise au point. *Ann Fr Anesth Réanim*, 2012; 31: 60-66."

o *Referinţă la carte*

Numele şi iniţialele autorului sau al autorilor, separate prin virgulă (regular). Titlul capitolului (regular) (numărul paginii sau paginilor citate). În: Titlul cărţii. Detalii privind Editorul. Editura, locul, anul editării.

Ex: „1. Belîi A. Gestiunea riscului şi siguranţa pacientului în anestezie şi terapie intensivă (p. 115-134). În: Recomandări şi protocoale în anestezie, terapie intensivă şi medicină de urgenţă. Editori: Săndesc D., Bedreag O., Păpurică M. Ed. Mirton, Timişoara, România, 2010."

o *Referinţa la Web*

Numele şi iniţialele autorului sau al autorilor, separate prin virgulă, sau denumirea deţinătorului de drept de autor (regular). Titlul. Numele site-ului. Disponibil la adresa: [URL]. Accesat pe: data.

Exemplu: „Agency For Healthcare Research and Quality (AHRQ). Production pressures. WebM&M. Disponibil la adresa: [http://webmm.ahrq.gov/case.aspx? caseID=150]. Accesat pe: 18.06.2010."

Pentru precizări şi informaţii suplimentare:
Serghei Popa, dr. şt. med., conf. univ.,
Redactor-şef
tel: +373 60907799
e-mail: editor.mjhs@usmf.md

articles or abstracts that have been published and are available through public servers. Any abstracts or unpublished data or personal items should not be included in the reference list, but may be included in the text and cited accordingly, indicating the involved researchers. It is of manuscript authors' responsibility to obtain the permission to refer to unpublished data.

References format

Authors are asked to provide at least one link for each citation (preferably PubMed).

o *Journal article reference*

Surname and initials of the author(s), separated by commas (regular). Title of article (regular). Abbreviated name of the journal (in italics), followed by the year, volume number: pages number (regular). Articles in press should be specified as "***In press***" (italic, bold), after the pages number. All the authors should be listed.

e.g.: "1. Belîi A., Cobâletchi S., Casian V., Belîi N., Severin G., Chesov I., Bubulici E. Les aspects pharmaco-economiques dans la gestion de la douleur perioperatoire. Mise au point. *Ann Fr Anesth Réanim*, 2012; 31: 60-66. "

o *Book reference*

Surname and initials of the author (s), separated by commas (regular). Title of chapter (regular) (cited page(s) number). In: Title of book. Details of the editor, publisher, place, year of publication.

e.g. "Belii A. Risk management and patient safety version anesthesia and intensive care unit (p. 115-134). In: Recommendations and Protocols in Anesthesia, Intensive care and Emergency medicine. Editors: Sandesc D., Bedreag O., Papurica M. Ed. Mirton, Timisoara, Romania, 2010".

o *Web reference*

Name and initials of the author(s), separated by commas, or Copyright holder (regular). Title. Site Name. Available at: [URL]. Accessed: date.

E.g.: "Agency for Healthcare Research and Quality (AHRQ). Production Pressures. WebM & M. Available at: [http://webmm.ahrq.gov/case.aspx? caseID = 150]. Accessed on: 18.06.2010".

For more details, please contact:
Serghei Popa, PhD, associate professor,
Editor-in-chief
tel: +373 60907799
e-mail: editor.mjhs@usmf.md