The Moldovan Medical Journal

The Publication of the Scientific Medical Association of Moldova

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Epidemiological features of septic nosocomial infections within various intensive care units

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Abstract

Background: Septic nosocomial infections are a major public health issue. Both the risk of contracting and the specificity of nosocomial pathology directly depend upon the type of inpatient settings, as well as on the institution-specific risk factors.
Material and methods: The study used a descriptive observation method based on a cross-sectional study. The present research documented and analysed retrospectively 687 follow-up records of patients admitted to different intensive care units.
Results: The study results found that the incidence of septic nosocomial infection within various intensive care units (ICU) differs, ranging between 24.68% up to 34.8%. The structure of nosological forms was dominated by severe infections as pneumonia – 50.7%, septicemia – 12.68%, surgical site infections – 12.60%, urinary tract infections – 8.45%. The polyetiological structure of pathogens varied depending on the types of ICU. Microorganisms of the genus *Staphylococcus, Acinetobacter, Clebsiella, Pseudomonas* and *Enterobacter* predominated in most gram-negative (87.25%) cases, being multi-drug resistant to antibiotics. The following risk factors for the development of nosocomial septic infections were identified: the widespread use of invasive devices in the treatment process, patient's comorbidities, polytraumas, vasopressors administration, the length of hospital stay within the ICU, etc. The clinical and economic effect is also important; hence the hospital stay length of patients with nosocomial infections was 2.2-2.5 times, the hospital stay cost per patient was 4.56 times, and the mortality rate was 4.55-8.43 higher compared to patients with no purulent nosocomial infections.
Conclusions: Septic nosocomial infections are an urgent issue for ICU admission, which requires the implementation of comprehensive programs to prevent morbidity and reduce microbial antibiotic resistance.
Key words: nosocomial septic infections, intensive care units, epidemiology, etiology, risk factors.

Introduction

Nosocomial infections (NI), also called healthcare-associated infections (HAIs), are a serious medical and socio-economic problem due to an increase in morbidity, the development of severe clinical forms, high mortality, and significant healthcare and economic impact [1-6].

Currently, the phenomenon of nosocomial infections is determined by several factors as the widespread use of complicated treatment methods, including the invasive ones, the multitude of infectious agents, the emergence of subpopulations of antibiotic-resistant bacteria, and the lack of vaccination [1, 7-13].

At the same time, the risk of contracting, as well as the specificity of nosocomial pathology, are directly dependent upon the type of the healthcare inpatient facility and on the specific risk factors of the institution [3, 6-8, 12].

In the hospitals of the Republic of Moldova, the real incidence of septic nosocomial infections in intensive care units has not been studied yet.

The specificity of intensive care units (ICU) is the concentration of both patients with serious conditions and healthcare providers within a limited space, as well as the massive use of invasive diagnostic and therapeutic methods, and high levels of immunodeficiency in patients. For these reasons, patients admitted to intensive care units are at higher risk of contracting and developing nosocomial infections. According to some studies conducted within intensive care units, about 45-60% of patients have some forms of nosocomial infection, which exceeds the incidence among patients within other inpatient facilities, the mortality being of 34-48% among patients with septic nosocomial infection, thus, resulting in a significant economic burden. The development of

nosocomial pneumonia, for example, leads to an increase in the duration of treatment within the intensive care unit by 10.3 days [2, 6, 11, 12, 14].

In hospitals of the Republic of Moldova, the real incidence of nosocomial septic infections in intensive care units has not been studied yet.

Material and methods

A descriptive observation method based on a cross-sectional study was used when describing the incidence of septic-purulent nosocomial infections, epidemiological and etiological features, risk factors and socio-economic consequences. In this regard, 688 follow-up records of patients admitted to various ICUs were documented and retrospectively analysed, namely, polytraumas (A), septic infections (B), heart defects (C). The microbial strains, that are the causative agents of nosocomial septic infections, were isolated, and the antibiotic sensitivity / resistance testing was carried out in medical laboratories according to the classical methods described by Galetchi P. and others (1997), Buiuk D., Negut M. (2009), as well as via the automated system VITEC-2 Compact [15, 16].

A retrospective descriptive analysis was carried out according to the methodology described in "General epidemiology. Fundamentals of evidence-based medicine" [17].

Results

The epidemiological analysis of the quantitative data obtained from the cross-sectional retrospective study of three types of intensive care units (tab. 1) revealed that the

development rate of nosocomial septic infections differs according to the profile of the inpatient facility, accounting for 24.6%, or 246.0‰ for "Polytrauma" ICU; 28.12%, or 281.2 ‰ – in "Sepsis" ICU; 34.1%, or 348.1 ‰ for "Heart Defects" ICU, the mean value being of 28.94% or 289.4 ‰.

Table 1. The incidence of septic infections within different types of intensive care units

ICU	No patients	Including septic infections			Mas	
		Abs	%	‰	%	‰
A	162	40	24.60	246.0		
B	288	81	28.12	281.0	28.94	289.4
C	158	55	34.81	348.1		

The structure of nosological forms was dominated by severe septic pathologies, such as pneumonia and bronchopneumonia – 44.74%, septicemia – 11.66%, urinary tract infections (UTIs) – 16.47%, and surgical site infections – 27.2% of cases (fig. 1).

At the same time, depending on the type of the inpatient care facility, a clear predominance of pneumonia and bronchopneumonia was revealed in the "Heart Defects" ICU – 57.74%, septicemia in the "Sepsis" ICU – 18.75%, urinary tract infections and wound infections – in the "Polytrauma" ICU – 23.1% and 41.7%, respectively (fig. 1 A, B, C, Mas).
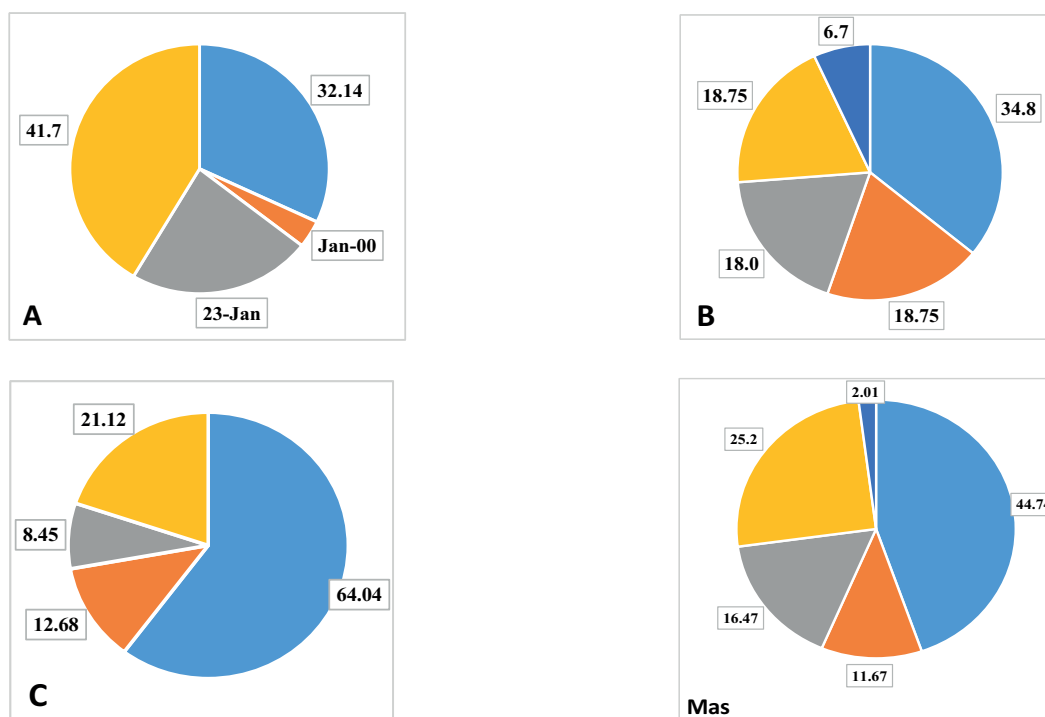
Fig. 1A, B, C, Mas. Distribution of nosological forms

■ Pneumonia and bronchopneumonia ■ Septicemia ■ UTI ■ Wound infection ■ Others

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Following the analysis findings of the pathogen spectrum of septic infections in the examined ICU patients, it was found that their structure is very diverse. For example, 18 types of microorganisms were the causative agents for septic infections within the "Polytrauma" ICU. The structure of pathogens was dominated by: *Kl. Pneumoniae* – 19.9%, *Acinetobacter spp* – 16.66%, *P. aeruginosa* – 14.5%, *Staphylococcus* strains – 17.7%, *E. faecalis* – 10.4% and *P. mirabilis* – 4.2%. The study of the antibiograms showed that the strains isolated from patients with septic infections were highly resistant to antibiotics in 68.19% of samples, had an intermediate resistance in 5.12% of samples and only 26.5% of samples were susceptible to antibiotics. High resistance to aminoglycosides – 71.16%, penicillins – 81.39%, cephalosporins – 88.18%, quinolones – 75.38% and carbapenems – 56.37% was revealed. As to the types of antibiotics, the strains were highly resistant to gentamicin – 77.5%, ampicillin – 77.16%, ceftazidime – 87.5%, ceftriaxone – 87.5%, cefepime – 88.23%, ciprofloxacin – 83.95 %, levofloxacin – 75.38%, and impinem – 70.9%, which are widely used in medical practice. In addition, it has been revealed that the microbial isolates exhibited multi-drug resistance to antibiotics. High multi-drug resistance was found in the following prevailing pathogens: *Kl. pneumoniae* – 78.95%, *Acinetobacter spp* – 81.25%, *P. aeruginosa* – 92.86% and *S. epidermidis* – 81.81%. It is also noteworthy that strains of *Staphylococcus* were methicillin-resistant in 52.0% of cases.

Microorganisms of the genus *Staphylococcus* (*aureus*, *epidermidis* and *haemolyticus*) dominated in the "Heart defects" ICU, accounting for 23.4% of the total number of strains isolated from patients with septic infections, followed by *Kl. pneumoniae* – 18.2%, *E. faecalis* – 18.2%, *P. aeruginosa* – 9.1%, *A. baumani* – 6.5% and *E. cloacae* – 5.2%. At the same time, it was found that *Candida* fungi (*albicans*, *krusei*, and *glabrata*), being determined in 7.8% of patients, played an important role as pathogens of nosocomial septic infections within the "Heart Defects"

ICU. The remaining 11.6% of microbial strains identified in the sources of septic-purulent infections belong to other types of microorganisms. Of the total number of isolated strains, 69.35% were multi-drug resistant to antibiotics: *Kl. pneumonia* – 78.0%, *Staphylococcus spp* – 56.6%, *Acinetobacter spp* – 87.25%, *P. aeruginosa* – 84.4%, *E. faecalis* – 53.0%, and *E. coli* – 88.9%. The microbial strains showed high resistance to aminoglycosides – 66.77%, penicillins – 81.25%, cephalosporins – 82.87%, quinolones – 70.59%, carbapenems – 48.55%, and depending on the types of antibiotics: to gentamicin – 77.50%, cefoperazone – 94.74%, tobramycin – 79.49%, ticarcillin – 91.67%, piperacillin – 89.19%, cefoperazone – 94.74%, ceftazidime – 87.88%, cefazolin – 85.71%, ceftriaxone – 88.24%, cefuroxime – 78.95%, cefepime – 66.67%, moxifloxacin – 84.0%, levofloxacin – 85.29%, meropenem – 53.66%, and vancomycin – 25.0%.

There were 24 types of microorganisms from septic ICU patients, among which *Kl. pneumoniae* – 28.3%, *P. mirabilis* – 15.09%, *P. aeruginosa* – 11.32%, *E. coli* – 9.04%, *E. faecalis* – 8.49%, *Staphylococcus spp* – 7.54%, and *A. baumanii* – 6.06%.

It is noteworthy that gram-negative microorganisms (*Kl. pneumoniae, A. baumanii, P. aeruginosa, E. coli, P. mirabilis*), which account for 87.25% of the total number of isolated strains, prevailed as causative agents of septic infections in all the intensive care units under study (fig. 2).
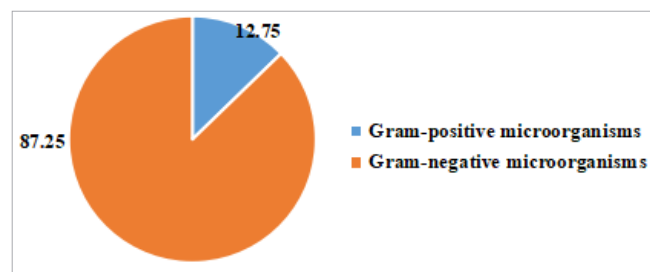
Fig. 2. The prevalence of gram-positive and gram-negative microorganisms in the etiological structure of septic infections within intensive care units
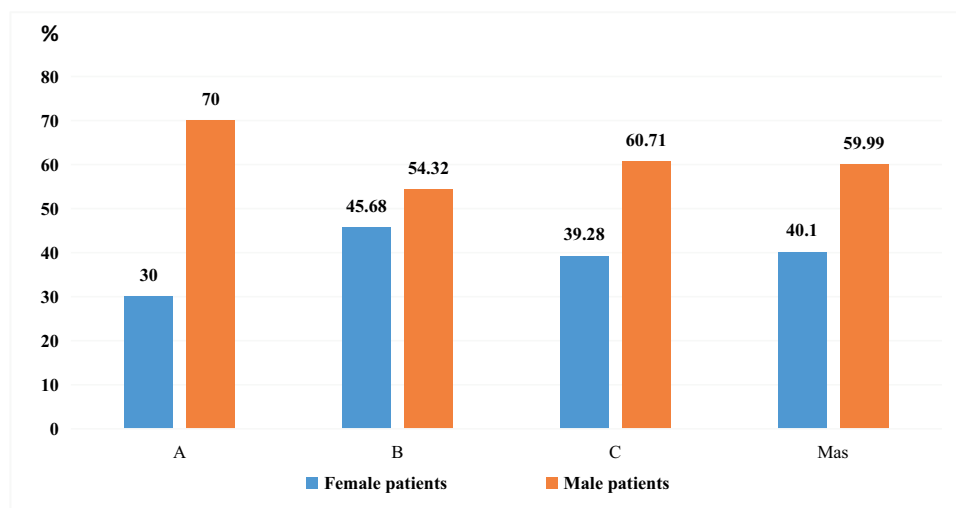
Fig. 3. The occurrence of septic infections depending on patient's gender

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The risk of developing nosocomial septic infections was higher in men, whose overall prevalence was 59.9% from the total number of patients compared to 40.1% in women, being common for all ICU types included in the study (fig. 3).

Obviously, people aged ≥60 years (48.4%) predominated among patients with septic infections, except for the "Heart Defects" ICU, where young people, up to one year old, predominated with a frequency of 33.33% of the total number diseases, as well as patients of working age viz. 20-39 years old admitted within the "Polytrauma" ICU, showing an occurrence of 37.5% of the total number of diseases (tab. 2).

The main risk factors for developing septic infections in patients admitted to "Polytrauma" ICU included the massive polytraumas found in 50.40% of patients, characterized by the presence of multiple wounds, including the open ones in primary sources – 78.50%, hence the prevalence of wound infections; emergency admission, being registered in 76.90%; horizontal patient position – 95.23%; artificial lung ventilation – 76.20%; vascular catheterization used in 96.60% of patients, including 68.29% – more than 48 hours; urinary catheterization – 90.47%, including 80.43% of patients – more than 48 hours; vasopressor administration – 80.95% (fig. 4A).

In the septic ICU, the use of a vascular catheter was found in 83.95% of patients with septic infections, including 63.33% of patients with ≥48 hours; urethral catheter – in 77.77% of patients, including 57.14% ≥48 hours, and 67.9% of patients were mechanically ventilated (fig. 4B).

In the "Heart Defects" ICU, the use of invasive devices in the treatment process (endotracheal tube for assisted lung ventilation – 38.5%, vascular catheter – 38.5%, urinary catheter – 40.2%, drainage of the chest and abdominal cavity – 39.1 and 66.7%, respectively, the use of nasogastric tube – 64.6%) was also highlighted as risk factors of developing septic infections (fig. 4C).

Table 2. Occurrence of septic infections according to the patients' age

ICU	Indices	Patients' age, years					Total
		≤1	1-19	20-39	40-59	≥60	
A	abs	-	-	15	7	18	40
	%	-	-	37.50	17.50	45.00	100.0
B	abs	-	1	6	18	56	81
	%	-	1.23	7.40	22.22	69.13	100,0
C	abs	25	11	5	13	21	75
	%	12.76	6.12	13.26	19.38	48.47	100.0
Total	abs	25	12	26	38	95	196
	%	12.76	6.12	13.26	19.38	48.47	100.0
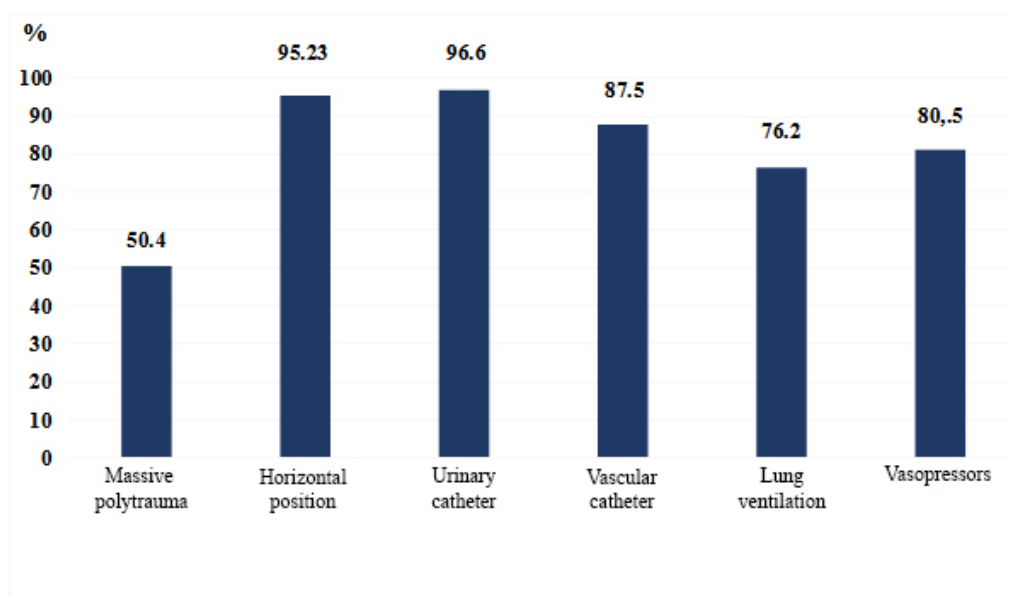
Fig. 4A. Frequency of invasive device use in critical care, type A
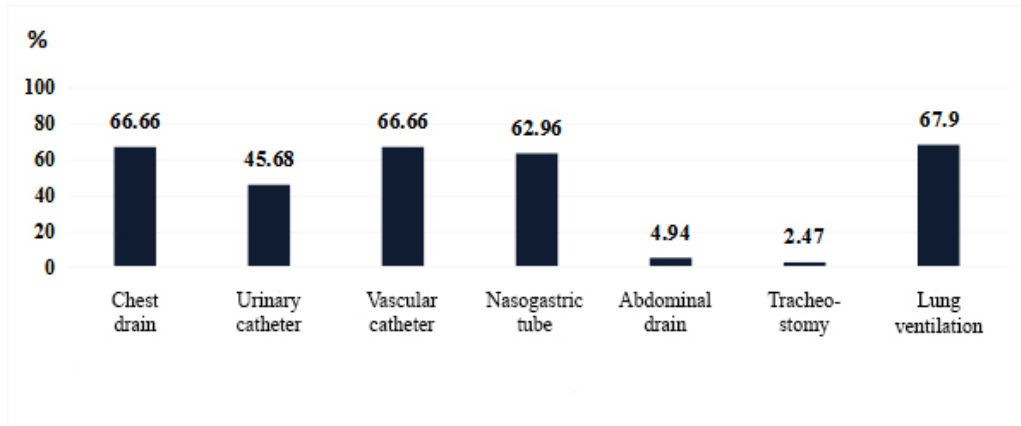
Fig. 4B. Frequency of invasive device use in critical care, type B
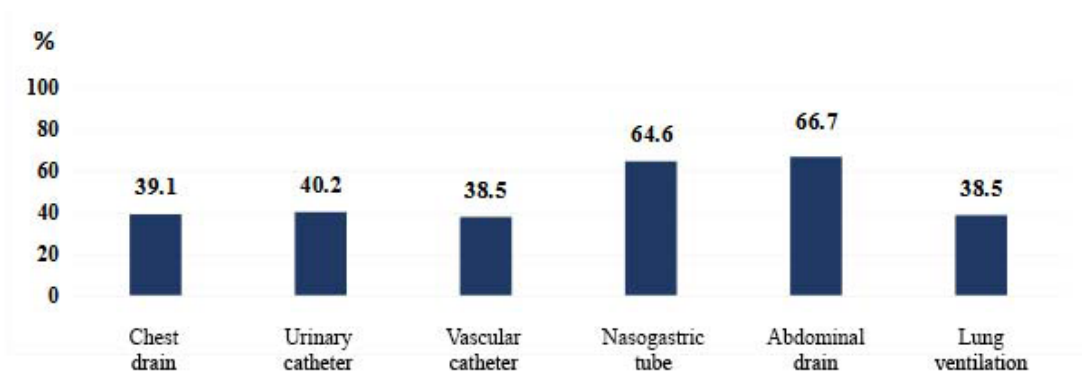
Fig. 4C. Frequency of invasive device use in critical care, type C

It has been established, for example, that endotracheal intubation of a patient showed an average increase of 23.68% for the risk of developing septic infections. At the same time, the risk got exponentially higher, the longer the duration of assisted lung ventilation was. If the duration of mechanical ventilation was up to 24 hours, the risk of developing pneumonia was 5.56%, if it ranged between 24 and 96 hours – 23.08%, and if mechanical ventilation was over 96 hours, the risk of developing in-hospital pneumonia increased up to 56.10% (fig. 5).
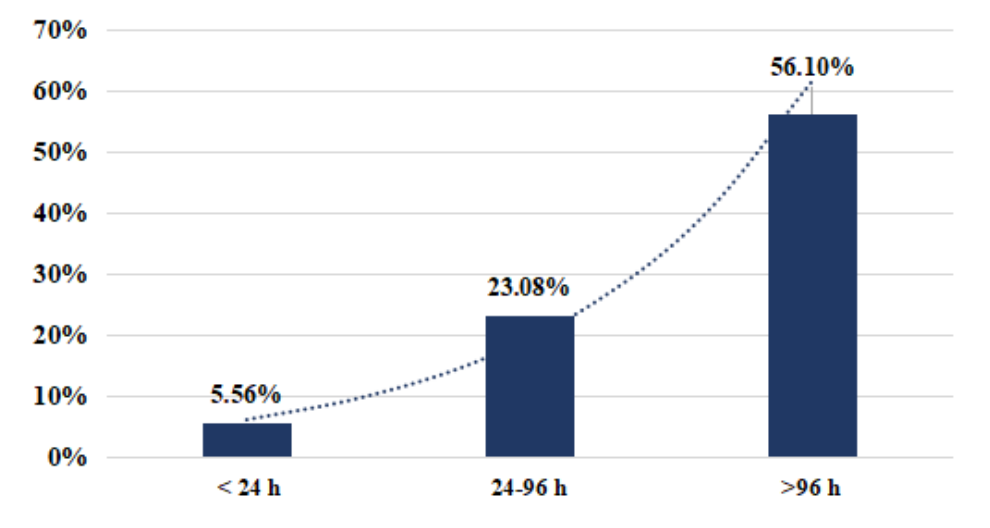
Fig. 5. Risk of developing nosocomial pneumonia depending on the duration of assisted ventilation

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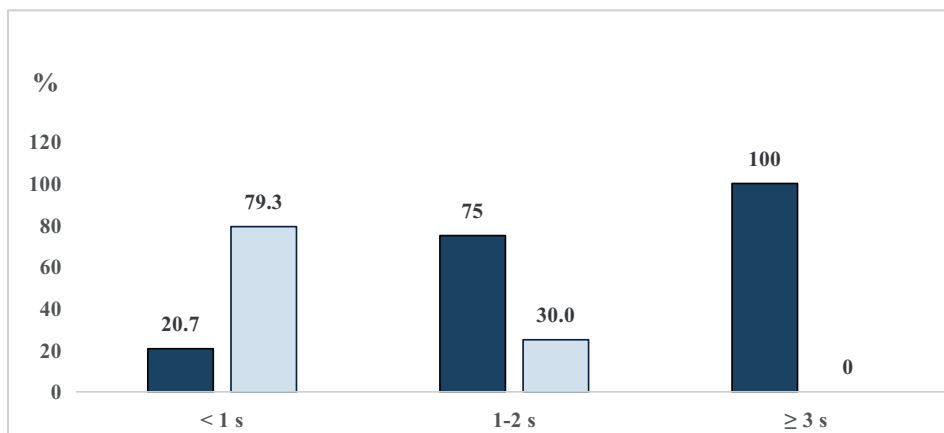
Fig. 6A. The incidence of septic infections depending on the patients' length of stay in the ICU, type A
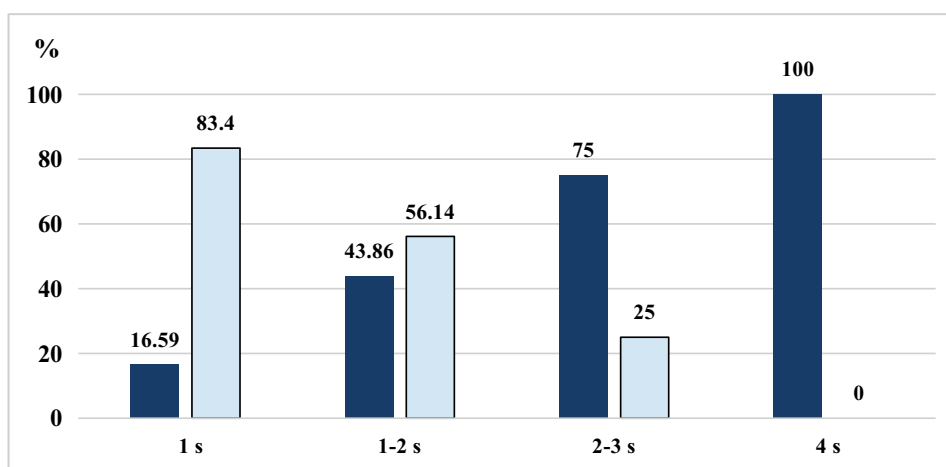
Fig. 6B. The incidence of septic infections depending on the patients' length of stay in the ICU, type B
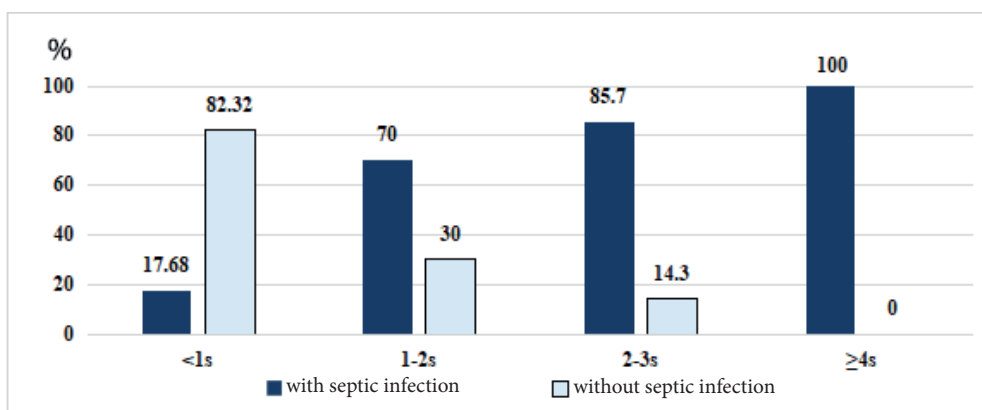
Fig. 6C. The incidence of septic infections depending on the patients' length of stay in the ICU, type C

A major risk factor of developing septic infections was the presence of comorbidities: chronic respiratory diseases – 32.35%, chronic cardiovascular diseases – 49.62%, hypertension – 45.04%, chronic kidney and liver diseases – 46.42% and 33.47%, respectively, diabetes mellitus – 23.49%, etc.

At the same time, the frequency of contracting and developing septic infections in the ICU is directly dependent on the patient's treatment duration within the respective inpatient facilities (fig. 6). In case of a patient's stay for up to one week within the ICU, following rehabilitation and intensive treatment, the rate of contracting and developing septic infections was ~ 18.12%, up to 2 weeks ~ 62.95%, up to 3 weeks ~ 86.9%, and up to 4 weeks – 100.0 %. This phenomenon was characteristic of all ICU types under study (fig. 6 A, B, C).

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Both clinical and economic impact, caused by septic infections in intensive care units is significant. It was found that the ICU length of stay in patients with nosocomial septic-purulent superinfections was 2.2 times, the patient's healthcare cost – 4.56 times, and the mortality rate was about 8.43 times higher compared to patients without nosocomial septic infections.

Discussion

The results of the present study on the real incidence of septic nosocomial infection, based on the model of three intensive care units confirm the specialized literature data on the relevance of the actual problem. High morbidity, severe forms of infection (pneumonia, septicemia, urinary tract infections, and surgical site infections), increased medical and socio-economic impact and require the implementation of comprehensive programs to reduce the risk of contracting and developing septic infections within the ICU.

A number of studies have shown that the microbial species that are commonly found as the causative agents of nosocomial septic-purulent infections show high multi-drug resistance to antibiotics used in hospitals, including the ICUs, which greatly complicates the therapeutic management of patients. The present study results confirm that one of the major problems facing medicine today is the high multi-drug resistance of hospital strains to antibiotics widely used in medical practice, hence a more tailored treatment, based on microbiological diagnosis and antibiogram, should be applied to patients with septic infections, especially in those admitted to intensive care units.

Conclusions

1. The incidence of nosocomial septic infections was found to be high within the ICUs under study, ranging from 248.6‰ to 348.0‰.
2. Severe forms of septic infections were predominant within various types of ICUs (pneumonia and bronchopneumonia – 44.74%, septicemia – 11.66%, urinary tract infections – 6.47%, and wound infection – 27.2%).
3. The etiological structure is clearly dominated by Gram-negative microorganisms (87.25%), including *Kl. Pneumoniae, A. baumanii, P. aeruginosa, E. coli, P. mirabilis*, which are multi-resistant to antibiotics.
4. The following predominant risk should be considered in contracting and developing septic infections: patient's advanced age and comorbidities, as well as the invasive procedures used (vascular and urinary catheterization, assisted pulmonary ventilation, thoracic and abdominal drainage, massive polytraumas, duration of intensive treatment, and vasopressor administration).
5. The clinical and economic impact in septic infections is significant due to an increase in the hospital length of stay by 2.2 times, the healthcare cost per patient by 4.56 times, and the mortality rate by 8.43 times.
6. The current situation requires the implementation of comprehensive programs to prevent morbidity and reduce antimicrobial resistance.

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VP conceptualized the idea and drafted the first version of the manuscript, IB, MC and CR collected the data. All the authors contributed to the study design, reviewed and approved the final manuscript.

Ethical approval
The research has gained a positive opinion issued by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 47 dated 12.04.2018).

Conflict of interests
The authors have declared no conflicts of interests.

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Bactericide polymers obtained from nitrofuran and chitosan derivatives

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Abstract

Background: In this study, the synthesis and characterization of chitosan polymeric materials grafted with nitrofuran derivatives – furaceline and isofural in terms of antibacterial properties and prolongation of their action were performed.
Material and methods: Synthesis of chitosan analog polymers with maleic anhydride was performed, followed by grafting of nitrofuran derivatives using ethyl chloroformate. The chemical composition of the obtained polymers was confirmed by FT-IR spectroscopy. Antibacterial study has been performed on a wide range of Gram-positive and Gram-negative microorganisms.
Results: Chitosan derivatives with a content of 30 mol% of maleic anhydride were obtained. To the analogous polymer "chitosan maleinized" the medicinal products isofural or furaciline with the help of the ethyl chloroformate were functionalized. By comparative analysis of the IR spectra of the final products with the IR spectra of maleinized chitosan and furacilin or isofuran was demonstrated the individual structure of the polymeric preparations "maleinized chitosan grafted with furacilin" / "maleinized chitosan grafted with isofural". The antibacterial substances, isofural and furaciline, among nitrofurans, being grafted with chitosan maleinized, keep their bactericidal activity in the limits of 75-300 µg/mL. The polymeric materials from chitosan maleate grafted with isofural or furacillin in a ratio of 70:30 have a prolonged antibacterial action (observation period 72 hours).
Conclusions: It has been found that isofural and furacilin, among nitrofurans, being grafted on chitosan polymeric material, retain their bactericidal activity and possess prolonged antibacterial action.
Key words: chitosan, furaciline, isofural, grafted copolymers, Gram-positive and Gram-negative microorganisms.

Introduction

Starting from the years 75-80 of the last century, the chemical-pharmaceutical direction for the elaboration of antimicrobial polymeric materials began to develop rapidly [1-3]. Pharmaceutical chemists were the first to believe that the future in in

creasing the effectiveness of antibiotics and other antibacterial preparations will return to their conjugation with polymeric materials and thus have a higher antimicrobial potential, with tens of times longer activity and reduced toxicity [3].

A special role in the synthesis of antimicrobial preparations on polymeric support have the researches that demonstrated the possibility of grafting of a series of antimicrobial drugs with dextran, polyvinyl alcohol, polyethylene glycol (PEG) and PEG derivatives [4]. Antibacterial preparations conjugated with polymeric materials possess relatively better antimicrobial activity.

It is known from the literature that in the years 1980-1990 were chemically coupled by covalent bonds the preparations with action on the nervous system, such as adrenaline, isoproternol and others with the synthetic copolymer of N-(3-oxypropyl) glutamine and N-aminophenylalanine.

The polymeric derivatives obtained showed activity in the initial catecholamines [5, 6].

In the literature are also described some polymers, such as the copolymer of N-vinylpyrrolidone with crotonic aldehyde, used for the grafting of antibacterial preparations [7, 8]. The authors showed that these compounds have a prolonged activity within 10-12 days, unlike the usual preparations that have activity for 1-2 days [8]. The use of polymeric preparations led to a reduction of the dose by 2-3 times, compared to the initial preparations.

In some publications there are described processes for grafting antibiotics from the penicillin group to synthetic copolymers, such as N-vinylpyrrolidone with crotonic or acrylic acid and from the styrene-butadiene block copolymer. The last preparation is also recommended for obtaining layers and films with antibacterial effect [9].

From the nitrofuran derivatives, a special role is played by N'-[(5-nitrofuran-2-yl) methylidene]pyridine-4-carbohydrazide, also called isofural [10]. This homologous antibacterial preparation, close to the chemical structure with furacillin but with more advanced bactericidal properties, was obtained by the classical condensation reaction of pyr-

idine-4-carbohydrazide with 5-nitrofuran-2-carbaldehyde [11]. The authors showed that the activity of this preparation is superior to that of other preparations in the nitrofuran group.

The aim of this paper is to investigate the possibility of grafting furacillin and isofural to the natural chitosan polymer, to confirm the chemical structure of the polymer-analogs obtained with the help of IR spectroscopy and to evaluate the antibacterial properties.

Material and methods

In this paper, the synthesis and research of polymeric materials with bactericidal properties from chitosan grafted with nitrofuran derivatives were performed.

Synthesis of polymers was carried out in two stages: in stage 1 the synthesis of the polymer-chitosan analog functionalized with maleic anhydride (MA) represented in fig. 1 was performed. The MA content was from 30 to 50 mol%.
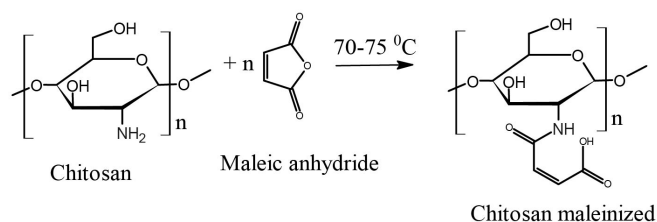
Fig. 1. Scheme of functionalization response
of maleic anhydride to chitosan

Grafting was carried out in a solution of N,N-dimethylformamide (DMF) with a chitosan concentration of 1.0% at 70-75 °C for 3 hours. The end of the reaction was confirmed by the thin layer chromatography. The reaction yield is about 80%.

In the second stage, the functionalization of isofural and furacilin in maleinized chitosan (step I) was performed.

Synthesis was performed according to the scheme shown in fig. 2 (step II).
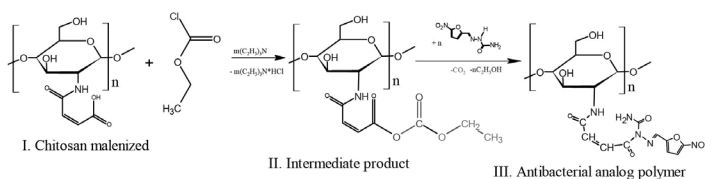
Fig. 2. Reaction schedule for functionalization
of furacilin/isofural in chitosan maleinized

Similarly, the isofural to maleinized chitosan was functionalized as follows: to the solution containing 1.0 g of maleinized chitosan dissolved in 50 mL DMF, at the temperature of 3-5 °C, with dropwise stirring were added 0.8 mL (7.8 mmol) of triethylamine and after 10 minutes 0.7 mL of ethyl chloroformate. After stirring for 30 minutes at 3-5 °C to the intermediate product (II) the solution of isofural in DMF (0.6 g dissolved in 10 mL DMF) was added. The resulting reaction product was stirred for another 3-4 hours at room temperature, then after concentrating the solution by vacuum distillation of the polymer the final polymer analog (III) was separated by sedimentation in hexane, then in diethyl ether.

The chemical structure of chitosan maleinized and other analogous polymers was investigated using IR spectroscopy. The BRUKER ALPHA platinum ATR spectrometer was used for the research (fig. 3).

The method for evaluating antibacterial properties

Evaluation of antibacterial properties of the synthetic origin substances (maleinized chitosan with isofural (70:30 mol%) and chitosan maleinized with furacilin (70:30 mol%) was realized applying the method of serial dilutions in liquid. As a nutrient medium 2% meat – peptone broth was used. As reference cultures were used Gram-positive and Gram-negative microorganisms (*Staphylococcus aureus* (t.
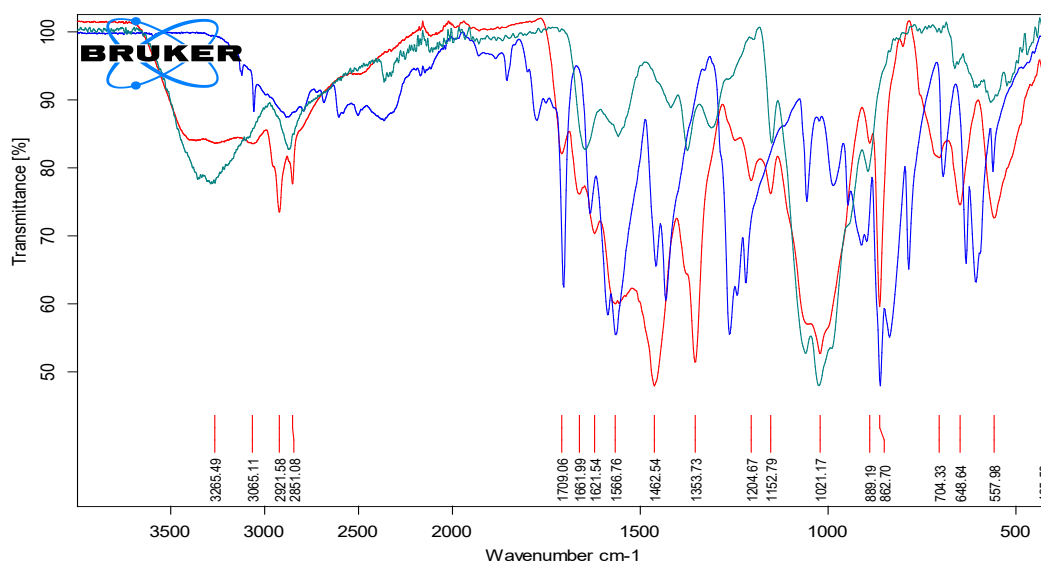
Fig. 3. FT – IR spectrum of chitosan maleinized compared to chitosan and maleic anhydride

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209), methicillin-resistant *Staphylococcus aureus* (MRSA) (t. NCTC 12493), *Enterococcus faecalis* (t. ATCC 19433), *Escherichia coli* (t. ATCC 25922), *Klebsiella pneumoniae* (t. 3534/51), *Proteus mirabilis* (t. ATCC 3177), *Acinetobacter baumanii* (t. ATCC 19606)).

Bacteriostatic activity was assessed in the absence of growth of microorganisms in the liquid nutrient medium. The bactericidal activity was evaluated based on the lack of growth of microorganisms on the solid nutrient medium – meat peptone agar, after the repeated sowing and ulterior thermostating during 24, 48 and 72 hours.

Results and discussion

1. Spectral analysis of the polymer-analogs synthesized

The IR spectra of chitosan (1), maleic anhydride (2) and chitosan maleinized (3) are presented in fig. 3. Figure shows in the spectrum of chitosan maleate the presence of new vibrations that are missing in the spectrum of chitosan

and in the spectrum of maleic anhydride. For example, vibrations $\nu = 3065$ cm^{-1} characteristic of the amide groups -NH-CO-. From the spectrum of malignant chitosan, new vibrations are also observed at $\nu = 1566$-1709 cm^{-1} characteristic of carbonyl groups. From the spectra there is also observed a massive increase in vibrations $\nu = 1353$ cm^{-1} characteristic of carbonate groups. In the spectrum of chitosan maleate we also notice the appearance of a massive band at $\nu - 862$ cm^{-1} characteristic of the double bonds in maleic anhydride grafted to chitosan macromolecules.

Fig. 4 shows the IR spectrum of the final chitosan maleinized polymer functionalized with furacillin, and the furacillin as a control sample.

Figure 5 shows the increase of the absorption bands 1540-1590 cm^{-1} characteristic of the amino (secondary) groups as well as the appearance of the new vibrations 3200-3300 cm^{-1} characteristic of the secondary and tertiary amide groups. The same changes were observed in the case of chitosan maleinized-isofural.
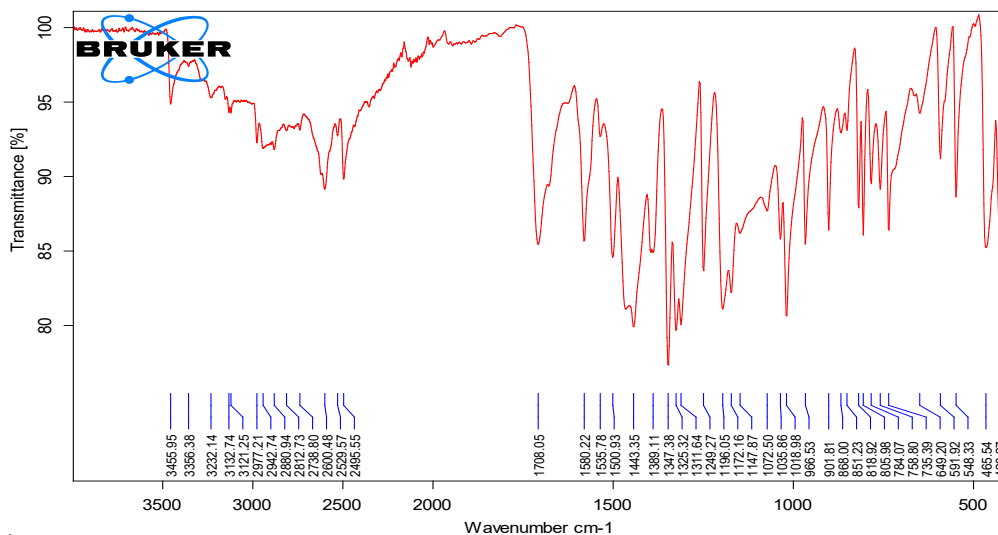
Fig. 4. IR spectrum of furacillin and furacillin grafted to chitosan maleinized (70-30 mol%)
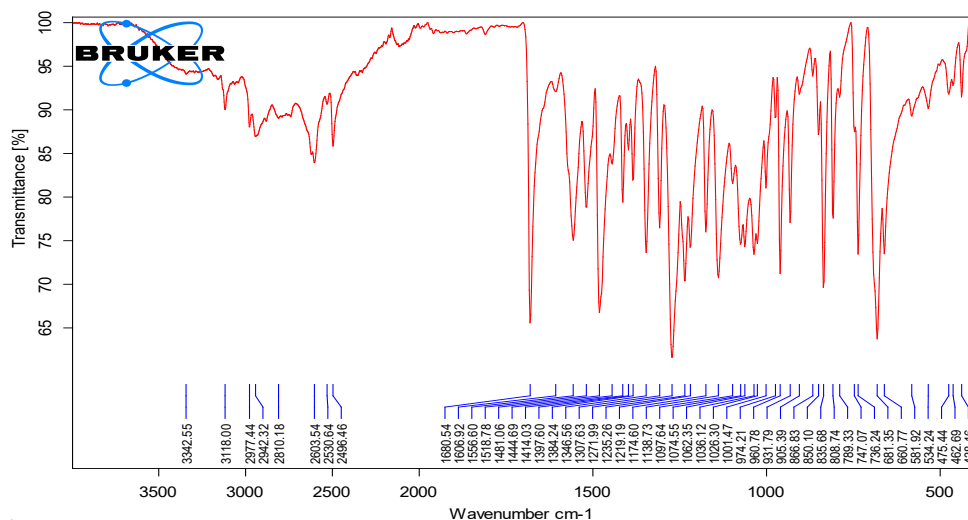
Fig. 5. IR spectrum of isofural (1) and isofural-grafted chitosan maleinized (70-30 mol%)

2. Evaluation of antibacterial action

As a result of the study of the antibacterial properties of the researched substances, it was found that both substances obtained from chitosan grafted with isofural or furacillin, possess antibacterial activity in a wide range of microorganisms, Gram-positive and Gram-negative (tab. 1 and 2).

The bactericidal activity of chitosan maleinized – isofural (70:30), after 24 hours of incubation on peptone agar, varies within the concentrations of 150 – ≥300 mcg/mL and constitutes for *S. aureus* (t.209) – 150 mcg/ml, and for the other species of gram-positive and gram-negative microorganisms – ≥300 mcg/mL.

The bactericidal activity of chitosan maleinized – furacilin (70-30), after 24 hours of incubation on peptone agar, varies within the limits of concentrations 150 – ≥300 mcg / mL and constitutes for *E. coli* – 150 mcg/mL, and for all other species of microorganisms – ≥300 mcg / mL.

As a result of the long incubation, 48 and 72 hours, of the cultures of microorganisms sown on peptonate broth, there have been obvious changes in the bactericidal activity of both substances over time.

The bactericidal activity of chitosan maleinized – isofural (70:30) in the condition of prolonged exposure increased for methicillin-resistant *Staphylococcus aureus* with 4 dilutions, from the concentration of the substance of 300 mcg/ mL to 18.75 mcg/mL, for *S. aureus* (t.209) – with two di-

Table 1. Bactericidal activity of the polymer of the analogue "Chitosan maleinized – isofural (70:30)" (minimum bactericidal concentration (µg / mL))

Test – Gram-positive bacterial cultures									
Dose (mcg/ml)	S.aureus (t.209)			MRSA (t.NCTC 12493)			E.faecalis (t.ATCC 19433)		
	24	48	72	24	48	72	24	48	72
300	-	-	-	-	-	-	±	-	-
150	-	-	-	±	-	-	±	+	+
75	±	-	-	±	-	-	+	+	+
37.5	±	-	-	±	-	-	+	+	+
18.75	+	+	+	±	-	-	+	+	+
9.37	+	+	+	+	+	+	+	+	+
Test – Gram-negative bacterial cultures									
Dose (mcg/ml)	E.coli (t.ATCC 25922)			K.pneumoniae (t.3534/51)			P.mirabilis (t.ATCC 3177)			A.baumannii (t.ATCC 19606)		
	24	48	72	24	48	72	24	48	72	24	48	72
300	±	-	-	+	±	±	+	-	-	+	-	-
150	+	±	+	+	+	+	+	+	+	+	+	+
75	+	+	+	+	+	+	+	+	+	+	+	+
37.5	+	+	+	+	+	+	+	+	+	+	+	+
18.75	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. Bactericidal activity of the polymer of the analogue "Chitosan maleinized – furacilin (70-30)"

Test – Gram-positive bacterial cultures									
Dose (mcg/ml)	S.aureus (t.209)			MRSA (t.NCTC 12493)			E.faecalis (t.ATCC 19433)		
	24	48	72	24	48	72	24	48	72
300	±	-	-	±	-	-	-	-	-
150	±	-	-	±	-	-	±	+	+
75	±	-	-	±	+	+	±	+	+
37.5	±	±	±	±	±	+	+	+	+
18.75	±	±	+	+	±	+	+	+	+
Test – Gram-negative bacterial cultures									
Dose (mcg/ml)	E.coli (t.ATCC 25922)			K.pneumoniae (t.3534/51)			P.mirabilis (t.ATCC 3177)			A. baumannii (t.ATCC 19606)		
	24	48	72	24	48	72	24	48	72	24	48	72
300	-	-	-	±	-	-	+	+	+	-	-	-
150	-	-	-	±	-	-	+	+	+	+	-	-
75	+	±	-	+	+	+	+	+	+	+	+	+
37.5	+	+	+	+	+	+	+	+	+	+	+	+
18.75	±	±	+	+	±	+	+	+	+	+	+	+

lutions, for *E. faecalis, E. coli, P. mirabilis* and *A. baumanii* – with one dilution, and for *K. pneumoniae* remained unchanged (Table 1).

The bactericidal activity of chitosan maleinized – furacilin (70-30) in the condition of prolonged exposure (72 hours) increased for *S. aureus* (t.209) by three dilutions, for methicillin-resistant *S. aureus* and *K. pneumoniae* – with two dilutions, for *E. coli* and *A. baumanii* – with one dilution, for *E. faecalis* and *P. mirabilis* remained unchanged (tab. 2).

Conclusions

1. Chitosan derivatives with a content of 30 mol% of maleic anhydride were obtained. To the analogous polymer "chitosan maleinized" the medicinal products isofural or furacilin with the help of the ethyl chloroformate were functionalized.

2. By comparative analysis of the IR spectra of the final products with the IR spectra of maleinized chitosan and furacilin or isofuran was demonstrated the individual structure of the polymeric preparations "maleinized chitosan grafted with furacilin" / "maleinized chitosan grafted with isofural".

3. The antibacterial substances, isofural and furacilin, among nitrofurans, being grafted with chitosan maleinized, keep their bactericidal activity in the limits of 75-300 μg / mL.

4. The polymeric materials from chitosan maleate grafted with isofural or furacilin in a ratio of 70:30 have a prolonged antibacterial action (observation period 72 hours).

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Authors' contributions
VP designed the research; DB did statistics; RR, LC interpreted the data; SR drafted the manuscript. All the authors revised the manuscript critically and approved the final version of the manuscript.

Ethics approval and consent to participate
The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, protocol No 46 of 12.04.2018.

Conflict of interests. No competing interests were disclosed.

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Open Access

Knowledge, attitudes and practices of neurologists regarding the management of chronic non-cancer pain in the Republic of Moldova

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Abstract

Background: Chronic pain is a public health problem due to its high prevalence, disability, and associated comorbidities. In the Republic of Moldova, there are no health policies or strategies regarding chronic pain, and the burden on the population is not known because it is not registered nor monitored by the state. The aim was to analyze the knowledge, attitudes, and practices of neurologists regarding the management of chronic pain in the Republic of Moldova.
Material and methods: Mixed observational study (qualitative and quantitative) was designed and carried out using a Knowledge, Attitudes, Practices research questionnaire. The study includes 50 neurologists, average age of 47.4±9.52 years, women (82%), and urban area (80%), interacting with chronic pain patients on a daily basis (86%).
Results: 42% of neurologists know about pain measurement tools, 40% of them use these pain measurement tools, 40% of neurologist know clinical guidelines, 92% of them practice pharmacological and non-pharmacological treatments. The neurologists (62%) presented negative attitudes about chronic pain patients. Just 18% of them have sufficient knowledge and skills to deal with such a patient, 64% of neurologists received training on chronic pain issues.
Conclusions: Neurologists have little knowledge of pain measurement tools and guidelines and don't use them, which makes chronic pain poorly addressed, evaluated, and treated at the national level. They recognize the right of the patient to live without suffering and to benefit from quality services focused on their needs, but consider patients difficult to approach, communicate and work with.
Key words: chronic pain, knowledge-attitudes-practices research, neurologists.

Introduction

Chronic pain is a public health problem due to its high prevalence, disability and associated comorbidities. The prevalence of pain varies from one country to another, being between 2-40% [1]. It is considered that globally 10% of the population are affected by chronic pain, it is around 60 million people that suffer, but national and regional studies indicate a prevalence of 20-25% [2].

A recent analysis demonstrated the pain prevalence in the USA of 12-20%, and in Europe – of 20% [3]. Prevalence greater than 40% was reported by studies conducted in Italy, France and Ukraine [3]. Pain brings suffering to individuals and challenges health systems, the economy and society: every year 1 in 5 Europeans is affected by chronic pain. This includes 153 million people with migraine or other chronic headaches, 200 million with musculoskeletal disorders and 100 million with chronic pain. The American Academy of

Pain Management reports that 57% of American adults have experienced chronic or recurrent pain in the past year. Of these, 62% suffer from pain for more than 1 year and 40% mention that they have permanent pain [4]. For these reasons, the American Congress declared 2001-2010 "The Decade of Pain Control and Research" and the Joint Commission for Accreditation and Attestation of Medical Organizations requires specialists to consider pain as the fifth vital sign, therefore mandatory to be measured and recorded [5].

In the Republic of Moldova, there is no health policy or strategy regarding chronic pain, the burden on the population is not known because it is not registered nor monitored by the state. Following the joint efforts of non-governmental organizations and specialists in the field, remarkable scientific progress was made: clinical research and PhD theses, monographs were edited, the national guideline on cancer pain was published, the law was

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amended for increasing access to opioid medications and changing university curricula to introduce palliative care [6-8]. At the Institute of Neurology and Neurosurgery, the pain centers were developed and professional societies, such as the Headache Society of the Republic of Moldova and the Moldovan Society for the Study and Management of Pain try to promote the problem of pain at the state and society level. For these reasons the proposed research is considered necessary.

The purpose of the research was to analyze the knowledge, attitudes and practices of neurologists regarding the management of patients with chronic non-cancer pain in the healthcare system of the Republic of Moldova.

Material and methods

In accordance with the aim and objectives outlined, mixed observational research (qualitative and quantitative) was designed and carried out. The knowledge and practices of neurologists regarding pain management and the attitude towards the patient with chronic non-cancer pain were evaluated using the structured questionnaire developed by the authors according to the criteria for KAP research (Knowledge, Attitudes, Practices) [9]. Quantitative study – a structured questionnaire with closed questions for self-completion was developed to conduct the study. The questionnaires were pretested and validated prior to the research. Qualitative study – in-depth interviews were conducted for the preliminary study and focus group to analyze the results. Two focus groups of 10 neurologists each were formed. For qualitative research there is no need for sampling, the number of people included in the research is determined by the purpose of the research.

Inclusion criteria: neurologists that give consent to participate in the study and treat patients with chronic non-cancer pain.

Exclusion criteria: neurologists not willing to consent for participation in study.

The questionnaire consists of 21 closed and open questions, with simple and multiple compliments. At the beginning of the questionnaire there is a preamble explaining the definition of chronic non-cancer pain and why that person is invited for the research. The questionnaire is structured in several compartments that aim to elucidate important aspects of the management of patients with chronic non-cancer pain in the healthcare system of the Republic of Moldova and are as follows: Frequency of consultations of patients with chronic non-cancer pain; Problems arising in the management of patients; Application of national/international guidelines for pain management; Knowledge about pathology and treatment; Non-pharmacological approach; Multidisciplinary approach; Referral of the patient within the healthcare system of the Republic of Moldova; Attitudes of doctors towards patients with chronic non-cancer pain; Training in pain/chronic pain; Needs for additional training of physicians in pain/chronic pain; Demographic data.

The individual and group interview was focused on several important topics: Working experience with patients with chronic pain; Knowledge about chronic pain; Current practices regarding patients with chronic pain; Attitudes towards patients with chronic non-cancer pain; and Barriers encountered in the management of these patients.

Results

The study group consists of 50 neurologists with average age of 47.4±9.52 years, mostly women (82%), from urban area – 80% vs. rural – 20%. 2% of respondents see patients with chronic non-cancer pain at least once a month, once a week – 12.0%, daily – 42.0%, several times a day – 44.0%. The vast majority of neurologists interact with chronic non-cancer pain patients on a daily basis (86%).

First, the knowledge about pain measurement tools was evaluated, whether doctors know and use them. As a result, 42% of the respondents know about tools to measure pain and only 40% of the surveyed neurologists measure pain during the consultation. This means that neurologists who interact most frequently with patients with chronic non-cancer pain have very little knowledge of pain measurement tools and the use of them less.

Analysis of physicians' knowledge of existing pain management guidelines demonstrated the following: 40% of respondents know a pain management guideline, of which they name a national guideline – 30% and/or an international one – 22.0%. In the Republic of Moldova there are two national pain treatment guidelines developed for neurologists on migraine and back pain management. Only 30% of the respondents knew at least one of them.

During the qualitative research, doctors mentioned that they do not know international guidelines because: "*I do not know English*" (B, 54 years), "*I did not consider it necessary to study any guideline because chronic pain is not recognized as a separate entity in the Republic of Moldova, and it is not coded and paid respectively*" (C, 59 years, focus group).

Respondents were assessed if they know that the patient with chronic non-cancer pain requires treatment with specific drugs (not only analgesics): 92% of respondents answered affirmatively to this question. Evaluation of the experience of using specific pain treatment, that is classified into several groups: antidepressants (including tricyclics), anxiolytics, anticonvulsants, and opioids, showed the following: 80% of doctors mentioned that they had prescribed tricyclic antidepressants to patients with chronic non-cancer pain, anticonvulsants were used by 88% of doctors, other antidepressants in 58% of respondents, anxiolytics in 76% and opioids just in 24% of the questioned doctors. 4% of the respondents did not indicate any class of these drugs, the majority of neurologists administered 4 most indicated drug classes (tricyclic antidepressants, anticonvulsants, anxiolytics, and other antidepressants) in 36% of cases and 3 drug classes in 28% of respondents.

The neurologists indicated opioids very seldom. The qualitative research elucidated the reason of that mentioned by doctors: "*we don't have enough knowledge and we are afraid of adverse reactions*" (M, 58 years old), "*we haven't prescribed opioids for years, because it was strictly monitored and it was forbidden, and now we don't know how to do it*" (P, 50 years old).

The knowledge of neurologists regarding minimally invasive and non-pharmacological methods of chronic pain management was evaluated. The methods were indicated according to international guidelines, regardless of whether they are available or not in the Republic of Moldova. 72% of doctors mentioned that they know that exercise and sports help with chronic pain management, physiotherapy – 72%, infiltrations – 82%, physical therapy – 78%, psychotherapy – 74%, electrical stimulation – 50%, magnetic stimulation – 50%, manual therapy – 54%, biofeedback – 54%, relaxation techniques – 74%, cognitive-behavioral therapy – 56%, yoga – 52%, acupuncture – 72% of respondents and 34% of those surveyed know all non-pharmacological methods mentioned in the questionnaire. Thus, the top methods were infiltrations, physical therapy and exercises, the methods that are available in the health system and patients with chronic pain benefit from them.

Doctors were asked to mention which of the non-pharmacological methods the patients were referred to: 62% of the respondents referred patients to physical exercises and sports, 74% – to physio procedures, 70% – recommended infiltrations, 74% – physical therapy, 40% – yoga, 64% – acupuncture, 56% – psychotherapy, 30% – electrical stimulation , 38% – magnetic stimulation, 34% – manual therapy, 22% – biofeedback, 36% – relaxation techniques, 34% – cognitive-behavioral therapy and 16% of the respondents referred to all the mentioned methods, although many methods are not available in the Republic of Moldova.

The high percentage of doctors who referred patients with chronic non-cancer pain to physical therapy and psychotherapy demonstrates that doctors know about the bio-psycho-social approach to chronic pain where biological, psychological and social factors must be evaluated and addressed and the patient needs the consultation of multidisciplinary teams that would work in coordination.

Unfortunately, in our country such teams are limited and referral to specialists is difficult. In qualitative research, respondents mention: "*If they are sent for physical therapy or psychotherapy, patients don't understand why they are sent and they don't go*" (K, 55 years old), "*When I tell patients that they need to go to psychotherapy, they don't believe me*" (F, 59 years old), "*Patients don't understand when I tell them that they have to deal with pain with other methods than drugs*" (A, 47 years old).

The reasons why neurologists did not refer patients to any non-pharmacological management method are: there are no available experts and methods in the Republic of Moldova (20%), there are no specialists and the respective methods in the district (8%), they do not know where to send the patient (2%) and they do not know that they should refer patients to specialists in the respective fields (6%). The most frequently mentioned barrier in accessing medical services by patients with chronic non-cancer pain was the availability of methods.

Neurologists were asked if they know that the patient with chronic non-cancer pain needs the consultation and treatment of a multidisciplinary team; 96% of the respondents know this fact and 96% of those surveyed mentioned that they sent patients with chronic non-cancer pain to the general practitioner (24%), to another specialist (32%), psychologist (72%), kinetotherapist (66%), physiotherapist (64%), pain specialist (34%) or osteopath (32%).

Multidisciplinary approach is when a team consisting of several specialists (neurologist, rheumatologist, traumatologist, neurosurgeon, physiotherapist, psychiatrist, nurse and social worker) works together in a well-coordinated way. In the qualitative research, the doctors mentioned that it is difficult to work in a team with a physiotherapist and a psychotherapist because the referral system is faulty, the approach of each specialist is different and they do not work to the same standards, for the same common goal – the treatment of chronic pain.

"*If I prescribe to the patient specific drugs for the treatment of chronic pain and additionally recommend physical therapy, then the patient comes in a few months and says that the physical therapist said to stop the drugs, to do only exercises*" (G, 57 years old), "*Sometimes the patient comes to the chiropractor or osteopath after months of procedures without proper investigations, because each specialist works according to their own preferences, or protocols if they are for that specialty*" (A, 45 years old), "*I often prescribe antidepressants to patients for 6 months and the primary care doctor does not follow the prescriptions, we lack interdisciplinary cooperation*" (S, 52 years old).

The attitude of neurologists towards the patient with chronic non-cancer pain was evaluated through a series of questions inserted in the questionnaire. 62% of the respondents mentioned that the patient with chronic non-cancer pain consumes time, emotions, resources, 2% – that the patient is incurable, 38% of doctors consider that patient very often aggravates the situation in order to obtain benefits, 62% – requests additional investigations and treatments, 32% – is non-compliant with indications and recommendations, 46% – does not want to actively participate in his own recovery, 62% – is poorly informed about the disease and treatment methods, 20% – is responsible (guilty) for his own situation (disease), 86% – has the right to live without suffering, and 66% – must benefit from access to medical services according to individual needs.

As can be seen from the responses of neurologists, the attitude is mixed; there are positive and negative statements. Doctors recognize the right of the patient with chronic pain to live without suffering and the right to benefit from services according to their needs. At the same

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time, it is mentioned that patients are difficult to approach: they consume time, resources and emotions; they are not compliant with indications and treatment. The patient is poorly informed about the disease and the available treatment methods, for that reason he does not want to actively participate in his own recovery and requests additional investigations and treatments. A remarkable percentage have an obviously negative attitude as they consider the patient incurable – so, it does not require effort and the use of resources; he is responsible for his own situation – so, the only person is to blame, respectively; does not require empathy and attention and very often aggravates the situation in order to obtain benefits – so, the patient is not believed, he pretends to get attention.

Neurologists have very little knowledge and less use of pain measurement tools, as well as of national and international pain management guidelines, making chronic pain a poorly addressed and undertreated nosology. They know the specific treatment of chronic pain and use most of the drug classes, less opioids for which they are reluctant. They know about the non-pharmacological methods and the multidisciplinary approach to chronic pain but mention that some methods are not available in the RM and others are difficult for patients to reach because of poor interdisciplinary collaboration. Neurologists recognize the right of the chronic pain patients to live without suffering and to benefit from qualitative services focused on their needs, but consider this type of patients difficult to approach, communicate, work with and worthy of blame for the situation in which they find themselves.

Only 18% of respondents state that they have sufficient knowledge and skills to work with patients with chronic pain, 74% of them consider that they have partial knowledge and skills and 8% – insufficient. In qualitative research, doctors mention: *"Knowledge is insufficient because chronic pain is not sufficiently taught either at the university or post-graduate level, we have to do self-education on our own outside the borders of the country"* (O, 38 years old), *"Practical skills for infiltrations or other minimally invasive techniques are taken from older colleagues or at international courses"* (L, 44), *"Communicating with these patients is a challenge for any doctor and we were not trained to face it"* (G, 47 years old).

Physicians were asked to mention what knowledge and/ or skills they still need for the management of patients with chronic non-cancer pain. They mentioned that it would be required:
- Additional theoretical knowledge in the management of patients with chronic pain – 50%,
- Additional practical knowledge in the management of patients with chronic pain – 52%,
- Additional knowledge about drug treatment of chronic pain – 50%,
- Additional knowledge about non-drug treatment of chronic pain – 66%,
- Practical communication skills with the patient with chronic pain – 32%,

- Practical skills of interaction with the relatives/family of the patient with chronic pain – 20%,
- Patient/family psychological counseling skills – 40%,
- Practical skills of minimally invasive interventions in the treatment of chronic pain – 30%.

Only 18% of respondents state that they do not require additional knowledge, others require at least one type of knowledge mentioned, most mentioned that they require all four types of knowledge – in 28% of interviewees. 46% of respondents do not need any type of skills, the rest need at least one type of practical skills for managing patients with chronic non-cancer pain. So, 82% of respondents would still need additional theoretical knowledge, 54% – additional practical skills.

64% of respondents state that they have received training related to chronic pain management from the refresher course given in the country, refresher course outside the country – 18%, national congresses – 34%, international congresses – 22%, lessons at the professional society – 52%.

When asked who they thought should be responsible for measuring, recording and treating pain, 86% said any doctor and 14% thought only a pain specialist should do this.

The doctors were asked to mention if the specialists in pain management are needed in the Republic of Moldova. 94% of respondents answered in the affirmative, from which 18% considered it should be in the form of a separate specialty, 34% – as additional skills obtained by any specialist and both forms mentioned 48%. 96% of respondents reiterated the need for a national chronic pain management guideline in the Republic of Moldova.

When asked who should be involved in the management of the patient with chronic pain, they mentioned the family doctor – 88%, pain management specialist – 90%, physiotherapist – 78%, kinetotherapist – 82%, psychologist – 88%, medical assistant – 68%, social worker – 66%, relatives and family – 80%, community – 66%. Only 60% of those surveyed mentioned that all mentioned above should be involved in the management of the patient with chronic pain.

Discussion

The research conducted demonstrated that neurologists who most frequently interact with patients with chronic non-cancer pain have little knowledge of pain measurement tools, both national and international guidelines, do not use them in daily practice, which causes chronic pain to be poorly recognized, evaluated and treated in the Republic of Moldova. In states with successfully implemented national pain management strategies, it has been demonstrated that any strategy will require the implementation of clinical guidelines that will reduce variations in the provision of medical services and will determine a consensus of specialists in the field. Pain management guidelines are believed to reduce disability by ensuring that the patient receives proactive treatment [10, 11].

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The neurologists in the present research know the modern methods of drug treatment of chronic pain, but show reluctance to prescribe some drugs, especially opioids. These data are also reported by other international studies where more specialists do not feel confident to prescribe opioids especially for chronic non-cancer pain [11, 12]. Not only opioids are poorly used, but also some antidepressants, even if these drugs are available, they are poorly used due to the lack of necessary knowledge [13].

Neurologists from the Republic of Moldova know the non-pharmacological methods of chronic pain management and the multidisciplinary approach, but they cannot provide the patient with that approach due to the lack of availability of several methods and poor interdisciplinary collaboration. Referral of patients to non-pharmacological methods and other specialists is difficult. The UK survey assessed 20 general practitioners to determine the most common reasons for headache patients to be referred to a specialist, and they noted: patient demand, particularly frequent referrals, difficult patient-doctor relationship and long consultation time [14]. Most commonly a general practitioner in Ireland will refer a patient with chronic back pain to a physiotherapist [15]. Research in Ireland included 293 general practitioners who mentioned that they would refer 59% of patients with back pain to a physical therapist because of the need to indicate exercises, posture modification but not for pain relief [15]. In Germany, general practitioners mentioned that patients with headache and neuropathic pain come to them, and those with back pain go directly to orthopedists-traumatologists. Among patients with chronic pain only 40% will go to the general practitioner regularly (39% – those with headache, 41% – back pain and 40% – neuropathic pain) [16]. Another study demonstrated that when the family doctor decides the referral to the specialist, but not the patient, the patient experience is more positive due to confidence in the established diagnosis, because the specialists are better trained and will increase the role of primary care in the management of chronic pain [17].

The neurologists in the Republic of Moldova mentioned that they feel unprepared to deal with the management of the chronic pain patient due to the lack of training and deficiencies in practical skills. The International Association for the Study of Pain states that the training of pain management specialists is below the need in both developed and developing countries. In an analysis of 19 institutions of higher education possessing 108 educational programs, pain education occupies <1% and veterinary students have the most hours. In a survey from the USA, 153 general practitioners mentioned that they did not feel sufficiently trained in some aspects of back pain to work effectively with these patients (50%). It may lead to an increase in the number of investigations and surgeries requested by these doctors [18]. Several studies that have assessed how prepared and trained family physicians feel to deal with the management of patients with chronic non-

cancer pain have determined that the vast majority do not feel prepared [18].

The neurologists in the present research recognize the right of the chronic pain patients to live without suffering and to benefit from quality services focused on their needs, but consider this type of patients difficult to approach, communicate, work with and worthy of blame for their situation. This data corroborates with international results: a recent study demonstrated that primary care specialists consider patients with chronic non-cancer pain to be difficult to approach and treat [19]. The biggest problem in dealing with these patients is communication. The vast majority of doctors feel difficulty in measuring the intensity of the pain perceived by the patient, which leads to the erroneous understanding of the patient's situation and expectations from the treatment, resulting in poor doctor-patient communication. This poor communication is the main cause of treatment failure mentioned by researchers [20]. The insufficient treatment of chronic non-cancer pain is a problem not only at the national level but also internationally – 2/3 of the specialists questioned at an international pan-European forum considered that the insufficient treatment of pain is a problem in their country [21].

Conclusions

1. Neurologists in the Republic of Moldova have very little knowledge of pain measurement tools and less use of them, as well as national and international pain management guidelines, which makes chronic pain a poorly addressed, evaluated and treated at the national level.

2. Physicians know the specific treatment of chronic pain and use most of the drug classes, less the opioids for which they are reluctant. They know about the existing non-pharmacological methods and the multidisciplinary approach to chronic pain, but they mention that some methods are not available in the Republic of Moldova and others are difficult for patients to access due to poor interdisciplinary collaboration, that is why patients cannot benefit from the approach to chronic pain management in terms of the biopsychosocial model.

3. Neurologists recognize the right of the patients with chronic pain to live without suffering and to benefit from quality services focused on their needs, but consider this type of patients difficult to approach, communicate, work with and worthy of blame for the situation in which they are.

4. Neurologists mentioned several barriers in the management of chronic non-cancerous pain, which can be grouped as system, clinical and communication. Physicians report that they have insufficient training in chronic pain management, requiring additional theoretical knowledge and practical skills to meet the demands of managing patients with chronic non-cancer pain.

5. Neurologists believe that every doctor should record, measure and treat pain, there is a great need of pain man-

agement specialists and a national guideline for pain management. They recognize that the management of chronic pain requires a wide involvement: specialist, patient, social worker, relatives, community, and government.

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Authors' contributions
OG designed the research, did statistics and interpreted the data; LR, SO, MS, SP, GC, IM drafted the manuscript and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

Ethics approval and consent to participate
The research protocol (No 1 of 27.02.2020) was approved by the Research Ethic Board of the *Diomid Gherman* Institute of Neurology and Neurosurgery and the tests have been done according to the contemporary principles in biological standardization of experiences and Declaration of Helsinki with further amendments (Somerset West Amendment, 1996).

Conflict of Interests
No competing interests were disclosed.

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The role of depression and anxiety in pain perception

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Abstract

Background: Depression and anxiety are associated with increased perception of pain severity. Because patients with a depressive disorder and anxiety often report pain, their sensitivity to experimental pain is controversial, probably due to differences in sensory testing methods and the lack of normal values.
Material and methods: The study was conducted on 140 selected subjects. The pain test was performed using a technique, called the submaximal effort tourniquet technique. Before the start of the study, a set of psychometric inventories and tests was prepared (visual analog scale, Beck Depression Inventory, Spielberger's State and Trait Anxiety Inventory).
Results: No differences in pain perception have been found in men and women as well as in relation to age, thus gender and age cannot be a predictor in pain perception. The anxiety has no effect on pain perception. The depression can be considered a predictor of pain intensity because a change in depression levels determines a change in pain intensity perception at the 3rd minute. If the depression category was changed from a patient with no depression to one with mild depression, pain intensity at minute 3 increased by approximately one point on the visual analog scale (B=.954, CI95% .200, 1.709, p=.014).
Conclusions: Depression can be considered a predictor in the evolution of pain perception. Not so much the depression score, but the increase in the severity of depression can predict the evolution of pain perception.
Key words: anxiety, depression, pain, visual analog scale, pain test.

Introduction

The International Association for the Study of Pain defines pain as "an unpleasant subjective feeling and emotional experience associated with actual or potential tissue damage", which is an interaction of psychological, emotional, behavioral, and social factors [1].

Pain is a subjective experience that is influenced by genetic, gender, social, cultural, and personal factors. This is accompanied by increased sympathetic and noradrenergic activity and a reduction in parasympathetic activity. Generally, an anxiety reaction develops due to acute pain. The link between mood disorders and acute pain has been shown to be increasingly significant because the link is bi-directional and, in both cases, they act as risk factors for each other. Depression and anxiety are associated with increased perception of pain severity, while the prolonged duration of acute pain leads to increased mood dysregulation.

Affective processes may interact with nociception and pain at different levels, namely, pain modulation, pain response, and pain behavior [2]. Negative emotional states, including fear and anxiety, are found to alter pain-related responses. A number of studies have shown an increase in pain and hyperalgesia, while others have reported inhibition of pain (analgesia) during stressful situations [2].

Several human experimental studies show that negative affective states, including anxiety, have been consistently associated with increased pain and hyperalgesia. However, there is evidence in the literature demonstrating that high levels of negative affect inhibit nociceptive and pain-like responses [3].

Pain is a common symptom in patients suffering from a depressive disorder. Both depression and chronic pain are common conditions in medical and psychiatric practice. Although depression and chronic pain can occur independently, they are often comorbid. In turn, chronic pain conditions trigger a depressed mood, which may finally meet the diagnostic criteria for a depressive disorder. Pain and depression are hypothesized to share common neuroanatomical pathways and neurobiological substrates, which could explain the increased vulnerability to pain complaints in depression and vice versa.

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Pains often co-occur with depression and anxiety and together represent a considerable social and economic burden. However, no systematic review has been conducted to examine the covariation between these conditions [4]. Data on the etiological factors underlying the co-occurrence of common pain in individuals with symptoms of anxiety and depression are very limited [5]. Most studies found that the covariation of pain with depression and/or anxiety was explained by genetic or both genetic and environmental factors [4].

As both depression and anxiety are associated with acute pain, the link between depression and acute pain is being studied more thoroughly. Although fewer data are published on anxiety and pain, the relationship is consistent across studies, as increased anxiety leads to increased perceived pain severity and decreased pain tolerance. Different studies show that anxiety, fear, and stress which have been shown to be mediators in the causal pathway between pain and disability can alter the pain threshold, demonstrating both increased and decreased pain threshold and pain tolerance [6].

Reports on the perception of experimentally induced pain in depressed patients are mixed, showing both increased and decreased pain threshold and pain tolerance in different studies. Because patients with a depressive disorder and anxiety often report pain, their sensitivity to experimental pain is controversial, probably due to differences in sensory testing methods and the lack of normal values [7].

The aim of the study was to analyze the role of depression and anxiety in pain perception in the experimental study.

Material and methods

The study was performed on 140 subjects selected out of 187 persons visiting the Department of Headache and Autonomic Disorders of the Institute of Neurology and Neurosurgery (Chisinau, the Republic of Moldova) from March 2018 to February 2022. They signed an informed agreement to be included in this study, which continued at the Department of Human Physiology and Biophysics of *Nicolae Testemitanu* State University of Medicine and Pharmacy. The subjects with acute or chronic cardiac or respiratory diseases were excluded.

Before beginning the study, a set of inventories and psychometric tests was prepared.

A visual analog scale (VAS) is used to assess the severity of a patient's pain. It is 10 cm long with 0= no pain, written at one end, and 10= most severe pain, written at the other. Patients are asked to mark where along the scale they would place the pain they perceived. The distance is measured in centimeters. The value shows the severity of pain perceived by the patient.

Beck Depression Inventory (BDI) was developed by Beck to measure a variety of symptoms of depression. It is a 21-item checklist that the patients fill in themselves. They select the most appropriate of the four choices.

Spielberger's state and trait anxiety inventory is made up of 40 questions and distinguishes between a person's state anxiety and their trait anxiety. The two forms of anxiety are separated in the inventory, and both are given their own 20 separate questions. When participants rate themselves on these questions, they are given a 4-point frequency scale. The frequency scales differ between the two types of anxiety.

The pain test was performed using a technique, called the submaximal effort tourniquet technique [8]. The pain was produced by a tourniquet which had been inflated around his upper arm to 200 mm Hg. The assessed parameter was pain sensation in 1st, 2nd and 3rd minutes after applying a tourniquet as chosen by the subject on the VAS.

Statistical analysis was performed using the software Statistical Package for Social Sciences version 26 (IBM SPSS 26). Descriptive statistics for numerical variables were presented by minimum, maximum, mean, standard deviation, median, 25th percentile, and 75th percentile. Descriptive statistics for discrete variables were presented by count, relative frequencies, and 95.0% CI for relative frequencies. Correlation analysis was performed using the Spearmen test, completed by bootstrap estimation of 95% CI. The form of relationships of potential predictors on pain perception at the third minute was estimated using regression analysis, with bootstrap being applied for model stability estimation.

Results

As can be seen in table 1, the research group included 140 subjects aged between 17 and 70 years, with an average age of 37 years (standard deviation 18 years), the median being 29 years, and interquartile range of 36 years. Of those included in the study, 55.7% (95% CI 47.4, 63.8) were males and 44.3% (95% CI 36.2, 52.6) were females. According to the methodology described above, the perceived pain intensity at 1, 2, and 3 minutes of painful stimulation was measured. The recorded values for the level of pain measured in the first minute ranged between 0 and 10 points on the VAS. The mean intensity at this time point was 4.9 points with a standard deviation of 2.3 points. The median of the recorded results was 5 points, and the interquartile range was 3.5 points. The pain at 2 minutes had values between 0 and 10 points with an average of 5.6 points, the standard deviation being 2.6. A median of 6 points and an interquartile range of 3 points were observed on VAS. In the research subjects, the pain at 3 minutes was between 0 and 10 points, with a mean intensity of 5.8 (standard deviation 2.7 points), the median being 6 points on the VAS, and the interquartile deviation of 4.5 points. The majority of those included in the research – 42.1% (CI95% 34.2, 50.4) did not have depression according to the Beck score. Another 24.3% (95% CI 17.8, 31.9) of study subjects had mild depression, and approximately one-third, 33.6% (95% CI 26.1, 41.7), had moderate depression according to the Beck scale score. The absolute values of this score

recorded in research participants varied between 0 and 24 points. The average of the recorded values was 6 points with a standard deviation of 6 points. The median value of the Beck test was 4 points, and the interquartile range was 7 points. The state anxiety level had values between 6 and 56 points with a mean of 31 points, the standard deviation being 11. A median of 30 points and an interquartile range of 16 points were observed.

Starting from the variables included in the study, in order to identify those that could show some relationships or interdependencies, the correlation matrix presented in table 2 was created. As can be seen, age had a statistically significant correlation coefficient only with the pain values measured at 1 minute (CC=.309, CI95% .158, .444, p<.001), at 2 minutes (CC=.356, CI95% .206, .496, p<.001) and at 3 minutes (CC=.263, CI95% .093, .419, p=.002). The gender of the people included in the study also correlated statistically significantly with the pain values measured at 1 minute (CC= -.223, CI95% -.379, -.065, .444, p=.008), at 2 minutes (CC=− .241, IC95% -.390, -.082, p=.004), at 3 minutes (CC=-.187, IC95% -.343, -.021, p=.027) at which, there were added the Beck test values grouped according to the degree of depression manifestation (CC=-.191, CI95% -.348, -.021), state anxiety (CC=-.170, CI95% -.331, -.002, p=.045) and trait anxiety (CC= -.188, CI95% -.347, -.024). Pain at 1 minute, in addition to those mentioned, correlated strongly with pain intensity at 3 minutes (CC= .632, CI95% .498, .738, p<.001). To the correlation coefficients described for the pain recorded at the 2nd minute, the one describing its relationship with the pain intensity at the 3rd minute was added (CC= .854, CI95% .774, .915, p<.001). The Beck test score transformed into an ordinal variable obviously correlated with the Beck test scale depression values in the form of a continuous variable (CC= .938, CI95% .921, .945, p<.001). Statistically significant correlations were also observed with state anxiety values (CC= .566, CI95% .440, .669, p<.001) and with those for trait anxiety (CC= .581, CI95% .448, .689, p<.001). The Beck test score included in the statistical analysis as a numerical variable, in addition to the described coefficients, correlated with state anxiety (CC= .569, CI95% .443, .672, p<.001) and with trait anxiety (CC=.587, CI95% .449, .699, p<.001). State anxiety, apart from the coefficients described above, correlated with trait anxiety (CC=.626, 95% CI .501, .726, p<.001).

Considering the correlation coefficients in table 2 and the complex relationships between factors, multivariate analysis was performed. Two models have been developed that aim to predict pain intensity at minute 3 of painful stimulation.

The first model included predictors of the pain variables at minute 1 and the Beck test score as an ordinal variable (tab. 3). Pain at minute 2 was not included in the model due to a strong correlation with pain at minute 1. As can be seen, 42.2% of the variability in pain intensity at minute 3 was explained by this model.

The coefficient of determination (Adjusted R Square) was 0.422, the sum of squares constituted 441,454 out of 1016,234 possible, which means that the proposed model explains almost half of the dispersion of the pain variable at minute 3. The null hypothesis (none of the parameters included in the model cannot predict the pain intensity value at minute 3 better than some arbitrary model) was rejected (F = 34.818, p = 0.000).

Table 1. Descriptive analysis of the research group. IBM SPSS 26 output

		Mini-mum	Maxi-mum	Mean	Stan-dard Devi-ation	Me-dian	The 25th percen-tiles	The 75th percen-tiles	Count	Col-umn N %	95.0% Lower CL for Column N %	95.0% Upper CL for Column N %
	Age	17	70	37	18	29	21	57				
Sex	F								78	55.7%	47.4%	63.8%
	M								62	44.3%	36.2%	52.6%
Pain 1 min		.0	10.0	4.9	2.3	5.0	3.0	6.5				
Pain 2 min		.0	10.0	5.6	2.6	6.0	4.0	7.0				
Pain 3 min		.0	10.0	5.8	2.7	6.0	3.5	8.0				
Beck test score	No depres-sion								59	42.1%	34.2%	50.4%
	Mild depres-sion								34	24.3%	17.8%	31.9%
	Average depression								47	33.6%	26.1%	41.7%
Beck test score		0	24	6	6	4	2	9				
State anxiety		6	56	31	11	30	24	40				
Trait anxiety		25	72	46	11	43	37	54				

Table 2. Analysis of correlations between measured variables. IBM SPSS 26 output

			Age	Sex	Pain 1 min	Pain 2 min	Pain 3 min	Beck test total score	Beck test grade of depr-ession	State anxiety	Trait anxiety
Age	Correlation Coefficient		1,000	-.088	.309	.356	.263	.028	.056	.065	.095
	Sig. (2-tailed)		.	.302	.000	.000	.002	.741	.515	.442	.262
	95% Confidence Interval	Lower	1,000	-.249	.158	.206	.093	-.132	-.108	-.113	-.056
		Upper	1,000	.075	.444	.496	.419	.179	.212	.237	.264
Sex	Correlation Coefficient		-.088	1,000	-.223	-.241	-.187	-.191	-.162	-.170	-.188
	Sig. (2-tailed)		.302	.	.008	.004	.027	.024	.056	.045	.026
	95% Confidence Interval	Lower	-.249	1,000	-.379	-.390	-.343	-.348	-.329	-.331	-.347
		Upper	.075	1,000	-.065	-.082	-.021	-.021	.007	-.002	-.024
Pain 1 min	Correlation Coefficient		.309	-.223	1,000	.848	.632	.062	.062	.047	.082
	Sig. (2-tailed)		.000	.008	.	.000	.000	.467	.467	.578	.338
	95% Confidence Interval	Lower	.158	-.379	1,000	.775	.498	-.115	-.115	-.106	-.086
		Upper	.444	-.065	1,000	.895	.738	.236	.241	.208	.245
Pain 2 min	Correlation Coefficient		.356	-.241	.848	1,000	.854	.080	.053	.077	.135
	Sig. (2-tailed)		.000	.004	.000	.	.000	.346	.533	.368	.111
	95% Confidence Interval	Lower	.206	-.390	.775	1,000	.774	-.091	-.127	-.091	-.036
		Upper	.496	-.082	.895	1,000	.915	.239	.218	.245	.299
Pain 3 min	Correlation Coefficient		.263	-.187	.632	.854	1,000	.105	.051	.077	.142
	Sig. (2-tailed)		.002	.027	.000	.000	.	.217	.551	.366	.095
	95% Confidence Interval	Lower	.093	-.343	.498	.774	1,000	-.069	-.134	-.083	-.034
		Upper	.419	-.021	.738	.915	1,000	.272	.219	.244	.306
Beck test score	Correlation Coefficient		.028	-.191	.062	.080	.105	1,000	.938	.566	.581
	Sig. (2-tailed)		.741	.024	.467	.346	.217	.	.000	.000	.000
	95% Confidence Interval	Lower	-.132	-.348	-.115	-.091	-.069	1,000	.921	.440	.448
		Upper	.179	-.021	.236	.239	.272	1,000	.945	.669	.689
Beck test score	Correlation Coefficient		.056	-.162	.062	.053	.051	.938	1,000	.569	.587
	Sig. (2-tailed)		.515	.056	.467	.533	.551	.000	.	.000	.000
	95% Confidence Interval	Lower	-.108	-.329	-.115	-.127	-.134	.921	1,000	.443	.449
		Upper	.212	.007	.241	.218	.219	.945	1,000	.672	.699
State anxiety	Correlation Coefficient		.065	-.170	.047	.077	.077	.566	.569	1,000	.626
	Sig. (2-tailed)		.442	.045	.578	.368	.366	.000	.000	.	.000
	95% Confidence Interval	Lower	-.113	-.331	-.106	-.091	-.083	.440	.443	1,000	.501
		Upper	.237	-.002	.208	.245	.244	.669	.672	1,000	.726
Trait anxiety	Correlation Coefficient		.095	-.188	.082	.135	.142	.581	.587	.626	1.000
	Sig. (2-tailed)		.262	.026	.338	.111	.095	.000	.000	.000	.
	95% Confidence Interval	Lower	-.056	-.347	-.086	-.036	-.034	.448	.449	.501	1.000
		Upper	.264	-.024	.245	.299	.306	.689	.699	.726	1.000

Table 3. Statistical data of multivariate analysis for model 1. IBM SPSS 26 output

Model Summary [b]									
					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.659 [a]	.434	.422	2.0558	.434	34.818	3	136	.000
a. Predictors: (Constant), Pain 1, Beck test score									
b. Dependent Variable: Pain 3									

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Table 4. ANOVA test for model 1. IBM SPSS 26 output

	Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	441.454	3	147.151	34.818	.000 [b]
	Residual	574.780	136	4.226		
	Total	1016.234	139			
a. Dependent Variable: Pain3						
b. Predictors: (Constant), Beck test score, Pain1, Beck test score						

The data in table 5 show that an increase in pain intensity at minute 1 by one point on the VAS causes an increase in pain intensity at minute 3 by 0.742 points on the VAS under conditions where the degree of depression was constant (B=.742, IC95 % .594, .890, p<.001). If the depression category was changed from a patient with no depression to one with mild depression, pain intensity at minute 3 increased by approximately one point on the VAS (B=.954, CI95% .200, 1.709, p=.014).

The elaborated model also respects the conditions for residuals and linear regression. The distribution of the residuals is normal, and the lack of associations between the predictive standardized values and the standardized residuals (fig. 1). All these together allow to consider the model as a functional one.

Model 2 initially included sex, age, first-minute pain intensity, and levels of reactive and trait anxiety as potential predictors. The data from table 6 show that about 40% of the variability of pain intensity at minute 3 was explained by model 2 (tab. 6).

The coefficient of determination (Adjusted R Square) was 0.407, the sum of squares was 435,678 out of 1016,234 possible (tab. 7), and which means that the proposed model explains approximately 0.4 of the dispersion of the pain variable at minute 3. The null hypothesis, according to which no parameter of those included in the model can predict the pain intensity value at minute 3 better than some arbitrary model, was rejected (F = 20.112, p < .001).

Table 5. Predictor coefficients for model 1. IBM SPSS 26 output

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	1.090	.611		1.783	.077	-.119	2.298
	Pain1	.742	.075	.640	9.906	.000	.594	.890
	Beck test score	.954	.381	.307	2.502	.014	.200	1.709
a. Dependent Variable: Pain3								
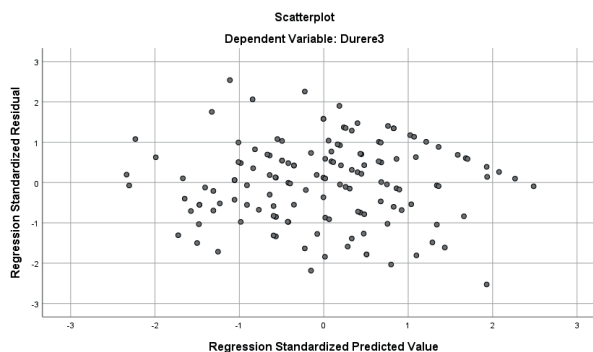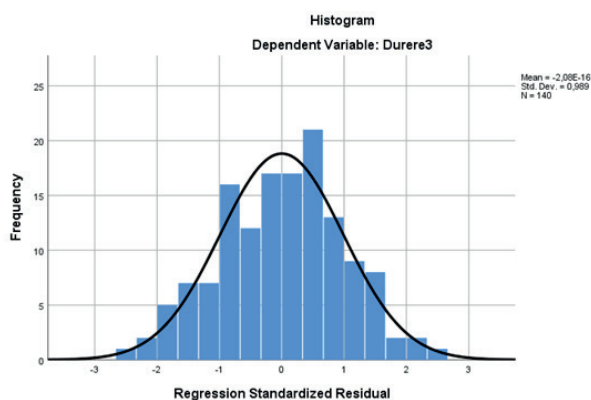
Fig. 1. Distribution of residuals (left); scatterplot of standardized predictive values and standardized residuals (right) for model 1. IBM SPSS 26 output

Table 6. Statistical data of multivariate analysis for model 2. IBM SPSS 26 output

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
2	.655 [a]	.429	.407	2.0815	.429	20.112	5	134	.000
a. Predictors: (Constant), Trait anxiety, Age, Sex, Pain1, State anxiety									
b. Dependent Variable: Pain3									

Table 7. ANOVA test for model 2. IBM SPSS 26 output

ANOVA [a]						
Model		Sum of Squares	df	Mean Square	F	Sig.
2	Regression	435.678	5	87.136	20.112	.000 [b]
	Residual	580.556	134	4.333		
	Total	1016.234	139			
a. Dependent Variable: Pain3						
b. Predictors: (Constant), Trait anxiety, Age, Sex, Pain1, State anxiety						

Table 8. Predictor coefficients for model 2. IBM SPSS 26 output

Coefficients [a]								
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
2	(Constant)	1.268	1.587		.798	.426	-1.872	4.407
	Pain1	.686	.081	.592	8.439	.000	.525	.847
	Sex	-.001	.002	-.022	-.326	.745	-.004	.003
	Age	.018	.010	.123	1.791	.076	-.002	.039
	1. State anxiety	.007	.023	.028	.309	.758	-.038	.052
	2. Trait anxiety	.012	.022	.049	.541	.589	-.032	.056
a. Dependent Variable: Pain3								
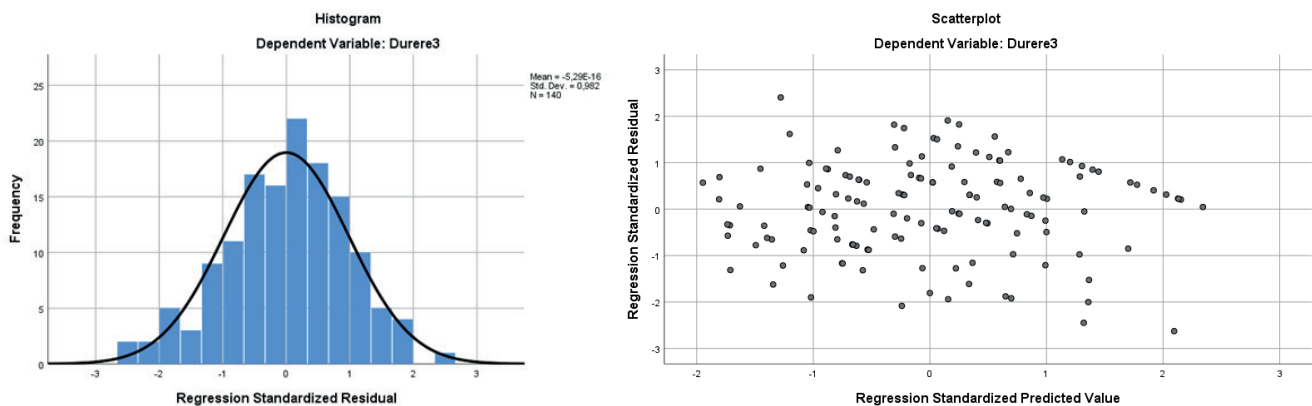
Fig. 2. Distribution of residuals (left); scatterplot of standardized predictive values and standardized residuals (right) for model 2. IBM SPSS 26 output

As can be seen in table 8, only pain intensity at minute 1 showed statistical significance and caused an increase in perceived pain at minute 3 by .686 points when it increased in intensity by one point on the VAS (B=. 686, CI95% .525, .847, p<.001). Therefore, the other parameters measured in the research subjects cannot be considered predictors of the level of pain at the 3rd minute at the time of the research.

It was also noted that the developed model fulfilled the two conditions of the linear regression for the residuals. Their analysis showed a nearly normal distribution and lack of associations between standardized predictive values and standardized residuals (fig. 2). All these together allow to consider the model as a suitable one.

It was proposed to examine in this study the role of depression and anxiety on pain perception. There were found no differences in pain perception between males and females as well as in relation to age, thus gender and age cannot be a predictor in pain perception. These results are consistent with recent data. Detailed analysis of the literature reports that gender-related differences in pain perception still exist, and they are explained by the diversity of methods used in pain modeling. Several papers which characterize pain perception caused by low temperature or high temperature show that the pain threshold is not different in women, while the pain threshold caused by ischemia is lower in women [9].

Several studies about pain show that anxiety rises

sensibility to experimental pain. Moreover, anxiolytic medication can reduce pain perception. However, gender does not influence pain perception. The conducted research shows that anxiety has no effect in the pain perception. Just like in the control group, the pain numeric score rises at the 1st and 2nd minutes and stays the same at the 3rd minute.

There was not found any correlation between depression and pain perception. Just like in the control group, the pain numeric score rises at the 1st and 2nd minutes and stays the same at the 3rd minute. The literature data about depression and pain perception are very controversial. Some data suggest that depression increases the pain threshold, while others show that there is no correlation between depression and pain perception. Analyzing all variables included in the study, it results that depression can be considered a predictor of pain intensity because the change in depression levels determines the change in pain intensity perception at the 3rd minute. If the depression category was changed from a patient with no depression to one with mild depression, pain intensity at minute 3 increased by approximately one point on the VAS (B=.954, CI95% .200, 1.709, p=.014).

Conclusions

Depression can be considered a predictor in the evolution of pain perception. Not so much the depression score, but the increase in the severity of depression can predict the evolution of pain perception.

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Pain experience in Parkinson's disease patients: preliminary results of a cohort study

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Abstract

Background: Pain is frequent in Parkinson's disease (PD) and has a great impact on life quality. The aim of the study was to establish the presence of the pain in a cohort of PD patients.

Material and methods: Study sample consisted of 102 consecutive PD patients, (mean age 61.51 ± 8.87 y.o.; disease duration 65.78 ± 41.34 mo.; 55 women and 47 men) evaluated for pain presence and divided into groups with and without pain: "PD pain +" and "PD pain -"

Results: The groups were similar according to ages (61.02 ± 9.61 vs. 62.51 ± 7.69 y.o.) and levodopa dose (729.85 ± 483.29 vs. 708.31 ± 357.50). Pain was present in 64 patients (62.7%) of all study group; more frequent in women (56.3% vs. 43.8%, p> 0.05), with motor fluctuations (72.4% vs. 27.6%, p> 0.05), with dyskinesia (64.0 % vs. 36.0%, p> 0.05) and restless leg syndrome patients (72.7% vs. 27.3%, p> 0.05). Akinetic-Rigid (0.83 ± 0.80 vs. 0.64 ± 0.56, p> 0.05), and quality of life scores (59.70 ± 25.46 vs. 53.84 ±35.76, p> 0.05) were insignificantly higher in "BP pain + "patients. They had longer disease duration (74.19 ± 39.99 y.o. vs 53.29 ± 41.06 y.o. p=0.017), higher depression (16.36 ± 11.97 vs. 8.09 ± 6.42, p=0.000), psychological (10.28 ±6.20 vs. 4.77 ± 2.82, p=0.000) and non-motor symptoms (66.27 ±39.25 vs. 46.68 ± 32.56, p=0.015) scores.

Conclusions: Pain is common in PD, especially in long disease associated with motor complications, depression and other non-motor symptoms.

Key words: Parkinson's disease, pain.

Introduction

Pain is recognized as a non– motor feature of Parkinson's disease [1] that can appear at any time during the disease course, from the prodromal to late stages [2]. Often the pain appears before the diagnosis of Parkinson's disease, and determines the investigations.

The prevalence of pain in the Parkinson's disease patients is higher than in controls, and could go to 40–75% [1]. PD patients are twice likely to suffer from chronic pain than age-matched non-PD control and also are more likely to receive prescription of analgesics than the general population [3]. Female gender, dyskinesia, postural abnormalities, motor complications and depression are the main predictors for the development of pain in PD [4].

Pain is a significant and troublesome non-motor symptom, experienced by Parkinson's disease patients as a range of different pain syndromes, varying in their cause, origin, location and chronicity [5]. It adversely affects health-related quality of life [6], but remains an under-diagnosed and properly under-treated symptom [5].

Pain is considered one of the most disabling non-motor symptoms in Parkinson's disease, with a strong impact on patients' quality of life, sometimes even greater than that of motor symptoms [7]. In the general population, chronic pain has shown a clear correlation with the severity of depression and reduced quality of life [8].

Although peripheral mechanisms may also contribute, recent studies have indicated that their role is not as important as of central mechanisms to abnormal pain processing [9]. It is presumed that in PD, disease-related states (motor complications, dystonia, rigidity and bradykinesia) as well as medical conditions (osteoporosis, rheumatic disorders) can trigger spontaneous pain which is then abnormally processed and results in painful manifestations in specific body parts [10].

Material and methods

The study was conducted on a cohort of consecutive patients diagnosed with PD at the specialized tertiary clinic with an expert in movement disorders. They were assessed on the basis of a structured questionnaire for general demographic and clinical data. The presence of pain was recorded as a subjective self-reported symptom by the patient. Based on this criterion, the patients were divided into two groups, with and without pain: (1) "BP pain +" and (2) "PD pain -". In all these patients motor

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(UPDRS III) and non-motor ((1) Non-Motor-Symptoms (NMS) Scale, (2) Scales for Outcomes in Parkinson's disease – Psychosocial Functioning (SCOPA-PS Scale), (3) Beck Depression Inventory Scale, (4) Montreal Cognitive Assessment (MoCA Scale) symptoms of Parkinson's disease were assessed; as well as (5) quality of life score Parkinson's Disease Questionnaire (PDQ-39). The data analysis was performed via statistical program StatsDirect, using descriptive, variation, and correlational analysis. Student's t tests or Mann-Whitney tests were used as appropriate. P values less than 0.05 were considered statistically significant.

Results

The study group consisted of 102 consecutive PD patients. The mean age in the cohort was 61.51 ± 8.87 y.o. with mean disease duration of 65.78 ± 41.34 mo. By sexes, PD patients, were distributed as follows: 55 were women (53.9%) and 47 were men (46.1%)). Pain, as a self-reported symptom was present in 64 patients (62.7%) of all the study group; and in 90% of cognitively preserved patients, according to MoCA (Montreal Cognitive Assessment test) scores ranging from 30 to 24. In this study pain was encountered more frequently in women (36p (56.3%)) than in men (28p (43.8%), p> 0.05).

No differences were established in the ages of "PD pain +" and "PD pain -" patients (61.02 ± 9.61 vs. 62.51 ± 7.69 y.o.) in the study, as well as in levodopa equivalent daily taken dose (729.85 ± 483.29 vs. 708.31 ± 357.50), in both groups.

Patients with motor complications were more prone to complain pain. So, the majority of the patients with motor fluctuations reported the presence of pain (21p (72.4%) vs. 8p (27.6%), p> 0.05). A similar situation was encountered in the subgroup of dyskinesia patients (16p (64.0%) vs. 9p (36.0%), p> 0.05). An expected result of the study was a significantly more presence of pain in the restless leg syndrome subgroup of patients (8p (72.7%) vs. 3p (27.3%), p> 0.05).

"PD pain +" patients exhibited an akinetic– rigid phenotype of disease, with higher Akinetic-Rigid Scores (0.83 ± 0.80 vs. 0.64 ± 0.56, p> 0.05), revealing more motor impairment. Also, they had a longer disease duration (74.19 ± 39.99 y.o. vs. 53.29 ± 41.06 y.o. p=0.017), compared to pain free patients.

Other non-motor symptoms were more prevalent in "PD pain +", objectified by higher Non-Motor-Symptoms scores (NMS: 66.27 ±39.25 vs. 46.68 ± 32.56, p=0.015), by contrast with "PD pain -" patients. PD patients complaining pain were more depressive, and had a higher psychological involvement, as highlighted by Beck and SCOPA-PS tests (Beck DI: 16.36 ± 11.97 vs. 8.09 ± 6.42, p=0.000), psychological (SCOPA-PS: 10.28 ±6.20 vs. 4.77 ± 2.82, p=0.000)

The quality of life scores (PDQ39: 59.70 ± 25.46 vs. 53.84 ±35.76, p> 0.05) were higher in "PD pain +" patients, but did not reach the statistical significance.

Discussion

The data on prevalence of pain in PD patients (62.7% of all the study group) are in line with the existing data that states a pain prevalence ranging from 40 to 75% in PD patients [1]. A complete consensus on the relationship between pain and gender in the literature does not exist [11]. There are studies reporting that pain is more frequent in females than males and there are also studies reporting that a pain-gender relationship in PD does not exist [12]. However, in some reports, with a large cohort of patients, aiming non-motor symptoms were assessed [13], pain was reported as more prevalent in PD females than in PD males, which is in agreement with the received study results.

There were similar ages (61.02 ± 9.61 vs. 62.51 ± 7.69 y.o.) between groups in the study. Younger age has been reported to be associated with pain in some studies [7], while disease progression has been reported as a risk factor for pain in PD in others [14].

There was not found significant difference in levodopa equivalent daily dose in "BP pain +" and "BP pain –" patients (729.85 ± 483.29 vs. 708.31 ± 357.50). While no correlation has been found between spontaneous pain and daily levodopa dose, some studies have reported that pain of variable quality and localization may fluctuate in intensity during OFF and ON states, particularly in presence of dyskinesia [9]. In this study pain was more prevalent in patients with motor fluctuations (21p (72.4%) vs. 8p (27.6%), p> 0.05), and with dyskinesia (16p (64.0%) vs. 9p (36.0%), p> 0.05). Some uncontrolled observations indicate that spontaneous pain may be minimized by strategies like continuous dopaminergic release and stimulation that usually improve levodopa-related motor complications [4]. Painful symptoms tend to worsen in PD patients who are off medication. For many individuals, however, pain sensations occur in strict relation to the motor fluctuations of the disease, and are ascribed to non-motor fluctuations [11].

Patients with pain in the present study had higher Akinetic-Rigid Score (0.83 ± 0.80 vs. 0.64 ± 0.56, p> 0.05) – indicator of a more severe disease. Another study found also significant correlations between UPDRS part II (p< 0.001), UPDRS part III (p = 0.002), rigidity (p = 0.001), bradykinesia (p = 0.001) and PIGD subscores (p = 0.009), dyskinesia (p = 0.001), wearing off (p = 0.001) with pain [15]. A positive relationship was established between chronic pain occurring in PD patients and the severity of the disease in the literature [12].

PD pain patients also had longer disease duration (74.19 ± 39.99 y.o. vs. 53.29 ± 41.06 y.o. p=0.017). A significant correlation was previously described between chronic pain, Hoehn & Yahr stage of PD and patients age [15]. No relationship between pain and age at diagnosis, disease duration, motor examination or PD stage was observed in several studies [14]. Thus, conflicting results are reported regarding the role of PD progression in the onset of pain, and this could be related to different pain

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subtypes occurring at different times in the course of PD. Thus, some studies reported that all types of pain are more prevalent in patients with late-stage PD than in early stages, while only nociceptive arthritic pain was more prevalent in early-stage PD [16].

Concerning other non-motor symptoms, the pain PD patients in this study had higher scores for: depression, psychological and non-motor symptoms. There is a bi-directional relationship among depression, common non-motor symptoms of PD and pain. Chronic pain may be a risk factor for depression, especially in the elderly people. Pain may negatively affect the prognosis of depression, and persistent pain may accelerate depression. From the other perspective, depression may affect the perception of pain, and may contribute to the pain becoming severe and refractory to treatment [17]. Results are inconsistent in the literature regarding pain-depression relationship in PD. In fact, depression is more common in PD patients suffering from pain and furthermore, PD patients with major depression suffer from more severe pain compared to patients without depression [18]. Also, pain is described as closely related to other non-motor symptoms as fatigue, daytime sleepiness, and sleep disorders.

Thus insignificantly, quality of life score PDQ39 was higher in "PD pain +" patients. It is known that, independently from the other motor symptoms of PD, chronic pain, accompanying the disease, adversely affects the daily living activities and quality of life of patients and becomes thus the cause of morbidity and disability. Another study, comprising 265 consecutive PD patients, reported that in patients with less than 6 years disease duration, pain was the fourth most discomforting non-motor symptom related to PD, while in patients with more than 6 years disease duration pain was reported to be the sixth most discomforting non-motor symptom related to PD [19].

Also, in BP, pain has been shown to be a major factor affecting quality of life related to physical and mental health [20] and also leads to reduced autonomy. A significant relationship between pain and depression has been reported in BP, suggesting that pain issues should be considered when treating BP patients with depression and vice versa. However, pain is an independent predictor of poorer quality of life, independent of its close relationship with depression. Pain has an effect on quality of life that is greater than motor impairment and comparable to the effect of motor complications [21]. So, pain is an important factor affecting the health-related quality of life, that's why prevention of chronic pain and / or treatment of the already existing pain may have significant effect in increasing PD patient quality of life.

Conclusions

Pain is common in patients with Parkinson's disease and affects women more than men. It is more prevalent in patients with longer disease duration, in those with more severe motor impairment, also in patients who have developed motor fluctuations. Pain is associated with high depression scores and is more prevalent in patients with other PD specific non-motor symptoms.

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LR conceptualized the idea, wrote the manuscript. LR, OG, SO and IO revised and finalized the text. All authors revised the manuscript critically and approved the final version of the manuscript.

Ethics approval and consent to participate
The research project was approved by the Research Ethics Committee of *Diomid Gherman* Institute of Neurology and Neurosurgery (protocol No 4, 29.09.2022).

Conflict of Interests
No competing interests were disclosed.

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Retinopathy of prematurity, the prevalence and risk factors in Moldova

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Abstract

Background: Retinopathy of prematurity (ROP) is a serious disease that affects premature infants and still represents the leading cause of blindness worldwide that can be prevented if detected earlier in time.
Material and methods: The ROP prospective observational study was performed enrolling all premature infants admitted to the Intensive Neonatal Care (INC) and Premature Care Unit from January 2020 to December 2021 with the gestational age (GA) of 32 weeks and less at birth and body weight (BW) of 2000 g and less. A total of 98 premature infants had retinal evaluation by indirect ophthalmoscopy starting with the five postpartum weeks followed every 7-10 days until 38 weeks and then every 2 weeks until 42-45 weeks. The severity of ROP was graded according to the International classification of ROP. The effects of GA and BW on the prevalence and severity on ROP were evaluated.
Results: Out of studied 98 infants, 36 patients (36.7 %) developed ROP stage 1 and 2, in one or both eyes, 3 (3.07%) infants developed stage 3. Out of these 3 premature infants with stage 3, one underwent avastin intravitreal injection with successful regression, 2 patients underwent laser photocoagulation treatment successfully.
Conclusions: The prevalence of ROP in this unit-based study was 36 patients (36.7 %). The most important risk factors: Low gestational age, and low body weight. Lower gestational age and body weight was a risk factor, as the greatest number (76%) 20 infants out of 26 with GA ≤ 29 weeks and BW ≤1000g developed ROP stage 1 and stage 2; 3.06% (3 infants) developed stage 3. Very important in preventing ROP vision loss, screening all infants at risk regardless of GA and BW as well as the duration of staying in INC represents the greatest priority.
Key words: retinopathy of prematurity, newborn, gestational age, risk factors.

Introduction

Retinopathy of prematurity (ROP) represents the leading cause of preventable worldwide blindness in infants. Currently we are facing a tremendous survival premature rate associated with increasing incidence of ROP.

Embryonic retinal arteries start to grow in the third month of the pregnancy and their development ends at birth. Therefore, the stages of the evolution of the eye are defective in prematurity and the growth of normal vessels is stopped. After premature delivery the process is associated with abnormal neovascularization of the retina.

International classification of ROP is a consensus statement that creates a standard nomenclature for classification of ROP [1]. The International Classification describes ROP by location zones and severity stages, as well as plus and preplus disease: Stage 1 – Demarcation line separates avascular retina from vascularized retina; Stage 2 – Ridge from demarcation line; Stage 3 – Extra retinal neovascularization; Stage 4-5 – Partial and total retinal detachment.

Currently the ROP is under constant epidemiological study around the world. GA and BW are the essential factors determining the ROP. According to Moldovan screening guideline all preterm babies with gestational age of 32 weeks and less, and body weight of 2000 g and less should be screened. According to the United Kingdom screening guideline body weight (BW) less 1500 g and gestational age (GA) less 32 weeks require indirect ophthalmoscopy. United States has validated new ROP screening criteria, BW less than 1500 g, and GA less than 30 weeks.

Material and methods

This was an institutional unit-based cohort prospective study of 98 preterm infants admitted to Intensive Neonatal Care and Premature Care Unit of the *Gheorghe Paladi* Municipal Clinical Hospital in Chisinau, the Republic of Moldova, from January 2020 to December 2021.

The examination was carried out according to the ROP guidelines recommended by Moldovan Ophthalmology Society: Preterm infants with GA ≤ 32 weeks, and BW ≤

2000 g, were examined. The severity of ROP was graded according to the International classification of ROP. The first examination was performed at 4-5 weeks postpartum under aseptic precautions in a temperature-controlled room. Infants with no ROP sign were examined every 2 weeks until 45 weeks. If ROP was found (demarcation line), examination was repeated every week until 45 weeks. Pupils were dilated with tropicamide 0.5% and phenylephrine 0.5% eye drops, pediatric eyelid speculum was used. Indirect ophthalmoscopy was performed. The 28D diopter condensing lens was used.

Results

A total of 98 preterm infants enrolled in this study from January 2020 to December 2021 screening data are shown in table 1. A total of 36 infants (36.7%) out of 98 have been detected with ROP. GA and BW show significantly different pattern between infants with and without ROP. In general, the proportion of ROP increased with lower BW and lower GA (fig. 1, 2). Lower gestational age and body weight were a risk factor, as the greatest number 20 infants (76%) out of 26 with GA ≤ 29 weeks and BW ≤1000 g developed ROP stage 1-2; 3 infants (3.06%) developed stage 3. While in infants' group with BW 1001 g – 1500 g 16 patients (24.6%) out of 65 developed ROP.

Fig. 1. Correlation BW-ROP

■ 750-1000g - 76%   ■ 1001-1500g - 24%
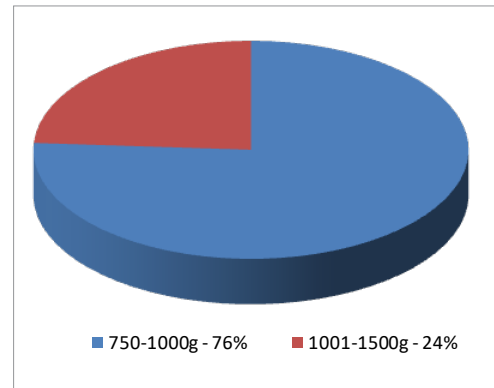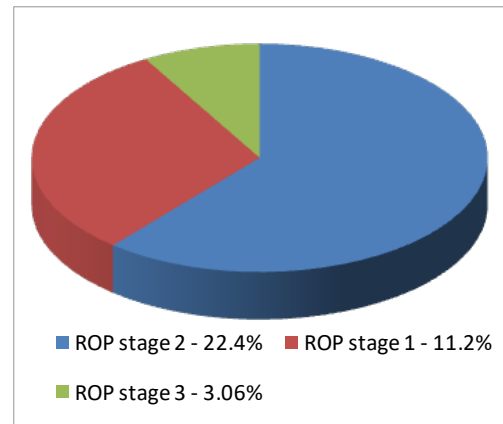
Fig. 2. ROP stage proportion

■ ROP stage 2 - 22.4%   ■ ROP stage 1 - 11.2%
■ ROP stage 3 - 3.06%

Table 1. Proportion of infants with different ROP stage

	No ROP	Total ROP	ROP Stage 2	ROP Stage 1	ROP Stage 3	AP-ROP	Number of infants examined
BW, g							
≤ 750		4		3	1	0	4
751–1000	6	16	11	4	1	0	22
1001–1250	25	10	6	3	1	0	35
1251–1500	24	6	5	1	0	0	30
1501–2000	7	0	0	0	0	0	7
Total	62 63.2%	36 36.7%	22 22.4%	11 11.2%	3 3%	0	98 100%
GA, weeks							
GA ≤ 26w		4		3	1	0	4
27-29 w	6	16	11	4	1	0	22
29-30w	27	10	6	3	1	0	37
31-32w	29	6	5	1	0	0	35
Total	62 63.2%	36 36.7%	22 22.4%	11 11.2%	3 3.06%	0	98 100%

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Discussion

The control of blindness in children is a priority within the World Health Organization Vision 2020 program. The World Health Organization Vision 2020 program defines ROP as "avoidable disease" [2]. After an improvement in child health care in Moldova, and ROP screening, rate of blindness decreased in our country.

The prevalence and treatment outcome of ROP in the world are affected by social factors, such as economic development, and the healthcare level of premature infants. With the establishment and improvement of ROP screening and treatment schedules in developed countries, the prevalence of ROP has been declining. On the other hand, the survival rate of very low BW premature infants and critically ill infants has been increasing. The prevalence of ROP in the USA increased from 14.70% in 2000 to 19.88% in 2012 [3-5]. The frequency of ROP was 2.4% in newborns weighing more than 2.500g and 30.2% in newborns with birth weight between 750-999g. So, the increasing number of ROP was associated with simultaneous decline in newborn mortality. In a national study in the UK between 1997-1999 the treatment rate was 59% while in 2013-2014 – 62.39% of newborns developed type 1 and 8.26% of infants had aggressive posterior ROP so the treatment rate was 2 times higher than previously estimated [6-9]. In Taiwan between 2002-2011 were reported 36.6% of infants with ROP.

According to recent data India accounts for 10% (about 280.000) of worldwide ROP related blindness due to low screening rate and low health care services of premature babies [10-14]. Five-year demographic profile of ROP in a tertiary institute in North India from 2013-2017 reveals 32.3% of infants with ROP from which 28% were detected with aggressive posterior ROP (AP-ROP). In Turkey, a study revealed during 2020-2022 the prevalence of ROP was about 16% [15-16]. While in China during 2016-2020 the ROP prevalence was 17.9%. As well as in Egypt during 2018-2020 the overall prevalence of ROP was 34.1% [17-18].

Importantly, in Italy in a prospective observational multicenter study between January 2008 – December 2009 there were registered 62.9% of preterm infants with ROP and 34% of infants required surgical treatment [19].

In Romania, according to an institutional publication the prevalence of ROP was estimated to be 40-50% with treatment rate 9%-16%. And at least 100 blind children born from 2002-2017 were attributed to missed screening [20].

Interestingly, for example in Japan, in an institutionally-based study between 2009 and 2011 there were registered 70.6% of ROP in infants born before 28 weeks with mean BW 779g at a neonatal intensive care unit Red Cross Sendai Hospital Japan [21].

Most screening guidelines were drawn up based on GA and BW which are the identified risk factors of ROP. Other factors, such as, long-term fluctuation oxygen therapy,

long intubation period, necrotizing enterocolitis, serious systemic diseases, intraventricular hemorrhage, multiple blood transfusions, and long-term hospitalization were reported to correlate to ROP [22-23].

More published research demonstrates the magnitude of this real public health care problem that persists not only in low-income countries.

The prevalence of ROP in this unit-based study was 36.7% which is relatively higher, and the treatment rate was 3% which involved patients with stage 3, two preterm infants underwent laser photocoagulation therapy, and one underwent intravitreal anti-vessels endothelial growth factor injection with no reactivation. Otherwise, there were 4 infants with stage 3 and there were not detected ROP stage 4 or stage 5 nor aggressive posterior ROP.

This study indicates that the prevalence of ROP increases gradually with the lower GA and BW. GA and BW are still the most important risk factors for ROP.

Conclusions

GA and BW are still the major risk factors in the evolution and severity of ROP. According to this institutional, unit-based study the prevalence of ROP was 36%. The proportion of ROP increased with lower gestational age and lower body weight, as the greatest number as 76% of infants with GA ≤29 weeks and BW ≤1000g developed ROP stage 1 and stage 2. There were not registered ROP stage 4 or 5 nor AP-ROP. The treatment rate in this study was 3%.

ROP screening criterion of BW ≤2000g, or GA ≤32 weeks proved to be the most effective in Moldova as well.

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Authors' contributions
LM conceptualized the study and drafted the first version of the manuscript, interpreted the data. EB and AC critically revised the manuscript. All the authors revised and approved the final version of the manuscript.

Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, proceedings No 01/26.08.2016.

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ORIGINAL RESEARCH

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Myopia progression in anisometropic amblyopia during combined treatment with orthokeratology and physiotherapy

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Abstract

Background: Myopia is labeled as one of the most common eye disorders, one of the most effective methods of its treatment being orthokeratological treatment. Anisometropic amblyopia continues to be treated by optical correction applied separately or in combination with occlusion or other therapies.
Material and methods: The study included 32 patients (64 eyes), who were divided by 8 patients (16 eyes) into 4 groups (2 primary and 2 control), depending on the presence or absence of amblyopia and the degree of myopia (small and medium). Subjects in the baseline group underwent combined treatment between orthokeratology and physiotherapy.
Results: The combined treatment resulted in the decrease of myopia according to the spherical equivalent by 60% in patients with amblyopia and mild myopia and from 90% in those without amblyopia. The values of the antero-posterior axis had a similar dynamic ($p <0.001$). In patients with moderate myopia its evolution decreased by 95% compared to patients with amblyopia – by 60%. The degree of anisometropia decreased by 10% ($p > 0.05$). Corrected visual acuity depending on the degree of amblyopia increased by 50% in cases with mild amblyopia and 150% in cases with moderate amblyopia ($p < 0.001$). The absolute volume of accommodation increased by 70% in patients with mild amblyopia and by 300% in patients with moderate amblyopia ($p <0.001$).
Conclusions: It is rational to apply refractive therapy in the treatment and prevention of acquired uncomplicated myopia, with an average index of quality of life of 93.1%, versus 39.3% for optical correction.
Key words: myopia, anisotropia, orthokeratology, physiotherapy, combined treatment.

Introduction

Myopia has been labeled as one of the most common eye disorders. Progression of myopia can lead to significant irreversible changes in the eyeball resulting in loss of vision. Complicated myopia is one of the main causes of invalidation as a result of ocular pathologies [1-5]. The frequency of myopia in developed countries of the world is 19-42%, reaching in some eastern countries 70% [6, 7]. The prevalence of eye diseases and their appendages among the population of the Republic of Moldova is represented by three diseases: cataract, myopia and glaucoma. The annual average prevalence of myopia is 45.5 cases per 10 thousand inhabitants. In the period 2007-2011 the given indicator was 1.5 times higher than in the period 2003-2006. The annual average incidence of myopia is 9.1 cases per 10000 inhabitants, with extreme values mA of 6.4 cases per 10000 inhabitants in 2006 and 11.8 cases per 10000 inhabitants in 2011. In the structure of the prevalence and incidence of eye diseases in children, most cases are due to myopia. The average annual prevalence of myopia is 93.9 cases per 10000 children. The annual average incidence of myopia

in children in the Republic of Moldova (2003-2011) is 23.4 cases per 10 thousand inhabitants [8]. Anisometropic amblyopia was clinically identified in 1743 by George Louis Leclerc, Count of Buffon, who proposed a treatment that is still applied today. Anisometropic amblyopia continues to be treated by optical correction applied separately or in combination with occlusion or other therapies [9]. In school-age children, the prevalence of anisometropia is 2.7% (age 7), up to 5.8% (age 9) [10]. According to the multiethnic studies of MEPEDS and Baltimore Pediatric Eye Disease (2011), the prevalence of anisometropia by grade was 20% – ≥0.50D, 3.8% – ≥1D and 0.7% – ≥2D. According to Vries' study of a group of anisometropic children (difference ≥2D), the prevalence of anisomyopics, anizohypermetropics and antimetropics was 20%, 70% and 10%, respectively [11].

The aim of the study was to assess myopia progression in patients with anisometropic amblyopia who have undergone orthokeratological treatment combined with physiotherapy.

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Material and methods

The study included 32 patients (64 eyes), who were divided by 8 patients (16 eyes) into 4 groups (2 primary and 2 control), depending on the presence or absence of amblyopia and the degree of myopia (small and medium). Subjects in the baseline group underwent combined treatment between orthokeratology and physiotherapy.

Physiotherapy consisted of performing for 10 days, every day, successively, a complex of physiotherapeutic procedures with an interval of 5-10 min between them. Initially it acts for 1 min. on each acupuncture point, selected from the general biologically active points GI4, GI11, E36, TR5, with electric current with an intensity of 25-60 mA of negative polarity and on the local acupuncture points V1, E1, VB1, BT5, V2, HT1, HT2, HT3, HT9 with electric current with intensity of 15-20 mA with positive polarity. Stimulation of the ciliary muscle with low-intensity helium-neon radiation laser is then performed for 4 min. The orthokeratological treatment consisted in the application for 3 years of orthokeratological contact lenses for night wear "Paragon CRT-100", made of HDS material (paflufocon B – fluorosilicone acrylate) with high oxygen permeability (OSO-ANSI Dk-100). The lenses were selected automatically by means of the program "CRT Topography Software" in the process of assessing the topography of the cornea using the device "Keratograph 4" (Oculus, Germany).

Patients in the control group wore monofocal aerial optical correction, which was changed according to possible refractive changes during the study.

The criteria for including patients in the study were: 8 years of age; the presence of the family factor; acquired axial myopia; anisometropic amblyopia; central fixation.

Objective data were collected by the following methods: visiometry, autorefractorkeratometry, pachymetry, corneal topography, non-contact Norn test, absolute accommodation volume, non-contact tonometry, anterior pole and posterior pole biomicroscopy, biometrics, optical coherence tomography of the optic nerve and region centers of the retina.

Results and discussion

Figure 1 shows the dynamics of the annual gradient of myopia progression (AGMP) according to the spherical equivalent and AGMP according to the anterior-posterior axis in patients with low myopia.

The data obtained in the study show that in the group with mild myopia – with the presence of amblyopia, after one year of applying the combined treatment, AGMP according to the spherical equivalent decreased from 0.93 ± 0.08 D to 0.13 ± 0.03 D (by 0.8 D; p<0.001), and in the control group, without amblyopia, this index decreased from 0.94 ± 0.08 D to 0.32 ± 0.7 D (by 0.62 D; p>0.05). After 2 years, in the main group, AGMP according to the spherical equivalent decreased compared to the initial data to 0.09 ± 0.02 D (by
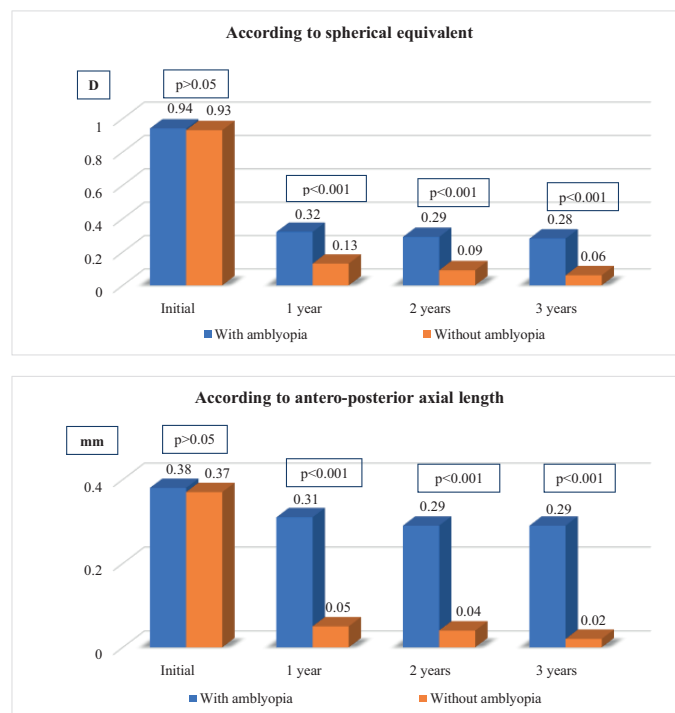
Fig. 1. Dynamics of the AGMP according to spherical equivalent and AGMP according to anterior-posterior axis in patients with mild myopia

0.84 D; p<0.001), while in the control group – to 0.29 ± 0.07 D (with 0.65 D; p>0.05). After 3 years, in the main group, AGMP according to the spherical equivalent decreased to 0.06 ± 0.01 D (by 0.88 D; p<0.001), compared to the control group, in which this index decreased to 0.28 ± 0.19 D (with 0.66 D; p>0.05).

In the group with moderate myopia and the presence of amblyopia, after one year of combined treatment, AGMP according to the spherical equivalent decreased from 1.54 ± 0.25 D to 0.19 ± 0.03 D (by 1.35 D; p< 0.001) compared to the control group, where this index decreased from 1.38 ± 0.29 D to 0.53 ± 0.08 D (by 0.85 D; p>0.05). During the following year, AGMP according to the spherical equivalent decreased to 0.04 ± 0.01 D (by 1.5 D; p<0.001) in the main group, compared to the control group – to 0.32 ± 0.07 D (by 1.06 D; p>0.05). After 3 years, in the main group, AGMP according to the spherical equivalent decreased to 0.03 ± 0.01 D (by 1.51 D; p<0.001), compared to the control group – to 0.32 ± 0.07 D (by 1.1 D; p>0.05). The difference between the data obtained in the main group and in the control group at the end of the study was statistically true (p<0.001), which demonstrates a higher efficiency of the combined treatment than the optical correction.

In the main group with mild myopia and the presence of amblyopia, after one year of treatment, the value of the AGMP according to the anterior-posterior axis decreased from 0.37 ± 0.08 mm to 0.05 ± 0.01 mm (by 0.32 mm; p<0.001), compared to the control group – from 0.38 ± 0.08 mm to 0.31 ± 0.06 mm (by 0.07 mm; p>0.05).

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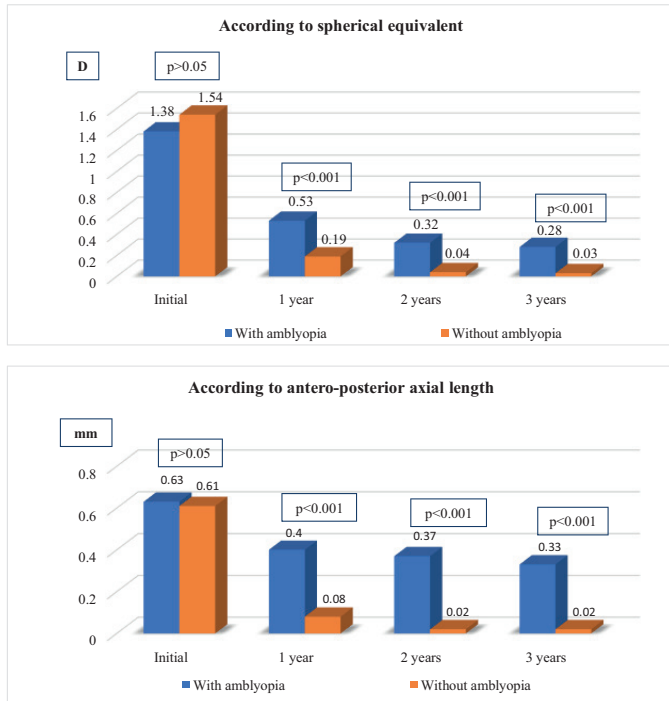
Fig. 2. Dynamics of the AGMP according to spherical equivalent and AGMP according to anterior-posterior axis in patients with moderate myopia

During the following year, the AGMP according to the anterior-posterior axis value decreased to 0.04±0.01 mm (by 0.33 mm; p<0.001) in the main group, compared to the control group – to 0.29±0.07 mm (by 0.09 mm; p>0.05). After 3 years in the main group, the AGMP according to the anterior-posterior axis value decreased to 0.02±0.01

Fig. 3. Dynamics of the AGMP according to the degree of amblyopia

mm (by 0.35 mm; p<0.001), compared to the control group, in which this index remained constant (p >0.05). The difference between the data obtained in both groups at the end of the study was statistically credible (p<0.001), which demonstrated a higher efficiency of the combined treatment compared to optical correction.

In the main group with moderate myopia and the presence of amblyopia, after one year of combined treatment, the AGMP according to the spherical equivalent value decreased from 0.61±0.14 mm to 0.08±0.02 mm (by 0.53 mm; p<0.001), compared to the control group – from 0.63±0.15 mm to 0.4±0.12 mm (by 0.23 mm; p>0.05).

During the following year, the AGMP according to the spherical equivalent value decreased to 0.02±0.01 mm (by 0.59 mm; p<0.001) in the main group and to 0.37±0.09 mm (by 0.25 mm; p>0.05) in the control group. After 3 years in the base group, the AGMP according to the spherical equivalent value remained the same (p<0.001), and in the control group it decreased to 0.33±0.08 mm (by 0.3 mm; p>0.05). The difference between the data obtained in the main group and in the control group at the end of the study was statistically true (p<0.001). This fact demonstrates a higher efficiency of combined treatment compared to optical correction.

Thus, in patients with mild amblyopia, AGMP according to the spherical equivalent decreased from 0.94±0.24 D to 0.32±0.08 D (by 0.58 D; p<0.001), and in those with amblyopia of moderate degree – from 1.38±0.39 D to 0.53±0.15 D (with 0.85 D; p<0.001). During the 2nd year of the study, the AGMP according to the spherical equivalent value decreased statistically insignificantly compared to the previous data in both groups: in patients with mild amblyopia – down to 0.29±0.08 D (by 0.03 D; p>0, 05); in patients with moderate amblyopia – down to 0.32±0.09 D (with 0.21 D; p>0.05). After 3 years of treatment, the AGMP according to the spherical equivalent value was 0.28±0.06 D in both groups, the difference between the groups being statistically insignificant (p>0.05).

In the same groups, with mild amblyopia, after one year of combined treatment, the AGMP according to the anterior-posterior axis value decreased from 0.38±0.11 D to 0.31±0.08 D (by 0.07 D; p<0.01), compared to patients with moderate amblyopia, where this index decreased from 0.63±0.18 D to 0.4±0.14 D (by 0.23 D; p<0.001). During the following year, the AGMP according to the anterior-posterior axis value decreased to 0.37±0.1 D in patients with low degree amblyopia (p>0.05) and to 0.29±0.08 D (p>0.05) in patients with moderate amblyopia. After 3 years, the AGMP according to the anterior-posterior axis value remained statistically unchanged compared to the previous data in both groups (p>0.05), but much more significant compared to the initial data. The difference between the data obtained at the end of the study in the two groups was statistically insignificant (p>0.05), which demonstrated a high efficiency of the combined treatment both in patients with amblyopia and in those without.

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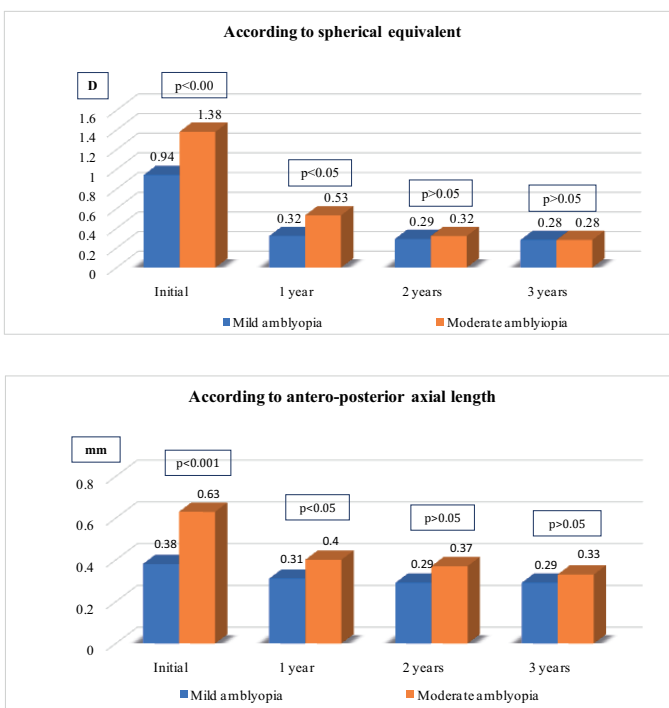
The study of the anisometropia value demonstrated a statistically significant decrease of this index under the influence of the combined treatment. Thus, initially it was 3.43±0.85 D and decreased in the first year of study to 3.22±0.64 D (p<0.001), in the second year – to 3.19±0 .34 D (p>0.05) and down to 3.18±0.28 D (p>0.05) in the third year of study.

A high direct correlation was also established between the annual gradient of myopia progression and the dynamics of the degree of anisometropia R2=0.8885.

Fig. 4. Comparative dynamics of the degree of anisometropia

Fig. 5. Dynamics of corrected visual acuity according to the degree of amblyopia

In both study groups, the increase of corrected visual acuity (CVA) was recorded following the application of the combined treatment. Thus, in the group with mild amblyopia the CVA increased from 0.63±0.12 to 0.87±0.15 after one year of treatment, to 0.95±0.21 and to 0.97±0.22 after 2 and 3 years of treatment, respectively (p<0.001). In the group with moderate amblyopia this index increased from 0.26±0.08 to 0.5±0.1, 0.62±0.12 and 0.72±0.15 after 1, 2 and 3 years of treatment, respectively (p<0.001).

In the group with mild amblyopia, after one year of combined treatment, the absolute volume of accommodation (AVA) value increased from 7.0±0.41 D to 11.4±0.67 D (by 4.4 D) compared to the group with moderate amblyopia, where this index had a less obvious dynamic (from 2.2±0.41 D to 4.6±0.48 D – by 2.4 D), the difference between the groups being statistically significant (p<0.001). After 2 years of study, the AVA increased up to 12.0±0.73 D in the group with low-grade amblyopia and up to 6.13±0.51 D in the group with medium-grade amblyopia; after 3 years – up to 12.0±0.78 D in the group with low-grade amblyopia and up to 9.5±0.58 D in the group with

Fig. 6. Dynamics of the absolute volume of accommodation according to the degree of amblyopia

medium-grade amblyopia. The difference between the data obtained in both groups at the end of the study was statistically insignificant (p>0.05).

Conclusions

1. The study demonstrated that in patients with amblyopia the annual gradient of myopia progression was statistically significantly higher compared to patients without amblyopia, regardless of the degree of myopia (on average by 0.74 D depending on the spherical equivalent and by 0.88 mm depending on the length of the eye globe axle).

2. It was determined that in patients with moderate amblyopia the annual gradient of myopia progression had a statistically significant increase (by 0.14 D and 0.15 mm) compared to patients with low degree of amblyopia.

3. The obtained results demonstrated that the application of the combined treatment decreases the degree of anisometropia by an average of 7.3%. A high direct correlation (r=0.88) was established between the annual gradient of myopia progression and the degree of anisometropia.

4. As a result of the combined treatment, the corrected visual acuity increased from 0.63 to 0.97 in cases of mild amblyopia and from 0.26 to 0.72 in cases with moderate amblyopia.

5. The study of the absolute volume of accommodation demonstrated the increase of this index in both groups, but more significantly in cases with mild amblyopia (up to 12 D) compared to the moderate myopia (up to 9.5 D).

6. For a more effective treatment of anisomyopic amblyopia, it is recommended to control the progression of myopia, taking into account its high correlation with the evolution of amblyopia.

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Authors' contributions
RB conceptualized the project and drafted the first version of the manuscript. VC and LD interpreted the data and critically revised the manuscript, GN collected the data. All the authors revised and approved the final version of the manuscript.

Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, protocol No 01, 24.08.2022. The informed consent was received from every patient.

Conflict of interests
No competing interests were disclosed.

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REVIEW ARTICLE

REVIEW ARTICLES

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Post-pulmonary embolism syndrome: long-term complications of pulmonary embolism

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Abstract

Background: People who survive an episode of pulmonary embolism (PE) have an increased risk of developing chronic complications even despite curative anticoagulant treatment. The association of dyspnea, low functional capacity, right heart failure, chronic thromboembolic pulmonary hypertension or chronic thromboembolic pulmonary disease is part of the notion of post-pulmonary embolic syndrome (PPES). Due to the fact that this syndrome is still not clearly described and mainly underdiagnosed, a poor awareness of the disease by patients and physicians leads to delaying specific treatment with unlikely improvement of quality of life for these patients. Chronic thromboembolic pulmonary hypertension is the most severe complication, which, if not diagnosed and not treated in time, can lead to fatal consequences. To improve the overall health outcomes of patients with acute PE, adequate measures to diagnose it and strategies to prevent long-term outcomes of pulmonary embolism are essential.
Conclusions: In this article, the data from the latest publications have been summarized to clarify the notion of PPES and its diagnostic algorithm.
Key words: thromboembolic pulmonary hypertension, post-pulmonary embolism syndrome, long-term outcomes.

Introduction

Following the researches carried out over the last 10 years, it has been demonstrated that less than half of the survivors of a pulmonary embolism do not return to their previous functional capacities. These patients in which persist right ventricle (RV) dysfunction continue to present with dyspnea, impaired functional status, diminished exercise capacity, becoming so limiting that they significantly reduce the quality of life (QoL) for many years ahead [1, 2]. The evolution of a patient with pulmonary embolism (PE) diagnosed and treated adequately, can be complicated by hemorrhages caused by anticoagulant treatment, recurrence of PE and long-term sequelae. People who survive an episode of pulmonary embolism are at increased risk of complications. In addition to the traditional complications (recurrent pulmonary embolism, anticoagulation-associated bleeding and cardiovascular events), the quality of life can be significantly affected by persistent dyspnea and sometimes disabling symptoms.

In this context, in order to systematize these patients,

the term post-pulmonary embolism syndrome (Post-PE syndrome or PPES) was proposed. Post-PE syndrome is defined by the presence at least 3 months after an acute episode, despite adequate anticoagulant treatment, of new or progressive dyspnea, a decrease in exercise tolerance and/ or an unexplained impairment of functional or mental status, by the existence of other comorbidities [3]. The notion of PPES includes chronic thromboembolic pulmonary hypertension (CTEPH) and chronic thromboembolic pulmonary disease (CTEPD), post-PE cardiac dysfunction (heart failure with persistent right ventricular impairment), and post-PE decreased functional capacity (physical deconditioning) [4, 5]. Decreased functional capacity includes changes in the ability to care for oneself, to perform usual household or work activities, following physical, cognitive or intellectual impairments. Such a patient will be included in the diagnosis of post-pulmonary embolism syndrome [6]. Recently a consensus was developed that helps an early identification of patients with late outcomes of pulmonary embolism and aims to improve their prognosis [3, 7, 8].

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Discussion

At the initial stage of diagnosis of the post-PE syndrome, it is important to exclude other comorbidities by performing early diagnostic functional tests, initiating their specific therapy in order to diminish the establishment of characteristic symptoms. The lack of clear guideline recommendations as well as inefficient application of diagnostic tests in clinical practice lead to a reported staggering diagnostic delay >1 year.

Chronic thromboembolic pulmonary hypertension is considered to be the most frequent and serious complication described in post-pulmonary embolism syndrome, which affects 2-4% of pulmonary embolism survivors [9]. An international CTEPH registry (Europe and Canada) indicated that 75% of patients with CTEPH had a documented antecedent history of acute pulmonary embolism [10]. The incidence of CTEPH after symptomatic acute pulmonary embolism is reported to range from 0.4% to 6.2% [11]. This complication, not treated in time, can even lead to death. To minimize the incidence of chronic complications after an episode of pulmonary embolism, it is important to identify and adequately treat patients with CTEPH as soon as possible. Chronic thromboembolic pulmonary hypertension is characterized by persistent obstruction of the pulmonary arteries by organized thromboembolic material associated with microvascular remodeling, which leads to an increased pulmonary vascular resistance, pulmonary hypertension (PH) and right heart failure [11]. Unilateral pulmonary artery obstruction represents a particular subset of CTEPH/CTEPD [12]. The mechanism of pulmonary hypertension in CTEPH is multifactorial. The most frequently discussed cause of pulmonary arterial hypertension is the persistence of the thrombus in the pulmonary arteries after an acute episode of pulmonary embolism. In a small subset of patients, a residual organized clot remains attached to pulmonary vessel walls. The complex pathophysiology of this entity primarily includes the failure of intra-arterial thrombus reabsorption, even in patients on adequate anticoagulant therapy, the pathophysiology of which remains not fully elucidated. Besides that, recent insights have revealed a small-vessel disease involved in CTEPH pathophysiology, including inflammation and infections that may be provocative for pulmonary hypertension (fig. 1).
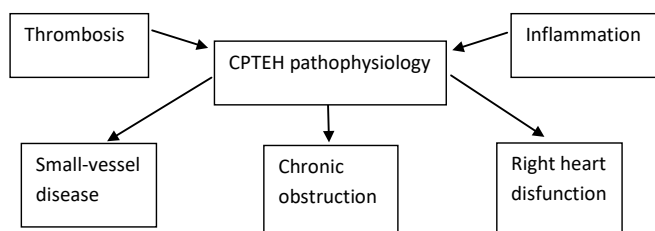
Fig. 1. Pathophysiology of chronic thromboembolic pulmonary hypertension (CTEPH) [13]

Lung ventilation/perfusion scintigraphy is the screening test of choice (fig. 2); a normal scan rules out CTEPH. For the diagnosis of CTEPH, a high-quality pulmonary angiogram is necessary to confirm and define the pulmonary vascular involvement. Prior making a treatment decision a right heart catheterization should be performed, in which a persistently pulmonary artery pressure greater than 20 mmHG presented over 3 months after an episode of pulmonary embolism, will establish the final diagnosis of CTEPH [11]. It is essential to diagnose chronic thromboembolic pulmonary hypertension in time to redirect them to a specific treatment. In addition to the medication widely used in patients with arterial hypertension, there is the possibility of performing invasive maneuvers: pulmonary endarterectomy with thrombus extraction or pulmonary balloon angioplasty, methods that have established a good long-term prognosis (improvement of hemodynamics and functional capacity) with an acceptable rate of complications in patients with CTEPH [14]. Interventional and medical treatment of CTEPH should be done in expert centres and aim to restore normal flow distribution within the pulmonary vasculature, unload the right ventricle and prevent or treat small-vessel disease.

Very similar to CTEPH, *chronic thromboembolic pulmonary disease (CTEPD)* involves similar vascular lesions without pulmonary hypertensive haemodynamics at rest (mPAP ≤20 mm Hg or 20 < mPAP < 25 mm Hg and pulmonary vascular resistance [PVR] < 240 dyn·s/cm^5) [16]. Both entities are characterized by the persistence of thrombi in the pulmonary arteries, physical deconditioning and a modified cardiopulmonary test (fig. 3). These criteria were established by the International Society of Thrombosis and Haemostasis, including dyspnea according to NYHA scale and abnormal 6-minute walk distance (6MWD) [6].

For example, patients with complete unilateral obstruction may present with normal pulmonary haemodynamic parameters at rest despite symptomatic disease. These patients are classified as having chronic thromboembolic pulmonary disease. CTEPH or CTEPD are potentially curable by pulmonary endarterectomy (PEA) or by balloon pulmonary angioplasty (BPA). However, more than half of patients are not eligible for surgery, or experience persistent or recurrent PH after PEA. According to some studies, pulmonary endarterectomy could improve function class and hemodynamic in patients with CTEPD [17].

Another form of chronic post-pulmonary embolism complication is *heart failure* with a primary involvement of the right ventricle. Incomplete recovery of the right ventricle following adequate treatment of acute pulmonary embolism occurs between 4 and 25% of cases [6, 15] and is defined by:

- The presence of an intermediate or high probability of pulmonary hypertension according to the European Society of Cardiology (ESC) criteria on echocardiography (the EACVI imaging guide);
- Hypokinesis or dilatation of the right ventricle (RV);
- Exertional dyspnea corresponding to NYHA II–IV.
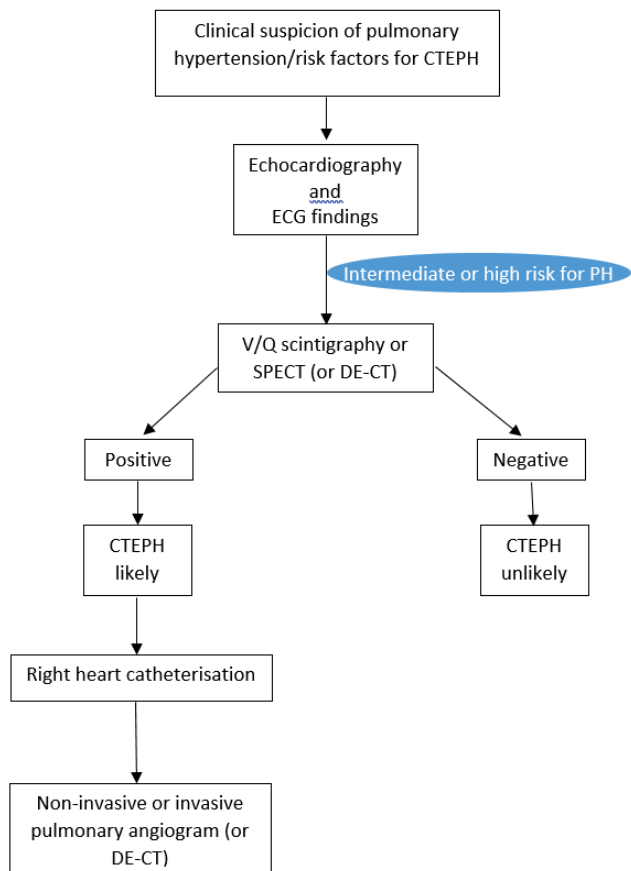
Fig. 2. Diagnostic imaging algorithm for CTEPH/CTEPD [12, 15].

ECG, electrocardiography; V/Q scintigraphy, ventilation/perfusion scintigraphy;
SPECT, single-photon emission computerized tomography;
DE-CT, dual energy computed tomography;
CTEPH, chronic thromboembolic pulmonary hypertension.

Left diastolic dysfunction is the most common abnormality detected on echocardiography (tab. 1). Adaptive

remodelling mostly occurs with RV wall hypertrophy. However, the RV is not capable of sustaining the long-term progressively increased afterload, thereby remodelling becomes maladaptive. Right ventricular failure leads to further RV dilatation, worsening LV filling, decreased LV stroke volume, all leading to right ventricular dysfunction [15].

Table 1. Proposed echocardiography algorithm for the assessment of RV function [18]

1.	Recommended screening test for known/suspected RV dysfunction	
2.	Useful for serial assessment, response to therapy	
3.	Parameters assessed	
	Dimensions	RV size
		Right atrial size
	ASE recommended for assessment of RV function (at least one)	Fractional area change
		S' (PW TDI)
		TAPSE
	RV index of myocardial performance	dP/dT
		Isovolumic acceleration
		Diastolic function (E/A, E/E')
	RV/pulmonary hemodynamics	PASP
		Right atrial pressure estimate (from IVC dimensions)
	Consider	PADP
		Pulmonary vascular resistance
	Additional parameters	RV free wall
		Septal strain
		Pulmonary acceleration time

Diagnostic Criteria	CTEPH	CTEPD
Symptoms	Exercise and resting dyspnea	Exercise dyspnea, or no symptoms
RHC	mPAP ≥25 mm Hg, PAWP ≤15	mPAP <25 mm Hg (usually 21–24), PAWP ≤15
RHC at exercise	—	Pressure-flow slope >3 mm Hg/L/min
V/Q scan	Mismatched perfusion defects	Mismatched perfusion defects
Angiography (CTPA or DSA)	Ringlike stenoses, webs/slits, and chronic total occlusions (pouch lesions, or tapered lesions)	Ringlike stenoses, webs/slits, and chronic total occlusions (pouch lesions, or tapered lesions)
CPET	—	mPAP/CO slope >1 (correlated with dead-space ventilation) and ventilatory equivalents for carbon dioxide slope >20[75]
TTE	Normal or enlarged RV and RA	Usually normal RV and (mildly enlarged) RA
Anticoagulation before diagnosis	At least 3 mo	At least 3 mo

Abbreviations: CO, cardiac output; CPET, cardio pulmonary exercise test; CTED, chronic thromboembolic pulmonary disease; CTEPH, chronic thromboembolic pulmonary hypertension; CTPA, computed tomography pulmonary angiography; DSA, digital subtraction angiography; mPAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; RA, right atrium; RHC, right heart catherization; RV, right ventricle; TTE, transthoracic echocardiography; V/Q, ventilation/perfusion.

Fig. 3. CTEPH and CTEPD characteristics [12]

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This dysfunction causes functional limitations. According to Dzikowska-Diduch O. et al., 34.2% of all symptomatic patients had echocardiographic confirmed diastolic dysfunction [19]. Studying a group of 845 survivors of an episode of pulmonary embolism, right ventricular dysfunction was determined in 17% of patients, which is comparable to the results of a meta-analysis performed by Sista A.K., where RV dysfunction was observed in 18% among patients [2]. If the electrocardiogram and NT-proBNP are within the normal limits, then the probability of relevant echocardiography abnormalities is low. The PEITHO trial, which included 219 survivors who were followed for 3 years after acute intermediate risk pulmonary embolism, demonstrated that 1 out of 7 patients develop chronic thromboembolic pulmonary hypertension or post-PE impairment (PPEI) [20]. Clinical and echocardiographic follow-up 6 months after acute phase may be useful for timely detection of late sequelae [20]. In another study, Stevinson et al. reported that echocardiography at 6 months detected right ventricular abnormalities, including dilatation and hypokinesia in approximately 25% (27/109) of patients, and functional limitation in nine of them [21]. Moreover, 41% of previously healthy patients had abnormal RV parameters on echocardiography or low functional capacity assessed by NYHA score or 6-minute walk test. PEITHO investigators suggested that lack of recovery or incomplete recovery of echocardiographic parameters of RV at 6 months may predict pulmonary hypertension [20]. A mechanism suggestive of subclinical and functional dysfunction of the RV may be right ventricle overload during acute pulmonary embolism [18]. Ciurzynski M. et al. found that survivors showed persistent abnormalities of RV function and morphology on echocardiography >1 year after PE, despite normalization of pulmonary artery systolic pressure [22].

The decrease in functional capacity is a complication that has as result the substantial impact on the reduction of the quality of life. In addition to the entities described above, such as dyspnea in chronic thromboembolic pulmonary hypertension and post-pulmonary embolism heart failure, other factors that lead to decreased functional capacity may be considered: anxiety, chest pain, post-thrombotic panic syndrome, and/or depression leading to decreased QoL in post-PE syndrome and general deconditioning [23, 24]. Deconditioning is defined as a loss of physical fitness due to failure to maintain an optimal level of activity or training [25]. Inactivity of any origin can lead to deconditioning, and in the patient with pulmonary embolism, the decrease in functional capacity is obtained by the vicious circle, represented by pain, anxiety, depression, dyspnea, together generating the lack of physical activity [23]. In the prospective ELOPE study, it was suggested for the first time that the deconditioning that occurs after acute pulmonary embolism appears to be the most likely explanation for the limitation of physical activity, mostly impaired circulatory or ventilatory function [1]. Exercise limitation on 1-month cardiopulmonary exercise test was predictive of worse functional outcome during follow-up, suggesting that identifying exercise-limited patients early after pulmonary embolism is of prognostic value, and that exercise rehabilitation interventions early after pulmonary embolism may have potential to improve long-term functional outcomes [1]. The role of inactivity was also highlighted in a cohort of intermediate– and high-risk pulmonary embolism patients, in which no significant association was found between patient-reported symptoms, pulmonary function, right ventricular dysfunction, and changes from cardiopulmonary testing [26]. Thus, the authors suggest that functional impairment appears to be attributable to general deconditioning rather than intrinsic cardiopulmonary limitation, and rehabilitation plays an important role for improving patient outcomes and quality of life. Other studies have also demonstrated clinical improvement after exercise training, further emphasizing the contribution of deconditioning in post-pulmonary embolism syndrome [27-30].

Mental health problems, such as depression, anxiety and panic syndrome that are related to venous thromboembolism are not negligible, contributing to functional deterioration after pulmonary embolism [23, 31, 32]. Such disorders as anxiety associated with pain could lead to less physical activity, with deconditioning and psychogenic functional limitation [33]. Identifying patients who require psychological counselling and treatment for depression or panic disorders, appears to have an incremental benefit for patients with pulmonary embolism [34].

Evaluation of patients with post-pulmonary embolism syndrome

For the evaluation of patients' symptoms with post-pulmonary embolism syndrome, such as dyspnea, fatigue, pain, decreased quality of life, a series of questionnaires and scores are available, summarized in table 2 [6]. There are two specific questionnaires available for patients with thromboembolic disease, but any available validated score can be used. The PVFS – Post-VTE Functional Status score [35, 36] can be used to assess dyspnea and the PEmb-QoL score (the validated Pulmonary Embolism Quality in Life) can be used to assess quality of life [37].

The PVSF scale is the most used questionnaire for scaling dyspnea in patients with post-PE syndrome. This scale is ordinal, has 6 steps ranging from 0 (no symptoms) to 5 (death, D), and covers the entire range of functional outcomes by focusing on limitations in usual duties/activities either at home or at work/study, as well as changes in lifestyle. The scale grades are intuitive and can easily be grasped by both clinicians and patients.

The post-VTE functional status is intended to be assessed: (1) at the time of discharge after a VTE diagnosis, (2) after 3 months, and (3) optionally after 12 and/or 24 months following a VTE diagnosis to check for recovery and degree of persisting disability. The 3-month follow-up

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Table 2. Validated scores and questionnaires for the assessment of subjective data and quality of life in patients with post-pulmonary embolism syndrome

Questionnaire	Parameter
PVFS – Post-VTE Functional Status	Dyspnea
Medical Research Council dyspnea Scale, original or modified (mMRC)	Dyspnea
PROMIS Short Form Dysnea Severity	Dyspnea
The modified Borg Dyspnea Scale	Dyspnea
the World Health Organization Functional Class	Dyspnea
PEmb-QoL (the validated Pulmonary Embolism Quality in Life)	QoL
QoL PROMs	QoL
PROMIS Short Forms for pain	Pain
The Checklist Individual Strength with fatigue severity	Fatigue
Patient Health Questionnaire– 9	Depression
Generalized Anxiety Disorder-7	Anxiety
Hospital Anxiety and Depression Scale	Depression, anxiety

corresponds to a routine visit performed by the treating physician for determination of the duration of anticoagulant treatment. The last time point is chosen as the functional status is expected to be stabilized in most patients and because it is the optimal moment for considering the presence of severe VTE complications, such as chronic thromboembolic pulmonary hypertension (CTEPH). The persistence of symptoms or an incomplete functional recovery after acute pulmonary embolism should trigger diagnostic tests to rule out CTEPH.

The second questionnaire, the PEmb-QoL (the validated Pulmonary Embolism Quality in Life) score, regards the quality of life of post-PE patients [1, 38]. It was proposed in 2009 and initially it was written in Danish, later translated into English. There are countries that have validated it, such as: China, Germany, France, and their experiences with it have been already published in PubMed. It contains nine subsections with various questions to which the patient must answer [38].

If the patient presents with symptoms and impaired functional status which can be included in the post-PE syndrome, it is a priority to rule out thromboembolic chronic pulmonary hypertension, because its early diagnosis and treatment leads to an improvement in the quality of life and further prognosis [4, 39].

The target population requiring diagnosis for chronic thromboembolic pulmonary hypertension comprises three groups [40-44]:
- ➢ Patients with pulmonary embolism and new-onset or progressive dyspnea, exertional dyspnea, oedema, palpitations, syncope, or chest pain;
- ➢ Those with a high pretest probability for CTEPH;

- ➢ Patients with signs of chronic embolism or RV overload on pulmonary angiography at the time of diagnosis of acute pulmonary embolism.

A non-invasive screening algorithm is shown in fig. 4.
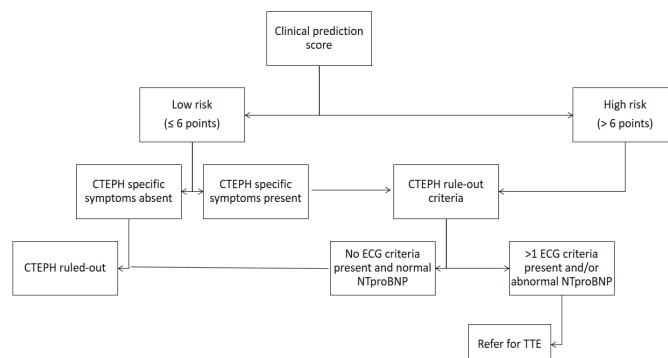
Fig. 4. Non-invasive early exclusion of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism: the InShape II algorithm [6, 45, 46].

CTEPH, chronic thromboembolic pulmonary hypertension,
ECG, electrocardiogram,
NT-proBNP, N-terminal-prohormone of brain natriuretic peptide,
TTE, transthoracic echocardiogram.

The clinical prediction score of chronic pulmonary hypertension after pulmonary embolism identifies patients with a higher risk of developing chronic thromboembolic pulmonary hypertension and was proposed by Klok FA et al. in 2016 [47]. It includes:
- Unprovoked pulmonary embolism +6 points;
- Known hypothyroidism +3 points;
- Onset of symptoms >2 weeks before of pulmonary embolism diagnosed +3 points;
- Right ventricular dysfunction on CT or echocardiography +2 points;
- Known diabetes -3 points;
- Thrombolytic treatment or embolectomy -3 points.

Exclusion criteria (roll-out) of chronic thromboembolic pulmonary hypertension include assessment of NTproBNP and ECG criteria of right ventricular overload. ECG criteria are considered:
- Paternal rSR' or rSr' in V1 derivation;
- R/S>1 in V1 with R wave > 0.5 mV;
- QRS axis > 90º.

If both parameters are normal, chronic thromboembolic pulmonary hypertension is considered excluded [6]. In retrospective studies applying the exclusion criteria of chronic thromboembolic pulmonary hypertension without any additional tests were considered to be safe [48, 49]. If chronic thromboembolic pulmonary hypertension is excluded, the subsequent diagnostic algorithm will be established individually. Diagnostic tests, such as transthoracic echocardiography (if not performed in the previous step), cardiopulmonary stress test, and imaging tests will be performed to assess the presence of persistent perfusion defects and residual clots (fig. 5) [6].

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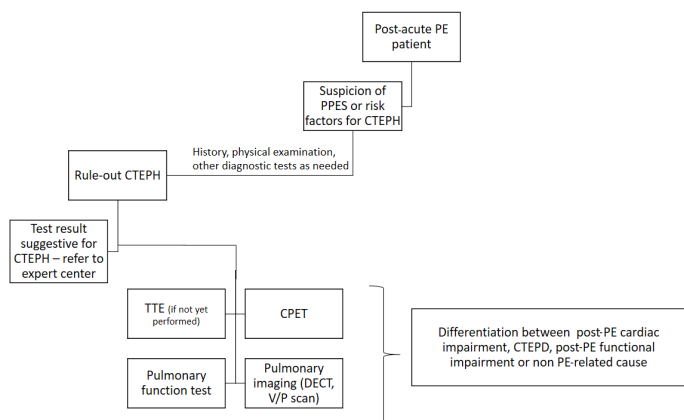
Fig. 5. Flow chart for follow-up 3 months after an acute PE for the detection of PPES

CPET, cardiopulmonary exercise test; CTEPD, chronic thromboembolic pulmonary disease; CTEPH, chronic thromboembolic pulmonary hypertension; DECT, dual-energy computed tomography; PE, pulmonary embolism; PPES, post–pulmonary embolism syndrome; TTE, transthoracic echocardiogram; V/Q, ventilation/perfusion.

Echocardiography can provide us with a series of useful information for the evaluation of patients with dyspnea in post-thrombebolic syndrome, first of all, the exclusion of pre-existing causes of dyspnea, such as left ventricular dysfunction established before acute pulmonary embolism event, congenital cardiac anomalies, valvulopathies, etc. In the 2022 ESC guidelines for the diagnosis and management of pulmonary hypertension, a series of echocardiographic parameters are proposed to be used in order to quantify patients with suspected pulmonary hypertension [50]. In addition to conventional echocardiography techniques, the new echocardiographic techniques, such as speckle tracking echocardiography and 3D techniques were proposed. The estimation of pulmonary pressure during exercise can be evaluated, but in most cases an increased pulmonary pressure may be present during exercise as a sign of left ventricle dysfunction. Echocardiographic parameters that can be used in the evaluation of the patient with probable PH [18, 51-53]:

- Increase in LV diameter (RVOT prox) in PLAX >35 mm;
- Dilatation of the right ventricle, VD/LV ratio >1 in apical 4 C;
- D shape of VD PSAX
- Distension of the inferior vena cava >21 mm and reduction of its collapse on inspiration (<50%) – subcostal window;
- Acceleration of RVOT time at the level of the pulmonary valve, >105 msec, PSAX;
- Reduction of LV fractional area <35% – Apical 4 chambers;
- Decrease TAPSE < 17 mm – M mode;
- Decreased velocity of the tricuspid ring (S′ <9.5 cm/sec), tissue doppler;
- Increase in AD area > 18 cm2 – apical 4 chambers;
- Increasing the maximum velocity of tricuspid regurgitation >2.8 m/s – continuous doppler;
- Estimated sPAP (>28 mmHg) – continuous doppler;
- Severe tricuspid regurgitation – apical 4C;
- VD dyssynchrony >25 msec – speckle tracking echo;
- Decrease in the deformation of the VD free wall <20% – speckle tracking echo;
- Decrease of the RV ejection fraction <45% – 3D echocardiography;
- Pericardial effusion in any sections.

If the echocardiography does not show signs of pulmonary hypertension, the cardiopulmonary exercise test is the next diagnostic step to quantify exercise limitation, but also with the aim of differentiating mechanisms of functional limitation.

If necessary, lung perfusion imaging is performed next. Patients with suspected chronic thromboembolic pulmonary hypertension or chronic thromboembolic disease should be referred to expert centers for specific imaging tests and right heart catheterization. Anemia, cancer and interstitial lung diseases, obstructive lung pathologies or other comorbidities that could cause dyspnea, not being related to the pulmonary embolism episode, should be excluded, and where appropriate, targeted treatment should be indicated [9]. Gleditsch et al. studied 26 survivors of pulmonary embolism evaluated by magnetic resonance imaging (MRI) before and after rehabilitation. In those whose dyspnea improved significantly, no important changes in cardiac parameters were detected on MRI. This fact could suggest the presence of functional dyspnea in some patients with post-pulmonary embolism syndrome [54].

In addition to all of the above, we should not ignore the screening tools for depression and anxiety, which are indicated in selected patients in the post-pulmonary embolism evaluation scheme. If the cause of the physical alteration in a patient after an episode of pulmonary embolism has not been detected, redirection to specialized centers for cardiopulmonary rehabilitation may be useful. Early rehabilitation through physical effort on individual programs would contribute positively to the functional recovery of patients with post-PE syndrome.

Conclusions

After an episode of pulmonary embolism, a large number of patients remain with functional limitations and persistent symptoms. Multiple studies and meta-analyses demonstrated that it is essential to diagnose post-PE syndrome earlier in order to have better long-term outcomes. There are algorithms proposed to objectify the symptoms after an episode of pulmonary embolism and to contribute to the correct and timely selection of patients with post-embolic pulmonary syndrome who require additional evaluations and specialized treatment. Since chronic thromboembolic pulmonary hypertension represents the most severe post-pulmonary embolism complication, earlier detection and treatment of this entity remains a priority.

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ND conceptualized the idea, IC and GS conducted literature review and wrote the manuscript, AG revised critically the manuscript. All the authors approved the final version of the manuscript.

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From non-specific low back pain
to chronic primary musculoskeletal low back pain: the evolving concept

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Abstract

Background: Low back pain (LBP) for many years is considered one of the most common conditions causing work absenteeism and long-term disability, with important implications for public health systems and economies. Pain generators of LBP are various, being distinguished specific and non-specific causative mechanisms. The term "non-specific" LBP remains ambiguous as potential sources of pain are supposed to be muscles or joints, but supplementary investigations do not correlate enough to explain the pain intensity and disability. The nociceptive and/or neuropathic mechanisms characteristic for acute pain tend to be influenced by central sensitization while pain chronification occurs, leading to new descriptor as nociplastic pain. Chronic low back pain, considered mostly non-specific, was mechanistically referred to primary musculoskeletal low back pain, the concept introduced in the new ICD-11 classification. The process of acceptance by the scientific medical community raised debates and discussions. The aim of the study was to analyze the evolving concept of non-specific low back pain to chronic primary musculoskeletal low back pain. A narrative literature review was carried out.
Conclusions: The term non-specific low back pain is used when the pain generators have not been accurately determined or cannot fully explain the existing symptomatology. Chronic primary musculoskeletal low back pain is better explained by central sensitization mechanisms and altered nociception, named nociplastic pain. Because of raised ambiguities regarding this concept further studies are expected to shed light on the problem.
Key words: non-specific low back pain, chronic primary musculoskeletal low back pain, central sensitization.

Introduction

Low back pain (LBP) for many years is considered one of the most common conditions causing work absenteeism and long-term disability, with important implications for public health systems and economies [1]. In accordance with the Global Burden of Disease study LBP was the leading cause of years lived with disability with estimated prevalence of 7.5% of the global population, or around 577 million people [2].

Low back pain is defined as *"pain in the area on the posterior aspect of the body from the lower margin of the twelfth ribs to the lower gluteal folds with or without pain referred into one or both lower limbs that lasts for at least one day"* [3]. Many potential anatomic sources, such as nerve roots, muscle, fascial structures, bones, joints, intervertebral discs, and organs within the abdominal cavity are known as pain generators for LBP [4]. Because of its complexity, diagnostic evaluation of the patient with LBP is a real challenge. Furthermore, identifying the anatomical source of pain is of major importance in determining management strategies. This is especially true

when there are specific pathophysiological mechanisms of non-spinal (hip conditions, diseases of the pelvic organs, aortic aneurysm) or spinal origin (herniated disk, spinal stenosis, fracture, tumor, infection, spondylarthritis) [5].

In cases of unidentified causes of LBP, it is referred to as non-specific one. According to International Association for the Study of Pain (IASP) this term is known as "Lumbar Spinal Pain of Unknown or Uncertain Origin". Over the years, authors provide references based on various studies about the lack of etiology of these conditions as being about 85-90%, compared to specific etiology [6].

Certain ambiguities in understanding the classification of LBP are created as well by the fact that some scientific sources classify this condition as mechanical and non-mechanical, or musculoskeletal and neurological [7, 8]. It is observed that most of the studies identified similarity between non-specific low back pain and mechanical or musculoskeletal LBP, taking into account the main underlying mechanisms. The source of musculoskeletal (non-neurologic) back pain is often non-specific and difficult to identify in most patients. It is considered that pain arises from degenerative spine changes and injury to

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local spinal structures, which include the vertebral column, ligaments, and surrounding muscles and soft tissues. In such cases the main pain generators are nociceptors localized in these structures.

At the same time, LBP could cover other spectrum of pain, such as neuropathic pain irradiating down the leg in case of radiculopathy and, in some cases, nociplastic pain, which is caused by amplification of pain in the CNS, especially when chronicity occurs. Frequently, these pain subtypes overlap (e.g., a patient with a herniated disc who has back pain can have radicular pain and diffuse symptoms outside pathoanatomical referral patterns) [9].

In the majority of cases acute low back pain is self-limiting, and prognosis is relatively favorable [10]. Chronic pain goes beyond the period of repair of tissue damage that occurred during the acute stage. According to published data, 2% to 48% (median, 26%) of patients with acute LBP in primary care settings become chronic [11]. Chronic pain is often non-specific, implying that there is no pathology or tissue damage or that the limited amount of pathology or tissue damage is not severe enough to explain the pain experience [12].

LBP is termed as chronic when persisting >3 months, being no longer considered as a symptom, but as a disease caused by numerous onset factors. A vast majority of patients (45–75%) report feeling pain 12 months after the onset of LBP, transforming the LBP into the most common musculoskeletal diseases among people with chronic pain [13]. According to the World Health Organization (WHO), 20–33% of the world's population have some form of chronic musculoskeletal pain [14]. The transition of acute pain to chronic is a dynamic process based on mechanisms of amplification of noxious and non-noxious stimuli, described as central sensitization (CS) [15, 16]. IASP definition of CS is "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input" [17]. The concept of CS can explain the discrepancy between the experienced pain severity, disability or other symptoms and the minor degree of tissue damage or suffering from pain in the absence of a clear origin of nociceptive input [12, 18].

The historical evolution of the various theories explaining the pain phenomenon, e.g., "the gate control theory", the concept of neuromatrix described by Melzac and others leads to the conclusion that chronic low back pain is not only an experience induced by the direct nociceptors painful stimulus but represents a multidimensional process with the involvement of biological, psychological, and social factors. From the biopsychosocial model of pain perspective, such factors as biological (genetic factors, comorbidities, etc.), psychological (anxiety, depression, cognitive beliefs, coping skills) and social (financial barriers, job satisfaction) are associated with the development of chronic pain, but at the same time can be the result of chronic pain [19, 20].

The recently published International Statistical Classification of Diseases and Related Health Problems (ICD) codes, ICD-11 identifies chronic pain as a stem code, with chronic primary pain a subcategory that can occur in one or more anatomical regions independently of identifiable biological or psychological contributors [21-23]. The low back pain has been devoted to primary chronic pain section as both diagnostic and treatment concepst have changed.

The aim of the study was to analyze the evolving concept of non-specific low back pain to chronic primary musculoskeletal low back pain.

The literature search was performed in PubMed, Web of Science and Scholar databases. The search terms were: `low back pain`, `chronic low back pain`, `classification chronic low back pain`, `definition chronic low back pain`. Articles were selected for the 20-year period and in English. The search for `low back pain` term returned 35.496 results, RCT`s – 3.112. The search for `chronic low back pain` term returned 12.545 results and RCT`s – 1.673. The search for `classification chronic low back pain` term returned 582 results, and `definition chronic low back pain` term returned 328 results. All the titles and abstracts were screened and suitable articles were extracted and analyzed. The principal themes are presented in the article.

Results and discussion

The Pain Task Force of the IASP defines chronic primary pain as pain in one or more anatomical regions that persists or recurs for longer than 3 months, and that is characterized by significant emotional distress (anxiety, anger/frustration or depressed mood) or functional disability (interference in daily life activities and reduced participation in social roles) [17, 22].

Diagnostic criteria for chronic primary pain [24].

Conditions A to C are fulfilled:

A. Chronic pain (persistent or recurrent for longer than 3 months) is present

B. The pain is associated with at least one of the following:

B.1 Emotional distress due to pain is present.

B.2 The pain interferes with daily life activities and social participation.

C. The pain is not better accounted for by another chronic pain condition.

Chronic primary musculoskeletal (MSK) pain is a stem category of chronic primary pain and is defined as chronic pain in the muscles, bones, joints, or tendons that is characterized by significant emotional distress (anxiety, anger/frustration or depressed mood) or functional disability (interference in daily life activities and reduced participation in social roles). Chronic primary MSK pain is divided into chronic primary low back pain, chronic primary cervical pain, chronic primary thoracic pain, chronic primary limb pain [22, 24].

The diagnosis of chronic primary MSK pain is appropriate independently of identified biological or psychological contributors unless another diagnosis would better

account for the presenting symptoms. The new classification of chronic primary MSK pain provides an opportunity to categorize, diagnose, and treat musculoskeletal pain conditions previously referred to as "non-specific" [22, 25]. Primary MSK pain is neither nociceptive nor neuropathic, as involvement of nociceptors or somatosensory system is not detected, but in which clinical and psychophysical findings suggest altered nociceptive function. Such mechanism was referred to a new concept of pain pathophysiology named nociplastic pain [22].

Nociplastic pain is defined as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain [26]. Kosek E. et al. [27] proposed clinical criteria to identify nociplastic pain affecting the musculoskeletal system (chronic primary MSK pain) (tab. 1):

Low back pain is a complex problem that includes conditions with different etiologies, evolution, and evolving mechanisms. Taken together, these mechanisms are a substantial burden on the patient, society and health care systems. The acute and subacute evolution of pain, with the duration of the pain phenomenon up to 6-12 weeks, is often related to "non-specific" or mechanical causes, when the involvement of the muscular and osteoligamentary systems (myofascial syndrome, zygoapophisal joints and intervertebral disc degeneration) lead to nociceptive pain. At the same time, mechanical low back pain could be specific in origin, as it is in disc herniation or lumbar spine stenosis with neuropathic pain component. The involvement of different structures responsible for onset of lumbar pain, but also different types of its evolution, outline various pain characteristics, such as nociceptive pain, neuropathic pain or nociplastic pain, and in some cases mixed forms of manifestation.

Table 1. Clinical criteria and grading for nociplastic pain affecting the musculoskeletal system [27]

1. The pain is:
1a. Chronic (>3 mo);
1b. Regional (rather than discrete) in distribution*;
1c. There is no evidence that nociceptive pain (a) is present or (b) if present, is entirely responsible for the pain;
1d. There is no evidence that neuropathic pain (a) is present or (b) if present, is entirely responsible for the pain. †
2. There is a history of pain hypersensitivity in the region of pain. Any one of the following:
Sensitivity to touch
Sensitivity to pressure
Sensitivity to movement
Sensitivity to heat or cold
3. Presence of comorbidities. Any one of the following:
Increased sensitivity to sound and/or light and/or odors
Sleep disturbance with frequent nocturnal awakenings
Fatigue
Cognitive problems, such as difficulty to focus attention, memory disturbances, etc.
4. Evoked pain hypersensitivity phenomena can be elicited clinically in the region of pain. Any one of the following:
Static mechanical allodynia
Dynamic mechanical allodynia
Heat or cold allodynia
Painful after-sensations reported following the assessment of any of the above alternatives.
Possible nociplastic pain: 1 and 4.
Probable nociplastic pain: all the above (1, 2, 3, and 4)‡

* – Musculoskeletal pain is deep, rather than cutaneous and regional, multifocal, or widespread in distribution (rather than discrete). In case of multifocal pain states that can be caused by different chronic pain conditions (e.g., shoulder myalgia and knee osteoarthritis), each chronic pain condition or pain region must be assessed separately.

† – The presence of a source of nociceptive pain, such as osteoarthritis, or of neuropathic pain, such as a peripheral nerve lesion, does not exclude the concurrence of nociplastic pain, but the region of pain must be more widespread than that which can be explained by the identifiable pathology.

‡ – The purpose of the grading system is to indicate the level of certainty that a patient has nociplastic pain and, as mentioned above, was inspired by the current grading system for neuropathic pain. However, because of the lack of clinically useful, reliable diagnostic tests to confirm the presence of altered nociception, currently nociplastic pain is graded as possible or probable but not definite. If future diagnostic tests are developed and validated, the introduction of the term "definite nociplastic pain" should be considered.

The scientific debates on back pain are mostly related to terminology. This is observed from the stage of understanding low back pain as "non-specific" with nociceptive mechanism, in which the involvement of muscles, joints and other osteoligamentar structures is plausible, to the notion of chronic primary MSK pain, in which non-structural pathophysiological processes are incriminated [21, 27, 28].

Given that scientists and clinicians consider that chronic low back pain is mostly non-specific, which in the new ICD-11 classification was mechanistically referred to primary lumbar MSK pain, discussions and questions arise regarding the mechanisms underlying its base. The classification of chronic pain and the concept of primary versus secondary chronic pain were introduced in ICD-11 following the known classification principles used for headaches and other conditions, e.g., insomnia and hypertension, aiming to delineate these conditions for a management as well-argued and approved based on the recommendation grades [21]. Acquainting physicians with the principles of this classification will facilitate the training of patients in understanding that it is not peripheral factors that are largely responsible for pain, but central ones, on which treatment methods will be targeted [25].

An understanding of pain classifications is important when discussing musculoskeletal syndrome pain due to its variable presentation [29]. The group of authors who introduced the concept of chronic primary MSK pain claim that not all regional pain conditions are solely due to tissue abnormalities but that some aspects can be mechanistically explained as sensitization of the nervous system. It is suggested that chronic primary MSK pain, arising in muscles, tendons, bones, and joints in the absences of anatomical changes, is best understood as "regional fibromyalgia" [25].

There is room for debate as to what extent can the degenerative changes of the spine be considered as an unknown cause of low back pain. Possibly they can represent a causative factor, but which cannot be treated and diagnosed at the moment? It has been advocated that in approximately 90% of cases of low back pain a clear cause was not identified, although most times the advanced diagnostic techniques (e.g., diagnostic blocks or electrodiagnostic testing), while studying the etiological aspects of low back pain, were not used. At the same time, when they were visualized in different groups of people, it was observed that the respective changes did not cause pain in all cases. Shifting to the chronic stage of pain, this uncertainty increases, as lumbar pain tends to remain regionalized, but more diffuse, and to be associated with other manifestations, such as sensitization of the painful area with allodynia or hyperesthesia and other comorbidities (sleep disorders, cognitive problems, and others).

It is known that any chronic pain is supposed to be acute at the beginning, and every acute pain has a lesional substrate, with a potential mechanical triggering factor. In an attempt to understand the mechanisms of low back pain, so far there is no clarity in the use of the term of "non-specific" pain.

Chiarotto A. and the authors mention the uncertainty of the term non-specific pain, considering the fact that changes in the structures that can generate pain, such as muscles, intervertebral joints, intervertebral disc, cannot be confirmed by medical history and clinical examination. For example, osteoarthritis of the intervertebral joints can undergo the same inflammatory changes as the joints of another level, but until now there are no diagnostic criteria for vertebral osteoarthritis [28].

Among the causes of non-specific LBP, myofascial syndrome is considered one of the most common, being named as regional myofascial pain, which does not have any neuroanatomical distribution [25]. Evolving to chronic low back pain the central pain processing mechanisms are incriminated to be responsible for pain in absence of clear origin of nociceptive tissue damage, resulting in nociplastic pain. Nociplastic pain is distinct from nociceptive and neuropathic pain, in which central sensitisation has been found to be present in many subgroups of patients [12]. Clinical criteria proposed by Kosek E. et al. to identify nociplastic pain provide an opportunity to establish the possible or probable diagnosis.

Comparing the old concept of myofascial pain syndrome and the new concept of musculoskeletal pain, the question arises whether it refers to primary or secondary chronic MSK pain. Scientific research data, including experimental ones on animals, are presented about the fact that repeated mild trauma to muscle tissue and fascia by intramuscular injection of a small amount of nerve growth factor induces central sensitization of spinal neurons through neuron-glia interactions and neuroinflammation [22]. The author discusses whether the myofascial syndrome is a primary or secondary chronic pain and suggests completing the proposed classification with the third component "chronic myofascial pain" which would emphasize the possibility of the existence of the somatic factor (muscles, fascia) in the primary MSK pain. This concept would broaden the understanding of the biopsychosocial model of pain with emphasis on the neurobiology of muscle and fascia innervation and central nervous system signal processing.

The scientific debates on back pain are mostly related to terminology, which is observed from the stage of understanding low back pain as "non-specific" with nociceptive mechanism, in which the involvement of muscles, joints and other structures is plausible, to the notion of chronic primary MSK pain, in which non-structural central pathophysiological processes are incriminated, defined as nociplastic pain [21, 27, 28]. A research group that has been studying these conditions supports the idea that previous terms for the characterization of chronic pain, such as "idiopathic" or "functional" are inappropriate and even misleading and should have been modified. Contrary to this, they consider the use of the term nociplastic pain not suitable enough, as long as "centralized pain", central

sensitization, and central hypersensitivity are already widely used, terms that are much better understood by nonpain specialists [30]. According to Rolf-Detlef Treede who makes a critical analysis regarding the concept of chronic primary musculoskeletal pain, it is concluded that "The attempts to integrate the mechanistic concept of "nociplastic pain" and the older term "myofascial pain" are interesting, but the conclusions are premature without further empirical evidence" [21].

Conclusions

The term non-specific low back pain is used when the pain generators have not been accurately determined or if they have been mentioned, they cannot fully explain the existing symptomatology. At the same time, the denial of specific causative factors in these cases is premature since there are no objective methods of confirmation. Chronic primary musculoskeletal low back pain, in which nociceptive or neuropathic components are not observed, is better explained by central sensitization associated with biological and psychosocial mechanisms. Nociplastic pain as the third mechanistic description of pain was introduced; also, clinical criteria for possible or probable diagnosis were proposed. The introduction of the concept of chronic primary MSK pain has raised ambiguities and has sparked debate over its terminology and understanding. The need for further studies is outlined in order to capitalize and appreciate the role of myofascial structures in chronic low back pain.

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Male fertility preservation at risk of gonadotoxicity

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Abstract

Background: Along with other aspects of male reproduction, fertility preservation has made significant advances in the past ten years. The overall survival rate for childhood cancer has greatly improved in recent decades, with a current 5-year survival rate of over 80%, compared to roughly 58% in the late 1970s. Many of the most common reproductive issues, such as cryptorchidism and hypospadias in newborns as well as testicular cancer and lower sperm quality in young adult males, have recently become increasingly common. Although the precise cause of these unfavorable effects on reproduction is yet unknown, it has been suggested that they may be related to the presence of common chemicals in the environment or exposure to specific drug classes during fetal life. Large progress has been achieved in recent years toward understanding the biology of male and female reproduction in both animals and humans and applying this information to the creation of methods for fertility preservation in a variety of clinical and ecological contexts.
Conclusions: A rapidly developing area, fertility preservation has a wide range of applications, from preserving the possibility of fertility in a child with cancer to preventing the extinction of an entire species. The emphasis on preserving fertility is now only placed on cancer patients who are of reproductive age, but its therapeutic importance may be extended to non-cancer patients as well.
Key words: prepubertal human testis, childhood cancer, gonadotoxicity, side effects, fertility preservation.

Introduction

Along with other aspects of male reproduction, fertility preservation has made significant advances in the past ten years. Due to cultural changes that have delayed motherhood and the quick pace of scientific advancements spurred by advances in cancer therapy and cryobiology, it is regarded as a necessary service in the medical field. It is widely acknowledged that cancer is a serious situation in which fertility preservation is a crucial step. By 2025, there will be more than 20 million new cases of cancer annually, according to global demographic and epidemiologic data [1-3]. Also, while finding a cure is still our top priority, we are confronted with a difficult treatment outcome since an increasing percentage of patients have poor quality of life due to the presence of a tumor and the following treatment. The biggest quality of life concern for young cancer survivors continues to remain infertility. As a result, fertility preservation techniques are essential as the long-term survival rate for cancer has increased. The 5-year relative survival rate for all cancers combined is currently nearing 70% among adults and >80% among children, according to recent studies [4]. The emphasis on preserving fertility is now only placed on cancer patients who are of reproductive age, but its therapeutic importance may be extended to non-cancer patients as well. As a result, far more extensive applications are anticipated soon.

The overall survival rate for childhood cancer has greatly improved in recent decades, with a current 5-year survival rate of over 80%, compared to roughly 58% in the late 1970s [5]. This showed that as a result of advancements, primarily because of improved chemotherapy treatments, a growing number of long-term pediatric cancer survivors has arisen. However, chemotherapy drugs do not just target malignant cells; they can also destroy healthy tissues unintentionally, which might have unfavorable repercussions. To improve the quality of life for childhood cancer survivors, research is increasingly focusing on minimizing harm to healthy organs. The negative consequences of therapy on fertility can be particularly concerning for younger people [6].

Many of the most common reproductive issues, such as cryptorchidism and hypospadias in newborns as well as testicular cancer and lower sperm quality in young adult males, have recently become increasingly common. Additionally, the timing of puberty has changed over time. Although the precise cause of these unfavorable effects on reproduction is yet unknown, it has been suggested that they may be related to the presence of common chemicals in the environment or exposure to specific drug classes during fetal life [7].

The increased awareness of long-term treatment-related toxicities that impact reproductive and endocrine

function is a result of improved children cancer survival rates [8]. For prepubertal boys who are not yet producing mature germ cells, sperm cryobanking is not practical prior to beginning life-saving medications, which presents a problem for fertility preservation in this cohort of patients [9]. Prepubescent males currently do not have any treatment choices that will safeguard and preserve their future fertility. Cryopreserving testicular tissues that contain spermatogonial stem cells (SSCs) before starting any gonadotoxic cancer therapy becomes a possible solution to this issue [10].

Fayomi A. P. and associates have shown that intracyto-plasmic sperm injection using autologous transplantation of cryopreserved prepubertal non-human monkey testis tissue can result in the production of functional sperm [11]. Applications using cryopreserved prepubertal human testis tissue are still at the experimental stage, despite the fact that this study is particularly positive and represents an opportunity for young boys.

Factors that affect the fertility of cancer survivors and their offspring: an overview

After cancer therapy, there is a great degree of variability in the risk and severity of infertility, which is influenced by numerous patient and therapeutic variables. Patient factors include aspects like age at diagnosis, length of time since treatment, sex, pretreatment fertility, and the location and stage of the cancer, while treatment factors include matters like drug type, administration route, location and dosage of radiation therapy, dose, and dose intensity [12]. Sterilization surgery could be necessary for the treatment of some malignancies. Additionally, by impairing potency and ejaculation, nonsterilizing surgery for malignancies of the bladder, prostate, and rectum may also have an impact on fertility [13].

Although there are obvious dose, medication, gender, and age-dependent effects, chemotherapy, and especially alkylating drugs like cyclophosphamide and procarbazine, can have a major impact on fertility [12, 13]. Whole-body radiation, radiation at or near reproductive organs, radiation at or near the pituitary gland, which produces FSH and LH, and other reproductive hormones, may all have a significant impact on fertility [12, 13].

In a large cohort study from the Childhood Cancer Survivor Study (CCSS), 6224 male subjects without surgical sterility aged 15-44 who were diagnosed with cancer before the age of 21 between 1970 and 1986 and survived for at least 5 years after diagnosis were examined for predictors of ever siring a pregnancy [14]. This investigation discovered a relationship between the dose of alkylating agents, the dose of radiation to the hypothalamus and pituitary, the dose of radiation to the testicles, the type of cancer, and the kind of chemotherapy [14]. Subjects who were not exposed to hypothalamic/pituitary/testes radiation or alkylating chemicals had a similar chance of siring a pregnancy to that of their control siblings. While a substantial difference in effect was seen based on the type of chemotherapy agent, increasing doses of each of these exposures were

significantly associated with a declining risk of siring a pregnancy. In contrast to those identified between the ages of 15 and 20, those diagnosed between the ages of 0 and 4 had an HR of siring of 1.80 (95% CI: 1.31-2.47), suggesting that earlier diagnosis may increase fertility [14].

Chemotherapy and radiotherapy in non-sterile cancer survivors who do not have cryopreserved sperm or embryos raise the possibility of treatment-related germline alterations having an adverse effect on progeny's health. Theoretically, these germline mutations could cause the child to have genetic abnormalities and a higher chance of developing cancer. This can be a typical worry for cancer survivors and a possible obstacle to wanting children [15]. Evidence demonstrating that cancer survivors are actually susceptible to treatment-induced somatic mutations that raise the chance of developing later malignancies may underline this issue [16].

However, research to date suggests that, if cases of hereditary cancer syndromes are taken into account, there is no increased risk of cancer in offspring of cancer survivors. After excluding people with hereditary cancer syndromes, a population-based study of cancer patients in Finland who were diagnosed before the age of 35 discovered that children born more than nine months after their parents' diagnosis did not exhibit an elevated risk of developing the disease in comparison to children of the cancer survivors' siblings [17]. Furthermore, stratifying by the child's cancer site did not reveal any appreciable increased risks. The risk of cancer increased, as anticipated, if hereditary cancer cases were not eliminated. Similar results were obtained from a population-based study conducted in the Nordic countries between 1943 and 1994, which excluded likely cases of hereditary cancers and found a nonsignificant risk of all cancer sites among children of cancer survivors [18]. When stratified by age at diagnosis and gender, the same result was obtained. Nearly every participant in this study had children, and follow-up on the children continued until they were 43 years old. The children were all born at least 8 years following their mothers' cancer diagnoses. The results of earlier research have also demonstrated that any increased risk of cancer in offspring results from familial aggregation rather than mutagenic effects [19]. However, the size of these investigations was constrained by the small case numbers and short follow-up periods.

Other signs of transgenerational genetic impacts, such as single-gene abnormalities (Mendelian disorders) and genomic instability, do not seem to be more prevalent in the offspring of cancer survivors [20]. Finally, according to the majority of research [21], there are no appreciable differences between male and female cancer survivors in terms of gender. Despite the fact that one major investigation did find a significantly altered ratio [22], the authors hypothesized that this difference was not brought on by a rise in deadly X chromosomal mutations but rather by the decreased testosterone levels. According to studies done on mice, chemotherapeutic drugs like cyclophosphamide may cause aneuploidy in oocytes, early

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embryonic death, and fetal malformation. However, it was hypothesized that these risks are most likely to occur in oocytes that are maturing at the time of exposure and that they are diminished by allowing enough time to pass between exposure and pregnancy [23]. Although aneuploidy in human spermatozoa has also been linked to chemotherapy, this connection has only been proven to be temporary, lasting less than 100 days [24].

It doesn't seem that the general health of children born to cancer survivors differs from what is expected. Children of cancer survivors did not exhibit an elevated risk of hospitalization compared to the control group after a median follow-up time to age 9.6, according to a population-based analysis of kids born in Denmark between 1977 and 2003 [25]. This remained true for all diagnoses associated with discharges, including those connected to injuries, infections, issues with any organ or metabolic system, and issues with the mind or behavior. Malignant and benign neoplasms were more likely to develop, although these risks were explained by inherited malignancies and greater surveillance, respectively.

Ethical discussions in approaching fertility preservation

Using previously frozen gametes or gonadal tissue, cancer survivors who have lost their reproductive ability could still want to have children. Due to the large spectrum of medico-social conditions that fertility preservation treats, some of which are highly unusual, patient care necessitates a personalized and multidisciplinary approach. Particularly, fertility experts who provide fertility preservation choices to cancer patients should be sufficiently educated and experienced in order to address the patient's treatment plan, prognosis, as well as unusual health risks for future offspring and the potential negative effects of pregnancy. Since these treatments are provided with the intention of protecting future fertility, there shouldn't generally be any ethical issues with offering them. In actuality, there are drawbacks: many options are still in the experimental stage; posthumous use of stored tissue or gametes has some legal repercussions; worries about the welfare of offspring due to an anticipated shorter life span of the parent; worries about the welfare of children born using gametes frozen after chemotherapy already started; and the possibility of cancer reseeding after transplanting cryopreserved tissue [26].

The five principle-based ethics serve as the basis for the majority of ethical norms employed in ethical analyses. These values include justice, beneficence, no malfeasance, autonomy, and authenticity.

Respect for humans, also known as autonomy, recognizes a person's right to have opinions, make decisions, and behave in accordance with their own personal values and beliefs. Informed consent and respect for privacy are based on this idea. This concept forms the basis for both reproductive rights and choices: if a woman's outlook for long-term survival is questionable, should fertility preservation be offered to her? Should a husband be allowed to use frozen embryos stored while his wife was still living and to use a member of her family to carry the embryos to term? In order to create an informed agreement, it is crucial to consider these possibilities and request disposal instructions for cryopreserved reproductive tissue, gametes, or embryos. Both beneficence and nonmaleficence may overlap, as in the case of a patient who wants to delay the start of chemotherapy treatments yet insists on undergoing fertility preservation against the advice of the oncologist. Justice is concerned with fairness and equality, i.e., the requirement to be fair in the burden-sharing and resource-distribution to all community members. The idea of fair treatment is frequently used, in particular, in circumstances when a choice must be made about the fair distribution of resources. However, the existing method of IVF, and specifically fertility preservation, is unfair. Insurance companies do not fund these procedures because many methods of fertility preservation are still considered experimental. The majority of patients are unable to obtain these treatments since they are only provided on an institutional grant basis or on a philanthropic basis, especially for low-income individuals [26].

Although there are numerous methods for preserving fertility, only embryo and sperm freezing have been shown effective; all other methods, such as oocyte and ovarian tissue freezing, *in vitro* oocyte maturation, and *in vitro* folliculogenesis, are still considered experimental. The collection and separation of spermatogonial cells from testicular biopsies, the freezing of testicular tissue for later transplantation or even xenografting, are being tested but remain extremely experimental for men when the option of semen cryopreservation is not accessible as for prepubertal boys. The right of both men and women to be informed about all alternatives for fertility preservation, their ramifications, including risks and costs, is important when utilizing experimental treatments. Additionally, as experimental techniques fall under the category of research protocols, institutional review boards should also review and approve them.

Restoring personal autonomy to persons who may eventually lose the ability to conceive is the main ethical justification for fertility preservation [27]. The possibility of both the parent and their progeny being affected makes it difficult to communicate danger information. Do no harm is a cornerstone of medical ethics. A team of medical oncologists, andrologist, reproductive endocrinologists, pathologists, and psychologists should ideally decide who is a candidate for fertility preservation, guided by documented protocols that may be communicated with patients [28]. False hopes shouldn't be given to patients. Alternative strategies, such as abstaining from intervention with the possibility of adoption or childlessness, should also be discussed.

Even for children, exposure to cancer treatments may lead to impaired future fertility. Children may be unable to comprehend these risks, but when they grow up, they

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could experience trauma from them. Since children's sexual immaturity restricts the options open to them for retaining their fertility, all of them are regarded as experimental. Testicular stem cell collection and cryopreservation with the intention of future autologous transplantation or *in vitro* maturation represent prospective techniques of fertility preservation for prepubertal boys who are unable to produce mature sperm. Someone might presume that fertility preservation for children is morally acceptable because it protects their reproductive autonomy and reduces morbidity (both reproductive and psychosocial) [29]. The primary ethical issue therefore relates to the procedures and methods required to safeguard fertility. The unique scenario of using children as both research subjects and patients leaves the provider vulnerable to possible technology abuse in the pursuit of a breakthrough [28]. It is advisable to involve several caregivers in the consent procedure to reduce this risk. In terms of medical research, children are a special and sensitive group. They lack the ability to give consent for research investigations, have reduced autonomy, and have diminished capacity to appreciate the risks and advantages of the research objectives. They need specific protection against possible rights violations that could happen during research investigations as a result [27].

How does chemotherapy treatment damage the prepubertal testis?

Today, chemotherapy and radiotherapy are both used as anticancer treatments with increasing success, and over the past 30 years, the survival rate has increased from less than 20% to around 80%. However, 10–100% of cancer survivors will have diminished semen parameters, and 15–30% will ultimately stay sterile over the long run, depending on the doses used and the length of the treatment. Any prediction of an individual's fertility is practically impossible because there is interindividual variability in the spermatogenetic recovery following any gonadotoxic treatment. Additionally, even while the initial course of treatment is established when beginning cancer therapy, the treatment plan may alter over time, making it much more challenging to determine the risk for sterility. Therefore, sperm cryopreservation should be made available on a regular basis to all male patients receiving gonadotoxic therapies. Since there are numerous techniques for obtaining sperm from patients who are post pubertal, age should not be a decisive factor. After a patient has been treated, assisted reproduction techniques, such as intracytoplasmic sperm injection, can be used to give the patient the best chance to father their genetically matched children. Testicular stem cell banking may be an option for boys in the prepubescent stage.

At all stages of life, radiation and chemotherapy have a high potential for damaging the testis. It is one of the most radiosensitive tissues, and radiation damage can result from either direct exposure to radiation or radiation that is diffused to other tissues [30]. Testicular damage following chemotherapy depends on the medication and dose [31,

32]. It's equally vital to consider what age a patient receives chemotherapy and radiation treatment. Prepubertal testis germinal epithelium could be less vulnerable to injury than adult testis, according to certain research [33]. However, if chemotherapy doses are calculated per square meter and radiation doses to the gonad given during childhood and adolescence are measured, some chemotherapy agents and radiotherapy doses that cause nonreversible azoospermia in those patients appear to be the same as those for adults [34]. In the testis, there are two significant endocrinologically active cells called Sertoli cells (SCs) and Leydig cells (LCs).

It is crucial to comprehend the precise processes by which various chemotherapy drug classes directly target and harm the prepubertal testis in order to aid in the development of preventative measures. Chemotherapy-related damage might significantly affect a patient's ability to conceive later in life, with possible implications for fertility as well as delayed sexual maturation [35]. The long-term viability of male germ cells, specifically SSCs, and of functional supporting somatic cells, is necessary for fertility [36]. Testis tissue biopsy is not typically done prior to or following chemotherapy treatment, hence research on direct injury to the testis is sparse in a clinical context. Studies employing this tissue should become more prevalent in the future because there has been a recent focus on cryopreserving prepubertal testis samples before the start of cytotoxic therapy for potential fertility preservation in the future. In fact, a recent study histologically investigated testis biopsies from prepubescent patients who were chosen for tissue cryopreservation due to the cytotoxicity of their cancer treatment regimens [37]. Though few have been conducted to date, animal studies have the potential to shed light on the gonadal toxicity of various medications and their mechanisms of action, as well as the effects of therapeutically relevant combination therapies. The research, both human and animal, concentrates on chemotherapy administered during the prepubertal stage, when the effects of the treatment can be observed afterward or deduced from examination of the adult testis later on.

Larger studies have confirmed earlier histological findings seen in early case reports, showing a link between testicular tissue damage and the use of alkylating drugs in treatment plans [38]. Particularly, testicular injury has been connected to the use of the cancer therapy medication cyclophosphamide in prepubertal animals. The studies included under "immediate evaluation" differed in the length of their analyses, looking at testicular injury both during and up to a year after the end of therapy, as well as soon before cessation or at the end of the treatment period. These studies have shown a dose- and time-dependent relationship between cyclophosphamide treatment and testicular injury. Where there is a reduction in the number of germ cells, resulting in Sertoli cell-only tubules, interstitial fibrosis, and basement membrane, treatment can shrink the testis overall [39]. Since comparison between the limited studies that are now available is limited due to

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the constraints previously mentioned as well as the variety of treatment regimens, it is difficult to determine a cut-off dose at which such harm is obvious. The duration of the treatment regimens may also affect how severe the impairment is, with shorter treatment times and higher cumulative doses lowering chemotherapy-induced damage [40].

The somatic and germ cells that make up the testis may react differently to chemotherapeutic medicines than one another. Chemotherapy-induced somatic cell damage may have a negative impact on germ cells and vice versa [41]. As evidenced by alterations in the gene expression of particular spermatogonial markers (MAGE A4 and CD9), it has been observed that cyclophosphamide targets both SSCs and more differentiated spermatogonia in the prepubertal testes [40]. With one study describing the occurrence of immature Leydig and Sertoli cells after cyclophosphamide treatment, this being reliable with the majority of papers that reported effects on germ cells [37].

Assessing pubertal/adult patients who received chemotherapy as children can reveal whether or not the prepubertal testes were injured as a result of the treatment. It can also reveal whether the testes have a chance of recovering and undergoing active spermatogenesis in the future. Sertoli cell only tubules were still present nine years following therapy in patients receiving relatively high doses of cyclophosphamide, resulting in serious testicular injury [42]. The disruption to the prepubertal testes may also depend on the length of the treatment regimen [43]. However, these variations may ultimately be caused by larger cumulative dosages or the age of the patient at the time of treatment, with younger patients possibly being more susceptible to a decreased tubular fertility index and suboptimal Sertoli and Leydig cell development [44, 45]. A case study of a 31-year-old man who received a cyclophosphamide-containing chemotherapy treatment as a child described somatic cell damage in the testis as a result of the chemotherapy, along with the presence of immature Sertoli cells; however, correlation cannot be concluded from a case report [46, 47].

Fertility-preservation methods

Depending on whether pediatric oncological therapy begins before or after puberty, different fertility preservation techniques are available [48, 49]. In addition to well-established metrics, experimental ones are also available.

The preventive treatment option for male adolescents and adults who have a reproductive issue or who are at risk of infertility is cryopreservation of semen. Recent studies have shown that only 39% of affected oncological patients are informed about the possibility of cryopreservation prior to a treatment that could be gonadotoxic [50, 51]. However, non-oncological conditions might also be connected to a surgical procedure that could be gonadotoxic or reduce the number of germ cells, which is a reason to talk about fertility preservation strategies like sperm cryopreservation.

The World Health Organization (WHO) also suggests that men who are interested in having an elective vasectomy be informed about preoperative sperm cryopreser-

vation [52, 53]. It is possible to retrieve and cryopreserve sperm from the testicular tissue in males who have azoospermia or are unable to ejaculate by performing surgical scrotal exploration and testicular sperm extraction, ideally microsurgically [49, 54, 55]. Retrograde ejaculation (post-traumatic, post-operative, or post-radiogenic) permits the cryopreservation of sperm from urine or following rectal stimulation in extremely uncommon circumstances [56, 57].

The only experimental alternative currently accessible for prepubertal boys or early teenage boys whose spermatogenesis is not yet complete is the excision of testicular tissue from the immature tissue in which the spermatogonial stem cells rest and can be cryopreserved.

All systemic treatments that may have gonadotoxic effects as well as all local treatments that may have an impact on gonadal function directly or via their regulatory mechanisms are indications for cryopreservation of sperm and testicular tissue. Additionally, while undergoing surgery that affects sperm deposition negatively over the long term (ejaculation and/or erection), it is medically necessary to preserve fertility. Cryopreservation is also advised for males who are engaged in a risky activity, such as military service, in nations where the use of cryopreserved semen samples is also permitted posthumously [49]. The creation of a reproductive reserve (also known as "social freezing") is theoretically attainable for every man [58].

Sperm Cryopreservation

Prior to any potentially fertility-damaging operation or exposure, an adult male or teenage male should collect and retain his ejaculate for fertility preservation. From the age of 13, Tanner 3, and a testicular volume of less than 10 mL, sperm can be cryopreserved using ejaculation, electrostimulation, and testicular biopsy or testicular sperm extraction (TESE) as a fertility reserve for subsequent assisted reproductive techniques.

The sample is taken while being masturbated. Rectal electrostimulation is an unusual approach for obtaining a semen sample, but it can be discussed, particularly in early to late adolescence. Testicular sperm cell extraction under anesthesia should be preferred in these situations because anesthesia is necessary in these circumstances and the ability to ejaculate is also a sign of maturity in adolescents during puberty development. This is because cryopreservation of stem cells is also an option when the germinal epithelium has not yet fully developed [56, 57].

The WHO advises that in order to increase the likelihood of conception, enough normal semen samples should be cryopreserved to last for at least 10 or more inseminations [52, 53]. The pooling of several samples has not proven to be helpful in the case of the extremely frequent limitations in ejaculate quality, both in oncological patients for fertility preservation and in infertile patients. With conventional sets for ejaculate cryopreservation, which contain 36 straws with 300 L of volume apiece, this is generally ensured. The possibility of creating a second depot should be discussed

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with the patient and made possible if the quality of the semen is noticeably lowered (either very few sperm or extremely few motile or vital sperm).

Cryopreservation of Immature Testicular Tissue

Under local or general anesthesia, and following the proper preoperative conversation with the patient, testicular tissue removal with the goal of testicular sperm extraction (TESE) is carried out after opening the scrotal skin and exposing the testicles on both sides. It is ideal to extract the testicle using microsurgical or microscopically assisted techniques (micro-TESE), but it is also possible to do so from different testicular locations (standard-TESE). In either case, the testicular blood flow must be preserved, and bleeding must be carefully controlled throughout the procedure. Multilocular tissue sample is sufficient in patients whose azoospermia is brought on by an obstruction.

Microsurgical epididymal sperm aspiration (MESA) may also be employed in specific circumstances (for uncorrectable obstruction). Regarding sperm production and postoperative scarring, fine needle aspiration is inferior to open testicular tissue removal and is not advised [55].

There is only the experimental option of eliminating spermatogonial stem cells (SSCs) using testicular biopsy in prepubertal boys because spermatogenesis has not yet begun [56, 57]. When taking testicular tissue for the cryopreservation of spermatogonial stem cells from prepubertal boys or early adolescents, the open biopsy is preferably performed only on one testicle. It is still experimental to cryopreserve surgically excised immature testicular tissue before puberty. It is currently not feasible for humans to undergo the further sperm maturation required from the testicular stem cells. There is also a chance of retransplanting cancerous cells, depending on the malignancy.

Conclusions

Although sperm cryopreservation from the ejaculate is a successful treatment, there is a 50% loss of critical cells. A testicular tumor affects 40% of patients who come in for sperm cryopreservation, followed by people with leukemia, lymphoma, or sarcoma [49]. 20% of all tumor patients are azoospermic at the time of the illness, or unable to ejaculate. The only preventative therapy option for these patients is surgical sperm extraction using testicular sperm extraction, ideally employing microsurgical methods. 60-70% of these individuals will have the opportunity to freeze fertile sperm thanks to this treatment [49].

Because patients with oncological diseases are frequently young when a partnership does not yet exist and frequently only reach the "cured" stage years after the end of therapy, the use of cryopreserved sperm samples is particularly beneficial for these individuals. Cryopreserved sperm is used in roughly 8–11% of patients, according to both recent and older studies [48, 59, 60]. Unfortunately, the severity of the sickness causes about 12% of patients to pass

away [59]. A meta-analysis found that 16% of cryodepots were damaged by the dissolution of the cryodepots due to a temporal recovery of spermatogenesis, which can be delayed for years at a time [60]. Nearly half of the men who used their cryodepots gave birth to at least one child, with intracytoplasmic sperm injection (ICSI) therapy having the best success rates [59, 60].

Applying the information, the risk of gonadal injury should first be assessed. Prior to beginning gonadotoxic therapy, fertility-preservation procedures are strongly advised if there is a high risk of gonadal toxicity. If there is a medium risk, fertility preservation should be explored. If there is a low risk, fertility preservation can also be discussed. The decision-making process should actively involve young patients.

It is important to thoroughly communicate the risk of a reproductive disease to those who are affected and their family members. Additionally, the dangers of fertility preservation techniques must be described, and future options like sperm donation (if legal in the relevant country) must be taken into account. It's crucial to remember that gonadotoxic therapies do not enhance the incidence of congenital defects or non-hereditary cancer in the progeny. After the oncological therapy is finished, precautions must be taken to guarantee appropriate pubertal growth. A fertility assessment should be performed after puberty or at the latest in young adulthood.

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Predictable severity biomarkers in Covid-19

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Abstract

Introduction: The recorded studies suggest that there is clear evidence-based association between various laboratory biomarkers and COVID-19 disease severity. These marker levels reflect the intensity of the cytokine-mediated hyperinflammatory response, which is strongly associated with a poor outcome of SARS-CoV-2 infection.

Conclusions: C-reactive protein is not only a systemic inflammatory marker, but also an important regulator of inflammatory processes. The level of this protein is positively correlated and can be widely used to predict the severity, prognosis and mortality in COVID-19 patients, additionally to vital signs monitoring, supportive care, oxygen therapy, ventilation and circulatory support. Procalcitonin is an indicator of disease severity, which can facilitate timely clinical decision-making, and determination of procalcitonin levels during COVID-19 patients' follow-up, as well as being used in assessing risk, predicting prognosis, and improving patient survival. The assessment of hematological laboratory parameters upon admission, which help in differentiating between severe and non-severe cases, high-risk and low-risk cases of mortality, allows raising awareness, monitoring and timely treatment of patients with COVID-19, as well as their early improvement of clinical condition. Inflammatory biochemical and hemocytometric measures are feasible, easily interpretable, and widely available biomarkers in most healthcare settings, favorable for being used in treatment and severity determination, in predicting clinical outcomes, and in the prognosis of patients with COVID-19. However, the assessment of the accuracy of these biomarkers needs to be determined in further more relevant worldwide studies, showing a more precise design, more accurate performance, and having larger sample sizes.

Key words: COVID-19, SARS-CoV-2, C-reactive protein, procalcitonin, biomarkers, severity prediction.

Introduction

The 2019 coronavirus disease (COVID-19) pandemic remains a scientific, medical, and social challenge. The complexity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lies in the unpredictable clinical course of the disease, which can develop rapidly, causing serious complications and death [1, 2]. The wide clinical range of SARS-CoV-2 infection varies from asymptomatic and mild to severe, critical and fatal. Asymptomatic forms account from 20% to 75% of confirmed cases of COVID-19 among overall population [3]. Most of the affected patients (81%) are mild and moderate cases, approximately 19% of patients develop severe (14%) or critical (5%) forms, the latter two being associated with respiratory failure, acute respiratory distress syndrome (ARDS), multiple organ failure syndrome, in-hospital care followed by poor prognosis and high mortality rates [4, 5].

Up to 30% of COVID-19 patients require hospital admission, whereas the disease was observed to progress in 19.6% of moderate cases, in 27.8% of severe cases, and in 66.7% of critical cases during the hospital stay. About 17-20% of patients are hospitalized within the intensive care unit (ICU). The overall mortality of COVID-19 patients is approximately 2.3%, and the mortality of patients admitted to the ICU is 50-61.5% [4-9].

Given the wide range of SARS-CoV-2 infection severity, it is critical to determine some laboratory biomarkers for risk stratification and outcome prediction in patients with COVID-19, as well as for identifying the patients who might provide objective criteria for disease evolution or rapid diagnosis of a condition resulting in severe complications and death, for supporting the clinical decision-making and the appropriate allocation of medical resources [7, 10-18].

Although, efficient indicators that predict the severity

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and progression of SARS-CoV-2 infection are not yet available, numerous studies have estimated various biomarkers (C-reactive protein, interleukin-6, lactate dehydrogenase, albumin, procalcitonin, white blood cell count, lymphocyte count, platelet count, neutrophil-lymphocyte ratio, C-reactive protein-albumin ratio, D-dimer, ferritin, cardiac troponin, renal biomarkers, etc.) to determine the possibility of predicting the progression and clinical outcomes of the condition, to evaluate the correlation of these biomarkers with disease severity and deaths caused by COVID-19 [2, 14, 15, 19-24].

The above listed laboratory biomarkers may be widely used in monitoring and predicting the disease outcomes and prognosis. However, the dynamic changes, the specificity and the sensitivity of these parameters for the diagnosis of SARS-CoV-2 infection and their correlation with the COVID-19 severity have not been fully studied and explained yet [2, 12, 25-28].

In the context of the aforementioned, the purpose of the present paper was to develop a narrative synthesis of contemporary studies on the correlation between the inflammatory biochemical indicators and hematometric markers with the severity, progression, prognosis and mortality of patients with COVID-19.

The publications were selected from the PubMed, Hinari, SpringerLink, and Google Search databases using the keywords: "COVID-19", "SARS-CoV-2", "C-reactive protein", "procalcitonin", "white blood cells", "lymphocytes", "neutrophils", "neutrophil-lymphocyte ratio", "platelets", "biomarkers", "severity prediction", which were used in various combinations to exploit the search results.

For an extended selection of bibliographic sources, the following filters were used: full-text articles, articles in English, articles published in 2020-2022. After processing the identified information and according to the search criteria, 724 full-text articles were selected. After excluding records not related to the purpose of the study, reviewing abstracts and full-text articles, 89 eligible original articles were selected with a variety of study designs, including editorials, descriptive synthesized articles, systematic and meta-analyses, and cohort studies that contained data on laboratory biomarkers found in patients with COVID-19, which have been qualified as possibly relevant for this synthesis.

In addition, bibliographic listings of the identified sources were searched to highlight additional relevant publications that were not found during the initial database search. After evaluating these sources, a total of 98 relevant publications were ultimately selected. The final bibliography of the present study included 98 articles, which were considered representative of the materials published on the topic of this narrative synthesis.

The information from the publications included in the bibliography was collected, classified, evaluated and synthesized, thus, highlighting the main aspects of the current view on the role of biochemical inflammatory indicators and hemocytometry markers in patients with COVID-19, their relationship and prediction of clinical outcomes, the severity and prognosis of SARS-CoV-2 infection.

In order to minimize the risk of bias within the present study, a thorough search of the databases was conducted to identify the maximum number of publications that were relevant to the study purpose. This present study also evaluated only those that met the criteria of validity, also used secure criteria for excluding articles from the study, and analyzed researches that showed both a positive result and those that do not highlight the benefit of determining inflammatory biochemical indicators and hemocytometry markers in COVID-19 patients.

If necessary, additional data sources were considered to clarify some concepts. Duplicate publications, articles that did not meet the purpose of the article and were not available for full review, peer review and commenting on articles, case series reports, articles with scarce or missing data on the values of the biomarkers under study, non-human studies and those on pediatric populations (<17 years) were excluded from the list of publications.

Results and discussion

The clinical spectrum of the SARS-CoV-2 infection varies greatly, which complicates diagnosis, prognosis and monitoring. Many patients with COVID-19 are asymptomatic or present mild symptoms. One subgroup of patients may initially develop severe disease form, while the non-severe form of the disease might worsen and evolve into a severe one followed by fulminant outcomes in other patients. On examination, subjective clinical symptoms can be more confidently interpreted using biological markers (biomarkers) that provide objective values during the COVID-19 development. The clinical course of the disease is unpredictable, which might suddenly develop, leading to critical clinical complications and even death [1, 2, 8, 12, 15, 16, 20, 29-32].

According to some scientists, systemic hyperinflammation or the "cytokine storm" plays a crucial role in the pathophysiology of SARS-CoV-2 infection [33, 34].

C-reactive protein (CRP). CRP is a non-specific indicator of systemic inflammation induced by various inflammatory mediators, occurring both in acute and chronic inflammation, as well as being an active regulator of the innate immunity. CRP is not only an excellent biomarker of inflammation, infection, and tissue damage, but is also directly involved in the pathological process: it contributes to the inflammatory response by releasing nitric oxide and producing cytokines. Thus, this protein plays a vital role in protecting against infections, preventing autoimmunity, regulating the inflammatory response [15, 20, 35-39] and is a useful marker for monitoring disease severity [20, 35-41].

CRP is typically absent in viral infections, while adaptive immunity is essential for eliminating the SARS-CoV-2 virus, thus, the macrophage activation syndrome may explain the high serum CRP level and how it contributes to disease progression in these patients [30]. Moreover, the strong inflammatory response that occurs in severe

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COVID-19 can cause a significant increase in CRP levels [37, 42].

One of the earliest responses to a viral or bacterial infection is the activation of acute phase reagents, including CRP, ferritin, serum amyloid A, albumin, procalcitonin, erythrocyte sedimentation rate, and proinflammatory cytokines [15, 19, 37].

The main response during the CRP acute phase in COVID-19 could be predicted based on the well-known behaviour of this protein in general and, specifically, in severe viral respiratory infections. Initially, CRP values were found to correlate with the lung lesion diameter, thus characterizing the severity of COVID-19 (mild, moderate, severe, or critical), and predicting poor clinical outcomes, as reported in many, mostly small, cohort studies published worldwide [13, 15, 19, 22, 34, 43, 44]. Two retrospective cohort studies suggested that CRP had better results than other parameters in predicting adverse outcomes in COVID-19 patients [17, 43]. Furthermore, the serum level of CRP upon admission was identified as a moderate differentiation factor for disease severity [43]. Additionally, a large-scale study and several systematic literature reviews and meta-analyses that evaluated the main clinical outcomes of severe COVID-19 have demonstrated the clinical and biological prognostic significance of CRP as a marker of disease activity, prevalence, severity and mortality rate of COVID-19 [1, 2, 33, 45].

CRP activates the complement system, an important component of the innate immune system, induces production of pro-inflammatory cytokines, improves phagocytosis and induces apoptosis, which, together with the inflammatory tendency of COVID-19 progression, can lead to severe outcomes [20, 30, 40, 46, 47]. IL-6 is the most significant cytokine and the main trigger of the "cytokine storm", mainly inducing CRP and directly correlating with this protein levels in COVID-19 patients. However, the CRP activity as a potentially inducing factor of an inflammatory status, resulting in severe COVID-19 evolution, has not been widely assessed yet [15, 19, 20, 30, 35, 38, 39, 40, 47].

The clinical significance of CRP in patients with COVID-19 was demonstrated in two single-centre retrospective studies in China. The studies revealed that most of the patients with severe forms had a significantly higher CRP level, compared to those with the non-severe ones: 100 mg/L versus 9.65 mg/L, respectively (p<0.001) [1, 43] and 57.9 mg/L versus 33.2 mg/L, respectively (p<0.001) [12, 22]. Another retrospective study found higher CRP levels in patients with COVID-19 and severe CT findings compared with those with moderate to mild CT findings. People who died from COVID-19 had higher levels of CRP (85.3 mg/L) compared to survivors and patients who were discharged (53.5 mg/L) [1]. A study from Iran reported that patients with CRP levels >64.8 mg/dl were more likely to develop severe complications, with a sensitivity of 70.05% and a specificity of 70.59%, which was being associated with hospital death in COVID-19 patients [48]. Additionally, high CRP levels correlated with some com-

mon complications among COVID-19 patients (shock, ARDS, acute kidney failure, and acute cardiac failure) and may be a promising biomarker for assessing the mortality rates [29, 44, 46, 48]. CRP level ≥220 mg/dl, measured in the first week of hospitalization, increases the risk of death by 7.73 times and the risk of venous thromboembolism by 2.17 times, thus showing statistically significant results (p<0.001) [49]. In order to distinguish patients with COVID-19 in whom the condition may worsen during treatment, a cohort study has justified the target values for laboratory tests performed upon admission. For CRP, this value was 14.3 mg/L [16].

In the early stage of COVID-19, CRP levels positively correlate with the lung lesions diameter and the severity of the disease [21, 26]. A study conducted in the United States found that significantly elevated CRP levels correlated with a poor prognosis for patient survival. Thus, the CRP level turned out to be a simple, fast and economical tool for assessing the lesion severity, which contributes to the choice of therapeutic options for patients with COVID-19 [1, 41]. Additionally, two studies from Turkey and Iran concluded that the inflammatory parameters, including CRP, were associated with the severity of SARS-CoV-2 infection and can be used as important risk factors for disease progression and mortality prediction [1, 48].

Therefore, multiple studies have determined a sudden increase in CRP levels among patients with severe COVID-19, compared to individuals with non-severe forms (mild and moderate) [1, 32, 37, 33, 50, 51]. Analysis of pulmonary changes assessed by computed tomography revealed a positive association with CRP levels. Moreover, high CRP levels are determined prior to the onset of lung lesions, showing a predictive value for disease severity. The higher the initial values of CRP, the more severe the lung injury and the chances of developing ARDS become imminent [20, 36, 47]. In addition, an inverse correlation between high CRP levels and a decrease in partial pressure of arterial oxygen in the PaO2/FiO2 ratio, which indicates that CRP is also a predictor of acute respiratory failure [47].

Therefore, CRP levels can be the most efficient and sensitive biomarker in predicting the disease progression, as well as in early diagnosis and appropriate management of COVID-19 complications [1, 2, 37, 47]. Since changes in CRP levels occur prior to lung damage, this indicator can be used clinically to predict the prognosis and severity of COVID-19 before its progression and the onset of clinical symptoms [20].

A systematic literature review and meta-analysis based on 25 retrospective cohort studies with a total of 5350 participants found a significant association of CPR with an increased combined poor outcome (mortality, severe COVID-19, ARDS, and need for ICU hospitalization), in severe COVID-19 patients, with the need for ICU admission, but not with mortality alone. The value of CRP ≥10 mg/L has a sensitivity of 51%, specificity of 88% and a positive probability ratio of 4.1, being suggested as the cutoff value of CRP. Serum CRP can be used not

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only as a prognostic marker but also for monitoring the improvement of COVID-19 condition [33].

Another systematic review and meta-analysis, conducted on 32 retrospective cohort studies that reflect data on 10491 confirmed COVID-19 patients, demonstrated an association between elevated CRP level (>10 mg/L) and combined poor outcome, which included ICU admission, oxygen saturation <90%, use of invasive mechanical ventilation, severe forms of the disease, and in-hospital mortality. Since the meta-analysis included studies from different geographical areas, the results provide global findings that can be generalized, while CRP can be used clinically as an early biomarker to identify individuals with increased risk, guide treatment and hospitalization needs, improve prognosis and reduce the mortality rate of COVID-19 patients [2].

In a systematic literature review, Yitbarek G. et al. found significantly higher mean CRP levels in patients with severe forms (81.28 mg/l) compared with patients with mild forms of COVID-19 (33.27 mg/l). /l). The same trend was found in all 15 retrospective cohort studies with a total of 15434 participants included in the review [1].

A systematic literature review and meta-analysis of 18 studies conducted on 3278 COVID-19 patients, including 732 patients with poor outcomes, and a retrospective cohort study of 456 patients with moderate COVID-19 form showed that high levels of CRP upon admission are associated with severe course of the disease, which are predictive of poor outcomes, such as hypoxia, need for ICU admission, need for mechanical ventilation, ARDS or death [17].

Another systematic literature review and meta-analysis of 16 eligible studies including 1896 survivors and 849 deceased patients with COVID-19 demonstrated a significant role of CPR in the outcome of SARS-CoV-2 infection. The deceased patients showed significantly higher concentrations of CRP compared to survivors. A significant association of CRP with mortality was found, while the persistence of high levels of this protein in individuals who died from COVID-19 suggests that CRP is a predictor for SARS-CoV-2 induced death.

The results of the other studies have also confirmed the correlation between CRP level, determined upon patient admission with the severity and mortality of SARS-CoV-2 infection, viz. the CRP level and length of stay were significantly higher in patients with severe forms compared to those with non-severe ones[16, 31, 37, 41, 47, 50, 52-54], the CRP level was higher among deceased patients compared to survivors [37, 43, 47, 48, 50, 53-55], as well as in patients hospitalized in ICU compared to those admitted to the COVID-19 unit [56]. A significant correlation between CRP concentrations and condition worsening in patients with non-severe forms of COVID-19 was observed [37]. A study from the UK demonstrated the significance of IL-6, being the most accurate predictor of death in patients with COVID-19, followed by CRP [1, 57].

Therefore, CRP levels can be an independent biomarker to determine the severity, unfavorable evolution, and mor-

tality in COVID-19 patients, regardless of comorbidities, age, and gender. Trends in the dynamics of CRP values, compared to the initial level, combined with the evolution of clinical manifestations and the need for therapeutic interventions, provide more prognostic data that contribute to careful management of patients. For every unit increase in CPR levels, the probability of developing a severe form of COVID-19 increases by 5% [43, 47, 52, 54, 57, 58].

In conclusion, most studies have shown that SARS-CoV-2 infection is characterized by an excessive inflammatory response, especially in the severe form of the disease. CRP is an independent, accessible and easy-to-interpret biomarker and a key regulator of inflammatory processes. The level of this protein positively correlates and can be used to predict the severity, prognosis and mortality of COVID-19 patients, as well as to early predict the probability of disease progression in asymptomatic cases and in patients with mild infections. To predict the prognosis and severity of COVID-19, CRP can be clinically used before the disease exacerbation and onset of clinical symptoms. The determination of the evolution of CRP levels during COVID-19 patient's follow-up may be of great importance to clinicians in stratifying patients for being transferred to ICU, early detection of severe cases, need for invasive mechanical ventilation, favourable disease progression, followed by improved prognosis.

Procalcitonin (PCT) is traditionally used as a marker of systemic inflammation in the diagnosis of bacterial infections and the severity of sepsis, compared to viral infections, supporting clinical decision-making on the use of antibiotics. Its role in predicting the severity of COVID-19 disease is still being assessed [11, 18, 35, 42, 59-63].

In healthy individuals, the normal PCT level averages 0.033 ± 0.003 ng/ml and is not determined by the methods used in clinical laboratories. In severe infections (bacterial, parasitic and fungal), PCT levels may exceed 100 ng/mL. In viral infections and non-specific inflammatory processes, the PCT level is normal or slightly elevated [10, 63-65].

Bacterial endotoxins and/or cytokines (interleukin-6 and tumor necrosis factor alpha) are well-known triggers for PCT synthesis. High levels of these cytokines have been reported in severe COVID-19 infections. For this reason, PCT can also increase in a hyperinflammatory condition of COVID-19 patients in case a bacterial pathogen is absent [10, 11, 64].

An increase in PCT serum is often observed in hospitalized patients with moderate or severe COVID-19 [10, 11, 35]. PCT synthesis can be stimulated by elevated proinflammatory cytokines, including interleukin-6, interleukin-1β, and tumor necrosis factor alpha [10, 66]. These mediators are greatly involved in the "cytokine storm" that is typical of the transition from the viremia to the hyperinflammatory stage in COVID-19, being characterized by the onset of respiratory symptoms and interstitial lung infiltrates, as shown on chest imaging [18].

Currently, there are more evidence-based associations between elevated plasma PCT concentrations with adverse COVID-19 outcomes. PCT is a predictive biomarker and an

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independent predictor of clinical and adverse outcomes in hospitalized patients with COVID-19, including moderate to severe and critical disease progression, need for ICU admission, need and duration of mechanical ventilation, and mortality rate [60, 64, 67, 68].

Patients with a baseline PCT level >0.1 ng/mL required significantly longer mechanical ventilation (averaging 5.6 days) than patients with a level ≤0.1 ng/mL (p=0.021), a level that can identify patients at risk for prolonged mechanical ventilation at admission. However, there was no significant difference in mortality at 28 days [69]. Two other retrospective studies found an association between PCT levels and the disease severity, but no correlations were found with in-hospital mortality, total length of hospital stay, or ICU length of stay. These results refute several previous studies that found that PCT levels correlated with ventilator duration and mortality [17, 70].

A series of systematic literature reviews, meta-analyses, cross-sectional and observational retrospective studies have demonstrated an association of elevated PCT levels (> 0.5 ng/mL) with COVID-19 disease severity, rapid disease progression, risk of sepsis, admission to the intensive care unit, use of invasive mechanical ventilation or even death. Patients with severe forms of COVID-19 showed a statistically significantly higher increase in PCT compared to patients with non-severe forms [5, 7, 10, 11, 13, 15, 17, 19, 22, 26, 42, 63]. Patients with non-surviving COVID-19 had higher initial PCT levels upon ICU admission compared to surviving patients [18, 65].

The results of a study showed that the mean serum PCT level was over 4 times higher in patients with severe COVID-19 compared to patients with moderate forms of the disease, and over 8 times higher in critically ill patients compared to patients with moderate forms of the condition. Among the discharged patients, both normal and abnormal PCT levels declined during recovery. However, in cases of death, serum PCT levels increased as the disease exacerbated. Thus, PCT may be an indicator of COVID-19 severity that may help determine the severity of patients infected with SARS-CoV-2, and PCT measurements during patient's follow up can be useful in predicting prognosis [68].

Two meta-analyses, which included 52 studies conducted on 6320 and 15296 patients, respectively, have shown a significant statistical association between PCT and the severity of COVID-19. Patients with a high PCT level had a greater chance of developing a severe form of the disease [24, 71]. Another meta-analysis, conducted on 49 studies with a total of 20211 COVID-19 patients, showed an association between 18 factors with a combined adverse outcome, which included death, severe COVID-19 forms, need for ICU admission and/or the need for mechanical ventilation. One of these factors was a high level of PCT that scored 4.8. However, elevated PCT has not been associated with patient's mortality [72].

Any significant increase in PCT (>0.5 ng/mL) is indicative of bacterial co-infection or the development of severe COVID-19 and a more complex clinical picture, whereas a slight increase in PCT (<0.5 ng/mL) is an important indicator for distinguishing between positive and negative patients with SARS-CoV-2 [10]. A recent meta-analysis showed that 63.7% of patients had severe COVID-19 among patients with elevated PCT values, compared with 27.0% of patients with negative PCT [11].

Thus, PCT is a promising prognostic biomarker of COVID-19 progression [71]. However, some studies have shown that the association between elevated PCT concentrations in COVID-19 patients with respiratory failure and mortality may be independent of bacterial co-infection [60]. Therefore, although some authors assume that this positive association reflects the presence of bacterial co-infection, currently there is no adequate evidence to support this hypothesis [11, 60].

A high PCT level at admission may indicate a more severe course of COVID-19 infection with adverse outcomes [11, 64, 70]. A meta-analysis that included 4 studies involving 1418 patients showed that elevated PCT levels increase the risk of severe COVID-19 by about 5 times [10, 68, 73, 74], whereas another meta-analysis conducted on 12 studies that included 2794 patients, of which 596 (21.33%) were patients with severe COVID-19, found that elevated PCT levels were associated with severe SARS-CoV-2 infection [66].

Moreover, a meta-analysis of 25 studies involving 5350 patients with COVID-19 found that elevated PCT levels were associated with an increased risk of poor combined outcomes, including mortality, severe acute respiratory distress syndrome (SDRA), need for intensive care unit admission, and disease severity. The subgroup analysis showed that elevated PCT was associated with an increased risk of mortality and severe COVID-19. A PCT level ≥0.5 ng/mL had a sensitivity of 88% and a specificity of 68% for poor combined outcomes in COVID-19 [33].

Another meta-analysis, which included 32 studies with 10491 COVID-19 patients under study confirmed that elevated PCT levels (>0.5 ng/mL) are associated with the COVID-19 severity and might be used in predicting disease exacerbation. Furthermore, high PCT level may increase the risk of poor combined outcomes by 6 times, which included ICU admission, oxygen saturation <90%, invasive mechanical ventilation, severe forms of the disease, and in-hospital mortality. The meta-analysis included studies from different geographical areas, thus, the results provide global conclusions that can be generalized, and PCT can be used in clinical practice as an early biomarker to improve management and prognosis, as well as reduce the mortality rate of patients with COVID-19 [2].

PCT is significantly associated with both mortality and probability of ICU admission of patients with COVID-19. As PCT levels increase, so does mortality. The obtained data suggest that PCT shows the severity of pneumonia during SARS-CoV-2 infection, which makes it possible to identify patients requiring ICU admission. These results contribute to a growing number of evidences for the usefulness of PCT in the context of COVID-19 infection to guide ICU management and proper resource allocation [74].

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As PCT levels increase among hospitalized COVID-19 patients, there is a significant trend (p<0.0001) of increased disease severity and lung parenchyma injury, determined by imaging and laboratory findings [26, 67].

High PCT levels in COVID-19 patients are associated with clinical, imaging and laboratory characteristics of disease severity and respiratory failure requiring a prolonged invasive mechanical ventilation, longer ventilation duration and increased risk of in-hospital death [60], although in some studies, an increase in PCT was strongly associated with hospital mortality only in older patients (>75 years) [67].

Thus, PCT may be an indicator of disease severity, may facilitate timely intervention, and should be used as a marker for risk assessment and prognosis in patients with COVID-19. Routine determination of PCT within ICU contributes to identifying COVID-19 patients at risk of developing early ARDS and clinical decision-making, while repeated PCT findings during the patients' follow-up may be useful in predicting prognosis, improving survival rate, and optimizing healthcare resources allocation, which may be quite limited in some countries.

Leukocytes. Leucocytes were generally normal or low upon the admission of COVID-19 patients [75]. As the disease progressed, a significant increase in the number of leukocytes was revealed, which is more common in severe COVID-19 compared to non-severe cases [5, 15, 18, 19, 22, 26, 75-78]. The leukocyte count was higher in the group of critically ill patients, compared to patients with severe or non-severe forms of the disease [54, 75]. Furthermore, the number of white blood cells (WBC) was significantly and directly associated with the severity of COVID-19, whereas the leucocytosis (>10×109/L), determined upon the admission of COVID-19 patients, caused a 3-fold higher risk of exacerbation and evolution to a severe form of the disease [15, 54, 75].

These results are supported by several systematic literature reviews and meta-analyses. A meta-analysis based on 52 studies that included 6320 patients with COVID-19 showed that patients with leukocytosis were more likely to develop a severe form, being admitted to the ICU [24]. Another meta-analysis conducted on 21 studies included 3377 patients with COVID-19 and showed a significant increase in white blood cell count among patients with severe and fatal forms of the disease, compared with patients with non-severe forms and survivors [10, 79, 80]. An optimal cutoff value (3.3 x 10^9/L) for the number of leukocytes for differentiating severe from non-severe forms of COVID-19 was also determined [77].

However, some studies have found a decrease in WBC count in both mild and severe cases [75]. A meta-analysis of 22 studies that included 3396 patients with COVID-19 also did not reveal significant changes in the number of leukocytes in patients with severe and non-severe forms of the disease upon ICU admission [7].

Thus, COVID-19 patients show normal or low WBC count upon admission that increases simultaneously with the disease progression. Leukocytosis is associated with an elevated number of neutrophils, tendencies to reduce lymphocytes, monocytes and eosinophils, which means clinical worsening and an increased risk of poor outcomes. However, in some severe cases of COVID-19, leukocytosis may be caused by viral/bacterial co-infections, corticosteroid administration, or *immune response variability.*

Neutrophils were mostly normal in non-severe COVID-19 patients, but increased in severe infections [7, 22]. A systematic literature review and meta-analysis of 22 studies involving 3396 patients with COVID-19 found a significant increase in neutrophil counts in patients with severe disease compared to patients with non-severe disease forms [7].

The possibility that neutrophilia is a predictor of disease severity and poor prognosis has been supported by several studies [15, 19, 75, 81]. Overall, neutrophil counts were statistically significantly higher in non-survivors than in survivors [18, 19, 75, 81] and in patients with severe cases compared with patients with non-severe forms of COVID-19 [15, 19, 75].

A meta-analysis of 52 studies involving 6320 patients with COVID-19 found that patients with neutrophilia were more likely to develop severe disease phenotype, being admitted to the intensive care unit, and were more likely to die. The prognostic neutrophil cutoff value for identifying patients at high risk of severe COVID-19 was ≥3.74×109/L [24].

In contrast to studies reporting neutrophilia, other smaller studies stated opposite conclusions. The latter did not find neutrophilia but a significant decrease in granulocytes (eosinophils, basophils, and neutrophils) in patients with severe COVID-19 compared to non-severe forms, normal and even low neutrophil counts in COVID-19 patients compared to healthy control group, though, when comparing the disease severity, the leukocyte count was much higher in patients with severe forms of the disease [75].

Thus, the number of neutrophils in blood is normal in patients with non-severe forms of COVID-19 and increases along with severe infections. Neutrophilia was significantly associated with the risk of disease progression to a severe phenotype, as well as the risk of ICU admission, and even death.

Lymphocytes. All studies reported lymphocytopenia, which is the most common hematologic abnormality and correlates with COVID-19 severity. In patients with a severe form of the disease, the number of lymphocytes is significantly reduced compared to patients with non-severe forms [5, 7, 18, 22, 26, 75, 76, 78, 82, 83]. Large-scale studies have shown that 83.2% of hospitalized COVID-19 patients had lymphocytopenia upon admission [75], which was more and less prominent in severe and critical cases (80–85.7%) [10, 27, 63, 84] compared to non-severe cases (25-44.4%) [10, 14, 27, 84].

These results were confirmed by three meta-analyses. A meta-analysis of 32 studies involving 10491 COVID-19 patients showed that lymphocytopenia is associated with a significantly higher risk of poor outcomes in hospitalized

COVID-19 patients [2]. Another meta-analysis conducted on 21 studies involving 3377 COVID-19 patients found a significant decrease in the number of lymphocytes among patients with severe and fatal forms of the disease compared to non-severe cases and survivors [10, 79]. A systematic literature review and meta-analysis performed on 22 studies involving 3396 COVID-19 patients showed a significant decrease in the number of lymphocytes in patients with severe disease compared with patients with non-severe forms [11].

The decrease in the number of lymphocytes upon admission was the most important and sensitive marker in predicting the severity, progression and outcome in patients with COVID-19 [15, 19, 75, 83]. More severe lymphocytopenia was predominantly present in non-survivors compared to survivors [18, 19, 48, 75, 82].

Thus, during the incubation and early stage of COVID-19 disease, the number of lymphocytes in peripheral blood is normal or slightly reduced. Lymphocytopenia, regardless of other indicators, comorbidities, age and gender, correlates with the COVID-19 severity and is a reliable indicator of early infection with SARS-CoV-2 and poor prognosis, as well as contributes to the assessment of disease progression. An association has been demonstrated between lymphopenia and the need for ICU care, the development of ARDS, and increased risk of death.

The neutrophil-lymphocyte ratio (NLR) constantly increases in patients with severe COVID-19, compared to patients with non-severe forms. In addition, the prognostic value of NLR as an independent predictor of severe forms and progression of SARS-CoV-2 infection has also been demonstrated [5, 15, 27, 75-78].

Several studies have highlighted a significant association of NLR with COVID-19 severity [10, 77, 82, 85]. A higher NLR upon admission was an independent predictor for the development of severe COVID-19, for poor clinical outcome among COVID-19 patients [4, 21, 75, 77, 82, 85] and for the lethal outcome due to COVID-19 [9, 76, 85, 86]. According to some study results, the NLR value equal to 2.973 upon admission, with a specificity of 66.8% and a sensitivity of 75.8%, may predict disease progression during hospitalization [10, 87], NLR value of 3.2-4.795, with a sensitivity of 56.3-88.3% and a specificity of 63.6-83.7%, may predict a severe COVID-19 form [5, 77, 88], and NLR value > 6, with a sensitivity of 86.7% and a specificity of 84.4%, predicts death due to COVID-19 (p <0.001) [88]. NLR, determined upon admission, was independently associated with death in patients with severe COVID-19, namely, the risk of death increased by 5.7% for each unit increase in NLR [89]. Therefore, the NLR value can indicate the COVID-19 severity and outcomes [5, 10, 77, 87, 88].

Small studies have reported high NLR in severe cases of COVID-19 [22, 75]. A study including a cohort of 452 patients with COVID-19 found that patients with severe infection (286 patients) had a statistically significantly higher NLR compared to patients with non-severe forms (5.5 and 3.2; p<0.001) [22].

A series of studies have suggested that an elevated NLR level is largely associated with the severity of COVID-19, being an independent biomarker of poor clinical outcomes, viz. the disease progression to severe, critical and even fatal ones [17, 19, 77]. Multivariate analysis showed that with an increase in NLR per unit, the risk of in-hospital death increases by 8%, whereas the deceased patients had a NLR ≥10.8 [86].

Two systematic literature reviews and meta-analyses conducted on 6 and 22 studies involving 828 and 3396 patients with COVID-19, respectively, found a significant increase in NLR values in patients with severe forms compared with patients with non-severe forms of the disease [10, 11, 90]. Another more recent and larger meta-analysis that included 64 studies involving 16205 COVID-19 patients found that NLR upon admission predicted both severity and mortality, and NLR >6.5 was associated with significantly higher mortality rates. The authors concluded that NLR is a consistent biomarker for predicting the disease severity (with a sensitivity of 80.2% and specificity of 75.8%) and mortality (with a sensitivity 78.8% and specificity of 73.0%) of COVID-19, regardless of age, gender, or comorbidities [91].

To improve the stratification and management of patients with COVID-19, a predictive model based on NLR and age was developed. The incidence of severe SARS-CoV-2 infection was only 9.1% in patients aged ≥50 years and NLR <3.13, while 50% of patients aged ≥50 years and NLR ≥3.13 developed a severe COVID-19 [75]. Around 94.5% of patients who died from COVID-19 complications had a NLR >5 [75, 81]. Due to the confirmed consistency and significance, high NLR can be used as an admission-screening tool to identify patients with increased risk of COVID-19 [75].

According to the results of a systematic literature review and a meta-analysis that evaluated 29 studies involving 4911 patients, NLR may be a better biomarker of systemic inflammation and COVID-19 severity than neutrophil or lymphocyte counts, analysed separately [15].

Therefore, elevated NLR results from an increase in the number of neutrophils and a decrease in the number of lymphocytes, which can be quickly calculated based on a routine blood test upon the patient admission. For patients with COVID-19, NLR has been shown to be an independent risk factor for severe forms of the disease, viz. there is a strong relationship between increasing NLR levels and COVID-19 severity. NLR is an early inflammatory marker that reveals severe and critical infection with SARS-CoV-2, as well as being an independent risk factor of in-hospital mortality for COVID-19 patients.

The NLR assessment can help identify individuals at high risk of contracting COVID-19, early detection, as well as in treatment of severe pneumonia in COVID-19 patients. This marker is very important, especially in areas where diagnostic tests are limited, which often creates difficulties in diagnosing SARS-CoV-2 infection. However, further research is needed to confirm these findings, to

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compare the predictive ability, as well as to determine the change in NLR depending on the treatment used.

Platelets. Platelet counts upon admission tended to be lower in severe cases of COVID-19 compared to non-severe cases [5, 7, 18, 19, 75], as well as in non-survivors compared to survivors [10, 18, 19, 28, 92].

Low platelet count has been identified as a prognostic factor and an indicator of the disease severity and clinical worsening during hospitalization [75]. Thrombocytopenia has been associated with an increased risk of severe disease, the need for ICU hospitalization, and mortality in patients with COVID-19 [10, 19, 28, 92]. Terpos et al. showed more frequent thrombocytopenia in patients with severe forms of COVID-19 (57.7%) compared to patients with non-severe forms (31.6%) [14]. Zhang et al. reported a platelet count <100×109/L in the last 24 hours before death in 63.2% of patients infected with SARS-CoV-2 [75, 81]. A large study conducted on 1476 COVID-19 patients, including 1238 (83.9%) survivors and 238 (16.1%) non-survivors, found thrombocytopenia in 306 (20.7%) cases, with a statistically significant increase in patients who died compared to survivors (72.7% vs 10.7%, p<0.001). The authors found a significant relationship between platelet count and COVID-19 mortality, with lower platelet counts associated with higher mortality. In patients with platelet counts of 0-50x109/L, 50-100x109/L, 100-150x109/L, and >150x109/L, the in-hospital mortality rate was 92.1%, 61.2%, 17.5%, and 4.7%, respectively (p<0.001) [28].

The role of thrombocytopenia as a clinical indicator of exacerbation and prognosis of patients with SARS-CoV-2 during hospitalization, as well as a predictor of severity and mortality of COVID-19 patients has been confirmed by systematic review and meta-analysis studies. A systematic literature review and meta-analysis of 22 studies, conducted on 3396 COVID-19 patients, found a significant drop in the number of platelets in patients with severe forms of the disease, compared to patients with the non-severe forms [7]. A meta-analysis that included 21 studies involving 3377 COVID-19 patients demonstrated a significant reduction in the number of platelets among patients with severe and fatal forms of the disease compared to non-severe forms and survivors [10, 79]. Another meta-analysis evaluated 9 studies involving 1779 COVID-19 patients, including 399 (22.4%) with severe forms, which suggested that thrombocytopenia is significantly associated with COVID-19 severity. A greater decrease in platelet counts was observed in deceased patients [14, 92].

However, some studies have not found significant differences in platelet counts between patients with severe and non-severe forms of COVID-19 [76].

Thus, patients with COVID-19 typically have normal or low platelet counts upon admission, though dynamic changes might occur during hospitalization. Several systematic reviews, meta-analyses, and studies have shown that thrombocytopenia is a predictor of severity and mortality in patients with COVID-19. Thrombocytopenia has been associated with an increased risk of over 5 times for severe forms of COVID-19. Platelet count monitoring may be useful as a clinical indicator of deterioration and prognosis in patients with SARS-CoV-2 during hospitalization.

In conclusion, the results of the present summary article show that there is clear evidence for an association between inflammatory and hematological laboratory biomarkers and the severity of COVID-19. These marker levels exhibit the intensity of the cytokine-mediated hyperinflammatory response and are strongly associated with poor outcome of SARS-CoV-2 infection. The greater the change in indicators, the greater the incidence of severe COVID-19 [58, 76]. They can be used as an adjuvant in clinical practice to guide treatment and hospitalization needs, to improve prognosis and reduce mortality, as well as to manage health care resources appropriately. The combined analysis of prognostic biomarkers contributes to a more accurate identification of the flare-up risks of severe COVID-19 resulting into unfavourable prognosis in patients with mild or moderate forms of the disease [2, 51].

Nevertheless, the interpretation of some study results present in this summary paper is limited due to the predominance of retrospective single-centre, small-sized sample studies due to the lack of consistency in determining the severity of the disease, lack of an accurate chronology of laboratory sample collection, lack of serial measurements of samples, as well as the termination of some studies without reporting the final results, since prompt data publication is required during a pandemic situation [19]. An assessment of the accuracy of these biomarkers needs to be determined in more relevant multi-centre studies with more precise designs, more in-depth performance, and larger sample sizes [2].

Conclusions

1. Inflammatory biochemical markers and hemocytometry markers are feasible, easily interpretable, and widely accessible biomarkers in most healthcare centres, as well as favourable for being used in the management of COVID-19 patients.

2. C-reactive protein correlates positively and can be used to predict the disease severity, prognosis and mortality in addition to vital signs' monitoring, supportive care, oxygen therapy, ventilation and circulation support in patients with SARS-CoV-2 infection.

3. In order to guide treatment and monitor patients, predict the prognosis and COVID-19 severity, C-reactive protein can be used clinically even prior to disease progression and the onset of clinical symptoms. Serial determination of C-reactive protein levels during the COVID-19 patients' follow-up has become an important diagnostic algorithm for admitting patients to the intensive care unit, early detection of severe cases, as well as for early implementation of invasive or non-invasive ventilation techniques, disease progression and outcome assessment.

4. Procalcitonin is a promising predictive biomarker for COVID-19 disease progression, whereas the association between elevated procalcitonin concentrations in COVID-19 patients with respiratory failure and mortality shows independence of bacterial co-infection.

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5. Procalcitonin may be an indicator of disease severity and may facilitate timely intervention. It should be used as a marker for risk assessment and prognosis in COVID-19 patients. Routine procalcitonin testing within the intensive care unit may identify patients with COVID-19 who are at risk of developing early acute respiratory distress syndrome and may facilitate clinical decision-making and serial measurements of procalcitonin that are useful in predicting prognosis and improving patient survival, as well as in optimizing medical resources allocation.

6. Leukocytosis, neutrophilia, lymphocytopenia, neutrophil/lymphocyte ratio, and thrombocytopenia have been identified as independent factors for poor clinical outcome in patients with COVID-19 and can be useful in predicting disease progression, need for ICU admission, and mortality rate.

7. The estimation of laboratory hematological parameters upon admission, used in differentiating severe cases from non-severe ones and high-risk cases from those with low mortality risk, allows raising awareness, proper follow-up and timely treatment of patients with COVID-19, as well as early improvement of their clinical condition.

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Unmet needs in the treatment of chronic lymphocytic leukemia and prospects for patients in the Republic of Moldova

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Abstract

Background: Chronic lymphocytic leukemia (CLL) is a malignant lymphoproliferative, monoclonal, indolent hemopathy characterized by pathologically increased synthesis of mature but immunologically dysfunctional B lymphocytes. CLL is considered the pathology of the adult with comorbidities, whose average age is 55 years, who is integrated into the work field. Despite advances in the field of medicine, CLL remains an incurable disease, but the use of targeted, personalized therapy with new agents' conditions not only increases the life span, but also improves the of quality life of these patients, ensuring social, family and professional integration. This fact presents a favourable socioeconomic impact, a valuable indicator especially in countries with a low and medium socio-demographic index. The identification of biomarkers and the advent of personalized therapies have transformed the way the disease is treated and changed the lives and quality of life of CLL patients.
Conclusions: CLL remains a current medical and socioeconomic problem, and the behaviour of patients with CLL remains a challenge for the health system in the Republic of Moldova. The implementation of cyto-genetic and molecular-biological diagnosis is important for the stratification of patients, the selection of optimal targeted therapy. CLL comorbidity is an independent indicator of treatment response, predisposes to adverse drug effects and reduces the quality of life of CLL patients. The individual approach to the patient with CLL, the administration of therapy according to international guidelines will give patients better chances of survival and a longer plateau of stabilization.
Key words: chronic lymphocytic leukemia, personalized therapy.

Introduction

Chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) is a malignant lymphoproliferative, monoclonal, indolent hemopathy characterized by increased pathological synthesis of mature but immunologically dysfunctional B-lymphocytes [1]. Every year, 191000 patients are diagnosed *de novo* worldwide [2]. The incidence in the Republic of Moldova is 2.2 per 100000 inhabitants, according to the National Cancer Registry.

CLL is considered the pathology of the adult, with an increase in the incidence of *de novo* cases with age. Worldwide, the average age of a patient with CLL is 55 years. CLL develops extremely rarely in children [3]. Population-based studies have shown that CLL presents epidemiologic, geographic, and ethnic variations [4]. It is most frequently diagnosed in the Western population, Caucasians, the United States of America. Lymphoproliferative pathology is rarely estimated in Asian countries (China and Japan) and India [5]. According to the data of the Hematology Department of the Republic of Moldova at the Oncological Institute, there are 508 patients with CLL (+144 patients with SLL), which is alarming, considering the decreasing number of the general population.

In the last 10 years, a number of new agents have been added to the therapeutic approach of CLL, including Bruton's tyrosine kinase (BTK) or Phosphatidylinositol-3-kinase delta (PI3Kδ), an antagonist of the antiapoptotic protein BCL-2, and novel anti-CD20 monoclonal antibodies, providing a period of varied survival of 2-20 years, with a median survival of 10 years [6]. In recent years, the identification of biomarkers and the advent of personalized therapies have transformed the way the disease is treated, which changed the lives of CLL patients, optimally integrating these new agents into the traditional treatment algorithm without overlooking or compromising the benefits of established treatments, especially chemoimmu-

notherapy. It is even more relevant in the era when most countries with universal health coverage are switching to chemotherapy-free regimens. Although chlorambucil monotherapy is considered palliative in these countries [7] it is often the only therapy available to most patients in low to medium socio-demographic index countries [8]. According to the same sources, the Republic of Moldova is placed among the countries with a medium-high socio-demographic index, as well as the neighbouring countries: Ukraine and Romania. Despite advances in medicine, CLL is still an incurable disease, but the targeted use of newer biological agents increases the duration and quality of life of these patients.

The literature search was performed using the search terms "chronic lymphocytic leukemia", "individualized treatment", "biological profile" and they were selected from databases, such as PubMed, Google Scholar, Scopus and Elsevier. The material was selected based on the studies published until 01/ 2023, which aimed to elucidate the new challenges of lymphoproliferative diseases. The articles which do not correspond to this article goal were excluded.

Analysis and discussion

Knowledge about CLL has undergone radical changes over the past decades and remains in constant evolution [6]. The stages of the development of CLL treatment options denote the close connection between the progress of the deeper study of the pathogenesis of this malignant hemopathy and the development of targeted treatment methods, which allows an individualization of the therapeutic behaviour of a patient with CLL (fig. 1).

Newer molecular techniques, clinical trials with new agents have led to a change in the natural history of this disease. Ongoing preclinical and clinical studies will reveal newer therapeutic targets and continue to improve patient medication outcomes, which will have a positive impact on

their quality of life [9]. Current treatment options cannot cure CLL, except for allergenic hematopoietic stem cell transplantation [10].

The clinical progression of CLL is heterogeneous and ranges from patients who require treatment immediately after diagnosis to others who do not require active therapy for many years, if at all [11]. The selection of first-line therapy in patients with CLL depends on the patient, patient preferences, the biological profile of the tumour, and the goals of the proposed therapy. Elderly patients, who constitute the representative group, often have one or more comorbidities (Charlson score ≥2), a fact that does not allow them to follow and tolerate chemotherapy [12]. According to the static data of the National Cancer Registry of the Republic of Moldova of 508 CLL patients and 144 patients diagnosed with small lymphocytic lymphoma, 345 (68%) are elderly people over 65 years old, and in 93 (27%) patients with CLL, 2 or more comorbidities were found. According to the National Bureau of Statistics, in 2021, the Republic of Moldova saw a decrease in the average life expectancy, which was 69.1 years, decreasing by 0.7 years compared to 2020 and by 11.3 years less compared to the average level of life expectancy at birth in the 27 EU countries in 2020 (80.4 years) [13]. According to the NCCN guidelines (Version 1.2023), the treatment strategy for elderly patients with comorbidities includes the administration of obinutuzumab in the first line of treatment [14], but to which patients from the Republic of Moldova still do not have access (tab. 1).

Obinutuzumab is a humanized type II anti-CD20 monoclonal antibody of the IgG1 subclass, in combination with chlorambucil is indicated for the treatment of adult patients with previously untreated CLL and comorbidities [15]. Clinical studies have confirmed the ability of obinutuzumab:

1960-1970
•,,wait and watch''
•Alkalyting agents (Chlorambucil,Cyclophosphamide)

1980-1990
•Purine Analogs (Fludarabine, Penthostatin, Cladribine)

2000
•Purine analogs+alkylators (Fludarabine+Cyclophosphamide)

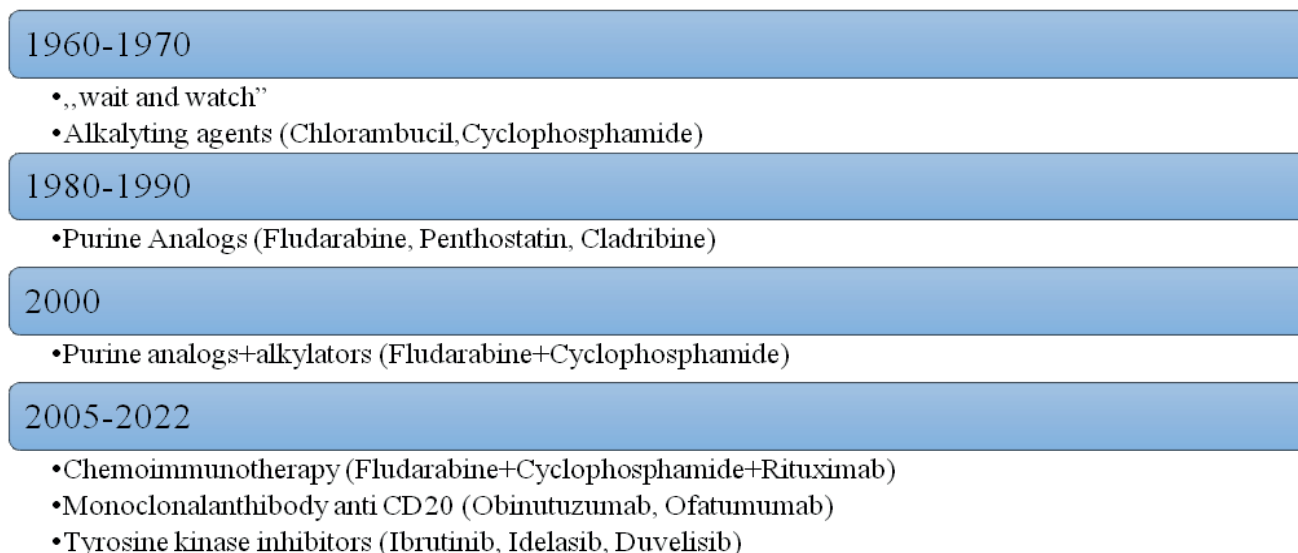| 2005-2022 |
| --- |
| •Chemoimmunotherapy (Fludarabine+Cyclophosphamide+Rituximab) |
| •Monoclonalanthibody anti CD20 (Obinutuzumab, Ofatumumab) |
| •Tyrosine kinase inhibitors (Ibrutinib, Idelasib, Duvelisib) |

Fig. 1. Historical progress of CLL therapy [6]

Table 1. Suggested treatment regimens CLL/SLLl without del (17p)/*tp53* mutation.
First line therapy, NCCN [15]

Preferred regimens	Other recommended regimens	Useful in certain circumstances
Acalabrutinib±obinutuzumab (category 1). Venetoclax+obinutuzumab (category 1). Zanubrutinib (category 1).	– Ibrutinib (category 1). – Bendamustine+anti-CD20 mAb. – Chlorambucil+Obinutuzumab – Obinutuzumab. – High-dose methylprednisolone (HDMP)+rituximab or obinutuzumab (category 2B; category 3 for patients <65 y without significant comorbidities). – Ibrutinib+obinutuzumab (category 2B). – Ibrutinib+ rituximab (category 2B). – Ibrutinib+venetoclax (category 2B).	(Consider for IGHV-mutated CLL in patients age <65 y without significant comorbidities) – FCR (fludarabine, cyclophosphamide, rituximab).

– To induce cell death and increases direct cytotoxicity [16, 17],

– To increase cytotoxicity of antibody-dependent lymphocytes and phagocytosis [16, 18],

– To reduce complement-dependent cytotoxicity [16],

– To initiate malignant B cell apoptosis.

The mechanism by which obinutuzumab induces cell death is explained by the fact that it binds to another part of the CD20 chain, unlike rituximab. In this way, an increase in antibody-dependent lymphocyte cytotoxicity and cell death is induced [19].

CLL11 is a phase III study comparing obinutuzumab with the standard treatment for patients with CLL (rituximab + chlorambucil), demonstrating that compared to the combination of rituximab + chlorambucil, the combination of obinutuzumab + chlorambucil, in patients with comorbidities in the first line of CLL therapy, is 3 times more likely to achieve complete remission [18, 20].

Chemotherapy being non-specific is associated with significant toxic effects, which is why the duration of treatment and its level are limited to older patients with a more deteriorated physical condition.

Research into the biology of CLL has profoundly improved our ability to identify patients at higher risk for disease progression and our ability to treat patients with drugs that selectively target distinct phenotypic or physiologic features of CLL. According to the recommendations of the ESMO Clinical Practice Guidelines, the therapeutic approach is mainly focused on the biological profile of the leukemic clone [21]. Thus, to guide the choice between chemoimmunotherapy or targeted treatments, testing for del(17p), TP53 mutations and immunoglobulin heavy chain variable status (IGHV) is a priority [22]. These researches, aimed at evaluating the biological profile, are listed in the national clinical protocol PCN-65, however they are not yet accessible to CLL patients in the Republic of Moldova [23].

Chemoimmunotherapy using anti-CD20 monoclonal antibodies, such as the fludarabine, cyclophosphamide, and rituximab regimen, remains the standard of care for CLL patients aged <65 years, in good health, and with low-risk prognostic factors [24]. Recent advances in the understanding of the pathogenesis of CLL have significantly improved the range of therapeutic approaches of the treatment of CLL. Therapeutic strategies targeting BCR signalling have been developed due to the pivotal role of BCR signalling in the pathogenesis of CLL. In addition, venetoclax, a BCL2 inhibitor, has significantly changed the therapeutic strategy for the treatment of CLL.

Regarding surface molecules, CD19, CD20 and CD52 have been extensively investigated as therapeutic target molecules in CLL. CD19 is a B-lymphocyte lineage-specific surface molecule (LB) involved in BCR signal transduction [25]. CD19 expression is restricted to the LB and hematopoietic stem cell lineage. T lymphocytes bearing a chimeric antigen receptor (CAR T cells) have been developed as a new cell therapy [26]. The efficacy of CAR T cells against CLL was first reported in 2011 [27-32].

CD20 is a surface glycoprotein expressed on mature LBs and its expression is restricted to the B-cell lineage. Most hematopoietic cells do not show this expression, therefore anti-CD20 monoclonal antibodies, such as rituximab, ofatumumab and obinutuzumab have been developed and used in the treatment of malignant tumours with mature LB [33]. Rituximab has revolutionized therapeutic strategies for mature B-cell malignancies, including CLL. Rituximab has been shown to be as effective and tolerable as monotherapy for non-Hodgkin's lymphoma [34]. Still, rituximab monotherapy was less effective against CLL [35]. In contrast to rituximab monoregimen, immunochemotherapy using riruximab, such as fludarabine, cyclophosphamide, and rituximab, is significantly more effective against CLL [36].

Ofatumumab monotherapy, a human monoclonal anti-CD20 antibody, is an effective, well-tolerated treatment for patients with fludarabine-refractory CLL [37]. The safety and efficacy of combination therapies and maintenance therapy using ofatumumab have been investigated in several studies [38-40].

A phase 1/2 clinical trial showed that obinutuzumab monotherapy is effective for patients with heavily pretreated refractory/relapsed CLL [41]. A randomized phase 3 trial demonstrated that the addition of obinutuzumab to chlorambucil significantly prolonged overall survival compared with chlorambucil monotherapy in patients with untreated CLL ineligible for intensive chemotherapy [42].

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Alemtuzumab is a humanized anti-human CD52 IgG1 monoclonal antibody. The efficacy of alemtuzumab has been investigated in previously treated [43] and untreated CLL patients [44], and the FDA approved it for the treatment of fludarabine-refractory CLL in 2001.

In addition to surface molecules, therapeutic strategies targeting BCR signalling have been developed to treat CLL. Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, is an orally bioavailable small molecule that covalently binds to the cysteine-481 residue of BTK. Ibrutinib has shown potent activity against previously treated CLL or CLL with TP53 aberrations [45-48].

New BTK inhibitors, such as acalabrutinib, tirabrutinib and zanubrutinib have been developed and their efficacy and safety profiles have been clarified in clinical trials [49-52]. Recent studies investigating acalabrutinib, a second-generation BTK inhibitor, confirmed the efficacy of combination therapy consisting of acalabrutinib and obinutuzumab in patients with *de novo* and relapsed/refractory CLL [53].

Idelalisib is a potent and selective PI3Kδ inhibitor [54, 55]. Oral idelalisib therapy showed a favourable safety profile and rapidly induced stable disease control in the majority of heavily pre-treated CLL patients [56]. Combination therapy with idelalisib and rituximab resulted in a higher overall response rate than rituximab monotherapy in patients with relapsed CLL [57, 58]. Duvelisib, a dual inhibitor of PI3Kδ and PI3Kγ, was approved by the FDA for relapsed or refractory CLL/small lymphocyte lymphoma in 2018 based on the results of the phase 3 DUO trial [59].

In addition to new drugs targeting BCR signalling pathways, such as BTK and PI3K inhibitors, the BCL2 inhibitor venetoclax has significantly changed the treatment of CLL. This BH3 domain sign prevents the interaction between BCL2 and BH3 and inhibits the anti-apoptotic effects of BCL2. The efficacy and safety of daily oral venetoclax for relapsed or refractory CLL was reported in a phase 1 dose-escalation trial [60]. A phase 2 trial of venetoclax monotherapy in patients with del17p relapsed or refractory CLL reported an overall response rate of 79.4% at a median follow-up of 12.1 months [61]. The recent phase 2 CLARITY trial investigating the combination of ibrutinib and venetoclax for relapsed or refractory CLL reported a high minimal residual disease (MRD) eradication rate [62]. Based on these trials, a MRD-guided treatment strategy may be the standard of care for CLL in the near future. Further studies will be useful to establish therapeutic strategies using such novel drugs and to improve clinical outcomes in CLL.

On December 2, 2022, in Chisinau, the Republic of Moldova, the advisory council of experts in the field of haematology oncology was held, entitled "Unsolved problems in the therapy of chronic lymphocytic leukemia and the prospects for patients from the Republic of Moldova".

It is important to note that the discussions at the end of this meeting involved all the participants who unanimously advocated a renewed approach to patients with CLL and the adaptation of treatment strategy according to international standards. The presence of authorities, representatives of the MoH and CNAM speaks of everyone's interest in solving patients' problems and improving their quality of life, by increasing funding for oncological diseases in general and CLL in particular.

Conclusions

1. CLL remains a current medical and socioeconomic problem in the Republic of Moldova, and the behaviour of patients with CLL remains a challenge for the health system in the country.

2. Implementation of cyto-genetic and molecular-biological diagnosis is important for the stratification of patients and the choice of the optimal therapeutic regimen, including target therapy.

3. CLL comorbidity is an independent indicator of treatment response, predisposes to adverse drug effects and reduces the quality of life of CLL patients.

4. The individual approach to the patients with CLL and the administration of therapy according to international guidelines will give patients better chances of survival and longer remission.

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Authors' contributions
IV designed the research, did statistics and interpreted the data, drafted the first version of the manuscript; TP conceptualized the project and designed the research, revised the manuscript critically; OK, IS interpreted the data, revised the manuscript critically; SB revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

Ethics approval and consent to participate
No approval was required for this study.

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BOOK REVIEW

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The monograph
"Aspects of patient safety in anaesthesia. Medication errors in anaesthesia and intensive care"
Print CARO Printing Company, Chisinau, 2023, 145 pages

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Patient safety is one of the biggest challenges facing healthcare around the world. Safety is the foundation on which quality and then patient-centered care is built. All combined they must meet the goal of the highest standards of care. The goals of safety and quality must be achieved before, during and after the application of anaesthesia.
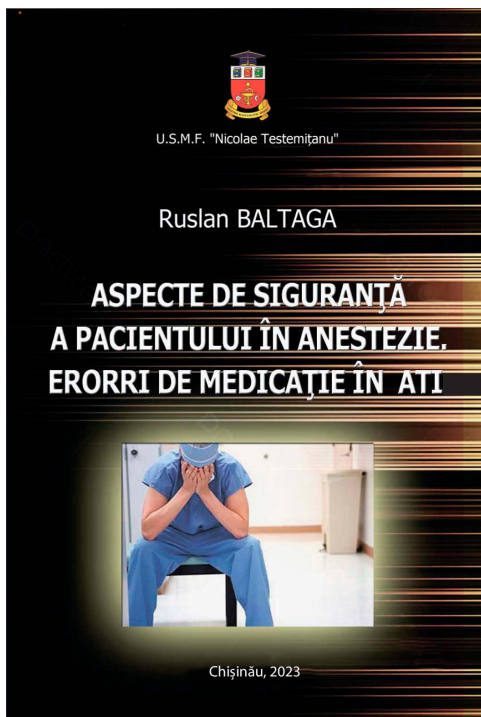
The concepts of quality and safety are closely interconnected and provide continuity. There is no common opinion as to the clear difference between them. Safety can also be seen as a quality issue and quality care must be based on safety. This is applicable more to medicine in general and less to anaesthesia. An important difference is that quality is usually measured in terms of success, while safety is measured in failures, especially catastrophic failures.

Success in achieving the desired outcome includes not only safe procedures, but also those that incorporate elements of evidence-based medicine, mainly because one's own experience is not sufficient in judging the positive or negative consequences of the procedure, or of the drug. Since anaesthesia is not a therapeutic procedure on its own, complete safety must be the primary goal of every anaesthesia. This can sometimes seem an impossible task.

To everyone's benefit, quality and safety have become increasingly important in modern medicine. Anaesthesiology and Intensive Care are frequently identified as an early adopter and promoter of patient safety principles, and held up as an example in dramatically improving outcomes.

In the Republic of Moldova, the concept of patient safety in anaesthesia is one of the research topics of *Valeriu Ghereg* Department of Anaesthesiology-Reanimatology No 1 of *Nicolae Testemitanu* State University of Medicine and Pharmacy and is coordinated by the author of this monograph. Society of Anaesthesiology and Reanimatology of the Republic of Moldova has adopted and is promoting Helsinki Declaration on Pateient Safety in Anaesthesia. In the frame of these activities a series of studies and implementation processes have been carried out to improve patient safety at the National level. This monograph is perfectly fitting the strategy to improve patient safety in anaesthesia, intensive care and related fields. The monograph is divided in two parts.:

In Chapter 1 – "Patient Safety in Anaesthesia" the patient safety issues are described starting with generalities and continuing with patient safety in Anaesthesia, Intensive Care. Combined literature review data and results of own studies "Implementation of WHO Surgical Safety Checklist and Global Pulsoximetry" which describes results of their implementation in the Institute of Emergency Medicine of the WHO Checklist together with its Pulse Oximetry component and the remote follow-up study results are presented as well. The results of the implementation of the checklist showed an improvement of the communication parameters within the anaesthesia and surgical team, as well as an improvement of the surgical treatment results (fewer septic and non-septic complications). In addition to the complex aspect of the Checklist items, the influence of pulse oximetry monitoring was studied (also implemented in all operating rooms, before the study there were only 2 functioning pulse oximeters at 22 operating tables). In the study, pulse oximeters had the possibility to record pulse oximetry parameters to all patients in all rooms. Data analysis showed a reduction in hypoxaemia episodes over time after implementation of surgical safety measures, including pulse oximetry in all operating rooms. These results stressed once more the importance of mandatory monitoring of patient during anaesthesia as an important component of patient safety. A study comparing oxygenation in local anaesthesia vs general anaesthesia is reported in this chapter. Oximetry values ranging from 93-98% are recorded more in general anaesthesia patients, and values from 90-92% are virtually identical in both types of anaesthesia. The explanation could come from the fact that although the above-mentioned values are within the normal range, nevertheless a tendency for better oxygenation is seen in regional anaesthesia, where usually (in the absence of complications) the airway manipulation is not involved.

Chapter 2 – "Medication errors" deals with the general issue of medication errors, especially in Anaesthesia and Intensive Care, where interest is increased due to several reasons: many drugs are administered with an effect on vital functions (effect on the degree of impairment of consciousness, impairment of airway permeability, respiratory function, cardiac function, muscle tone, etc.) and therefore with the potential for complications, including fatal ones. The chapter describes general issues, nomenclature, classification, risk factors, prevention strategies. The literature review is supplemented by a series of own studies on medication errors. The study "Reporting medication errors" involved anonymous questioning of all employees of an ICU department (specialist doctors, resident doctors, nurses) about medication errors. One of the main conclusions of the study is that the phenomenon of medication errors exists in anaesthesiology services in the Republic of Moldova, the qualification and professional status of the medical staff do not influence the frequency of medication errors. Other aspects, such as safety of use of cardiotonics and vasopressors, safety of neuromuscular blocking agents, safety aspects of regional anaesthesia are described.

In conclusion the monograph covers a wide range of patient safety aspects in anaesthesia, which is a modern concept in medicine. A systematic approach to problem description, identification of solutions and proposal of practical recommendations are included in the monograph and will serve as a good guide for further studies and for practical implementation of patient safety good practices in anaesthesia, intensive care and related fields.

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