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Welcome to the Moldovan Medical Journal!

The Moldovan Medical Journal is an international scientific double-blind peer reviewed periodical edition, 4 per year, of the Scientific Medical Association of the Republic of Moldova designed for specialists in the areas of medicine, dentistry, pharmacy, social medicine and public health. From its debut the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development.

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ORIGINAL RESEARCHES

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Antibiotic susceptibility and factors involved in virulence and persistence of *Acinetobacter baumannii* strains

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Abstract

Background: *Acinetobacter baumannii* has emerged as a medically important pathogen because of the increasing number of infections produced by this organism over the preceding three decades and the global spread of strains with resistance to multiple antibiotic classes. Recently, a particular attention has been drawn to the study of the microbial persistence properties and their correlation with the rate of elimination from the source of infection, as well as the prognosis of the disease progression.
Material and methods: There were examined 53 strains of *A. baumannii*, isolated from patients with trophic ulcers. The bacteriological examination, as well as tests on determining both the persistence factors and the antibiotic susceptibility of the isolated strains were carried out according to the current method.
Results: *A. baumannii* strains were highly resistant to all antibiotics tested, 38 (71.7%) showed multidrug resistance. The studies regarding the persistence factors of *A. baumannii* strains, revealed that 100% exhibited an antilysosyme activity, 78.0% – anticomplementary activity, 73.6% – produce biofilms, 58.5% – hemolytic activity, 28.3% and 13.2% – lecithinase and plasma coagulation activity, respectively.
Conclusions: Isolated strains showed higher level of antimicrobial resistance and multiple persistence factors. The study results proved that treatment of trophic ulcers is still a major problem, requiring rational monitoring and management strategies.
Key words: *Acinetobacter baumannii*, trophic ulcers, antibiotic resistance, persistence factors.

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Introduction

The genus *Acinetobacter* includes aerobic Gram-negative and non-fermenting cocobacilli, which are widely spread in nature and might be commensal or conditionally become pathogenic to humans. The most common species to cause infections is *A. baumannii*, being the most frequently isolated from clinical specimens, followed by *A. lwoffii*, *A. haemolyticus* and *A. johnsonii*. These bacteria have been long considered as low-virulent strains with a reduced pathogenic potential, however, today they play an important role in colonization and infections of immunocompromised patients. These strains are able to colonize the skin and airways of patients, particularly of those admitted within intensive care units, where high bacterial incidence has been recorded (up to 75%) [1, 2].

These bacteria are recognized as disease-causing agents, involved in healthcare-associated infections, displaying high morbidity and mortality rates in critically ill patients. These patients are more prone to germ transmission due to invasive medical procedures, the widespread use of a variety of antimicrobial agents, particularly the broad-spectrum ones and inappropriate infection control [3].

The infections associated with different types of immunodeficiencies, virulence and antibiotic resistance of the *Acinetobacter* strains, make up 28.3% – 84.3% of mortality cases among patients. Currently, of particular concern is the selection of some *A. baumannii* strains that can express serum bactericidal resistance to various factors and biofilm-forming capacity, thus often being involved in bacteremia associated with high mortality rate (up to 75%) [3-6].

The treatment of *Acinetobacter* spp. infections is often challenging, since the bacteria show an intrinsic resistance to many antimicrobial agents and due to a variety of mechanisms, as well as have an extraordinary ability to develop resistance to all classes of antimicrobials, used in the treatment of gram-negative bacillus infections [4, 7].

It is well recognized that Acinetobacter species have a natural resistance to cephalosporins of generations 1-2 and aminopenicillins. The bacterial resistance to other classes of antibiotics is due to various mechanisms as, enzyme inactivation (penicillinases, cephalosporinases, carbapemenases, amino acid acetyltransferases), changes in the target sites (Penicillin Binding Protein), impaired membrane permeability, and activating the pumps [8].

The worldwide spread of multidrug-resistant or pan-resistant Acinetobacter baumannii strains has increased dramatically since 1990. The World Health Organization (WHO) has included these bacteria in the group of highly infectious agents that would spiral out of the antibiotic control [1].

According to the 2011-2014 European surveillance network EARS-NET (European Antimicrobial Resistance Surveillance – Net-work) data, the highest antibiotic resistance levels of gram-negative bacilli have been reported in southern and eastern Europe. The highest resistance level was recorded in species of the genus *Acinetobacter* [9].

Acinetobacter baumannii is one of the most serious *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, A. baumannii, Pseudomonas aeruginosa,* and *Enterobacter species* (ESKAPE) organisms, as declared by the WHO that can escape the effect of antibacterial drugs [1].

Although trophic ulcers are a major health problem, the current methods of treatment are sometimes not enough and often lead to amputation due to misunderstanding regarding microbiology of these infections or methods of their eradication.

Although it is well known that the associated microbial infections might significantly worsen and delay the healing processes of trophic ulcers, recent research studies suggest that microbial biofilms highly contribute to their chronicity. Over 90% of chronic wounds infections are related to biofilms, whereas only 6% occur in acute wounds [10]. The protective and hostile nature of these biofilms makes the treatment of these infections extremely difficult.

At present, there are evidences to suggest that the ability of bacteria living within cells (e.g. macrophages) and biofilm formation are associated with persistent infections [11].

One of the causes of bacterial long-term persistence in the host organisms is the multiple factors that inactivate the antimicrobial mechanisms of the immune system. It is also well known that the microbial persistence potential determines the length of their stay in the macro-organism, whereas its suppression by antimicrobial drugs leads to a reduced infectious potential of the microorganism [12, 13].

The study was aimed to conduct and evaluate the antibiotic susceptibility and the persistence factors of *A. baumannii* strains isolated from trophic ulcers.

Material and methods

The study group included 53 strains of *A. baumannii*, isolated from trophic ulcers. Solid Oxoid culture media were used to isolate *A. baumannii*: Columbia Blood Agar Base with 5% sheep blood, MacConkey Agar.

The identification was assessed by classical biochemical tests and confirmed via VITEK 2 COMPACT automatic system.

The Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing , whereas the *in vitro* antibiotic test results were interpreted according to 2019 EUCAST (European Committee on Antimicrobial Susceptibility Testing) [14].

The *A. baumannii* strains were tested for four classes of antimicrobial drugs: aminoglycosides (gentamicin, amikacin, tobramycin), carbapenems (meropenem, imipenem), fluoroquinolones (levofloxacin, ciprofloxacin) and sulfanylamides (trimethoprim-sulfamethoxazole). *A.baumannii* strains that showed resistance to three or more different classes of antimicrobials were classified as multidrug-resistant (MDR) *A. baumannii* [15].

The lecithinase activity was assayed on the egg yolk salt agar, the hemolytic activity – on a blood agar plate, the plasma coagulase activity – on an inoculated culture into a sterile citrate plasma and the anti-lysozyme and anti-complementary activity we determined according to the method described by Bukharin O. et al. [13, 16, 17].

Detecting the anti-complimentary activity

The microbial suspension was inoculated on a surface of 1.5% agar plate; by using the bacteriological loop (the optical density of the microbial suspension corresponded to Mac Farland turbidity standard 1.0). The petriplates were inoculated at a temperature of 37°C during 18-24 hr. in order to manifest biological properties of bacteria. Furthermore, the studied cultures were inactivated in chloroform vapors for 10 minutes, then the plates were covered with a second layer of 1.5 ml of agar and 1 µl complement, so that the final concentration of the compliment corresponded to 20; 10 and 5 UH / ml, respectively. The petriplates were incubated in the inverted position at 37 ° C for 1 hour to develop the bacterial anti-complementary action and products of their activity. Then the plates were coated with a third layer of 0.7% agar containing 0.1 ml of bacterial suspension based on the indicator culture of *Escherichia coli* ГИСК 212, which showed an increased sensitivity to the bactericidal action of complement system. The plates were incubated at 37°C for 18-24 hr. to detect bacteria-mediated complement inactivation. Anti-complementary activity was assessed by the presence of indicator culture growth areas surrounding the tested bacterial cultures, where complement inactivation occurred.

Detecting the anti-lysozyme activity

The tested strain was cultured on a sloping agar for 18-24 hours at 37°C, then transferred into peptone broth and cultured at 37°C for 6 hours. The optical density in peptone broth was adjusted to 0.15, which corresponded to $1x10^8$ CFU/ml.

Simultaneously, the lysozyme suspension was prepared in peptone broth with 12.5µg /ml concentration. The use of

a higher concentration of lysozyme might inhibit the bacterial growth, whereas lower lysozyme concentrations do not allow identification of this phenomenon.

100µl of lysozyme broth was added in the wells of a plate, designed for enzyme-linked immunosorbent assay, at a 12.5µg/ml concentration, to which 25µl of microbial suspension was added. 100 µl of peptone broth and 25µl of microbial suspension were added into the control wells (2 wells), which were incubated for 4 hours. The optical density was measured over 2 and 4 hours. The results were read using an ELISA reader, whereas the optical density (OD) was measured at a wavelength of 600 nm (A600).

The strains were distributed according to the extent of this phenomenon, based on the following criteria:

1. Low expression levels of antilysosyme activity: K <0.49;
2. Mean expression levels of antilysosyme activity: K in the limits of 0.5-2.49;
3. High expression levels of antilysozyme activity: K> 2.5.

Determining biofilm production

Biofilm production by isolated strains was quantitatively determined using the microtiter plate method [17]. For the purpose of study, 150µl of peptone broth and 15 µl of bacterial suspension were added to a 96-well plate and adjusted to the 0.5 McFarland turbidity standard (respectively 1.5 x 10^8 CFU/ml), which were previously prepared from 18-24 hour bacterial culture and grown on 5% blood agar. The plates were coated and incubated for 24 hours at 37°C. Subsequently, the level of adhesion of the tested strains to inert substrate was determined by removing the content from each well and then rinsing five times with sterile saline and fixing with cold methanol for 5 minutes. After removing of the methanol, the dried plates were stained with 0.1% violet crystal solution for 30 minutes. The excess stain was removed by washing and the stained biofilm was resuspended in a 33% glacial acetic acid solution. Thus the obtained suspensions were used to determine the optical density (OD), based on the spectrophotometric absorbance readings at 570 nm colored suspension (A570). The tests were performed in duplicate.

The optical density cut-off value (ODc) is defined as the average OD of negative control + 3x the standard deviation (SD) of negative control. Biofilm formation by the tested strains was assayed and classified according to the adsorption of the violet crystal dye. The isolates were classified into four categories: non-adherent (OD ≤ ODc), poor adherent (ODc <OD ≤ 2xODc), moderately adherent (2xODc <OD ≤4xODc) and strongly adherent (4xODc <OD).

The reference strains *A. baumannii* (BAA-747) were used for quality control. EpiInfo 2000 was used in statistical data analysis.

Results and discussion

The present study assessed the antimicrobial susceptibility profiles of *A. baumannii* strains isolated from trophic ulcers (fig. 1-4).

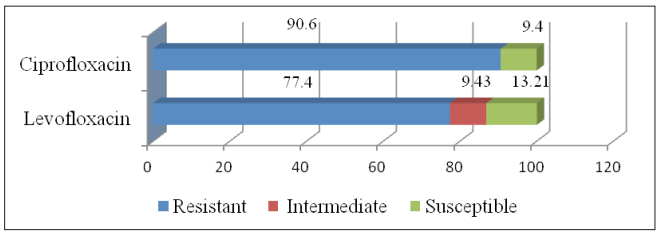

Fig. 1. Antibiotic susceptibility testing of *A. baumannii* strains to fluoroquinolones

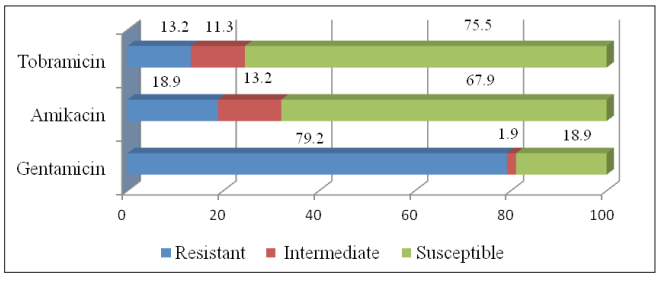

Fig. 2. Antibiotic susceptibility testing of *A. baumannii* strains to aminoglycosides

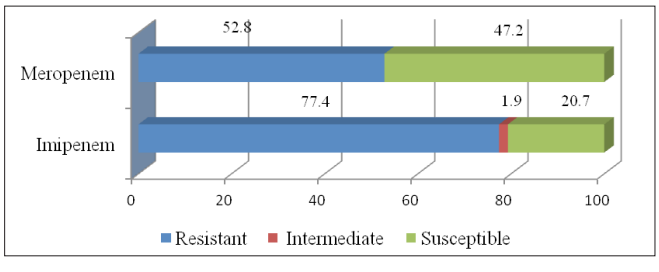

Fig. 3. Antibiotic susceptibility testing of *A. baumannii* strains to carbapenems

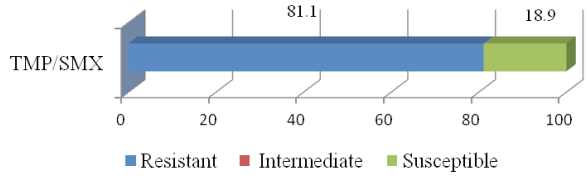

Fig. 4. Antibiotic susceptibility testing of *A. baumannii* strains to sulfanylamides, TMP/SMX – trimethoprim/sulfamethoxazole

The data analysis of the previous figures revealed that *A. baumanii* strains, isolated from trophic ulcers are highly resistant to most antibiotics. Of 53 strains, 38 (71.7%) were multidrug-resistant. Higher antibiotic susceptibility was registered to tobramycin 40 (75.5%) and amikacin 36 (67.9%).

The resistance of *A. baumannii* strains to carbapenems (imipenem – 77.4% and meropenem – 52.8%) is of major concern. The World Health Organization stated that carbapenem-resistant *Acinetobacter baumannii* infections have become a major public health challenge and a serious health threat in future [18].

The present study also assessed one of the persistence factors of *A. baumannii* strains viz. the anti-lysozyme acti-

vity. The anti-lysozyme activity (the ability of microorganisms to deactivate muramidase, which is an essential link in non-specific resistance) is very common among various isolates [19]. Of the 53 *A. baumannii* strains isolated from trophic ulcers, 53 (100%) showed an anti-lysozyme activity. A high expression level of anti-lysozyme activity (K> 2.5) was recorded in 16 (30.2%) strains, a mean expression level (K0.5-2.49) – 24 (45.3%) strains and a low expression level (K <0.49) was revealed in 13 (24.5%) of strains.

Hemolysin was another persistence factor studied, which acts as an exotoxin that may lead to chronicity of various infectious process [20]. Hemolytic activity was recorded in 31 (58.5%) *A. baumannii* strains isolated from trophic ulcers.

Lecithinase was present in 15 (28.3%) *A. baumanii* strains and plasmocoagulase – in only 7 (13.2%) strains. Lecithinase is an exoenzyme that acts upon phospholipids (lecithin) within the muscle fiber membrane, erythrocytes and other cells. Plasmocoagulase is a proteolytic enzyme that leads to septic thrombi formation and exerts an antiphagocytic effect by spreading within the body. These enzymes are aggressive factors for the microorganisms [20].

The ability to deactivate the complement system of the macroorganism leads to a microbial persistence within the infectious focus, which develops into chronic processes [19]. Lipid A and serine protease of *A. baumannii* exhibit an anticomplementary activity, thus providing a long-term bacterial survival inside the host organism [21, 22].

Of the 53 strains studied, 50 (94.3%) showed anti-complementary activity, of which 39 (78.0%) strains inactivated the complement at a concentration greater than 15 CH50 / ml, 8 (16.0%) – at a concentration of 5-15 CH50 / ml and 6 (12.0%) – at a concentration of 5 CH50 / ml. Only 3 strains (5.7%) did not inactivate the complement.

Biofilm-forming ability of *A. baumannii* clinical strains is crucial for their survival inside the macroorganism (even in antibiotic treatment) [23].

Of the 53 *A. baumannii* strains tested – 39 (73.6%) produced detectable biofilms. As regarding the biofilm status, 15 (38.5%) of the isolates were determined as strong biofilm forming, 6 (15.4%) – moderate biofilm forming and 18 (46.1%) – produced weak biofilms, out of the 39 strains. Similar data have been reported by a series of studies, which reported that over 74% of *A. baumannii* clinical strains produced biofilms [24, 25].

The correlation between the biofilm formation ability and MDR strains was also studied (tab. 1). Of the 38 MDR *A. baumannii* strains – 14 (36.8%) produced strong biofilms, 4 (10.5%) – moderate biofilms, 17 (44.7%) – weak biofilms and 3 (7.9%) – produced no biofilms.

Table 1

Correlation between MDR and Non-MDR strains of *A. baumannii* and biofilm formation ability by using the phenotypic method

Types of biofilm	MDR	Non-MDR	Total
Strong biofilm producer	14 (26.4%)	1 (1.9)	15 (28.3%)
Moderate biofilm producer	4 (7.5%)	2 (3.8%)	6 (11.3%)
Weak biofilm producer	17 (32.1%)	1 (1.9%)	18 (34.0%)
No biofilm producer	3 (5.7%)	11 (20.7%)	14 (26.4%)
Total	38 (71.7%)	15 (28.3%)	53 (100%)

A significant association between MDR and biofilm formation ability was reported (P <0.001).

Conclusions

The research findings underline the importance of an effective surveillance of antimicrobial resistance of *A. baumannii* strains, thus suggesting the appropriate use of antimicrobials in order to prevent the emergence of bacterial resistance to these specific drugs.

The present study proved that the most expressed pathogenicity factors in *A. baumannii* strains, isolated from trophic ulcers, are lysozyme inactivation, complement and biofilm formation. The assessment of virulence and persistence factors will help the practitioners to effectively manage these infections, providing a more effective control of appropriate antimicrobials and thus resulting in a reduced mortality and morbidity rates in patients.

The study results justify the development of new strategies for the prevention and treatment of *A. baumannii* infections.

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Adhesive diseases in children. Prevention, diagnosis and treatment strategies

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Abstract

Background: The adhesive disease and its associated complications, caused by adhesive mechanical bowel obstructions, mainly related to genetic causes and disorders of cell homeostasis, is one of the major health problems due to its diverse impact on the growing organism.
Material and methods: The clinical study was conducted on a group of 50 children aged 1 month – 18 years old with abdominal adhesion disorders, complicated by bowel obstruction, following a surgical intervention. Apart from the assessment of the anamnesis, clinical, and imaging manifestations, traditional bio-humoral homeostatic imbalance markers, endotoxemia levels, associated complications, and comorbidities, the blood inflammatory and excessive fibrosis biomarkers were assessed at different clinical periods of the pathological process.
Results: A non-randomized pro- and retrospective study was carried out to assess the epidemiological, clinical and paraclinical, histopathological, evolutionary, preventive and treatment charcteristics in order to determine the major complication triggers following a surgical intervention on the small intestine, colon, appendix or uterus , as well as to highlight their possible peculiarities in children.
Conclusions: This study completed the clinicians' views on intraperitoneal adhesion pathophysiology, thus confirming the importance of the microbial factor, inflammatory mediators, activation of humoral systems due to organic cellular lesions, the extension of the inflammatory response, as well as the genetic factors depending on the acetylation phenotype in children.
Key words: children, adhesive disease, diagnosis, treatment.

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Introduction

Adhesive disease and its complications, mainly caused by a mechanical bowel obstruction, is one of the major health problems due to its diverse impact on the growing organism [1, 2]. The high- incidence disability due to this pathology is still a topic under discussion. Currently, there is no unanimously accepted standard for the diagnosis, treatment and prophylaxis strategies in children. The actuality of this issue is determined by its high frequency, polymorphism of clinical manifestations, its severity and unpredictable evolution, poor prognosis, and associated complications. Recent studies have defined the long-lasting challenges related to this intra-abdominal complication. However, the latest successful researches in pathophysiology and molecular biology have opened new avenues in the assessment and prophylaxis of adhesion processes, by assessing the genetic factors, impaired cell metabolism and homeostasis that encodes the body's susceptibility to this complication [3, 4].

Adhesion formation is a risk factor following all the abdominal surgeries, occurring in up to 100% of cases. Adhesions are more commonly to occur after the surgeries performed on the small bowel, colon, appendix, or uterus. Adhesions are less common in surgeries on the stomach, bladder or pancreas. To date, there are no anatomical and clinical criteria to reduce postoperative complications, as well as there are no preventive measures to avoid adhesion processes, especially in children. The surgical diagnosis and management of abdominal diseases have an indisputable role in enhancing the early or supportive treatment. Despite some progress, there are still many questions, which arise the constant interest of the scientific world [5].

Additionally, a better understanding of the adhesion processes, which result in mixed bowel obstruction and might potentially evolve into multiple organ failure and intestinal failure, could help in developing a more effective approach in order to reduce complications, disabilities and the number of deaths. In the last decades, the nosological classification of adhesion processes has been carried out by describing their main clinical, imaging, histopathological and evolutionary patterns [6].

The purpose of the study. Complex clinical and paraclinical examination methods (biochemical, bacteriological, imaging, and histopathological assessment) were used to develop and optimize the diagnostic schemes, prognosis,

treatment, and prophylaxis in pediatric adhesive disease and its complications.

Material and methods

The clinical study was carried out at *Natalia Gheorghiu* Scientific Center of Pediatric Surgery, in collaboration with the Biochemistry Laboratory of *Nicolae Testemitanu* State University of Medicine and Pharmacy on a group of 50 children aged from 1 month to 18 years old, diagnosed with abdominal adhesion complicated by bowel obstruction, who underwent surgical interventions during 2011-2019. The research project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (favorable opinion dated of 13.05.2015, minutes No 55). The anamnesis, clinical and imaging data, traditional bio-humoral homeostatic imbalance markers, endotoxemia levels, associated complications and comorbidities were assessed, as well as the following biomarkers: the level of nitric oxide (NO) metabolites and serum concentration of protein-bound hydroxyproline [7]; serum levels of interleukin 1β (IL-1β) via ELISA method, by using the "Imunoteh" test kit, USA; C-reactive protein (PCR) was estimated via the Beckman test kit, Ireland; the serum sialic acid (SA) and ceruloplasmin concentration was assessed via the photometric assay [8]; the intensity of the acetylation processes necessary to establish the acetylation phenotype was assessed by determining the N-acetyltransferase activity [9]; the serum copper level was determined via ELITEH test kit, France. The comprehensive extemporal biochemical assessment was performed in all patients from the study group, at different clinical and evolutionary pathological stages of the clinical course viz. inpatient and preoperative care, the 4th postoperative day, at time of discharge and follow-up period.

The morpho-histopathological assessment was carried out in 46 patients, who underwent a surgical intervention for intraperitoneal adhesions, in order to assess the prognosis, medical and surgical management, and prophylaxis of adhesive bowel obstruction. This study included macro- and microscopic examination of various abdominal adhesions, as well as in purulent peritonitis, acute appendicitis (catarrhal, phlegmonous, and perforated gangrenous), intra-peritoneal organ involvement, etc. The biopsy samples were retrieved from different anatomical sites, namely from occluded intestinal segments, mesentery, omentum, enterostomic portions, vermiform appendix, and intraperitoneal lymph nodes. Thus, the microscopic examination revealed fragments of bowel, omentum, adhesive bands, and vermiform appendix. The fragmented pieces had from 3-4 to 10 tissue sections. Tissue sections were stained with hematoxylin -eosin and picrofuxin by Van-Geison's method, whereas a 10% formalin solution was used to fix and study the samples under microscope with a 7 – eyepiece, using the 10x objective.

The test findings were evaluated via the Microsoft Office Excel program; the Student-test was applied to process the statistical mean values and Fisher criterion for coherent selections. To determine the statistical significance, the P value should have been less than 0.05 [10].

Results

The study included patients diagnosed with adhesive bowel obstruction. The non-randomized pro- and retrospective study was carried out to assess the epidemiological, clinical and paraclinical, histopathological, evolutionary, preventive and treatment peculiarities, as well as to determine the major complication triggers and their possible features with regard to currently available medical literature. The study group included 50 patients. Sex distribution pattern revealed a higher male to female ratio, viz. 2: 1. According to the place of residence, most children were from rural areas – 85% compared to those from urban areas – 15% of patients aged 6-12 years. The patients who were enrolled within the study were diagnosed with bowel obstruction, following a postoperative adhesive bowel obstruction and strangulation. These patients required a surgical intervention and were divided based on endotoxemia levels. Twenty patients, who suffered from adhesive bowel obstruction but showed successful conservative treatment outcomes, were excluded from the study.

Symptoms at admission included abdominal pain – 100%, intestinal motility disorders, dilated intestinal loops, hyperleukocytosis, intestinal transit disorders – 100%, vomiting – 100%, fever – 70%, tachycardia – 100%, tachypnea – 90%, etc. The time intervals from the onset and time of admission was 24-48-72 hours. Most children from the present study were hospitalized with severe and extremely severe abdominal adhesive pathological process, accounting for 42% of the total number of studied patients. Thus, 68% of patients were admitted with late clinical-evolutionary stages, later than 24-48-72 hours from the onset, which influenced the subsequent evolution, prognosis and the disease-specific survival rate.

It should be mentioned that patients with intraperitoneal inflammatory processes, hospitalized within the first 24 hours after the disease onset made up 58% of cases, over 36 hours – 24%, and over 2-7 days – 10%. Two patients underwent relaparotomy on the 5th postoperative day, followed by inter-intestinal band excision.

A severe disease onset was reported in 92.6% of cases, manifested by abdominal pain syndrome in 82.5% of cases, nausea and repeated vomiting episodes – 70%, insomnia – 58%, and anorexia – 70%. Constipation was present in 47% of patients aged over 3 years old and 26% of patients showed no changes in bowel movement frequency and transit patterns. 27% of patients had loose stools, thus being hospitalized within the Intestinal Disease Units in 9% of cases. 4% of children aged up to 3 years, were primarily diagnosed by the family doctor as experiencing a reaction to teething. These children were admitted at the late stages of the disease at specialized Surgery Departments.

54% of patients exhibited mild abdominal pain due to

the underlying acute respiratory viral infection, thus being late referred to surgery departments. 90% of patients had local peritoneal signs (muscular defense, abdominal bloating, lack of intestinal transit, etc.). Abdominal asymmetry was present in 49% of patients with intraperitoneal pain syndrome, whereas 18% of cases had a specific tense abdomen, 90% – positive peritoneal signs, 39% – different endotoxemia levels (fever, hyperemia, tachypnea, impaired skin microcirculation; 12 children showed a confusional state related to primary central nervous system (CNS) involvement and clinical features of a systemic infection, hemodynamic changes and homeostasis; 8 children exhibited coagulopathy with bleeding manifestations (systemic hemorrhage and thrombocytopenia).

Paraclinical examinations revealed hyperleukocytosis in 88.8% of patients, leucopenia – 16.4%, thrombocytopenia – 10.2%, an increased ESR – 86.2%, anemia – 66.6 %, hypoproteinemia and dysproteinemia – 71.2%, hypertransaminasemia – 20.1%, low prothrombin index – 74.4%, high fibrinogen concentration – 58.6%, and hydroelectrolytic disorders were found in 91.1% of patients. Imaging scans included abdominal ultrasound exam, barium sulfate contrast of gastrointestinal (GI) transit time, computed tomography (CT) (in 5 patients), which allowed to diagnose a positive intestinal obstruction.

Acetylation phenotype was determined in all 50 patients. The studies revealed that of the 50 patients, who underwent clinical and biochemical assessment, 32 (65%) patients exhibited fast acetylators, whereas 18 (35%) – slow acetylators.

Some biochemical and immuno-biochemical changes were particularly highlighted in children with different acetylation phenotypes.

The findings presented in table 1 show that the nitric oxide (NO) metabolite level was significantly higher at admission time, thus exceeding 3-10 times the control values. At the same time, the highest NO values were recorded in adhesive processes, following an apendicectomy, as well as in fecal peritonitis (almost 10 times higher compared to that of the control group). However, this index gradually decreased on the first day postoperatively and subsequently, showing its minimum values of 2.2-2.8 times higher compared to the control group, which is considered as a reference range for the 9th-10th day of hospital discharge.

Similar, though milder changes were recorded in dynamic assessment for serum cytokine IL-1β, the data obtained (tab. 2) showed that serum IL-1β concentration was significantly higher at admission, viz. exceeding 3-7 times the control group values. At the same time, the highest serum IL-1β values were recorded in adhesive processes in fecal peritonitis and in intraperitoneal adhesive processes (almost 7 times higher compared to that of the control group). However, this index gradually decreased on the 1st day postoperatively and subsequently, the minimum values exceeding 1.6-2.1 times higher than the control group indices, which is considered as a reference range for the 9th-10th day of hospital discharge.

The serum C-reactive protein (CRP) level increased significantly at admission time, exceeding 4-12 times the control values and maintained high values on the 1st and 3rd day postoperatively. However, it gradually decreased on the 5th day postoperatively, whereas at discharge, the CRP level was 1.4-2.6 times higher than the reference values, though showing no statistical relevance (tab. 3).

Table 1

Dynamic alterations of serum nitric oxide metabolites levels (NO, μmol / l) in abdominal adhesive processes of different origin

Research stages	Disease types				
	A. Acute phlegmonous appendicitis	B. Adhesive processes after appendicectomy	C. Adhesive processes in fecal peritonitis	D. Adhesive bowel obstruction	E. Intra-peritoneal adhesions
At admission	2.1 ± 0.36** 280%	7.4 ± 1.12** 987%	8.1 ± 0.95*** 1080%	4.3 ± 1.02** 573%	3.1 ± 0.44*** 413%
1st day, postoperatively	2.9 ± 0.56** P_1>0.5 387%	6.3 ± 1.14** p_1>0.5 840%	7.4 ± 0.86*** p_1>0.5 987%	4.5 ± 0.89** p_1>0.5 600%	3.7 ± 0.41*** p_1>0.5 493%
3rd day, postoperatively	3.5 ± 0.81** p_1<0.05 467%	3.8 ± 0.45*** p_1<0.01 507%	8.5 ± 0.72*** p_1>0.5 1138%	3.6 ± 0.45** p_1>0.5 480%	3.1 ± 0.46** p_1>0.5 413%
5th day, postoperatively	1.7± 0.09** p_1>0.5 227%	2.1± 0.38** p_1<0.01 280%	7.3 ± 0.31*** p_1>0.5 973%	2.3 ± 0.51** p_1>0.5 307%	2.8 ± 0.37** p_1>0.5 373%
At discharge	2.1 ± 0.32** p_1>0.5 280%	0.94± 0.17 p_1<0.001 125%	1.86 ± 0.24** p_1<0.001 248%	1.3 ± 0.42 p_1<0.05 173%	1.7 ± 0.29* p_1<0.05 227%
Control group	0.75 ± 0.08 100%	0.75 ± 0.08 100%	0.75 ± 0.08 100%	0.75 ± 0.08 100%	0.75 ± 0.08 100%

Note: Statistical significance if compared to control values - * - p <0.05; ** - p <0.01; *** - p <0.001; if compared to the 1st stage - p_1<0.05; p_1<0.01; p_1<0.001.

Table 2

Dynamic alterations of serum cytokine IL-1β levels (μg / l) in abdominal adhesive processes of different origin

Research stages	Disease types				
	A. Acute phlegmonous appendicitis	B. Adhesive processes after appendicectomy	C. Adhesive processes in fecal peritonitis	D. Adhesive bowel obstruction	E. Intra-peritoneal adhesions
At admission	44.6 ± 6.81* 231%	98.4±10.26** 510%	136.4 ±11.35*** 707%	75.1 ± 12.27* 389%	131.7 ± 10.71** 682%
1st day, postoperatively	57.9 ± 7.53** p_1>0.5 300%	101.2 ± 11.82*** p_1>0.5 524%	118.6 ± 8.72*** p_1>0.5 615%	87.5 ± 10.34** p_1>0.5 453%	140.4 ± 18.12*** p_1>0.5 727%
2nd day, postoperatively	48.1 ± 6.81* p_1>0.5 249%	91.7 ± 9.75** p_1>0.5 472%	89.4 ± 6.91** p_1<0.01 463%	75.6 ± 8.91** p_1>0.5 392%	108.1±11.87*** p_1>0.5 560%
5th day, postoperatively	34.1 ± 9.17 p_1>0.5 177%	40.1 ± 6.68* p_1<0.01 208%	52.7 ± 5.86** p_1<0.001 273%	55.2 ± 10.13* p_1>0.5 286%	58.6 ± 14.21* p_1<0.01 294%
At discharge	26.5 ± 5.84 p_1>0.5 173%	31.1 ± 3.11 p_1<0.05 161%	31.7 ± 2.42 p_1<0.001 164%	40.7 ± 9.77 p_1<0.05 211%	32.8 ± 10.35 p_1<0.001 170%
Control group	19.3 ± 4.5 100%	19.3 ± 4.5 100%	19.3 ± 4.5 100%	19.3 ± 4.5 100%	19.3 ± 4.5 100%

Note: Statistical significance if compared to control values - * - p <0.05; ** - p <0.01; *** - p <0.001; if compared to the 1st stage - p_1<0.05; p_1<0.01; p_1<0.001.

Table 3

Dynamic alterations of serum C-reactive protein (CRP) level (mg / l) in abdominal adhesive processes of different origin

Research stages	Disease types				
	B. Acute phlegmonous appendicitis	C. Adhesive processes after appendicectomy	D. Adhesive processes in fecal peritonitis	E. Adhesive bowel obstruction	F. Intra-peritoneal adhesions
At admission	20.2 ± 4.36** 388%	41.7±8.16*** 802%	62.0±7.83** 1192%	9.8±2.43 188%	29.3±8.72** 563%
1st day, postoperatively	25.1 ± 5.74** p_1>0.5 419%	36.9±6.72*** p_1>0.5 710%	54.3±5.6*** p_1>0.5 1044%	25.3±7.51* p_1<0.05 710%	38.5±6.92*** p_1>0.5 740%
2nd day, postoperatively	21.8 ± 3.78** p_1>0.5 419%	29.1±5.89** p_1>0.5 560%	37.8±4.86** p_1<0.05 727%	19.7±4.12** p_1<0.05 302%	36.1±9.16** p_1>0.5 694%
5th day, postoperatively	12.4 ± 3.06* p_1>0.5 238%	15.7±5.36** p_1<0.05 302%	18.1±2.31** p_1<0.001 348%	14.7±7.34 p_1>0.5 348%	16.7±5.36** p_1>0.5 321%
At discharge	10.7 ± 6.91* p_1>0.5 206%	13.4±7.26 p_1<0.05 258%	9.7±1.16* p_1<0.001 187%	7.2±2.33 p_1>0.5 138%	12.3±6.58 p_1>0.5 237%
Control group	5.2 ± 0.1 100%	5.2±0.1 100%	5.2±0.1 100%	5.2±0.1 100%	5.2±0.1 100%

Note: Statistical significance if compared to control values - * - p <0.05; ** - p <0.01; *** - p <0.001; if compared to the 1st stage - p_1<0.05; p_1<0.01; p_1<0.001.

Alterations in serum sialic acids and protein-bound hydroxyproline (tHP) levels in children with dynamic adhesion processes, depending on acetylation phenotype and treatment approach are shown in Table 4. Therefore, the levels of sialic acids and tHP at admission and on the 1st day postoperatively were significantly higher than those from fast-acetylators control group. However, this index decreased on the 3rd day postoperatively, having its minimum values on the 9th-10th day, at discharge. A similar dynamic pattern was recorded for slow acetylators, although the serum sialic acids and tHP levels were lower than in fast acetylators, whereas the highest concentration was recorded on the 1st day postoperatively, which decreased to normal values at the time of discharge (tab. 4).

The serum copper concentration in slow acetylators showed a statistically significant increase in the early stages, having the highest values on the 5th day postoperatively, followed by a decrease on the 10th -15th day, at discharge (tab. 5). The serum copper level in fast acetylators increased on the 1st postoperative day, then decreased to its lowest values on the 5th day postoperatively and afterwards returned to its control values.

The level of ceruloplasmin, a copper-containing protein in slow acetylators elevated after surgery, showing the hi-

Table 4

Dynamic alterations of serum sialic acid and protein-bound hydroxyproline (tHP) levels in children with serum abdominal adhesive processes of different origin, depending on acetylation phenotype and treatment approach

Indices	Serum sialic acid level		Serum protein-bound hydroxyproline (tHP) level	
Research stages	Slow acetilators	Fast acetilators	Slow acetilators	Fast acetilators
At admission	3.2±0.18*** 152%	3.5±0.21*** 167%	131.7±6.54* 122%	136.9±8.42* 126%
1st day, postoperatively	3.8±0.25** 181% p>0.5	3.9±0.28** 186 % p>0.5	139.8±10.82* 129% P>0.5	161.1±15.47** 149% p>0.5
3rd day, postoperatively	3.7±0.17*** 176% P>0.5	3.4±0.19** 162% P<0.05	116.8±11.47 117% P>0.5	136.5±12.02* 126% P>0.5
5th day, postoperatively	2.3±0.21 114% P<0.05	2.5±0.19 119% P<0.05	98.9±7.82 110% P<0.05	112.2±7.31 122% P>0.5
At discharge	2.2±0.11 100%	2.3±0.17 114%	102.3±7.15 110%	110.3±8.12 119%
Control values	2.1±0.19 100%	2.1±0.19 100%	108.3±6.18 100%	108.3±6.18 100%

Note: Statistical significance if compared to control values - * - p <0.05; ** - p <0.01; *** - p <0.001; if compared to the 1st stage - p_1<0.05; p_1<0.01; p_1<0.001.

Table 5

Dynamic alterations of serum copper and ceruloplasmin levels in children with serum abdominal adhesive processes of different origin, depending on acetylation phenotype and treatment approach

Indices	Serum copper level, μM/l		Serum ceruplasmin level, mg/l	
Research stages	Slow acetilators	Fast acetilators	Slow acetilators	Fast acetilators
At admission	27.1±2.1* 132%	25.4±2.4 124% p>0.5	377.3±26.8 107%	381.3±28.9 108% p>0.5
1st day, postoperatively	38.6±3.4** 188%	36.1±3.8** 176% p>0.5	446.5±25.6 126%	397.6±41.2 112% P<0.05
3rd day, postoperatively	32.7±4.1** 160%	33.1±2.4** 161% p>0.5	380.7±19.8 108%	371.3±25.7 105% p<0.05
5th day, postoperatively	31.2±4.1* 152%	23.8±2.1*** 116% p>0.5	368.3±21.2 104%	361.5±19.7 102% p<0.05
At discharge	24.8±3.1 121%	22.7±2.4 111% p>0.5	363.8±24.6 103%	359.4±21.9 101% p>0.5
Control values	20.5±1.4 100%	20.5±1.4 100%	354.1±22.7 100%	354.1±22.7 100%

Note: Statistical significance if compared to control values - * - p <0.05; ** - p <0.01; *** - p <0.001; if compared to 1st stage - p_1<0.05; p_1<0.01; p_1<0.001.

Table 6

Bacteriological study

Microbial flora	Bacterial agent	Abs.	P±ES,%
Gr. -	E. coli	25	50±5.4%
	Ps. Aeroginosa	5	10±1.2%
	Kl. Pneumonae	7	14±2.9%
	Proteus	4	8±1.4%
Gr. +	St. aureus	13	26±1.7%
	Str. Epidermidis	8	16±0.4%
	Candida albicans	10	20±2.8%
	Intestinal dysmicrobism, grade I-IV	30	60±5.7%
	2 bacterial association	12	24±1.3%
	3 bacterial association	18	36±0.8%
Anaerobes	Clostridium	5	10±0.8%
	Antibiotic-resistant strains	8	16±1.8%

ghest values on the 5th day postoperatively, then returned to normal ones at discharge. In fast acetylators, the dynamics of ceruloplasmin was similar to that of slow acetylators, howe-ver, being statistically irrelevant (tab. 5).

The bacteriological studies were carried out according to the developed algorithm and the study design, presented in table 6, followed by the diagnosis and monitoring of the bacterial flora within the abdominal cavity, blood and wound. The results showed a predominance of Gr. Flora - 50% and polybacterial associations – 60%.

Thus, patients with diffuse purulent-fibrinous peritonitis that was related to an underlying long-lasting and persistent intestinal paresis, developed grade I-III dysbiosis soon after surgery, being determined at all levels of GI tract. The following pathogens were reported in 3 patients aged 2-5 years with ileo-stomata: enteroccocus and Klebsiela strains. The prophylaxis of intestinal dysbiosis was corrected via selective decontamination methods that included probiotics (Linex, Opefera, Acidolac, Lactobex Baby). A reduced pathogenic population and small amounts of lactobacteria

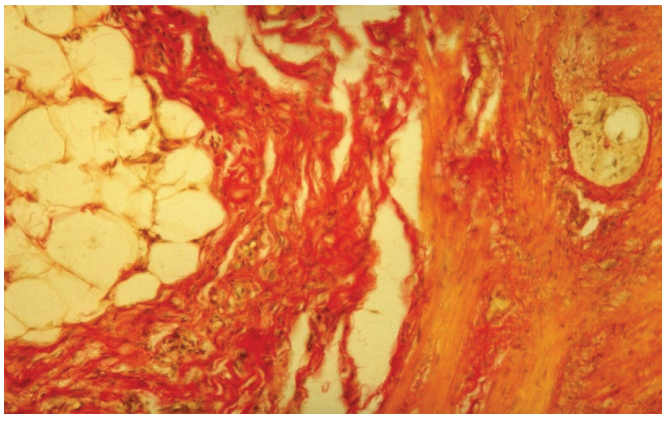

Fig. 1. Productive perivisceritis evolving into sclerosis, collagen fiber bundles with solitary fibrocytes. Van Gieson staining. 10-x eyepiece, 20-x objective.

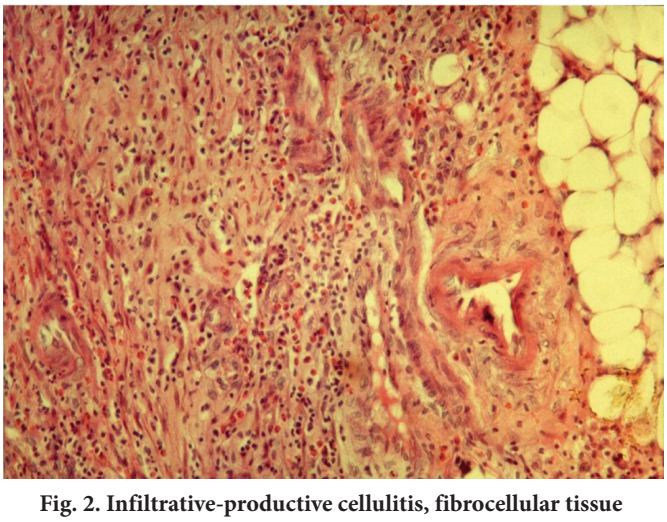

Fig. 2. Infiltrative-productive cellulitis, fibrocellular tissue with solitary or group lipocyte inclusions. Hematoxylin-eosin staining. 10-x eyepiece, 20-x objective.

allowed optimizing the time of stromal closure, which was performed on the 2nd and 4th month after the application. This therapeutic approach along with antiadhesion drugs (Serrata, Longidaza 3000ME, rectal suppositories, Cuprinil per oral, Fermencol – electrophoresis and local gel, as well as the use of anti-inflammatory and immunomodulatory medication – Wobenzym) allowed optimizing the stromal closure time that was performed on the 2nd and 4th month after their application by latero-lateral (2) and termino-terminal (1) anastomoses, followed by positive treatment outcomes at early and late stages. The children were aged from 2.3-5 years old.

Microscopic histopathological assessment of the biopsied material revealed presence of celluloid fat, crossed by intermediate layers of connective tissue, divided into irregular-sized cellulo-adipose lobules, often round or oval-shaped. The connective spaces between the cellulo-adipose lobules varied in thickness, being made up of connective fibers that were parallel to a significant number of fibrocytes (fig. 1 and 2).

There was found a number of areas with swollen and homogenized connective fibers, located within a homogenized ground substance with the high number of fibrocytes. The blood vessels were unevenly dilated and hyperemic. The cellulo-adipose tissue that was adjacent to the infiltration areas exhibited an increased concentration of fibroblasts (massive leukocyte infiltraion), which proliferated from the connective spaces. The analysis of the obtained findings regarding the small bowel portion, severe inflammatory changes of the peritoneum and intraperitoneal organs, as well the high level of body sensitivity to determine the inflammation degree was carried out. Subsequently, a more in-depth study was performed on the biopsy samples of the intraperitoneal adhesions, retrieved from the same anatomical and pathological sites, as well as on the neuronal damage of the Auerbach plexus.

Most of hospitalized children with abdominal adhesions were admitted with severe or extremely severe health condition that made up 71.5% of all patients included within the study group. According to the research data, 78.9% of patients were hospitalized in late clinical evolutionary stages, which had an extremely negative impact on further evolution and the prognosis of the disease.

It should be noted that a significant number of children (70%) were reported with concomitant cardiovascular, respiratory, renal-urinary, and musculoskeletal disorders, diabetes mellitus, acute mesadenitis, phlegmonous diverticulitis, Flexner dysentery, hemorrhagic vasculitis, parasitic diseases (enterobiosis, ascariosis, and hydatidosis), post-traumatic sequelae, etc.

Antiadherent treatment. The complex antiadherent treatment was started intraoperatively with a mild adhesiolysis, peritoneal cavity lavage, 200 ml of 5% aminoapronic acid solution intraperitoneally + 2 ampoules of thrombin and abdominal cavity drainage with glove lamellae in the right iliac fossa.

The antiadherent treatment approach included collagen synthesis inhibitors – Cuprinyl-penicillamine and bacterial collagenase – *Clostridiopeptidase A* for removing collagen fiber formation. Penicillamine was administered from the 2nd-3rd day postoperatively, doses were based on the child's age: 0.15 / 1 capsule – in children up to 5 years, 0.3 / 2 capsules – in children aged from 5 to 12 years old and 0.4 / 3 capsules – over 12 years, once / per day. The treatment lasted from 10-14 days. No side effects were reported regarding the use of penicillamine.

Clostridiopeptidase A was used as ultrasonophoresis with iruxol, a combination of clostridiopeptidase A and chloramphenicol. Ultrasonophoresis with iruksol was applied on the anterior abdominal wall on the 2nd-3rd day after surgery. The ultrasound UTP-1 device used a pulsating mode, at one pulse /second frequency and 0.4 W / cm intensity. The course of treatment included up to 10 procedures.

Children with adhesive intestinal obstructions, who underwent laparotomy, were given postoperative treatment on the 1st-2nd day by using electrophoresis or collalizine injections nearby the plague, followed by galvanization, instead of ultrasonophoresis+iruksol.

The antiadherent treatment scheme also included Fer-

mencol, containing a series of highly active collagenases that enabled the hydrolysis of both collagen peptide bonds and polysaccharides that form the connective tissue. 15 procedures of electrophoresis with Fermencol gel were applied on the postoperative wound. Longidase 3000ME with immunomodulating and collagenolytic effects was also applied.

Treatment of adhesive syndrome. The complex treatment approach of the adhesive syndrome was developed at Natalia Gheorghiu Scientific Center of Pediatric Surgery, IMPH IMC that included the following major principles:

- Food intake suppression;
- Rebalance of the hydroelectrolytic, acid-base, and metabolic disorders under constant and individual monitoring;
- Use of related detoxification methods (plasmapheresis);
- Antibacterial therapy;
- Immunocorrection therapy;
- Surgical treatment;
- Relapse prevention.

According to the assessed changes, the minimal resuscitation treatment was based on the following complex investigations: clinical, imaging, biochemical, and bacteriological tests, leukocyte intoxication index, etc., thus justifying the following therapeutic algorithm in the preoperative stage (to reduce the endogenous intoxication and improve critical organ functioning, particularly of the GI tract):

- Rebalancing of hydroelectrolytic and metabolic disorders;
- Anemia correction;
- Antibiotic therapy in providing resolution of the infectious process (cerulopasmine, aminoglycosides, metronidazole, etc.);
- Use of hepatoprotective agents;
- Gastric and bowel decompression;
- Fever treatment;
- Symptomatic medication (vasoactive, corticosteroid, and cardiotonic drugs).

The most appropriate rebalancing approach was surgery, followed by patient detoxification and fast recovery. The surgical method of choice and its duration depends on the diagnosed condition, the evolutionary stage, complications, the patient's biological field, etc.

Tactical and technical aspects of surgical interventions / repeated interventions were aimed:

- To undergo repeated laparotomy or relaparotomy with peritoneal cavity drainage using glove lamellae;
- Adhesiolysis;
- To remove obstruction of the GI tract and provide proper bowel movement;
- To restore the digestive tract integrity by applying intestinal anastamosis (term-terminal, termino-lateral, and latero-lateral);
- To eliminate the source of peritoneal contamination and restore the abdominal cavity.

To achieve these purposes, a primary surgery or a repeated laparatomy is required, the latter could be performed via the surgical or median approach. Previous laparotomy was considered as the optimal access pathway.

The postoperative treatment is based on the following objectives:

- Nasogastric intubation (reduces abdominal distension, avoids repeated vomiting and prevents from entering into the upper respiratory tract, improves pulmonary ventilation);
- Oxygen therapy in patients with clinical signs of respiratory failure;
- Clinical and biological follow-up and specific treatment adjustments;
- The preoperative antibiotic therapy is continued based on clinical criteria, then modified depending on the antibiotic-susceptibility testing results;
- Stimulation of intestinal peristalsis (Prozerini, Cerucal, and Quamatel IV solution);
- Selective decontamination (Linex, Opefera, Acidolac, Probiotic, Lactobex Baby, Ferzim plus, AERIS, etc.);
- Hydroelectrolytic and metabolic rebalancing;
- Early patient mobilization;
- Hygiene and dietary regimen aimed to resume the patient's natural diet;
- Patients' local evolution (peritoneal, abdominal) and overall condition follow-up;
- Hemodynamic monitoring (pulse rate, respiration, temperature, blood pressure, central venous pressure, diuresis, etc.);
- Immunocorrection therapy by using amino acids, which increase protein synthesis and decrease proteolysis, as well as administration of essential polyene fatty acids Omega3, Fish Oil Jr;
- Antiadherent treatment according to the developed schemes and symptomatic medication;
- Plasmopheresis, if required.

The most difficult stage of the above mentioned treatment approach is the antibacterial one, due to its long-lasting antibiotic administration (before surgery), followed by the formation of antibiotic-resistant strains, high virulence of microbial agents against the underlying low-level resistance of the growing body, detection of microbial associations, and higher incidence of allergic reactions. Preoperative patient preparation for improving and restoring vital functions should not last less than 2 hours and not exceed 6 hours.

Discussion

The study was based on a complex multi-planar analysis of clinical observation data, comprehensive laboratory and imaging methods, morphology, mathematical assessment of marker indices in basic pathologies, as well as on medical and surgical treatment outcomes in 50 children aged from 1 month -18 years old, who underwent abdominal surgery.

It should be mentioned that all children were diagnosed via minimally invasive methods, namely, X-ray, imaging, and laboratory investigations, taking into account their medical history, type of surgical intervention, primary dis-

ease, and previous abdominal surgeries, in order to find the best treatment solution in terms of the type, clinical picture, clinical and evolutionary stage, and acute phase patterns of the disease. Since suspected complications are the first information-gathering step of the diagnostic process, all patients with abdominal surgical infections require a careful medical interviewing. Moreover, most apparently irrelevant details related to less specific clinical manifestations and minimal changes found in usual investigations might be essential for establishing an early effective diagnosis and prevention, as well as justify an adequate treatment, based on currently available and accepted disease diagnosis, prevention and management [11, 12].

As regarding the pathology-related problems, they arose from numerous factors involved in the etiopathogenesis of these pathological conditions, which might cause major changes in different homeostatic systems of the growing body [13].

It should be noted that further studies are required to assess the risk factors, in order to develop specific preventive measures and a differentiated individualized treatment [14, 15].

The comprehensive study of the anamnestic data regarding the basic condition and its associated complications allowed concluding that the clinical features were characterized by an acute onset and various clinical symptoms. It should also be mentioned that the severe patient's condition developed considerably on a previously underlying disorder, which made it difficult to properly assess the child's condition. Furthermore, most patients had previously undergone surgical treatment in the late stages of destructive acute appendicitis and appendicular peritonitis, which, apart from challenges in establishing the primary diagnosis and medical-surgical approach, contributed significantly to being associated with complications or conditions that required relaparatomy.

The development of complications is mainly due to the late diagnosis of acute appendicitis, errors in the medical and surgical treatment of intestinal obstruction, inadequate clinical and evolutionary assessment of peritonitis, inadequate abdominal cavity drainage, and inappropriate use of enterostomal therapy, which resulted in adhesion development, long-lasting tertiary peritonitis, etc., a fact being reported by a number of authors [16].

Patients enrolled within the study had a precarious biological field due to various causes and associations, as well as a clinical picture, characterized by an acute onset, severe signs of endotoxicosis (grade II-III), endotoxic shock, peritoneal sepsis, fever, chills, etc. Moreover, the severe child's condition largely developed on an underlying pathology, thus making it difficult to assess the clinical and evolutionary stages of the disease. Most patients exhibited severe fecal peritonitis, perforated gangrenous appendicitis, and intestinal obstruction (both mechanical bowel obstructions and intestinal invagination). These children had signs characteristic of endogenous intoxication, septic and toxic shock, and severe microcirculatory disorders, followed by multiple organ failure.

The obtained findings indicated that the main sources of endogenous intoxication in generalized intra-abdominal infections are both the large peritoneal surface and the GI tract pathology, in case of intestinal failure. The endogenous infection of the patient with generalized peritonitis showed a "primary endogenous" character, thus contributing to selective decontamination of the digestive tract in patients with peritonitis and abdominal sepsis. Therefore, the bacteriological assessment is crucially important for all stages of acute peritonitis management in children, namely in the diagnosis, postoperative antibacterial treatment and early diagnosis of postoperative complications based on the developed algorithm.

Recent studies have provided increasingly more evidence on the importance of intestinal microbiota in the pathogenesis of peritonitis and in the onset of the adhesive diseases. This topic was given special attention since intestinal microbes might play a key role in the development of infants and their immune system due to the immunological, metabolic and neurological benefits offered to the growing body. The specialized literature has reported that intestinal microflora and intestinal microbes are essential for the normal development of the child, since these help in food digestion, vitamin K and B12 production , metabolism of xenobiotics, anti-pathogen protection, stimulation or modulation of the immune system , as well as provide control over the hypothalamic pituitary adrenal axis [17].

The imbalance of the normal gut microbiota, also called dysbiosis, can lead to gastrointestinal disorders, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and different systemic manifestations, such as blood clotting and nerve conduction disorders due to vitamin K and B12 deficiency. There are evidences suggesting that several intrinsic and extrinsic factors, such as genetic variation, diet, stress, and medications, particularly antibiotics, can dramatically interfere with gut microbiota, resulting in abdominal dysbiosis [18, 19]. It has been proven that the imbalance of the normal gut microbiota, dysbiosis, and the immune response of the associated mucosa play a key role in the development of inflammatory bowel disease (IBD), peritoneal inflammation, endogenous intoxication, and abdominal and systemic sepsis, being obviously involved in the intimate pathogenic, pathochemical and immunobiochemical mechanisms of excessive fibrous tissue formation, thus in developing adhesive diseases among children [20].

Complicated evolution of acute peritonitis is often caused by both multifactorial association of the bacterial agents and the antibiotic-resistant strains that increase the incidence of postoperative complications, which require new conceptual approaches and decision-making attitudes [21, 22].

There are current studies that prove the benefits of therapeutic strategies for managing intestinal dysbiosis via administration of antibiotics, prebiotics, probiotics and fecal microbiota transplantation for IBD [23].

The concomitant disorders found in children, increased the risks associated with anesthesia, surgery, and postoperative accidents and complications, thus resulting in costly treatment and poor prognosis. Therefore, there is a stringent need to identify the main approaches in prevention of

adhesive diseases in order to minimize the financial impact on healthcare systems [24].

Currently, it is highly important to improve the study aspects related to disease pathogenesis, predicting factors for developing abdominal adhesion formation and differentiated treatment approaches [25]. Therefore, it is worth mentioning that changes found in complete blood count and routine plasma biochemical markers (total protein level and fractions, liver function tests, blood coagulation tests, ionogram data) of the patients included within the study helped to assess the severity degree of total fecal and purulent peritonitis, intestinal obstructions, spontaneous intestinal fistulas, perforated gangrenous appendicitis in advanced clinical and evolutionary stages. The bacteriological investigations allowed monitoring the main causative pathogens of intestinal adhesive disease.

Following the analysis of pre- and retrospective study data, based on patient's medical observation records, surgical protocols and investigation data, including specially developed study aassessment of molecular mediators of intestinal fibrosis (inflammation markers – NO, IL-1β, CRP, sialic acid, protein-bound hydroxyproline, ceruloplasmin, and serum Copper levels) in children with different acetylation phenotypes. The peculiarities of these changes were also established.

An increased serum nitric oxide (NO) level relates to the presence of an acute inflammatory process that might produce the abdominal adhesion formation. NO plays a crucial role in synthesizing from L-arginine by nitric oxide syntheses (NOSs). NO involves immune responses via cytokine-activated macrophages, which yield high NO levels. Selective NO biosynthesis inhibitors and synthetic arginine analogues have been reported to treat NO-induced inflammation [26].

The present study showed a sudden increase in serum IL-1β values at admission, with the highest levels being recorded in fecal peritonitis and intra-peritoneal adhesive processes, which later on gradually decreased due to the treatment applied, thus having minimum values at time of patient's discharge. Elevated serum IL-1β values suggest the presence of an acute inflammatory process, which is responsible for abdominal adhesion formation. IL-1β, also known as leukocyte pyrogen, endogenous leukocyte mediator, mononuclear cell factor, and lymphocyte activation factor, is an important mediator of variety of cell activities, including cell proliferation, differentiation, and apoptosis. [27]. Induction of cyclooxygenase-2 (PTGS2/COX-2) by this cytokine in the central nervous system (CNS) is found to produce inflammatory pain hypersensitivity. [28]. The excessive IL-1 production is harmful and contributes to developing inflammatory diseases, autoimmune encephalomyelitis, rheumatoid arthritis, gout and other disorders. [29]. It has also been suggested that the release of IL-1β, mediated by inflammasomes, might be a strictly cytolysis-induced event due to necrosis or pyroptosis. Pyroptosis or caspase 1-dependent cell death is initiated by a cascading activation of inflammasomes, which leads to the release of IL-1β [30].

The C-reactive protein (CRP) marker of acute inflammatory response was also assessed within the research, showing significantly higher values in the clinical and evolutionary stages of the pathological process. Moreover, the high values found in the early research stages, although slowly decreasing due to the treatment applied, were still elevated at time of discharge in both post-apendicectomy adhesive processes and fecal peritonitis, thus indicating the persistence of an inflammatory process. CRP is produced as a homopentameric protein, termed native CRP (nCRP) predominantly in liver hepatocytes, but can also be synthesized by smooth muscle cells, macrophages, endothelial cells, lymphocytes and adipocytes. nCRP can dissociate at sites of inflammation and infection into five separate monomers, termed monomeric CRP (mCRP). CRP plays an important role in inflammatory processes and hosts responses to infection, including complement pathway, apoptosis, phagocytosis, nitrogen oxide (NO) release, and cytokine production, primarily of interleukin-6 and TNF-α [31]. Therefore, the level of serum CRP reflects the intensity of abdominal inflammatory processes in children with adhesion processes.

Sialic acids are involved in different biological events, such as cell adhesion, immunity and inflammation, while the increasing cellular inflammatory responses due to sialic acids removal from ligands or cell surface receptors, show the clear functions of sialic acids in negative regulation of cellular inflammation [32]. Thus, the high level of serum sialic acids recorded in dynamics among children with adhesion processes is related to desialized cell surfaces, thus proving that the inflammatory processes were more intense in fast acetylators compared to slow ones.

The level of protein-bound hydroxyproline (tHP) in our study was a truly intensifying collagen synthesis marker. Thus, fibrous tissue at hospitalization and on the first postoperative day was significantly higher in fast acetylator group compared to slow acetylators. However, this index decreased in both groups on the 3rd postoperative day onwards, showing its minimum values at discharge. Such a dynamics in tHP values can be explained by administration of early collagenolytic preventive treatment in rapid acetylators, a fact confirmed by recent studies [11].

The obtained results indicated an increase in ceruloplasmin and serum Cu level content in slow acetylators postoperatively, showing maximum values on the 5th postoperative day, then returning to normal range at time of discharge. The dynamics of fast acetylators indices was similar to that of slow acetylators, though they were no statistically relevant. As it is known, ceruloplasmin is an alpha 2 globulin that binds copper and is synthesized in hepatocytes. Ceruloplasmin is important for its biological functions in removing excessive catecholamines and serotonin via oxidation, as well as in inhibiting both serum histaminase and the oxidation of lipids in the cell membrane due to its anti-inflammatory and antioxidant action. Moreover, it has been found that macrophage-derived ceruloplasmin contributes significantly to protection against inflammation and tissue injury in acute and chronic experimental colitis [33]. The functional significance of ceruloplasmin, as well as a number of copper-containing enzymes, such as cytochromoxi-

dase, monoamine oxidase, tyrosinase, and superoxidismutase, were found to exert a strong antioxidant effect of annihilation on superoxide radicals, thus converting them into oxygen and water.

It can be concluded that the complex of specially selected biochemical examinations indirectly reflect the characteristics of collagen biosynthesis in children with different acetylation phenotypes, thus highlighting the role of acetylation in the development of intraperitoneal adhesion processes in children. The specifically rapid type of acetylation is characteristic for children with postoperative adhesive diseases. Adhesive intestinal obstruction more commonly occurred in fast acetylators compared to the group with slow acetylators. It has been proven that collagen synthesis was faster in fast acetylators associated with peritoneal inflammation, destructive appendicitis and due to surgical traumas, followed by a marked endotoxemia. Therefore, a major genetically determined adhesion process developed. It should be mentioned that adhesive intestinal obstructions in slow acetylators exhibited milder symptoms and lower levels of endotoxicosis, dehydration, and acid-base balance changes [34].

This study results are similar to data obtained by Golubeva M.N., who reported that children with fast acetylation phenotypes (greater than 76%) exhibited a higher rate of adhesion formation than lysis of adhesions in peritonitis [35]. The peritoneal injury in these patients leads to a marked intra-abdominal adhesion formation. However, slow acetylation phenotypes (less than 76%) showed a slower acetylation process. These children had poorly marked or absent intra-abdominal adhesion processes , following a severe or repeated peritoneal trauma. The study of Yakovleva O.A. et al. proved the importance of genotype and phenotype assessment of N-acetyltransferase as a predictor of bronchopulmonary diseases [36].

Acetylation is known to be crucial for metabolism. Acetylation is the ability of the body (genetically determined) to metabolize compounds that contain amino groups. Currently, there are more than 200 genes responsible for the metabolism of xenobiotics, defined as foreign substances that enter the body through different pathways, the N-acetyltransferase gene being one of them, which encodes the N-acetyltransferase. The enzyme activity might occur in the liver and different tissues, which further divides people into two groups, namely fast and slow acetylation phenotypes. The recent pharmacogenetic studies have established the heterogeneity of the human population in terms of the ability to metabolize drugs and other xenobiotics, which largely determines the efficacy and safety of the therapy performed.

The antiadhesion treatment was continued in outpatient care conditions due to some assessed biochemical parameter deviations, which persisted even at 20-25 days postoperatively.

The biopsy samples retrieved from different segments of the intraperitoneal cavity during surgery have provided important data for all stages of the evolutionary adhesion processes. The local and general reactive manifestations of the adhesive processes pose a significant threat on the growing body like in peritoneal hyperemia that is accompanied by the release of large amounts of biologically active substances into the blood flow. Furthermore, this leads to vascular stasis and a subsequent triggering of all mediating local inflammatory responses, which generally impair the overall condition, vital organs and proper system functioning. In turn, these different-origin aggressions are accompanied by functional impairment of the affected region. In this regard, further researches should be carried out to highlight the molecular mechanisms of intestinal fibrosis that would determine the main factors contributing to fibrotic process in general and particularly in intestinal fibrosis [37-39].

All the anatomical and pathological adhesion types were made up of a fibro-conjunctival axis with dilated capillaries. The adhesion processes lead to a pathological development of a major type of acute intestinal obstruction, mainly sited within the small bowel. Most of adhesions caused obstructions and repeated obstructions, which turned into an acute intestinal occlusion.

It can be concluded that the study group included complicated cases, from a clinical point of view, especially in patients, who required not only a complex and special diagnosis, but also a proper assessment of the biological field.

The assessment of homeostatic changes enabled confirming the crucial role of homeostasis rebalancing, by eradicating the pathological focus and resorption of necrotic tissues, as well as removing the excessive tissue proteolysis and exogenous substances, thus providing optimal regeneration conditions.

Therefore, the complex analysis of some pathophysiological mechanisms at different clinical and evolutionary stages in pediatric acute abdominal surgical pathology led to the development of a differentiated therapy, surgical approach + intensive pre-, intra- and postoperative therapy resulting in the most effective methods of treatment.

The obtained study results proved that the strictly individualized management of pre-, intra- and postoperative medical and surgical treatment allowed improving the previous poor prognosis, by reducing serious complications, as well as decreasing morbidity and mortality rate in acute intraperitoneal inflammatory surgical pathology.

The surgical treatment requires case-by-case individualized approach, taking into account the clinical, X-ray, ultrasound, and laboratory data, as well as the biohumoral features in terms of their severity and form. The beneficial effects of this treatment strategy are confirmed by a series of clinical studies [40-43].

It might be concluded that postoperative adhesions occur, following almost any abdominal surgery, whereas 20% of patients may have repeated episodes of adhesive obstructions, 80% of cases might experience possible relapses after appropriate conservative treatment, and 5% might report adhesive bowel obstructions, which do not improve symptoms even in using enemas or prokinetics , thus a relaparatomy is required. Prevention of adhesion formation requires early diagnosis, appropriate surgical treatment, and careful hemostasis in anastomosis, performed by using monofilament threads.

Further in-depth studies on the peculiarities of pediatric adhesion diseases are necessary in order to develop new strategies for prevention, diagnosis and effective, possibly differentiated and customized treatment.

Conclusions

1. The obtained study results were based on a group of 50 patients aged 1 month-18 years during 2011-2018 years, which showed that hospital morbidity rate, due to adhesive bowel obstruction, tends to steadily increase, whereas the clinical features, complications and challenges in diagnosis is still a medical and surgical topic of interest. This present study has completed the current data on the etiopathogenesis of intraperitoneal adhesion processes, thus confirming the crucial role of the microbial factor, inflammatory response mediators, cell and humoral immune activation, extension of the inflammatory process and the genetic factors due to the acetylation phenotype in children.

2. The dynamic methods of the research based on a series of modern, clinical, histological, biochemical, and bacteriological techniques allowed assessing the clinical and evolutionary disease stages, as well as the severity degree of its associated complications. The high level of postoperative inflammatory mediators indicated an exacerbation of the inflammatory process and tissue hypoxia, thus being a risk factor in the development of intraperitoneal adhesion processes and resulting in poor prognosis due to a recurrent acute intestinal obstruction. The comprehensive diagnostic methods used via modern paraclinical techniques allowed predicting their role in the development, evolution and prognosis of intraperitoneal adhesion process.

3. A conservative and differentiated treatment of abdominal intestinal obstruction in acute adhesion disease was used at first. The surgical treatment approach depended on the intraoperative condition, which allowed identifying the surgical techniques. However, all cases were likely to have a relapse of intestinal obstruction, especially in children with fast acetylation phenotype, therefore, a 2-4 year follow-up is required for patients who underwent surgery, via regular clinical, imaging, and laboratory investigations and antiadherent treatment (Serrata, Fermencol, Longidase, Collalysin + Wobenzym – anti-inflammatory, immunomodulatory drugs).

4. The scientific problem solved within this research refers to complementary data on clinical, paraclinical, and imaging peculiarities, as well as on the biochemical markers, particularly of N-acetyltransferase, which provided comprehensive information on the acetylation type, thus justifying the use of medical techniques.

5. In conclusion, further in-depth studies of this issue are required, particularly on the pathophysiology of collagen synthesis, the prophylaxis of the adhesion process and targeted antiadherent treatment. The use of Longidase, Fermenchol, Cuprinil and anti-inflammatory drugs over the last years has proved to reduce the inflammatory response, adhesion formation in patients operated on the abdominal cavity. Although 100% of patients who underwent surgery develop adhesion processes, the antiadherent treatment might substantially reduce the number of cases associated with acute intestinal obstruction, followed by surgical treatment. Therefore, a rational conservative treatment will reduce both the morbidity and mortality rates in acute adhesive intestinal obstructions.

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Authors' contribution
JB drafted the first manuscript, LB acquired and interpreted the data, VB interpreted the data, EG designed the trial and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
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Conflict of Interests
The authors have no conflict of interests to declare.

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Opportunities of Fenspiride anti-inflammatory therapy in patients with chronic obstructive pulmonary disease

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Abstract

Background: Currently, bronchodilator medication recommended in the basic treatment of stable chronic obstructive pulmonary disease (COPD) does not have direct anti-inflammatory properties. According to recent researches Fenspiride has a multi-functional effect on various links of the inflammatory process in the respiratory tract. The purpose of this study was to assess the clinical efficacy and safety of Fenspiride in the treatment of COPD in remission.
Material and methods: The research was performed on a group of 42 patients with COPD in stages GOLD 1 and GOLD 2. Depending on the treatment applied, patients were divided into 2 groups. The first group received Fenspiride with basic treatment and the second group only basic treatment. The main symptoms, pulmonary ventilation indexes and exercise tolerance were assessed in all patients.
Results: The results obtained showed that Fenspiride improves the symptoms of the disease, pulmonary ventilation and increases patients' exercise tolerance.
Conclusions: Fenspiride is an effective and harmless remedy that can be used for the anti-inflammatory treatment of stable COPD in stages GOLD I and II.
Key words: chronic obstructive pulmonary disease, pulmonary ventilation, exercise tolerance.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a common pathological condition characterized by persistent, usually progressive, irreversible or only partially reversible limitation of airflow, associated with an inflammatory response of the airways and lung tissue, as a result of exposure to harmful or gaseous particles [1, 2].

COPD has become a major medical, social, and economic problem for global health systems due to the dizzying rise in both prevalence, and morbidity and mortality [2, 3].

The effectiveness of treatment aimed at stopping the progression of COPD largely depends on the possibilities of attenuation of the chronic inflammatory process as the main link in the pathogenetic chain of the disease [2]. Currently, bronchodilator drugs are recommended in the basic treatment of stable COPD, medication, which does not have direct anti-inflammatory properties.

Current pharmacological treatment of stable COPD reduces the symptoms, frequency, and severity of exacerbations, but does not change the long-term decline in lung function and patient health. This dictates the need to look for new medications, which would increase the effectiveness of existing therapy [4], which refers primarily to the treatment of bronchial inflammation that is resistant to corticosteroids in most patients [5].

Some perspectives in the anti-inflammatory treatment of COPD are associated with the use of Fenspiride, which according to multiple recent experimental and clinical researches have a multi-functional effect on various links of the inflammatory process in the respiratory tract [6, 7].

The purpose of the study consists in the assessment of the clinical efficacy and safety of Fenspiride in the treatment of patients with stable COPD, grade GOLD 1 and GOLD 2 over 6 months.

Material and methods

The study included 42 patients (38 men and 4 women) with a diagnosis of stable COPD. The age of the patients ranged from 46 to 55 years (mean age = 49.3 ± 2.8 years). The average disease duration was 14.3 ± 2.5 years, and the smoking index was equal to 11 packs / year. Patients did not have major concomitant illnesses, which could lead to their exclusion from the study. Depending on the treatment applied, patients were divided into 2 groups, comparable by age, gender, duration of illness and smoking. The patients in group I (baseline) (n = 20, 18 men and 2 women) were administered Fenspiride (Eurespal, manufacturer "Servier",

France) at a dose of 80 mg twice daily per os, based on Salbutamol treatment, if necessary. The patients in the control group II (n = 22, 20 men and 2 women) received only Salbutamol treatment when needed. All patients underwent clinical, laboratory and instrumental investigations, including spirometry and chest radiography.

To assess the functional capacity of patients with COPD, exercise tolerance was determined using the 6 minute walk test (6MWT) [8]. After completing the 6 minutes of walking, the distance walked by the patient was measured and a questionnaire for the subjective evaluation of fatigue and dyspnea felt (Borg scale) was applied to each studied patient [9].

The statistical analysis was performed using the program "STATISTICA for Windows, Version 11". Data were presented as mean ± standard deviation. The two-tailed statistical criterion "Student" was also used to evaluate the data before and after treatment. A "p" value of less than 0.05 was considered statistically significant.

Results and discussion

The main clinical symptoms (shortness of breath, cough, and sputum expectoration) signaled a genuine decrease already after 3 months of treatment. After a 6-month course of treatment, compared to the intermediate stage of the study, a further reduction in the severity of all clinical symptoms was observed (fig. 1).

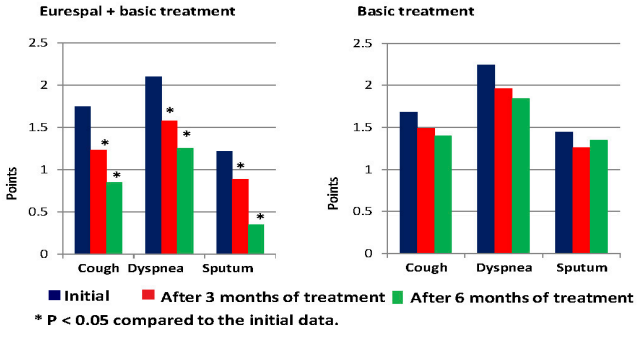

Fig. 1. Dynamics of the clinical symptoms after 6 months of treatment.

The positive dynamics of clinical symptoms took place against a background of improved pulmonary ventilation indexes. After 3 months of treatment, the maximum expiratory volume in the first second (FEV1) increased significantly in group I and over the next 3 months this index continued to increase, but its increase was not statistically significant. At the end of the treatment course, a statistically significant increase of the FEV1 / vital capacity ratio was reported, compared to the initial data and the results obtained at the end of the first 3 months of treatment. In group II, the same indexes, calculated after 3 and then 6 months of treatment did not show significant changes (fig. 2).

The assessed shortness of breath before and after the walk test also changed. The degree of dyspnea at the end of

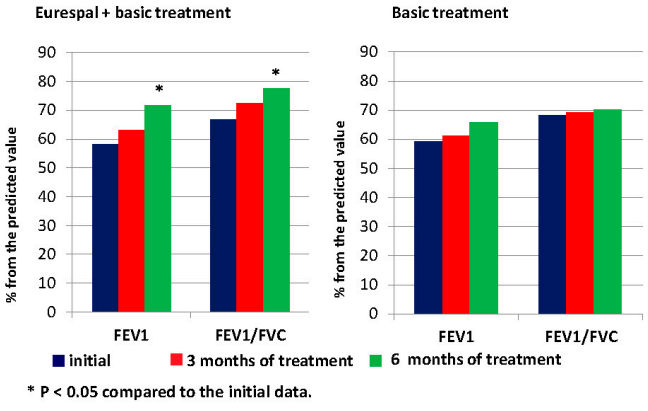

Fig. 2. Dynamics of the indices of external respiration function after 6 months of treatment.

the walk test examined after 3 and 6 months of treatment was more pronounced in group II (fig. 3).

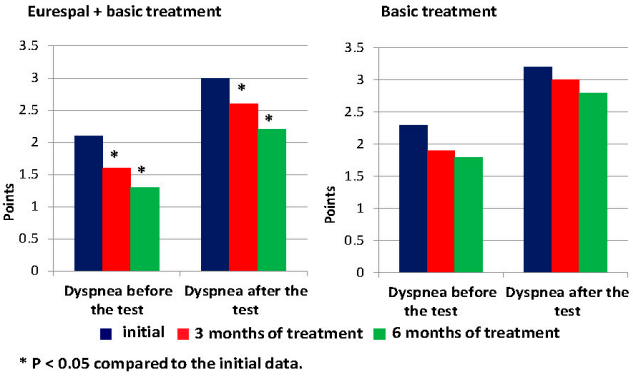

Fig. 3. Dynamics of dyspnea on physical exertion according to the data of the 6-minute walk test.

According to a 6-minute walk test, the administration of Fenspiride in the complex treatment of patients with COPD also contributed to increased exercise tolerance. The distance walked by the patients of the first group after 3 months of treatment increased by 32.5% (from 296.1 ± 18.3 m to 392.3 ± 17.4 m, $p < 0.05$), and by the end of treatment the increase in the distance walked was insignificant, 3.1% by the 6th month, compared to the 3rd month.

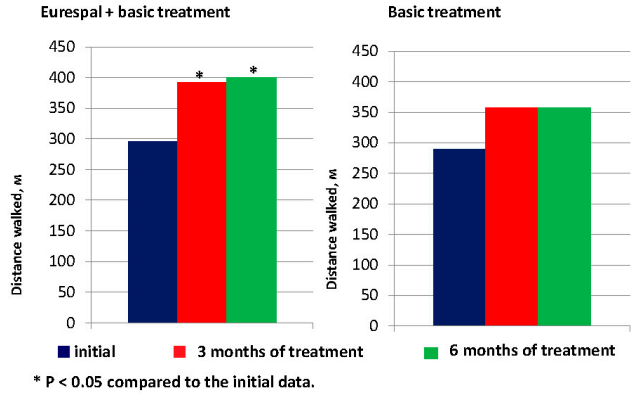

Fig. 4. Dynamics of the exercise tolerance after 6 months of treatment.

In group II this indicator did not change significantly: after 3 months of treatment the distance walked increased by 23.5% (from 289.5 ± 16.8 m to 357.4 ± 15.7 m, $p > 0.05$), and over 6 months – by 2.4% compared to the 3rd month (fig. 4).

The frequency of use of salbutamol in the group of patients taking Fenspiride was significantly lower not only in relation to the reference values ($p < 0.05$), but also in comparison with the similar values in group II, which showed a positive dynamic, but insignificant throughout the treatment.

The doctor's assessment of the tolerance of Eurespal treatment showed no adverse reactions during the study, except in two cases (nausea and headache), which did not require drug correction.

The results obtained in the current study are consistent with the investigations of other authors [5, 7].

Conclusions

The study results revealed that the group of the patients who underwent treatment with Fenspiride in combination with Salbutamol, if necessary, showed significant decrease in main symptoms severity, improved pulmonary ventilation indexes and increased exercise tolerance. This allows us to conclude that Fenspiride is an effective and harmless remedy that can be used for the anti-inflammatory treatment of stable COPD in stages GOLD I and II.

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Authors' contributions

VC conceptualized the project and drafted the first manuscript. SM interpreted the data. SN and CM critically revised the manuscript. All authors revised and approved the final version of the manuscript.

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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. The informed consent was received from every patient.

Conflict of Interests

No competing interests were disclosed.

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Nr. 1 ÎN LUME ibuprofen[2]

Rezumatul caracteristicilor preparatului Brufen. Certificatul de înregistrare în Republica Moldova nr.25825 din 09.10.2019, valabil până pe 09.10.2024. Denumirea comercială. Brufen 600 mg granule efervescente în plicuri nr.30. Compoziția. 1 plic conține ibuprofen 600 mg. **FORMA FARMACEUTICĂ.** Granule efervescente. Granule de la mici până la mai mari, de culoare albă, cu aromă de portocală. **Indicații terapeutice.** Artrita reumatoidă, inclusiv artrita reumatoidă juvenilă sau boala Still, spondilita anchilozantă, osteoartrita ș.a. artropatii nereumatoide (seronegative) artrita gutoasă acută. Afecțiunile reumatice nearticulare și periarticulare, precum sindromul umărului înghețat (capsulita), bursita, tendinita, tendosinovita și a durerile lombare. Traumele țesuturilor moi (luxațiile și entorsele), pentru atenuarea durerilor ușoare și moderate (dismenoreea primară, durerile dentare și postoperatorii, durerea după epiziotomie, durerea după naștere și pentru atenuarea simptomatică a durerilor de cap, inclusiv a migrenelor). Tratamentul febrei. **Doze și mod de administrare.** Administrare orală. *Adulți și adolescenți cu vârsta peste 12 ani (≥ 40 kg):* 1200-1800 mg/zi, divizată în câteva prize. Pentru unii pacienți poate fi suficient 600-1200 mg pe zi. În cazuri severe și acute poate fi util de crescut doza până la finisarea fazei acute. Doza zilnică maximă nu trebuie să depășească 2400 mg în câteva prize; în caz de necesitate doza poate fi crescută până la 3200 mg cu supraveghea minuțioasă a stării pacientului. Copii. Contraindicat copiilor sub 12 ani. **Contraindicații.** Hipersensibilitate la substanța activă sau la oricare dintre excipienți; este contraindicat pacienților cu astm bronșic, urticarie ori reacții de tip alergic în urma administrării de acid acetilsalicilic sau alte AINS; insuficiență cardiacă severă (NYHA IV); insuficiență hepatică severă; insuficiență renală severă; tendință crescută de sângerare sau sângerare activă; antecedente de sângerări gastrointestinale ori perforații, în urma terapiei cu AINS; colită ulcerativă, boala Crohn, ulcer peptic recidivant sau hemoragie gastrointestinală (două sau mai multe episoade distincte de ulcer sau hemoragii diagnosticate) prezente sau în antecedente; trimestru trei de sarcină. **Reacții adverse.** *Tulburări ale sistemului nervos:* frecvente - cefalee, ameţeli; *tulburări gastrointestinale: frecvente* - dispepsie, diaree, greață, vomă, dureri abdominale, meteorism, constipații, melena, hematemeză,hemoragii gastrointestinale; *afecțiuni cutanate și ale țesutului subcutanat:* frecvente - erupții; *Tulburări generale:* frecvente - fatigabilitate. **Categoria de eliberare.** Cu prescripție medicală. Informația completă despre preparat se găsește în REZUMATUL CARACTERISTICILOR PREPARATULUI Brufen granule efervescente din 22.07.2020.

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If this recovery takes more than 6 months, the patient should not receive MAVENCLAD® any more. **Contraindications:** Hypersensitivity to cladribine or any of the excipients, infection with human immunodeficiency virus, active chronic infection (tuberculosis or hepatitis), initiation of treatment in immunocompromised patients, including patients currently receiving immunosuppressive or myelosuppressive therapy, active malignancy, moderate or severe renal impairment or in pregnancy and breastfeeding. **Precautions:** Cladribine's mode of action is closely linked to a reduction in lymphocyte count. The effect on lymphocyte count is dosedependent. Decreases in neutrophil count, red blood cell count, haematocrit, haemoglobin or platelet count have also been observed in clinical studies, although these parameters usually remain within normal limits. Lymphocyte counts must be determined prior to therapy in Years 1 and 2, and at 2 and 6 months after start of treatment in each treatment year. Cladribine can reduce the body's immune defence and may increase the likelihood of infections. HIV infection, active tuberculosis and active hepatitis must be excluded before initiation of cladribine. Screening for latent infections, including tuberculosis and hepatitis B and C, must be performed prior to initiation of therapy in Years 1 and 2. A delay in initiation of cladribine should also be considered in patients with an acute infection until the infection has been adequately treated. Vaccination of patients negative for varicella zoster virus antibodies is recommended prior to initiation of MAVENCLAD® therapy; treatment with MAVENCLAD® must be postponed for 4 to 6 weeks to allow for the full effect of vaccination to occur. The incidence of herpes zoster was increased in patients on cladribine; anti-herpes prophylaxis should be considered during Grade 4 lymphopenia. Cases of progressive multifocal leukoencephalopathy have been reported for parenteral cladribine in patients treated for hairy cell leukaemia with a different treatment regimen. In the clinical study database of cladribine in MS (1,976 patients, 8,650 patient-years) no case of PML has been reported. Baseline magnetic resonance imaging (MRI) should be performed before initiating MAVENCLAD®. Malignancies were observed more frequently in cladribine-treated patients than in patients who received placebo in clinical trials. In patients who require blood transfusion, irradiation of cellular blood components is recommended prior to administration to prevent transfusionrelated graft-versus-host disease. In patients previously treated with immunomodulatory or immunosuppressive medicinal products, the mode of action and duration of effect of the other medicinal product should be considered prior to initiation of MAVENCLAD®. When switching from another MS medicinal product, a baseline MRI should be performed, usually within 3 months. Use of MAVENCLAD® is not recommended in patients with moderate or severe hepatic impairment. MAVENCLAD® contains sorbitol. Patients with hereditary problems of fructose intolerance should not take this medicinal product. **Fertility, pregnancy, lactation:** Before initiation of treatment in both Years 1 and 2, women of childbearing potential and males who could potentially father a child should be counselled regarding the potential for serious risk to the fetus and the need for effective contraception. In women of childbearing potential, pregnancy must be excluded before the initiation of MAVENCLAD® in Years 1 and 2, and prevented by use of effective contraception during cladribine treatment and for at least 6 months after the last dose. Women using systemically acting hormonal contraceptives should add a barrier method during cladribine treatment and for at least 4 weeks after the last dose in each treatment year. Women who become pregnant during therapy with MAVENCLAD® should discontinue treatment. Male patients must take precautions to prevent pregnancy of their partner during cladribine treatment and for at least 6 months after the last dose. Cladribine could cause congenital malformations when administered during pregnancy. Studies in animals have shown reproductive toxicity. MAVENCLAD® is contraindicated in pregnant women. It is not known whether cladribine is excreted in human milk. Because of the potential for serious adverse reactions in breastfed infants, breastfeeding is contraindicated during treatment with MAVENCLAD® and for 1 week after the last dose. **Undesirable effects: Very common:** lymphopenia. **Common:** oral herpes, dermatomal herpes zoster, decrease in neutrophil count, rash, alopecia. **Very rare:** tuberculosis. Prescribers should consult the Summary of Product Characteristics in relation to other adverse reactions. **Marketing authorisation holder:** Merck Europe B.V. Gustav Mahlerplein 102, 1082 MA Amsterdam Olanda.

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The clinical pattern of patients with recurrent stroke

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Abstract

Background: Recurrent strokes account for about 25% of all strokes that occur annually. Studies show varying recurrence rates, ranging from 7% – 20% at 1 year to 16% – 35% at 5 years. Establishing a clinical pattern of patients with recurrent stroke could optimize the management strategy of this pathology.
Material and methods: A retrospective observational study was conducted that included 60 patients with primary stroke (n=30) and recurrent stroke (n=30). The severity of stroke was assessed using the National Institute of Health Stroke Scale scale and the degree of neurological disability – using the mRS scale. Predictive factors, post-stroke infectious complications and compliance with primary and secondary prophylaxis measures were also investigated. For the statistical analysis of the data, the Student's t test was performed for two independent samples.
Results: In the primary stroke group the mean age was 63.7±2.0 years, whereas in the recurrent stroke group it was 68.8±1.42 years. Statistically significant differences between groups were recorded for age (p=0.043), dyslipidemia (p=0.020), post-stroke infectious complications (p=0.032), cerebellar deficit (p=0.029), cognitive deficit (p=0.020) and neurological disability (p=0.003). Also, 93.33% of patients with atrial fibrillation following anticoagulant treatment as a secondary prophylaxis were under coagulated.
Conclusions: Elderly patients with poor risk factors control will be prone to experience a stroke of moderate severity, which will involve a moderate-severe degree of post-stroke disability, expressed by motor, sensitivity, verbal, cerebellar and cognitive deficit, as well as post-stroke infectious complications of the respiratory and urinary tract.
Key words: recurrent stroke, predictive factors, clinical pattern.

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Introduction

Recurrent stroke is the onset of a repeated ischemic or hemorrhagic event in the brain, which lasts more than 24 hours and occurs after an initial stroke, regardless of the territory in which it occurs [1].

Recurrent strokes account for about 25% of all strokes that occur annually [2]. The incidence of stroke recurrence is high despite developments in primary and secondary preventive treatment [3, 4]. The cumulative incidence of stroke recurrence at 5 years varies between 16 and 30% in Western countries [1, 5–8]. Studies show varying recurrence rates, ranging from 7% – 20% at 1 year to 16% – 35% at 5 years [8, 9].

Stroke recurrence is closely and significantly related to increased mortality and morbidity [10, 11]. The risk of recurrence varies depending on the type of cerebrovascular disease and risk factors [12]. Risk factors for recurrent stroke are similar to those for primary stroke and include modifiable and non-modifiable factors.

The analysis of 30 studies suggests that hypertension is the main risk factor for recurrent stroke. During the period up to a recurrent stroke there is a number of changes in the cardiovascular system, including cerebral circulation such as vascular remodeling, inflammation, oxidative stress and baroreflex dysfunction, contributing to the pathogenesis of stroke in hypertension [13].

Age, diabetes, dyslipidemia and atrial fibrillation are other predictive factors for stroke recurrence, but they are presented in heterogeneous proportions in various studies [12].

Small vessels disease as compared to large artery atherosclerosis was associated with a lower chance of recurrence. Moderate level of evidence was found for a lower risk of an undetermined cause of stroke as compared to large artery atherosclerosis. No predictive imaging factors were found based on CT or ultrasound. A moderate level of evidence for the prediction of recurrent ischemic stroke based on MRI was found for multiple lesions, multiple stage lesions, multiple territory lesions, chronic infarcts, and isolated cortical lesions. A limited level of evidence was present for the association between white matter lesions and stroke recurrence [14].

The underlying mechanisms of stroke recurrence are not well known, and the etiology may be multifactorial. Epide-

miological and prospective studies have focused on predictive factors and the frequency of stroke recurrence, but not enough attention has been paid to this phenomenon on a case-by-case basis. Thus, the approach to the subject of predictability of stroke recurrence becomes complicated in the absence of a well-defined clinical pattern.

Elucidating the aspects related to the predictive factors of stroke recurrence in order to establish a clinical pattern of patients with recurrent stroke could optimize the management strategy of this disease.

Material and methods

A retrospective observational study was conducted that included 60 patients with primary stroke (n = 30) and recurrent stroke (n = 30), admitted in the Stroke Unit of the Institute of Neurology and Neurosurgery of the Republic of Moldova in January 2019 – December 2019.

The inclusion criteria in the study were: patients with primary ischemic and hemorrhagic stroke and patients with recurrent ischemic and hemorrhagic stroke, confirmed by CT or MRI. The exclusion criteria from the study were: patients with ischemic and hemorrhagic stroke who died.

On admission stroke severity was assessed by NIHSS scale (National Institute of Health Stroke Scale), that is composed by 11 items and it is used to quantify the neurological deficit caused by a stroke. Thus, the NIHSS score between 0 – 4 points corresponds to a mild stroke, the NIHSS score between 5 – 15 points – to a moderate stroke, the NIHSS score between 16 – 20 points – to a moderate-severe stroke, and the NIHSS score between 21 – 42 points corresponds to a severe stroke.

The post-stroke infectious complications that occurred in these patients during hospitalization were also investigated.

The mRS scale (*modified Rankin Scale*) was used to measure the degree of disability or dependence in the daily activities of people who have experienced a stroke or other causes of neurological disability. This is an ordered scale from 0 to 6: a score of 0 points meaning no symptoms, and 6 points – the maximum score meaning dead.

Carotid Doppler ultrasound was used to identify the different degree of stenosis or vessels occlusion.

The following risk factors were investigated: hypertension (by stage), diabetes, dyslipidemia (according to total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides), smoking, cardiovascular disease (atrial infarction, myocardial infarction and valvular heart disease) and obesity (according to BMI).

For the statistical analysis of the data, the standard descriptive statistics kit was used through the data analysis of EXCEL and the Student's *t* test for two independent samples was performed on IBM SPSS Statistics 26.0. The use of the standard descriptive statistics kit facilitated the calculation of mean values and standard deviations, and the Student's *t* test for two independent samples allowed the calculation of the p value for each element investigated in this study.

Results

The research included a total number of 60 patients with stroke – 33 women (55%) and 27 men (45%), the women:men ratio being 1.2:1. The mean age of the patients was 66.25±1.71 years, with limits between 44 and 92 years. Patients were divided into 2 groups as follows: the first group – 30 patients with primary stroke and the second group – 30 patients with recurrent stroke. Table 1 presents the general characteristics of study populations.

Table 1

General characteristics of population study

Parameters	Primary stroke group	Recurrent stroke group	p
Age, years	63.7±2.0	68.8±1.42	<0.05*
Women, n	18	15	>0.05*
Men, n	12	15	>0.05*
Women:Men	1.5:1	1:1	>0.05*

Note: Statistical test applied: * - t Student for two independent samples.

In the primary stroke group the mean age was 63.7±2.0 years, unlike the group with recurrent stroke where the mean age was 68.8±1.42 years, which determined the presence of statistically significant differences between groups (p=0.043; p <0.05).

Regarding the sex criterion, in the primary stroke group the women:men ratio was 1.5:1, and in the recurrent stroke group the women : men ratio was 1:1 (p=0.44; p> 0.05).

The results obtained after the analysis of risk factors are shown in tab. 2.

Table 2

Analysis of recurrent stroke risk factors/predictors

Parameters	Primary stroke group	Recurrent stroke group	p
Hypertension	- stage 1: 3.33% - stage 2: 30% - stage 3: 66.67%	- stage 1: 0% - stage 2: 23.33% - stage 3: 76.67%	0.30 (p>0.05)*
Diabetes	20%	40%	0.094 (p>0.05)*
Dyslipidemia	26.67%	40%	0.020 (p<0.05)*
Obesity	- N/O: 73.33% - class 1: 10% - class 2: 10% - class 3: 6.67%	- N/O: 56.67% - class 1: 10% - class 2: 23.33% - class 3: 10%	0.28 (p>0.05)*
Cardiovascular disease	- AF: 13.33% - VHD: 3.33% - MI: 6.67%	- AF: 36.67% - VHD: 13.33% - MI: 13.33%	0.016 (p<0.05)*
Smoking	26.67%	33.33%	0.58 (p>0.05)*

Note: AF – atrial fibrillation; N/O – Normal weight/Overweight; MI – myocardial infarction;
VHD – valvular heart diseas. Statistical test applied: * - t Student for two independent samples.

Regarding the severity of the stroke, moderate strokes were more common in both groups and there were no statistically significant differences between the NIHSS scores recorded in both groups of patients (10.1 points *vs* 11.7 points; p=0.17, p>0.05). The mRs score was of a statistically significantly higher average degree of disability (3.6 points *vs* 2.9 points; p=0.003, p<0.01) in patients with recurrent stroke, assigning a moderate-severe degree of neurological disability to these patients. The data are presented in tab. 3.

Table 3

Assessment of stroke severity and neurological disability of patients

Parameters	Primary stroke group	Recurrent stroke group	p
NIHSS score	10.1±0.78 points	11.7±0.85 points	0.17 (p>0.05)*
mRS score	2.87±0.17 points	3.57±0.15 points	0.003 (p<0.01)*

Note: NIHSS – National Institute of Health Stroke Scale; mRS – modified Rankin Scale.
Statistical test applied: * - t Student for two independent samples.

Analysis of clinical data revealed that patients with recurrent stroke had a significantly higher proportion of cognitive deficit (20% *vs* 10%, p<0.05) and cerebellar deficit (30% *vs* 16.7%, p<0.05), as well as post-stroke infectious complications of the respiratory tract (29% *vs* 16.1%, p<0.05) and urinary tract (16.1% *vs* 6.5%, p>0.05). These results are shown in tab. 4.

Table 4

Evaluation of patients clinical data

Parameters	Primary stroke group	Recurrent stroke group	p
Motor deficit	90%	96.67%	0.30 (p>0.05)*
Sensitivity deficit	63.33%	63.33%	1.0 (p>0.05)*
Visual deficit	3.33%	10%	0.30 (p>0.05)*
Cognitive deficit	10%	20%	0.020 (p<0.05)*
Cerebellar deficit	16.67%	30%	0.029 (p<0.05)*
Aphasia	46.67%	60%	0.30 (p>0.05)*
Pneumonia	16.13%	29.03%	0.032 (p<0.05)*
Urinary tract infections	6.45%	16.13%	0.078 (p>0.05)*

Note: Statistical test applied: * - t Student for two independent samples.

The evaluation of paraclinical data, namely the US Doppler examination found in patients with recurrent stroke a more advanced degree of atherosclerosis than in patients with primary stroke (53.3% *vs* 30.3%, p=0.069, p>0.05).

The level of compliance with the measures of primary and, especially, secondary prophylaxis was assessed, and in order to objectify the patients' words, the cases of patients with atrial fibrillation were investigated. Most often, in patients with atrial fibrillation/atrial flutter following anticoagulant treatment with vitamin K antagonists, INR should be between 2 and 3. Thus, of the 15 patients with atrial fibrillation, 14 (93.33%) had the INR<2 and only 1 patient (6.67%) had an INR between 2 and 3. Patients having an INR value < 2 get an insufficient dose of anticoagulant and thrombi can form in the heart cavities causing strokes. These results require a review of patients' risk factors control and of the gaps in the application of secondary stroke prevention measures. The data were plotted in fig. 1.

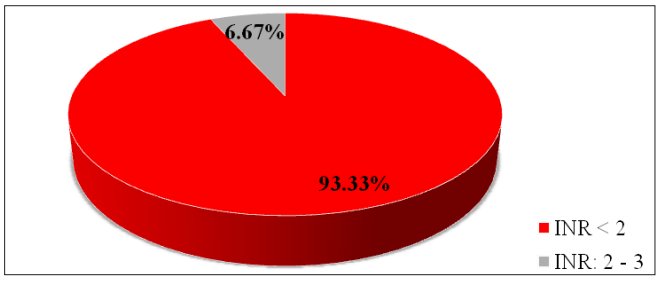

Fig. 1. Control of coagulability by INR in patients with atrial fibrillation following anticoagulant treatment with vitamin K antagonists.

Investigation of stroke recurrence predictors, as well as clinical and paraclinical data was the pivotal element of our research to establish a clinical pattern of patients with recurrent stroke. This was systematized in fig. 2.

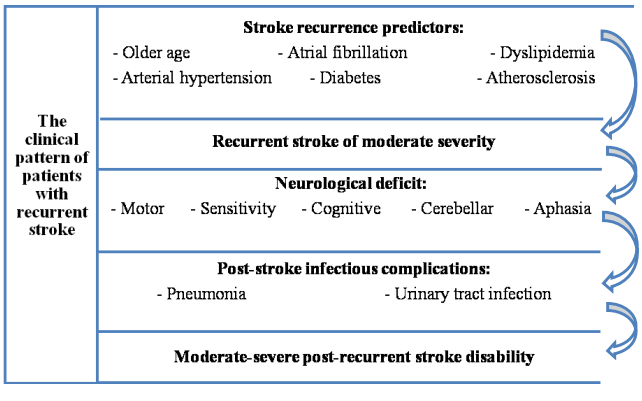

Fig. 2. The clinical pattern of patients with recurrent stroke.

Discussion

The results of our study highlighted the following predictors of stroke recurrence: older age, persistence and/or progression of hypertension, diabetes, dyslipidemia, cardiovascular diseases(especially atrial fibrillation), advanced atherosclerosis and incorrect/inadequate risk factors control, confirming the data of other current papers [15-18].

The novelty of our research concerns the clinical aspects of recurrent stroke compared to those of primary stroke. The association of predictive factors and the newly discovered clinical elements allow shaping the clinical pattern of the patient with recurrent stroke. Regarding the stroke severity, moderate-grade strokes were more common in both groups. However, patients with recurrent stroke had a higher degree of disability than patients with primary stroke. Motor and sensitivity neurological deficits were registered with the same frequency in both groups of patients, but cognitive and cerebellar deficits were more common in patients

with recurrent stroke, their proportion doubling compared to that in patients with primary stroke. Currently there are no studies that integrate the clinical part of this disease.

It was observed that the majority of patients (93.33%) were exposed to the risk of recurrence of stroke due to non-compliance with secondary prophylaxis measures and inadequate/incorrect control of risk factors. A study assessing knowledge about risk factors in high-risk patients found that only 42% of patients with a history of stroke were aware of their own risk of recurrence and only 27% of them reported to their physician [19].

Conclusions

Elderly patients with poor control of atrial fibrillation, dyslipidemia, stage 3 hypertension, diabetes mellitus and class 2–3 obesity will be prone to experience a stroke of moderate severity, which will involve a moderate-severe degree of post-stroke disability, expressed by motor, sensitivity, verbal, cerebellar and cognitive deficit, as well as post-stroke infectious complications of the respiratory and urinary tract.

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Authors' contribution
EM designed the study and revised the manuscript critically; MC collected the data, drafted the first manuscript. Both authors revised and approved the final version of the manuscript

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No approval was required for this study

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The authors have no conflict of interests to declare

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Stress before and after surgery in patients with laparoscopic treatment of gallstone disease and inguinal hernia

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Abstract

Background: Laparoscopic surgery for combined surgical pathology demands technique of simultaneous operations. The technique of simultaneous treatment of inguinal hernia (IH) and gallstone disease (GD) has been developed and tested.
Material and methods: Prospected parameters were: heart rate (HR), variation range (ΔX), mode of the amplitude (AMo), and duration mode (Mo). The level of Index of Nervous Tension (INT) was evaluated by Baevsky method for estimating stress level and tension of sympathetic nervous system. Parameters were compared between control group (No1, n=76 one operation for IH) and simultaneous surgery group (No 2, n=58 IH+GD). In all cases laparoscopic transabdominal periperetoneal alohernioplasty was performed.
Results: Heart rate was increasing after surgery, maximum after 2 h (by 26.3% and 23.3%, p>0.05); the ΔX in both groups decreased after 2 h (by 12.4% and 12.1%, p<0.05) and after 2 days (5.3% and 6.8%, p<0.05); Mo did not differ in both groups (p>0.05); the dynamics of the AMo increased with a maximum after 2 h (by 20.2% and 20.6%, p<0.05); the INT rate was increasing up to 2 hours postoperative (by 93.6% and 93.4% (p<0.05)). All indicators were back to normal rates within two days and did not differ in both groups.
Conclusions: No difference in the level of tension in sympathetic nervous system and the degree of centralization of heart rate regulation was registered in both groups. Our developed technique has been shown safe and effective.
Key words: laparoscopy, gallstone disease, inguinal hernia, simultaneous.

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Introduction

Gallstone disease is a common surgical pathology. Each year gallstone disease is diagnosed in 1-2% of adult population in USA and EU [1]. Chronic cholecystitis is well-treated by using laparoscopic surgical treatment. Nowadays laparoscopic cholecystectomy is the main recommended tactic for use [2]. Also, it is shown that early laparoscopic cholecystectomy is the most acceptable approach if complications are considered (mechanic jaundice, choledocholytiasis, gallbladder cancer etc.) [2]. At the same time 27% of males and 3% of females develop a groin hernia at some time of life [3]. Up-to-date fast-track surgery strategic demands minimal hospital stay time. Considering all these factors it is acceptable to perform laparoscopic cholecystectomy simultaneously with other operations if comorbid cholecystitis is diagnosed. One-staged treatment reduces total cost of treatment because of reducing terms of general hospital stay. The main problem of simultaneous operations may become their additional trauma and duration, which theoretically may influence the surgical stress and rate of postoperative complications [4]. These factors demand additional equipment of operating rooms and skills for the surgeon. It is necessary to use technique that provides minimum trauma and invasiveness of surgical interventions, anesthesiological support and management of patients in the postoperative period [5]. This prompted us to develop a new laparoscopic technique for simultaneous surgical treatment of inguinal hernia gallstone disease (GD). Under these conditions, because of increasing admeasurement of surgical trauma, one of the important criteria for the effectiveness and safety of simultaneous operations compared to mono-interventions is the severity of operational stress [6]. Adaptive adjustment of the cardiovascular system is one of the first to note while talking about stress. Stress can be valued through the variability of heart rate [6, 7].

Aim of the work: Using the variability of cardiac rhythm to evaluate the intensity of postoperative stress in patients that underwent transabdominal preperitoneal patch technique (TAPP) and simultaneous laparoscopic cholecystectomy. To compare their result with the one in patients that underwent only TAPP.

Material and methods

Sample includes patients that underwent TAPP (control group No 1, n=76) and patients that underwent TAPP (as one of recommended procedure for inguinal hernia (IH) repair [8, 9]) and simultaneous cholecystectomy (research group No 2, n=58). Patients underwent surgical treatment during 2015-2019 year time period. Survey and all the operations were performed in the Volynian regional clinical hospital in the laparoscopic surgery unit. In every case laparoscopic transabdominal preperitoneal alogernioplastic was performed. In group No 1 in each case standard technique was used. In group No 2 in each case our developed technique of simultaneous operations was used. Selection into groups was performed exclusively on the principles of surgical comorbidity. All patients underwent routine conservative treatment before and after surgery. The average length of stay of the patient in the hospital before surgery was 1 hospital day.

Exclusion criteria: patients with progressive coronary heart disease in combination with severe heart failure and severe chronic kidney disease, isolated obliterating atherosclerosis of the vessels of the lower extremities, chronic pulmonary diseases in the acute stage, cancer of various localization.

To measure the operative stress by a variation of pulsogram, was used cardiocomplex "CardioLab +" (designed and manufactured by HAI-MEDICA, Kharkiv/Ukraine) for all patients in the control group and the main group the day before surgery and 1, 2, 3, 6, 12 hours and 1, 2 and 3 days postoperatively. The recording was performed in the patient's ward in a supine position not earlier than after 7-10 minutes of adaptation to this position. At least 100 cardio intervals were recorded with subsequent determination of the main statistical characteristics according to the method of R.M. Baevsky [10-12].

Recorded data was used to determine heart rate (HR), variation range (ΔX) – the difference between the maximum and minimum duration of cardio intervals, mode of the amplitude (AMo) – the percentage of the most common cardio intervals, as well as their duration – mode (Mo).

According to the obtained data, the voltage index of regulatory systems, index of nervous tension (INT) was calculated by R.M. Baevsky, which reflects the degree of centralization of heart rate control: INT = AMo / (2 ´ Mo ´ DX).

Estimation of the probability of differences between the control and main groups was performed using the nonparametric Mann-Whitney test.

Results and discussion

Data analysis was performed. According to the results of the research there was no statistically significant difference in heart rate between groups.

Records of heart rate are shown in tab. 1 and fig. 1.

Heart rate was increasing and reached its maximum in 2 hours after surgery in both groups (26.3% in group 1 and 23.3% in group 2, p>0.05). Subsequently, in both comparison groups, the indicator decreased and, starting from the 12 hours of postoperative period, did not differ statistically significantly from the value of the indicator before surgery (p>0.05). The same reactions on pain and trauma were registered in other researches [13, 14]. Also common reaction was shown in patients after cholecystectomy [15]. It is noteworthy that the value of heart rate in all periods of the postoperative period in the group of patients that underwent TAPP with laparoscopic simultaneous cholecystectomy and patients that underwent only TAPP did not differ significantly (p>0.05).

Table 1

Heart rate of patients in both groups (Me (LQ; Uq) – median and upper and lower rate)

Period of measurement	Control group (No 1)	Research group (No 2)	p
Day before surgery	72.2 (64; 75)	71.7 (66; 75)	>0.05
Postoperative period			
1 hour	85.8 (81; 90)	83.6 (78; 87)	>0.05
2 hours	91.2 (83; 96)	88.4 (82; 92)	>0.05
6 hours	83.6 (77; 89)	84.2 (77; 88)	>0.05
12 hours	75.9 (71; 81)	76.1 (68; 78)	>0.05
1 day	74.8 (68; 77)	75.5 (67; 78)	>0.05
2 days	72.2 (66; 76)	72.8 (67; 77)	>0.05
3 days	70.8 (65; 75)	71.4 (66; 75)	>0.05

Note: here and further *p* – possibility of difference for rate in the control group from the research group

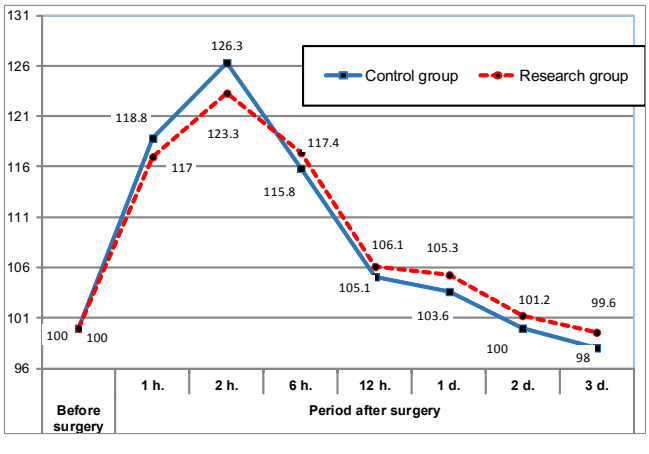

Fig. 1. Heart rate dynamics (in percent to the control level)

(Note: here and further * – differences for the terms before operation are statistically possible p<0.05).

Analysis of the variation scale (ΔX) (tab. 2, fig. 2) had shown that before the operation the indicator did not differ significantly between the control and the main groups (p>0.05). Worth to notice that intraoperative and postoperative (up to 2 hours) heart rate and its variations in both groups were similar between groups as well as from other studies [16]. HR levels shown in this study are widespread during IH operations [17].

Table 2

ΔX rates of patients in both groups (Me (LQ; Uq) – medianand upper and lower rate)

Period of measure-ment	Control group (No 1)	Research group (No 2)	p
Day before surgery	0.152 (0.144; 0.151)	0.148 (0.139; 0.151)	>0.05
Postoperative period			
1 hour	0.142 (0.134; 0.154)	0.134 (0.127; 0.139)	>0.05
2 hours	0.118 (0.109; 0.134)	0.114 (0.110; 0.124)	>0.05
6 hours	0.144 (0.137; 0.155)	0.139 (0.128; 0.147)	>0.05
12 hours	0.150 (0.139; 0.158)	0.142 (0.131; 0.151)	>0.05
1 day	0.153 (0.140; 0.164)	0.142 (0.137; 0.144)	>0.05
2 days	0.144 (0.136; 0.151)	0.138 (0.131; 0.152)	>0.05
3 days	0.175 (0.167; 0.189)	0.171 (0.151; 0.177)	>0.05

The dynamics of the ΔX in the postoperative period groups was wavy in both groups with the first period of decrease after 2 hours (12.4% in group 1 and 12.1% in group 2, p<0.05) and after 2 days (5.3% in group 1 and 6.8% in group 2, p<0.05). After 3 days, the rate in both groups increased and in the control group became significantly higher than before surgery (15.1%, p<0.05) as well as in the research group (15.5%, p<0.05) which was expectable for normal adaptation process [18]. It is remarkable that common data was shown for cholecystectomy performed laparoscopically without TAPP [15, 19].

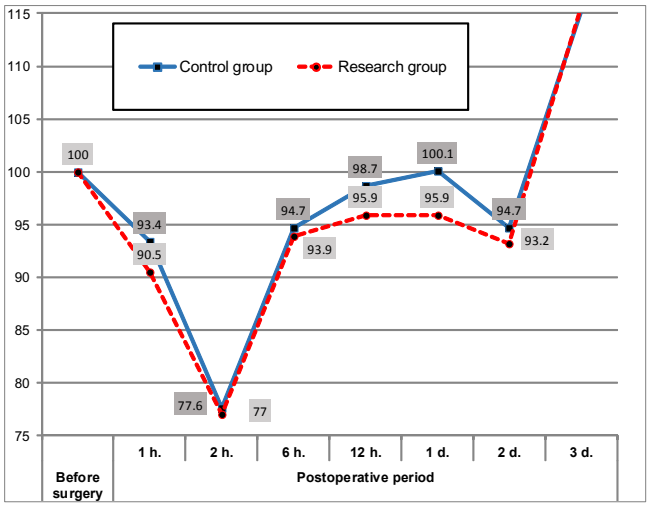

Fig. 2. ΔX (in percent to the control level)

There were no significant differences between the comparison groups during the postoperative period (p>0.05).

Furthermore, the dynamics of the *Mo* was similar to the value of heart rate (tab. 3, fig. 3). The day before the operation, the value of *Mo* between the control and main groups did not differ significantly (p>0.05).

In the dynamics, the value of *Mo* decreased in both groups up to 2 hours of the postoperative period compared with the preoperative level (20.2% in group 1 and 20.6%, p<0.05). This is characteristical for traumatic events [18]. Subsequently, the rate increased and starting from 12 hours of the postoperative period. It did not differ statistically significant from the preoperative level (p>0.05).

Table 3

***Mo* rates of patients in both groups (Me (LQ; Uq) – median and upper and lower rate)**

Period of measure-ment	Control group (No 1)	Research group (No 2)	p
Day before surgery	0.851 (0.811; 0.904)	0.857 (0.793; 0.908)	>0.05
Postoperative period			
1 hour	0.711 (0.647; 0.756)	0.720 (0.674; 0.791)	>0.05
2 hours	0.679 (0.627; 0.717)	0.681 (0.625; 0.733)	>0.05
6 hours	0.727 (0.666; 0.771)	0.724 (0.651; 0.769)	>0.05
12 hours	0.796 (0.731; 0.825)	0.833 (0.768; 0.899)	>0.05
1 day	0.827 (0.755; 0.879)	0.822 (0.744; 0.867)	>0.05
2 days	0.849 (0.777; 0.885)	0.841 (0.767; 0.891)	>0.05
3 days	0.868 (0.807; 0.941)	0.857 (0.812; 0.910)	>0.05

Comparison of the control and main groups in the postoperative period did not reveal significant differences in the value of Mo (p>0.05).

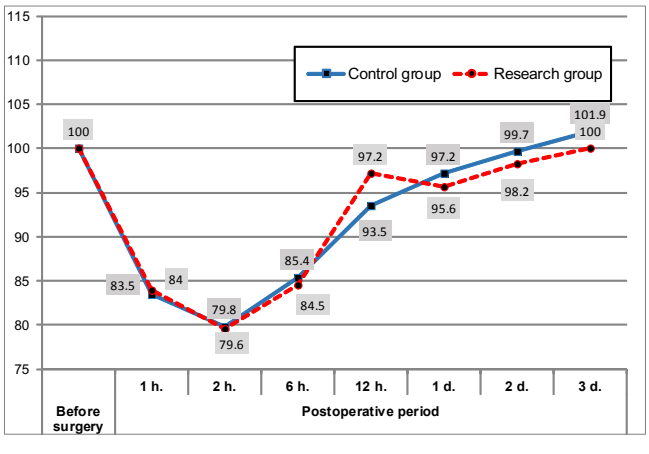

Fig. 3. *Mo* (in percent to the control level)

Table 4

A*Mo* rates of patients in both groups (Me (LQ; Uq) – median and upper and lower rate)

Period of mea-surement	Control group (No 1)	Research group (No 2)	p
Day before surgery	36.1 (32.2; 37.9)	38.5 (34.7; 38.9)	>0.05
Postoperative period			
1 hour	42.8 (38.8; 43.6)	44.4 (40.7; 44.9)	>0.05
2 hours	44.8 (40.0; 47.6)	46.1 (42.7; 47.3)	>0.05
6 hours	41.8 (37.0; 44.6)	43.2 (40.2; 46.4)	>0.05
12 hours	39.6 (35.5; 42.8)	41.0 (37.7; 41.9)	>0.05
1 day	38.2 (34.8; 41.6)	38.5 (35.1; 41.1)	>0.05
2 days	36.0 (34.7; 38.2)	36.2 (35.0; 41.0)	>0.05
3 days	35.1 (31.8; 37.4)	35.9 (34.4; 40.1)	>0.05

The value of A*Mo* also did not differ significantly (tab. 4, fig. 4) in the preoperative and late postoperative period between the control and experimental groups (p>0.05).

The dynamics of the studied indicator increased in postoperative period compared to the preoperative period level with a maximum after 2 hours (24.1% in group 1 and 19.7% in group 2, p<0.05). Subsequently, the indicator decreased and, starting from 12 hours of the postoperative period, in both comparison groups reached the level of the preoperative period (p>0.05).

Comparison of the A*Mo* in the postoperative period did not reveal statistically significant differences between the control and main groups (p>0.05). The recovery time in both groups is comparable to recovery after operation of laparoscopic inguinal hernia repair [20-22] but slightly longer than recovery after open hernia repair with spinal and paravertebral anesthesia [23].

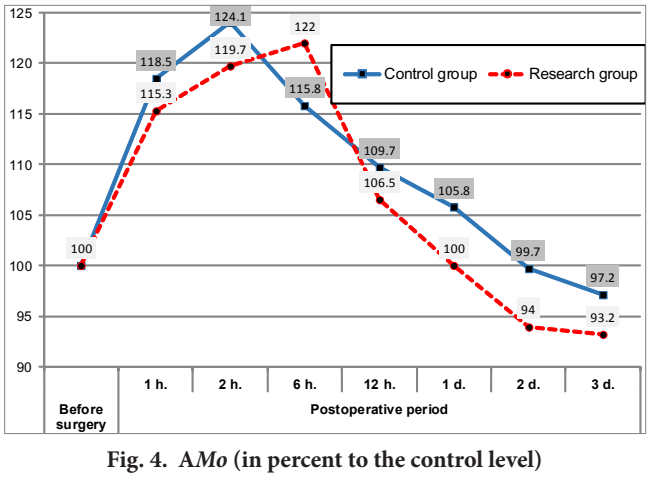

Fig. 4. A*Mo* (in percent to the control level)

An integral measure of the tension of adaptation mechanisms is the value of INT.

Surveys showed that the value of INT in the preoperative period (tab. 5, fig. 5) between the control and main groups did not differ significantly (p>0.05).

Table 5

IN rates of patients in both groups (Me (LQ; Uq) – median and upper and lower rate)

Period of measurement	Control group (No 1)	Research group (No 2)	p
Postoperative period			
1 hour	212.1 (171.4; 251.4)	218.2 (187.4; 207.4)	>0.05
2 hours	251.9 (211.4; 307.6)	281.1 (231.7; 340.4)	>0.05
6 hours	195.7 (157.4; 231.2)	201.4 (174.3; 258.1)	>0.05
12 hours	172.1 (141.7; 201.4)	185.4 (152.8; 221.9)	>0.05
1 day	152.2 (131.4; 179.2)	165.9 (152.9; 211.3)	>0.05
2 days	174.8 (132.7; 209.4)	192.1 (152.4; 224.8)	>0.05
3 days	116.4 (96.2; 127.4)	125.3 (108.2; 142.7)	>0.05

The IN rate was increasing up to 2 hours in the postoperative period compared to the preoperative level: in the control group – by 93.6%, in the research group – by 93.1% (p<0.05). After 6 hours INT decreased in both groups and did not differ significantly from the preoperative level (p>0.05). There was a repeated increase in the value of INT after 2 days in both experimental groups. However, the obtained result compared to the preoperative level was statistically unlikely (p>0.05).

Comparison of the control and main groups in the dynamics of the postoperative period did not reveal statistically significant differences (p>0.05).

It has been shown that optimal recovery time for laparoscopic hernia repair is about 3 days [24-26]. It means that IN decreasing to preoperative levels in both groups in 3 days does not get beyond normal recovery for IH repair even with simultaneous cholecystectomy [27]. Both groups had normal recovery process [28].

Thus, the analysis of statistical indicators of the variation pulsogram in the preoperative period did not reveal significant differences between the control and research groups, which indicates the representativeness of the observation groups.

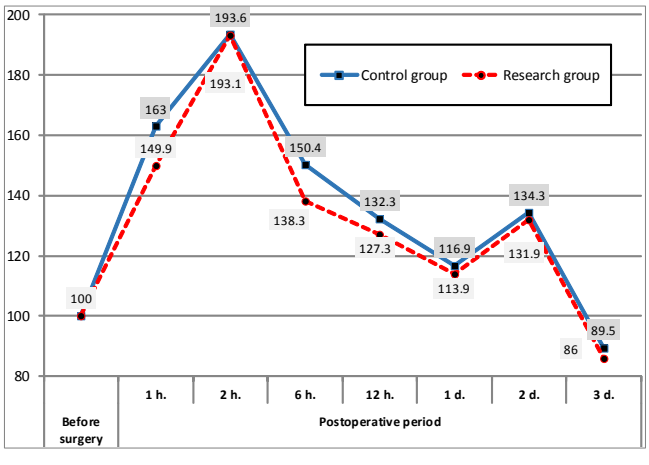

Fig. 5. INT (in percent to the control level)

In the postoperative period up to 2 hours after surgery the indicators values of Mo, ΔX decreased and AMo and INT increased. This indicates an increasing of the activity of the sympathetic nervous system and increasing of the centralization of heart rate control, which indicates an increasing of the stressor effects of surgery. Obviously, this fact is associated with a decrease in the effect of drugs used for anesthesia.

By the 12 hour in the postoperative period, the deviations reached the preoperative level in both groups. Noteworthy is the moderate statistically significant decrease in the value of ΔX and the tendency to increase the INT after 2 days in the postoperative period, which is the evidence of a delayed response of the body to surgical trauma. By the day 3 after surgeries, all applied statistical indicators of mathematical analysis of heart rate reached the preoperative level. There is common information about rehabilitation during simultaneous treatment of gallstone disease [29] where 2.55+0.89 days of hospital stay are shown. Length of stay also didn't differ between groups and data from studies in which only TAPP [30] and only laparoscopic cholecystectomy were per-

formed [31]. Analysing from anesthesiologycal approach it is completely clear that intraoperative heart dynamics during simultaneous operation was in normal zone comparing to the control group and data from other studies [32].

However, despite the types of surgical interventions performed, both laparoscopic transabdominal periperitoneal alohernioplasty and simultaneous cholecystectomy and only TAPP in the postoperative period at all times caused almost the same deviations of the studied parameters, which were not statistically significant between groups of patients. This fact indicates that the degree of stress for both types of surgical interventions is almost the same for the level of tension in sympathetic nervous system and the degree of centralization of heart rate regulation, regardless of the volume and duration of interventions and indicates the safety and viability of simultaneous operations for treatment of hiatal hernia and gallstone disease. Considering recommendations of Hernia Group that inguinal hernia should be repaired as fast as possible [30] as well as cholecystitis [33] because of their complications and risks [34, 35] possibility of simultaneous treatment without negative recovery outcomes is remarkable.

Conclusions

1. Mono-intervention for inguinal hernia and simultaneous operations for inguinal hernia and gallstone disease using our developed technique of simultaneous laparoscopic operations are accompanied by almost identical deviations of statistical indicators of variability of the network rhythm in the postoperative period in all terms of observation.

2. The absence of statistically significant differences in the dynamics of the postoperative period between the groups of patients with mono- and simultaneous interventions on the level of tension in sympathetic nervous system and the degree of centralization of heart rate regulation indicates the same level of postoperative stress and indicates the safety and viability of simultaneous technique.

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MH and IM conceptualized the project and drafted the first manuscript. AP interpreted the data. ID critically revised the manuscript. All authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of *I. Horbachevsky Ternopil National Medical University*, proceedings No 60 of 26.10.2020. The informed consent was received from every patient.

Conflict of Interests
No competing interests were disclosed.

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Lactobacillus rhamnosus, and *Lactobacillus reuteri* with estriol in the treatment of vaginal dysbiosis pathologies

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Abstract

Background: A healthy vagina is characterized by hydrogen peroxide and acid-producing lactobacilli, which are crucial to maintain the physiological vaginal ecosystem and their depletion speeds up bacterial overgrowth with pH elevation, salidase and amine production, leading to the observed signs and symptoms of vaginal dysbiosis. An effective treatment should be combined of lactobacillary flora and low doses of estrogen to maintain the physiology of the vaginal epithelium.
Material and methods: This is a prospective randomized study that included 218 patients, who were divided into 2 groups according to the treatment regimen, performed between October 2018 and December 2019 at the Department of Obstetrics and Gynecology of *Nicolae Testemitanu* State University of Medicine and Pharmacy. Patients selected were divided into L_1 (120 patients assigned to treatment with the combination of two microorganisms *Lactobacillus rhamnosus* and *Lactobacillus reuteri* with estriol 0.03 mg vaginal pessaries) and L_2 (98 patients who were given *Lactobacillus rhamnosus* in vaginal capsules). The treatment regimen for both groups was the same – 1 pessary or 1 capsule once in 24 hours, in the evening, for 12 days. Patients were evaluated before treatment (visit 1) and on day 13 of treatment (visit 2) and 1 month after the end of treatment (visit 3).
Results: Of the 120 women included in the study in the first group and 98 in the second group, a significant improvement (normocenosis) according to the research physician and patients was found in 93.3% (112) patients of group 1 and 71.4% (70) patients in group 2, satisfactory improvement (consistent with the intermediate type of biocenosis) in 5% (6) patients of group 1 and 15.3% (14) patients from group 2, unsatisfactory result 1.7% (2) patients from group 1 and 13.3% (13) of patients of group 2 (later relapse was noted in these patients).
Conclusions: Probiotic treatment with vaginal *Lactobacillus rhamnosus, Lactobacillus reuteri* and low doses of estriol seems to be useful in hindering bacteria growth especially after antibiotic therapy; therefore this intervention may be considered a new prophylactic treatment for preventing recurrence of bacterial vaginosis, in particular in high-risk patients.
Key words: bacterial vaginosis; *Lactobacillus rhamnosus*, vaginal flora, estriol.

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Introduction

Vaginal eubiosis is characterised by a beneficial lactobacillus-dominated vaginal microbiota. In contrast, vaginal dysbiosis is characterised by an overgrowth of multiple anaerobes and is associated with an increased risk of adverse urogenital and reproductive health outcomes [1]. A healthy vagina is characterized by hydrogen peroxide and acid-producing lactobacilli, which are crucial to maintain the physiological vaginal ecosystem and their depletion speeds up bacterial overgrowth with pH elevation, salidase and amine production. Lactobacilli, particularly those producing H_2O_2, play a pivotal role in controlling the microenvironment of the vagina and in inhibiting the overgrowth of potentially pathogenic organisms [2]. Possible mechanisms of this protection include inactivation of pathogens by different Lactobacillus products (lactic acid, H_2O_2 and bacteriocins), competition for epithelial cell attachment sites and stimulation of the local immune system [3].

Several preclinical *in vitro* and *in vivo* studies have shown that *Lactobacillus rhamnosus* and *Lactobacillus reuteri* have antibacterial, antifungal and anti-inflammatory properties, as well as anticarcinogenic, antigenotoxic, antimutagenic and immunomodulating properties. *Lactobacillus reuteri* have the ability to produce antimicrobial molecules, such as organic acids, ethanol, reuterin. Due to their antimicrobial properties, *Lactobacillus reuteri* can inhibit the colonization of pathogenic microbes and reconstruct the host microflora [4].

To maintain a healthy vaginal ecosystem or restore its disruption, sufficient estrogen levels, an intact mature vaginal epithelium and physiological lactobacterial microflora are required. Thus, a combination of beneficial lactobacilli and estrogen is a topical treatment [5, 6].

Currently, in European countries there is a tendency to reduce the use of local estrogens [7]. Given the pathogenesis of vaginal atrophy, estrogen therapy is the gold standard in treatment. Of the three natural estrogens of the human body, estriol has the shortest half-life and the least biological activity. Estrogens, estrone (E1), estradiol (E2), and estriol (E3) are female sex hormones that are usually determined in the human body. While E2 and E1 can be reversibly metabolized, E3 cannot. Like all estrogens E3 stimulates the proliferation and maturation of the vaginal epithelium. However, in E3, sensitivity to receptors is lower (about 10 times) than in E2, and thus, it cannot induce the development of estrogenic effects on the endometrium, bone, and breast tissue in physiological concentrations [8-11].

Vaginal estrogens are more effective in eliminating genitourinary symptoms than oral drugs, since lower doses are required due to the lack of hepatic metabolism, and a high level of estrogen induces direct vaginal response [10]. Thus, topical administration of E3 in the treatment of vaginal diseases is, in general, preferable, since this hormone is safer for local administration than other estrogens and has a more pronounced proliferative reaction than when taken orally. This is especially important if systemic hormone replacement therapy with estrogen is not required [9]. Estriol has a positive effect on the microflora of the vagina due to an increase in colonies of lactobacilli and a decrease in enterobacteria [8, 11-14].

Material and methods

A prospective randomized study was performed that included 218 patients. The patients were divided into 2 random groups, according to the treatment they received: L_1 – 120 patients and L_2 – 98 patients. The study was performed at the Department of Obstetrics and Gynecology of *Nicolae Testemitanu* State University of Medicine and Pharmacy between 14.10.2018-16.12.2019.

Patients in L_1 were given a combination of two microorganisms *Lactobacillus rhamnosus* and *Lactobacillus reuteri* with estriol 0.03 mg vaginal pessaries and patients in L_2 were given *Lactobacillus rhamnosus* in vaginal capsules. The treatment regimen for both groups was the same – 1 pessary or 1 capsule once in 24 hours, in the evening, for 12 days. Intravaginal washings or topical use of certain therapeutic or other substances were prohibited during treatment, and patients were advised to abstain from sexual intercourse for 8 hours after administration of the medication. Simultaneous treatment was allowed only in patients with hypertension.

The inclusion criteria in the study were: the age of patients 20-45 years, patients who needed to restore normal vaginal microflora and those who received local or systemic antibacterial therapy for the treatment of infectious diseases of internal or external genitalia; recurrent chronic genital infection; the period from the end of antibacterial therapy till the patient's address for microflora recovery treatment was less than 1 month; informed consent to comply with the requirements of the drug administration protocol and the time of examination.

The exclusion criteria from the study were: hypersensitivity to the medication, benign or malignant tumors of the uterus, vulva and vagina (including intraepithelial neoplasia of the vagina, endometriosis) and mammary glands, vaginal bleeding of indefinite etiology, use of any drugs with a vaginal administration one week before the enrollment in the study (spermicides, intimate gel, vaginal washings); sexually transmitted diseases (trichomoniasis, gonorrhea, ureaplasma, genital herpes, chlamydia, human immunodeficiency virus HIV, AIDS); enrollment in other studies in the last 30 days; pregnancy and lactation; the patient's refusal to participate in the study.

The efficacy treatment was evaluated taking into account the dynamics of clinical symptoms, the gynecological valve examination of the genitalia and the results of the microscopic examination. Clinical efficacy was analyzed according to the dynamics of symptoms on a generally accepted scale in four points (0 points – no complaints, 1 point – insignificant, 2 points – moderately expressed, 3 points – expressed). Dynamics of objective clinical signs (vaginal discharge, hyperemia) according to the results of the vaginal examination (assessment on a scale according to the degree of manifestations: absence – 0, medium – 1, moderate – 2, expressed – 3). Dynamics of the results of smear microscopy (Gram) of the pathological vaginal discharge before treatment (visit 1) and on day 13 of treatment (visit 2) and 1 month after the end of treatment (visit 3), pH assessment of vaginal fluid.

The results of the study were processed using the STATISTICA 7.0 and the Microsoft Office software package, Microsoft Excel (2007), using parametric and nonparametric statistical criteria.

Results and discussion

The mean age of the patients included in the study was 36.6 ± 6.84 years in L_1 and 33.9 ± 6.05 years in L_2. The main criteria for inclusion in the study was the need to restore the vaginal flora after antibacterial therapy (bacterial vaginosis, candidiasis, nonspecific vaginitis). The data are presented in table 1. Before treatment, the patient of the study groups presented the following complaints: vaginal discharge 53.3% (64) in L_1 vs 50% (49) in L_2, $p > 0.05$; vaginal odor 14.2% (17) in L_1 vs 12.2% (12) in L_2, $p > 0.05$; itching and burning 6.7% (8) in L_1 vs 6.1 %(6) in L_2, $p > 0.05$; discomfort 45.2% (54) in L_1 vs 47.95% (47) in L_2, $p > 0.05$; dyspareunia 14.2% (17) in L_1 vs 12.2% (12) in L_2, $p > 0.05$.

During the gynecological examination before treatment, the vaginal mucosa was of a pale pink color, without a vascular pattern in most patients in the first and second group. Moderate hyperemia of the vaginal mucosa and cervicitis were determined in 18.3% (22) patients of the first group and in 18.4% (18) in the second group, ($p> 0.05$). The intensity of hyperemia of the vaginal mucosa at the beginning of the study in L_1 was 0.38 ± 0.78 points and 0.37 ± 0.77 points in patients of L_2. At the second visit, mucosal hyperemia was absent in all patients in the first group, thus a significant

Table 1

Vaginal dysbiosis pathologies of the patients from the studied groups before enrollment in the study

	L_1 n=120		L_2 n=98		P
	n	%	n	%	
Bacterial vaginosis	36	30	31	31.6	>0.05
Vulvovaginal candidias	14	11.7	8	8.2	>0.05
Nonspecific vaginitis	70	58.3	59	60.2	>0.05

decrease in the frequency of this symptom was observed (p<0.05), which was also confirmed by the positive dynamics of clinical symptoms (complaints – lack of itching, burning, normal vaginal discharge or their absence). In 10.2% patients of the second group, mild hyperemia and vascular pattern were observed, a significant decrease in the frequency of this symptom was also observed (p<0.05). However, in L_1, compared with the L_2, a significantly more decrease in cases of vaginal hyperemia was observed (p<0.05). At the 3rd visit in the first group, moderate hyperemia was detected in 4 (3.3%) patients, severe hyperemia – in 2 (1.7%) patients; the total score for the intensity of hyperemia was 0.11 ± 0.51. In dynamics, compared with visit 1, a significant decrease in cases of vaginal hyperemia was observed (p <0.05). In the second group, moderate hyperemia was also detected in 4 (4.1%) patients, severe hyperemia – in 7 (7.1%) patients, the total score characterizing the intensity of hyperemia was 0.29 ± 0.85 (tab. 2). The results (the 1st and the 3rd visits) in the second group showed statistically significant differences (p<0.05). However, in the first group, compared with the 2nd group, a more significant decrease in cases of hyperemia was observed (p<0.05).

In both groups in patients who demonstrated severe vaginal hyperemia, at the third medical appointment the vaginal mucosa was edematous and in those patients a relapse of the previous vaginal dysbiosis was detected and appropriate treatment was prescribed.

The microscopic examination for detecting vaginal biocenosis was performed before and after treatment. In both groups key cells, yeast cells, pseudomycelia, and fungal

Table 2

Hyperemia of the vaginal mucosa in dynamics

Medical appointment	Intensity	L_1 n=120		L_2 n=98		p
		n	%	n	%	
1	Moderate	22	18.3	18	18.4	>0.05
	Absent	98	81.7	80	–	
	Total score	0.38±0.78		0.37±0.77		
2	Absent	120	100	90	91.8	<0.05
	Medium	0	–	10	10.2	
	Total score	0		0.10±0.30		
3	Absent	120	100	87	88.7	<0.05
	Moderate	4	3.3	4	4.1	>0.05
	Expressed	2	1.7	7	7.1	<0.05
	Total score	0.11±0.51		0.29±0.85		

Table 3

The number of leukocytes in the vaginal smear in dynamics under the influence of treatment

Number	L_1 n=120			L_2 n=98		
	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)
£ 10 in f/v	58 (48.3)	110 (91.7)	112 (93.3)	43 (43.9)	64 (63.9)	82 (83.7)
11-20 in f/v	62 (51.7)		2 (1.7)	55 (56.1)	34 (34.7)	4 (4.1)
>20 in f/v	–	–	6 (0.5)	–	–	12 (12.2)

spores were absent in all patients but was detected a leukocyte reaction from medium to moderate severity (tab. 3).

At the first medical appointment, in 58 (48.3%) patients in L_1 were detected up to 10 leukocytes in the field of vision and in 43 (43.9%) women in L_2. In 62 (51.7%) patients in group 1 and 55 (56.1%) patients in group 2 were revealed 11 to 20 cells in the field of view, (p> 0.05). At the second visit, a decrease in the number of white blood cells in the visual field in dynamics was revealed: up to 10 white blood cells in the visual field were observed in 110 (91.7%) patients of group 1 and in 64 (63.9%) patients of group 2. In 10 (8.3%) women in group 1 and 34 (34.7%) in group 2 were revealed 11 to 20 cells in the field of view, (p<0.05). At the 3rd visit, up to 10 leukocytes in the field of vision were observed in 112 (93.3%) patients of group 1 and 82 (83.7%) patients in group 2, (p <0.05). In 2 (1.7%) cases in group 1 and in 4 (4.1%) cases in group 2, 11 to 20 cells were detected in the field of view, (p> 0.05). In 6 (0.5%) cases in group 1 and 12 (12.2%) cases in group 2, the number of leukocytes was more than 20 in the field of view, (p<0.05). Comparison of the results between the groups in dynamics revealed the predominance of patients in the first group with a low white blood cell count compared to the second group.

Microscopy of the vaginal discharge at the first medical appointment revealed a predominance of cases with a large number of superficial epithelial cells in the field of view in both groups: 82 (68.3%) cases in L_1 and 63 (64.3%) cases in L_2. In 38 (31.7%) cases in L_1 and 36 (36.7%) cases in L_2 was revealed a moderate number of epithelial cells. Differences between groups were not statistically significant (p> 0.05). At the second visit, the number of epithelial cells decreased: a small number of cells was detected in 98 (81.7%) patients of group 1 and 69 (70.4%) patients in group 2. In 14 (11.7%) patients in group 1 and 25 (25.5%) patients of group 2 was revealed a moderate number of cells. Differences between groups were statistically significant, (p <0.05). A large number of cells was revealed in 8 (6.7%) participants in group 1 and in 4 (4.1%) patients in group 2. Differences between groups were not statistically significant (p> 0.05). At the 3rd visit, the number of epithelial cells in vaginal smears decreased in most patients and corresponded to the phase of the menstrual cycle. A small number of cells was detected in 102 (85%) patients of group 1 and in 65 (66.3%) patients of group 2, (p <0.05). In 10 (8.3%) participants of the group 1 and in 20 (20.4%) patients of group 2 was revealed a moderate number of cells (p <0.05). A large number of cells was detected in 6 (5%) participants of group 1 and in 13 (13.3%) patients of group 2, differences between the groups were statistically significant (p <0.05). The dynamics of the number of epithelial cells in the vaginal smears under the influence of treatment is presented in table 4.

In the studied groups none of the patients showed normocenosis before treatment – the normal state of the vaginal microbiota (the prevalence of lactobacilli, the absence of gram-negative microflora, the number of leukocytes up to 10 in the f/v, epithelial cells, respectively, the phase of the menstrual cycle). An intermediate type of vaginal biocenosis (moderate or insignificant number of lactobacilli, gram-

Table 4

The number of epithelial cells in the vaginal smear under the influence of treatment

Number	L_1 n=120			L_2 n=98		
	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)
Small (unique)	0	98 (81.7)	102 (85)	0	69 (70.4)	65 (66.3)
Moderate	38 (31.7)	14 (11.7)	10 (8.3)	36 (36.7)	25 (25.5)	20 (20.4)
Considerable	82 (68.3)	8 (6.7)	6 (5)	63 (64.3)	4 (4.1)	13 (13.3)

Table 5

Vaginal biocenosis under the influence of treatment

Type of vaginal biocenosis	L_1 n=120			L_2 n=98		
	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)
Normocenosis	0	108 (90)	112 (93.3)	0	65 (66.3)	70 (71.4)
Transitional	36 (30)	12 (10)	6 (5)	24 (24.5)	29 (29.5)	15 (15.3)
Dysbiosis	84 (70)	0	2 (1.7)	62 (63.3)	4 (4.1)	13 (13.3)

Table 6

The dynamics of the pH of the vaginal discharge under the influence of treatment

pH	L_1 n=120			L_2 n=98		
	Visit 1 n/%	Visit 2 n/%	Visit 3 n/%	Visit 1 n/%	Visit 2 n/%	Visit 3 n/%
3.5-4	0	108 (90)	112 (93.3)	0	65 (66.3)	70 (71.4)
4.1-4.5	36 (30)	12 (10)	6 (5)	24 (24.5)	29 (29.5)	15 (15.3)
>4.5	84 (70)	0	2 (1.7)	62 (63.3)	4 (4.1)	13 (13.3)

positive cocci, gram-negative bacilli; leukocytes, monocytes, macrophages, epithelial cells, complaints and clinical manifestations) was detected in 36 (30%) cases in group 1 and 24 (24.5%) cases in group 2. Vaginal dysbiosis (a significant decrease or complete absence of lactobacilli, abundant polymorphic gram-negative and gram-positive strain and coccal flora, variable white blood cell count – up to 20 in the f/v) was assessed in 84 (70%) cases in group 1 and in 62 (63.3%) cases in group 2, p> 0.05. Vaginal biocenosis before and after treatment is presented in table 5.

The dynamics of the pH of the vaginal discharge corresponded to the dynamics of changes in the biocenosis of the vagina. When comparing the results, a significant increase in the acidity of the pH of the vaginal discharge and an increase in its acidic protective function were observed in group 1 when compared with group 2. The differences between the groups were statistically significant (p <0.05). The dynamics of the pH of the vaginal discharge in the study groups are presented in table 6.

Of the 120 women included in the study in the first group and 98 in the second group, a significant improvement (normocenosis) according to the research physician and patients was found in 112 (93.3%) patients of group 1 and 70 (71.4%) patients in group 2, satisfactory improvement (consistent with the intermediate type of biocenosis) in 6 (5%) patients of group 1 and 14 (15.3%) patients from group 2, unsatisfactory result in 2 (1.7%) patients from group 1 and 13 (13.3%) of patients of group 2 (later relapse was noted in these patients).

Conclusions

Throughout the study period, the clinical symptom monitoring data and the results of microscopic examination allowed us to state the fact of the clinical efficacy of the treatment with the combination of *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and estriol in most patients, expressed in improving the overall well-being of the patients, namely the disappearance or reduction of itching, burning, pathological discharge, discomfort, vaginal hyperemia, pH of the vaginal discharge, as well as reducing the severity of the leukocyte reaction and normalizing the morphological picture of the vaginal smear.

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Authors' contribution
LP – conducted/performed the laboratory work; AN – designed the research, did statistics and interpreted the data; NC – conceptualized the project and revised the manuscript critically; MB – interpreted the data and drafted the manuscript; VC – draf-ted the manuscript. All the authors revised and approved the final version of the manuscript.

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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 14 of March 17, 2017.

Conflict of interests
No competing interests were disclosed.

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Silent cardiovascular risk factors among medical students

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Abstract

Background: Dyslipidaemia and obesity are recognized modifiable cardiovascular risk factors, a major health challenge of the 21st century. Youth obesity and lipid abnormalities are insufficiently documented. Asymptomatic young people tend not to appeal to health services, but they may be at high cardiovascular risk.
Material and methods: A cross-sectional study was performed on 138 male medical students. All the participants underwent the anthropometric and clinical examination. The obtained data were statistically processed by using the "Statistica 6.0" software program. The difference was considered statistically significant with $p < 0.05$.
Results: It was established that 34.1% of respondents had excessive fat accumulation, 5.1% were defined as obese, according to BMI criteria. According to ethnic- and sex-specific WC cut-offs, 14.5% subjects were centrally obese. Out of the cohort of medical students, 34% had at least one abnormal lipid parameter. The low HDLc was the most prevalent dyslipidaemia in all the students – 12.3%. The results of the survey showed that rural young males were more likely to manifest lipid abnormality – 38.3% versus 22.8% for urban area, also rural origin was associated with a higher rate of central obesity – 16%.
Conclusions: Young men from Moldova have an alarming rate of asymptomatic dyslipidaemia and obesity. Our findings support the need of early general preventive efforts targeting young population at high risk.
Key words: obesity, dyslipidaemia, students, cardiovascular risk factor.

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Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally, and one of the major health challenges of the 21st century. Despite significant progress in promoting cardiovascular health, in the Republic of Moldova, CVDs are responsible for 59% of total mortality [1]. Worldwide, more than 1.9 billion adults, 18 years and older, are overweight, of these over 650 million are obese [2].

Dyslipidaemia and obesity are considered as independent modifiable cardiovascular risk factors. Although the asymptomatic atherosclerosis starts in childhood, clinically it occurs in adulthood and it is well-documented that obesity and dyslipidaemia have the tendency to be tracked into adulthood [3, 4].

Early identification of cardiovascular risk factors has a crucial clinical importance, as many of these are reversible and thus, their effects can be influenced, or even eradicated. Therefore, the targeting at high-risk group in a preclinical stage is important for using promt population-wide strategies and will substantially contribute to reducing high costs of CVDs management.

National and global statistics show an increased prevalence of cardiovascular risk factors among the young population (18-30 years) [5-8], as well as the increasing occurrence of fatal cardiac events in people previously undiagnosed with cardiovascular disease [9, 10]. There are epidemiological studies dedicated to conventional cardiovascular risk factors in childhood [11], as well as in adulthood [12], their profile in young people being insufficiently documented. This age group presents scientific interest, because it is marked by a series of biological and psychosocial changes and is certainly emerging as the crucial period of transition from adolescence to adulthood. Trends to adopt an unhealthy lifestyle due to persistent psychoemotional stress, lack of time for a regular, balanced diet and physical activities, sleeping less at night enhance the susceptibility to obesity and dyslipidaemia, followed by all morbid consequences [13, 14]. During this period, such events can occur as leaving the family environment, starting university studies, professional involvement in the workplace, changing marital status, all contributing to a state of vulnerability for young people in the context of behavioral risk factors [15]. Young people tend not to use medical services, because at the initial stage they are asymptomatic. That is why it is decisive to delimit the group of patients with increased cardiovascular risk at the presymptomatic stage, in order to implement early prophylactic strategies.

The aim of our study was to assess the spectrum of conventional cardiovascular risk factors in the cohort of medical students.

Material and methods

A cross-sectional study, involving 138 young males, medical students from faculties of Medicine, Pharmacy and Dentistry of *Nicolae Testemitanu* State University of Medicine and Pharmacy (SUMPh), aged between 18-29 years was performed. The study received *Nicolae Testemitanu* SUMPh Research Ethics Committee approval (21.02.2011). All the participants had the informed consent signed before filling in the STEPS (WHO) questionnaire. All the participants underwent the anthropometric and clinical examination at the University Clinic of Primary Health Care. Selected anthropometric indices were: height, weight, BMI (body mass index), WC (waist circumference). BMI was calculated according to the WHO formula: BMI (kg/m2) = weight (kg) / height2 (m^2) WHO [16]. Venous blood samples were drawn after an overnight fasting and serum was separated into aliquots and stored at −70°C until analysis. The period before freezing did not exceed 6 hours. Serum lipids were evaluated in the Laboratory of Biochemistry, *Nicolae Testemitanu* SUMPh. Serum TG (triglycerides) concentrations were determined using the enzymatic-colorimetric method (Triglycerides Mono SL NEW, ELITech Clinical Systems, France). Total cholesterol (TC) concentrations were measured by the enzymatic cholesterol esterase-cholesterol oxidase method (Cholesterol SL ELITech Clinical Systems, France). The estimation of HDLc (high density lipoprotein cholesterol) concentrations was performed using precipitation method (HDL Cholesterol ELITech Clinical Systems, France). Serum LDLc (low density lipoprotein cholesterol) concentrations were estimated using Friedwald formula (applied for the TG values <4.5 mmol/l): LDLc(mmol/l) = TC – HDLc – (TG/2.2) [17]. Non-HDLc (non high density lipoprotein cholesterol) was calculated as follows: nonHDLc = TC – HDLc (mmol/l). The participants were grouped according to the anthropometric parameters cut-off values for the interpretation of the obtained data (Table 1). According to the international classification of BMI [16], the sample was grouped into two categories, BMI≤24.9 kg/m2 (not overweight) and BMI≥25.0 kg/m^2 (overweight or obese).

Young male participants with WC parameters above the cut-off values were considered centrally obese and those with below cut-off values were considered with no central obesity.

Table 1

Cut-offs used for the anthropometric parameters interpretation.

Cut-off	Anthropometric parameter		
Below	BMI_1group: BMI<25 kg/m^2	WC1 group:	WC ♂ <94 cm WC ♀ <80 cm
Above	BMI_2group: BMI≥25 kg/m^2	WC2 group:	WC ♂ ≥94 cm WC ♀ ≥80 cm

Lipid parameters' values above the cut-offs were interpreted as abnormal and associated with elevated cardiovascular risk. Only for the HDLc the values below than the cutoffs were interpreted as associated with high cardiovascular risk (tab. 2). The obtained data were statistically processed by using the "Statistica 6.0" software program. The M values, their standard deviation were estimated. The difference was considered statistically significant with $p < 0.05$.

Table 2

Cut-offs used for the lipid parameters interpretation

Cut-off	TC	LDLc	HDLc	nonHDLc	TG
Below	<5.2 mmol/l	<3.4 mmol/l	<1.0mmol/l ♂) <1.3mmol/l ♀)	<3.8 mmol/l	<1.7 mmol/l
Above	≥5.2 mmol/l	≥3.4 mmol/l	≥1.0mmol/l(♂) ≥1.3mmol/l ♀)	≥3.8 mmol/l	≥1.7 mmol/l

Results

The analyzed young male cohort was characterized by the following values of anthropometric parameters: height 177.55 ± 6.09 cm, weight 75.24 ± 12.22 kg, waist circumference 83.30 ± 9.30 cm and BMI values were 23.83±3.38 kg/m^2.

In the study, 91 subjects had values below 25 kg/m^2 (65.9%) and 47 persons (34.1%) had values above 25 kg/m^2, out of studied population, 5.1% were defined as obese, according to BMI criteria. According to ethnic- and sex-specific WC cut-offs, 118 (85.5%) were centrally non-obese and 20 (14.5%) subjects were centrally obese.

All the averages of the lipid parameters values showed normal ranges, according to the interpretation threshold values. The TC content was 4.21 ± 0.58 and ranged from 2.93 to 6.89 mmol/l. The TG level varied between 1.08 and 2.68 mmol/l, with an average of 1.46 ± 0.24 mmol/l. LDLc concentrations were 2.26±0.53 mmol/l, serum concentrations of nonHDLc were of 3.12±0.53 mmol/l. At the same time, in young participants, high density lipoproteins concentrations were 1.23±0.23 mmol/l, ranging from 0.71mmol/l to 2mmol/l.

Out of 138 surveyed medical students 34% had at least one abnormal lipid parameter; all were defined as having dyslipidaemia. The most common type of dyslipidaemia was low levels of high-density lipoprotein, documented in 17 cases (12.3%), followed by high levels of non HDLc (11.6%), high levels of triglycerides (8.7%) and 5.8% accounting for high levels low-density lipoprotein.

The majority of respondents originated from rural areas 81 (58.7%), aside from that rural origin was associated with a higher rate of central obesity – 16%, versus 12.3% for urban respondents ($p>0.05$). Applying WHO criteria for BMI, it was estimated that 34.6% residents from rural area were overweight or obese, versus 33.3% for urban residents, with no statistical significance. Also the results of the survey showed that rural young males were more likely to manifest lipid abnormality – 38.3% and 22.8%, respectively.

Discussion

In similar studies performed in Slovakia, Jordan and Turkey, involving young students, the prevalence of overweight / obesity, based on BMI parameter, ranged from 17.0% to 47.4% [18-23]. At the global initiative of the World Health Organization, the first national Survey Study STEPS (2013) [19] dedicated to the assessment of risk factors for chronic non-communicable diseases was set up. It was carried out among the young population (18-29 years old) and revealed an average BMI value of 24.4 kg / m^2 for young men [19]. Also the results of the study established that 56% of the general population was overweight, 23% of the participants were obese, and 29.4% of the population have serum total cholesterol levels exceeding the reference values. Based on the results we can conclude that the population of Moldova leads an unhealthy lifestyle. Peltzer K et al. conducted a study involving 15746 students from 22 universities in different countries (mean age – 20.8 years). It was attested that 22% of subjects were overweight / obese, predominantly young men – 24.7% of men [14]. A recent study conducted in Italy, based on the survey of 734 students with a mean age of 21.5 ± 2.9, highlighted a rather alarming prevalence of overweight (24.2%) and obese (4.2%) men [24]. In Russia, the prevalence of obesity among young men was 3.7% and 24.4% of participants were overweight [14]. Ana Belén Cutillas et al. assessed the anthropometric parameters of 223 students and the analysis showed that 24.2% of young male participants were overweight and 4.2% were obese [25]. The authors of the study, which evaluated 968 students in Brazil, reported absolutely concerning data in this context. It was established that the prevalence of overweight / obese men was 39.1% / 12% [26]. In Mexico, evaluating 620 young students aged 18-24, the following results were reported: the prevalence of male obesity – 10.6%, and female obesity accounted for 11.1% [27].

The STEPS study conducted in the Republic of Moldova revealed that every sixth respondent was overweight and 22.9% – obese. The proportion of obese women (28.5%) was 1.6 times higher than that of men (17.8%). The average BMI recorded was 26.6 kg / m^2. In the national surveillance study on non-communicable diseases performed in the group of subjects aged 18-29 years, the mean BMI was 23.9 [19].

The Bogalusa Heart study shows that attested adiposity in young adults aged 19-26 is a major cardiovascular risk factor, which contributes to the development of the abnormal lipid profile pattern, and serum total cholesterol levels were lower in men. Additionally, serum LDLc concentrations were shown to be higher in men than in women, but HDLc was higher in women than in men [28]. A multicenter epidemiological study of coronary risk factors (CARDIA study) in the 19-26 age group showed that average LDLc concentrations were higher in men, but there were no statistically significant differences between the sexes for serum concentrations of total cholesterol. The mean HDLc concentration was lower in men than in women. The average concentration of TG was higher in men [29]. Another study conducted in the population of Finnish children and young adults showed that 4-12% of men have LDLc with values above 4.0 mmol / l, and 4.6% of men recorded HDLc values below 1.0 mmol / l [30].

Both studies (Bogalusa Heart and CARDIA) proved that high concentrations of serum lipids in young adulthood are associated with cardiovascular entities in late adulthood, and levels of lipoproteins tend to track from childhood to adulthood [28, 29]. Therefore, early preventive programs are decisive in order to develop healthy lifestyles [31].

Conclusions

Young men from Moldova have an alarming rate of asymptomatic dyslipidaemia and obesity. Our findings support the need of early general preventive efforts targeting young population at high risk.

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SG drafted the first manuscript; AB, CB – laboratory tests realization; SG, AB, CB, IV – result interpretation and conclusion elaboration; VI – designed the study and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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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 2, 21.02.2011.

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Authors declare no financial or non-financial conflict of interests.

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The individual variability of the arterial "corona mortis"

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Abstract

Background: *"Corona mortis"* is usually referred to in the majority of the bibliographical sources as an arterial anastomosis, formed after the connection between the pubian branch of the obturator artery and the obturator branch of the inferior epigastric artery. The metaphoric name for this anatomical variant describes the severity of the complications that may manifest after its lesion in case of a herniotomy or in the surgical interventions that are conducted on the acetabulum, with anterior surgical access pathways. The incidence of this anastomosis variates between 12-80%, its individual aspects and morphological characteristics were not mentioned in literature.

Material and methods: We have conducted a descriptive, retrospective study, based on which we studied the branches of the external iliac artery and the anterior trunk of the internal iliac artery on 197 angiographies, that were obtained from the database of the Vascular surgery section of *Timofei Mosneaga* Clinical Republican Hospital, Chisinau, the Republic of Moldova archive in order to determine the incidence of "*corona mortis*" and its individual variability based on gender, age and laterality.

Results: *"Corona mortis"* was identified in 39.08% cases, from which 30.96% were in the male gender and 8.12% in the female gender, the majority of patients being in the 61-70-year age group. The classical variant of "*corona mortis*" called *Lambda*, was identified in 70.13% ofcases, the *circle* type – in 27.27% and the *laurel wreath* type – in 2.60%.

Conclusions: The knowledge of the uncommon anatomical variants of "*corona mortis*" is vital, because their lesion may lead to severe complications during surgical interventions in the pubic region.

Key words: *"corona mortis"*, inferior epigastric artery, obturator artery.

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Introduction

Annually, in the entire world, more than 2 million of hernioraphies are conducted as a result of inguino-femoral hernias [1].

Laparoscopically, this intervention can be made through the transabdominal preperitoneal access, where the surgeon should know in detail the anatomical particularities of the preperitoneal space and especially the dangerous zones that present vulnerabilities like "*corona mortis*".

The metaphorical name for this arterial branch, explains the severity of the surgical complications that may manifest after its lesion [2].

"Corona mortis" is usually referred to in the majority of the bibliographical sources as an arterial anastomosis, formed due to the connection between the pubian branch of the obturator artery and the obturator branch of the inferior epigastric artery, localized posterior from the superior branch of the pubic bone, at variable distances from the pubian symphysis [3-5].

Some authors consider "*corona mortis*" as an origin variant of the obturator artery [6], others – as a variable vascular anastomosis (arterial or venous), where the connection branches and the topographical regions are different [7-10].

According to the studies that were conducted recently, the venous type of "*corona mortis*", if damaged during surgical interventions may lead to postoperatory hematomas. In case of herniotomies, the arterial "*corona mortis*" can be damaged, causing minimal but persistent hemorrhage, that can lead to death [11-13].

At the same time, "*corona mortis*" also represents a danger in case of hip fractures, just as surgical interventions on the acetabulum, with anterior access [14].

In the literature, the incidence of the arterial anastomosis "*corona mortis*" variates between 12-80%. When it comes to the type (form) and its individual aspect, there is no information at the moment [15].

Because of this, the main goal of our study is to determine the incidence of the anatomical variants of arterial "*corona mortis*" and its individual variability depending on age, gender and laterality.

Material and methods

We have conducted a descriptive, retrospective study, and on its basis we studied the branches of the external iliac artery and the anterior trunk of the internal iliac artery on 197 angiographies, obtained from the database of Vascular surgery section from *Timofei Moșneaga* Clinical Republican Hospital, Chisinau, the Republic of Moldova archive. The inferior limb angiographies were made on all the patients in the study poll in order to identify and evaluate the peripheric occlusive syndrome. The femoral and iliac arterial systems were studied from 3 aspects: anterior, posterior and lateral.

The patients were grouped based on their gender and age (tab. 1).

Table 1

The repartition of the study poll based on gender and age

Age (years)	Male gender	Female gender
20-30	2	0
31-40	1	1
41-50	6	1
51-60	50	5
61-70	85	15
71-80	15	13
≥81	2	1
Total	**161**	**36**

The analysis of the angiographic records included the study of the origin of the internal and external iliac arteries, their branching, path and topographical relationship towards neighbouring blood vessels, just as the arterial anastomoses that are visible in the pubic region. We determined their morphological, topographical and individual particularities.

The criterion for the identification of "*corona mortis*" on angiographies constituted the arterial anastomosis between the internal and external iliac arterial systems, located posteriorly from the superior branch of the pubic bone in the Retzius space, it crosses the superior branch of the pubic bone, just as the anatomical variants that may pose a threat in case of herniotomies.

Using the *RadiAntDICOM Viewer 3.42. software* we evaluated the morphometry of the mentioned arteries, the obtained data was stored, processed and statistically analysed using the Microsoft Excel and Statistics 6.0 softwares.

Results

"Corona mortis" was identified on 77 angiographies (39.08% of cases), from which 61 (30.96%) were of the male gender and 16 (8.12%) of the female gender. Their repartition depending on the age category and gender was included in table 2.

Depending on the laterality, "*corona mortis*" was determined: bilaterally in 34 cases (27 – male gender, 7 – female gender); unilaterally on the right side – 16 cases (11 – male gender, 5 – female gender); unilaterally on the left side – 27 cases (23 – male gender, 4 – female gender), this information may be found in table 3.

Table 2

The repartition of the identified patients with "*corona mortis*" depending on gender and age

Age (years)	Male gender	Female gender
20-30	0 (0%)	0 (0%)**
31-40	1 (1.30%)	0 (0%)
41-50	3 (3.90%)	0 (0%)
51-60	17 (22.08%)	3 (3.90%)
61-70	35 (45.45%)	5 (6.49%)
71-80	5 (6.49%)	7 (9.09%)
≥81	0 (0%)	1 (1.30%)
Overall	**61 (79.22%)**	**16 (20.78%)**

**For this group there were no patients for the study poll.

Table 3

The repartition of the identified patients with "*corona mortis*" depending on gender and laterality

Localization and gender	Number	%
MR	11	14.28%
ML	23	29.87%
MB	27	35.06%
FR	5	6.50%
FL	4	5.19%
FB	7	9.10%

*MR – male, right side; ML – male, left side; MB – male, bilateral; FR – female, right side; FL – female, left side; FB – female, bilateral.

The classical variant of "*corona mortis*", named *Lambda*, that is between the branch of the inferior epigastric artery and the oburator artery's branch was detected in 70.13% of the identified cases of "*corona mortis*". We named it *Lambda* because of the similarity between the angiographic image and the corresponding letter from the Greek alphabet. It has an ascendent pathway when it is located posteriorly from the superior branch of the pubic bone and a descendent pathway when it is located anteriorly.

Depending on the internal diameter of the anastomosing arteries (the internal diameter was measured in the point where the arteries are passing through the superior branch of the pubic bone) we differentiated between *Lambda major* (the internal diameter is greater than 3 mm), that was identified in 3 cases (3.90%) and *Lambda minor* (the internal diamter is not greater then 3 mm) – we identified this form in 51 cases (66.23%).

For example, we can see the angiographic images of a patient of male gender, that is 64 years old, and has a *Lambda major* type "*corona mortis*", unilaterally on the left side with an internal diameter of 3.62 mm (fig. 1) and a patient of female gender, that is 74 years old, for whom we have established bilaterally the *Lambda minor* type "*corona mortis*", its

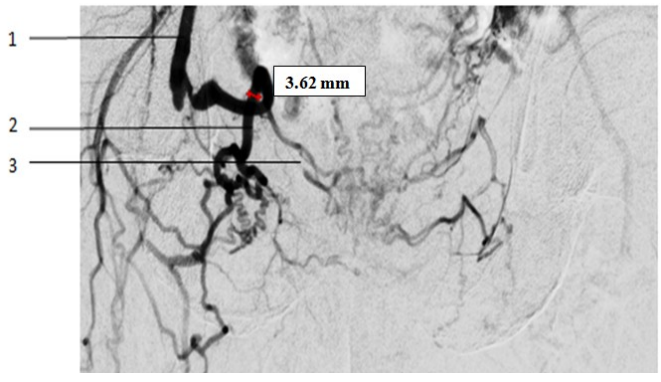

Fig. 1. ***Lambda major*** **type, unilateral. 1. External iliac artery. 2.** ***Lambda major*** **type. 3. Poupart's ligament.**

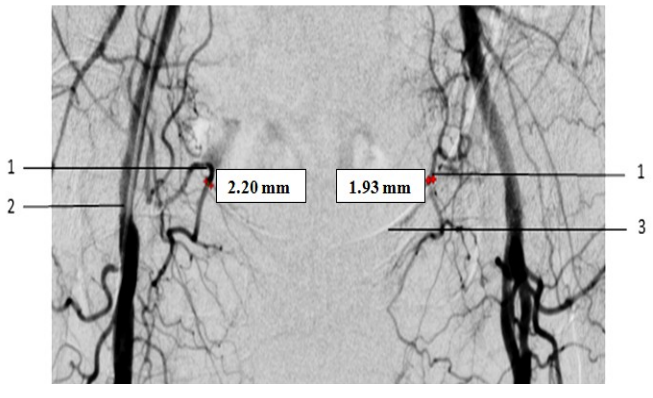

Fig. 2. Bilateral ***Lambda minor*** **type. 1.** ***Lambda minor*** **type. 2. External iliac artery. 3. Poupart's ligament.**

internal diameter constituted 2.20 mm on the left side and the one on the right side – 1.93 mm (fig. 2).

Circle type *"corona mortis"* was determined in 27.27% of cases, from which the *closed* type was identified in 7 cases (9.09%) and the *open* type in 14 cases (18.18%).

The name of this variant comes from the suggestive morphological pattern of the anastamotic or origin arterial branches. The *closed circle* type represents an arterial anastomosis that makes a connection between the external and internal iliac arterial systems, where both of the arterial origin spots are present. The *open circle* type represents an origin variant of a branch, that is not omologated in the anatomical nomenclature, that originates from the femoral or external iliac artery, this being the reason why we consider it as a distinct type of *"corona mortis"*.

In Figure 3 we can observe an angiographic image of a male gender patient, that is 56 years old and has a *closed circle* type *"corona mortis"*, the internal diameter of the anastomotic artery, superiorly, constituted 1.45 mm, inferiorly at the level of the femoral artery – 1.80 mm. At the same time, the same patient, had a *Lambda minor* type *"corona mortis"* with an internal diameter of 1.14 mm in the place where it was crossing the superior branch of the pubic bone (fig. 4).

Laurel wreath type *"corona mortis"* was identified only in 2 cases (2.60%). This representing an intersystemic anastomosis between the external iliac arteries.

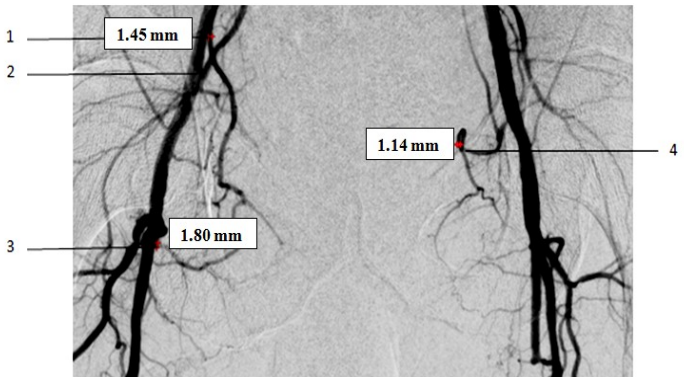

Fig. 3. Bilateral "*corona mortis***". Left side –** ***Closed circle*** **type, right side –** ***Lambda minor*** **type. 1. Superior origin spot. 2. External iliac artery. 3. Inferior origin spot. 4.** ***Lambda minor*** **type.**

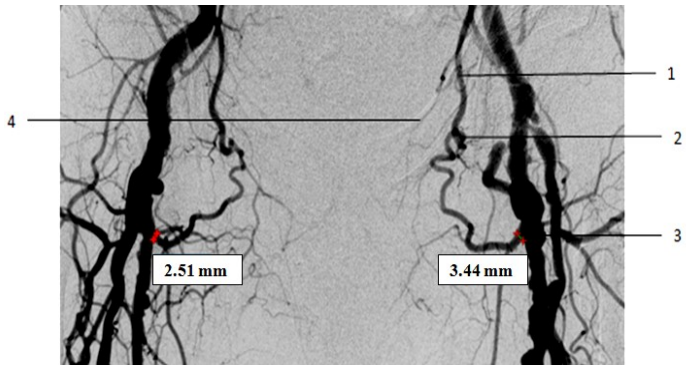

Fig. 4. Bilateral "*corona mortis***". Both sides –** ***Closed circle*** **type. 1. Inferior epigastric artery. 2.** ***Closed circle*** **type. 3. Inferior origin spot. 4. Poupart's ligament.**

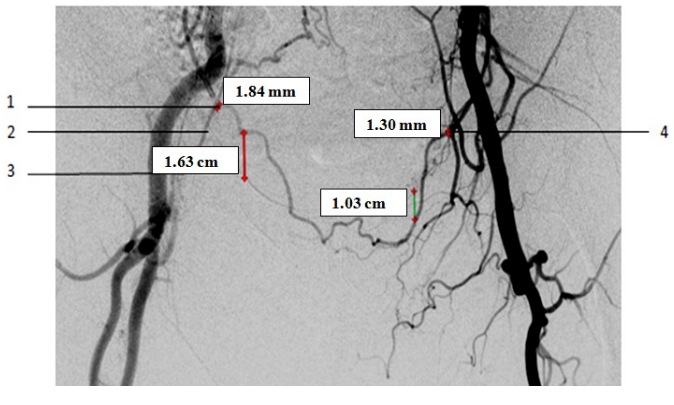

Fig. 5. ***Laurel wreath*** **type. 1. Left origin spot. 2. Inferior epigastric artery. 3. Poupart's ligament. 4. Right origin spot.**

In one of these cases, the anastomotic artery from the left side was located superiorly, at 1.63 cm distance from the Poupart's ligament, on the right side – 1.03 cm inferiorly from this ligament. On the left origin spot, the external iliac artery had an internal diameter of 1.84 mm, on the right side – 1.30 mm (fig. 5).

Laurel wreath type *"corona mortis"* doesn't correspond to the usual topography, but represents an anatomical variant that may pose a threat in case of herniotomies and other surgical interventions in this region because it is closely located towards the Poupart's ligament.

Discussion

"Corona mortis" was studied by some authors on cadaveric materials, others used angiographic records for this goal.

Rusu M, et al. [10] using the dissection method, studied 40 hemipelvises, obtaining an incidence of 65% for classical "*corona mortis*", Perandini S. [6], using an angiographic study poll of 300 hemipelvises determined an incidence of 30%.

Smith J. conducted an analysis of the anatomical studies that were made by other authors and mentioned a prevalence of 1-43% for this anatomical variant, for angiographic studies – up to 29% and for the endoscopic studies – 10-22% [16].

Though, the majority of authors don't consider the *open circle* type "*corona mortis*" as an anatomical variant of connection, but only as a variant of origin for the inferior epigastric artery, some of them mention it in the literature [5, 8, 9].

Talalwah WA. [3] relates that the *open circle* type*"corona mortis"* is nothing else but aberrant arteries that haven't involuted during the intrauterine development of the embrio, in which the vasculogenesis processes have continued.

We have to remark about different opinions that we confronted in the literature about the internal diameter of the anastomosing arteries, the majority of them relating about its importance, the value for this indicator was not greater than 1 mm [17, 18].

In the majority of cases, that were identified by us, the diameter of these arteries constituted 1.4-4 mm, though from the clinical aspect of view we have to consider that during the surgical interventions there are branches that have a smaller internal diameter [19].

Darmanis et al. [20] identified only 5 cases of "*corona mortis*", using the anterior pathway of access for the surgical interventions made on the pelvis, having 492 patients in the study poll. Letournel E. [21] encountered only one type of "*corona mortis*" in his practice, its branches had a sufficiently great diameter, because of which the necessity of applying a ligature on this branch was persistent.

Though, the literature regarding the clinical practice is controversial with the angiographic and cadaveric studies, the knowledge of the morphological, topographical and individual particularities of the "*corona mortis*" will lead to the avoidance of the surgical complications that regard the damage of this anatomical variant during surgical interventions in the pubic region.

Conclusions

1. The most frequent type of "*corona mortis*" is the classical one, *Lambda minor* – according to the conventional classification that was described in this study.

2. "*Corona mortis*" was situated billaterally aproximatively in half of the patients with the identified anatomical variant on the angiographic records.

3. The uncommon types of "*corona mortis*" need to be known in order to avoid complications during the surgical interventions that regard the pubic region.

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Authors' contribution
ZZ described the classical and uncommon types of "*corona mortis*"; DC has put into evidence the topographical, morphological and individual particularities of this anatomical variant; EC conducted the literature review.

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Ethics approval and consent to participate
The research protocol was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 2 of 27.10.2016).

Conflict of Interests
The authors have no conflict of interests to declare.

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Chromosomal abnormalities in men with azoospermia

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Abstract

Background: Infertility affects about 15 percent of all couples attempting pregnancy, with the man responsible in approximately half the cases. Azoospermia is detected in up to 8% of male infertility situations. The prevalence of chromosomal abnormalities is increased in azoospermic men.
Material and methods: We performed cytogenetic analysis in a group of 128 infertile men with azoospermia from the Republic of Moldova during 2013-2018 period. Karyotyping was performed on peripheral blood lymphocytes according to standard methods of G-banding of metaphase chromosomes. For reporting the results, the 2016 *International System of Cytogenetic Nomenclature* was used.
Results: Chromosomal variations were identified in 48 infertile men with azoospermia. In 38 cases were found abnormalities of gonosomes and in 10 cases abnormalities of autosomes. The most common sex chromosomal abnormality was Klinefelter syndrome: in 21 (55.3%, 95CI 47.23-63.37) cases homogeneous form 47,XXY and in 4 (10.5%, 95CI 5.52-15.48) cases mosaic form. Y-chromosome aberrations were also identified: in 7 (18.4%, 95CI 12.11-24.69) cases was noticed duplication of distal arm 46,XYqh+ and in 3 (7.9%, 95CI 3.53-12.27) cases deletion of the same arm 46,X,del(Y). Additionally, 45,X/46,XY and 46,XX karyotypes were found.
Conclusions: 38% of the studied group have chromosomal variations that may explain the origin of infertility. All men with azoospermia should be offered cytogenetic screening followed by appropriate genetic counseling before infertility treatment.
Key words: infertility, azoospermia, chromosomal abnormalities.

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Introduction

Globally, it is estimated that about 15% of couples of reproductive age face fertility problems [1]. Infertility affects both men and women, and in about half of the cases a male factor can be identified [2]. The term "male infertility" is not a clinically defined syndrome, but rather a collection of heterogeneous conditions, most commonly caused by disorders of spermatogenesis, clinically manifested by asthenozoospermia, teratozoospermia, oligozoopermia and azoospermia [3].

Azoospermia is found in about 8% of infertile men and 1% in the male population [4]. The role of genetic factors in the pathogenesis of male infertility has become increasingly recognized by reproductive specialists. The individual's genome contributes to infertility by influencing the anatomy of the urogenital tract and physiological processes, including hormonal homeostasis, spermatogenesis and sperm quality. The most common genetic causes of azoospermia are chromosomal abnormalities and their frequency is negatively correlated with the concentration of sperm. In azoospermic patients the prevalence of reported chromosomal variations was between 15% and 25%, depending on the subgroup of azoospermic men studied [5]. Chromosomal abnormalities in infertile men can be numerical or structural, with the involvement of sex chromosomes or autosomes [6].

The introduction of assisted reproduction techniques such as intracytoplasmic sperm injection (ICSI) and microsurgical sperm extraction (micro-TESE) presents an option for infertile couples to overcome the factor of male infertility [7]. The use of these ICSI and micro-TESE techniques can overcome the barrier in the process of natural fertilization, but there are many concerns about the safety of ICSI and the likely transmission of genetic abnormalities to offspring [8].

Before resorting to assisted reproduction techniques, cytogenetic examination is mandatory to detect the cause of male infertility with severely affected spermiogram. The identification of an abnormal karyotype as well as chromosomal polymorphisms should lead to a comprehensive genetic counseling, which should include all information about the individual type of abnormality, its clinical relevance, possible inheritance / transmission, genetic risk for offspring [9]. This allows infertile couples to make an informed decision when opting for medically assisted reproduction. Therefore, cytogenetic screening continues to

Table 1

Distribution of chromosomal abnormalities in men with azoospermia, years 2013–2018

Years	Abs. No men with azoospemia	The average age/ years	46,XY		Karyotype with chromosomal variations	
			Abs. No	%, 95$_{CI}$	Abs. No	%, 95$_{CI}$
2013	22	35	15	11.7%, 95CI 8,86-14.54	6	4.7%, 95CI 2.83-6.65
2014	23	35	13	10.2%, 95CI 7.53-12.87	8	6.3%, 95CI 4.16-8.44
2015	22	33	12	9.4%, 95CI 6.82-11.98	10	7.8%, 95CI 5.43-10.17
2016	21	33	12	9.4%, 95CI 6.82-11.98	9	7.0%, 95CI 4.74-9.26
2017	22	26	13	10.2%, 95CI 7.53-12.87	8	6.3%, 95CI 4.16-8.46
2018	18	32	13	10.2%, 95CI 7.53-12.87	5	3.9%, 95CI 2.19-5.61
Total	128	32	80	62.0%, 95CI 58.22-66.78	48	38.0% 95CI 5.33.22-41.78

remain a good practice for proper diagnosis, treatment, evaluation and prognosis [9, 10].

The aim of the study: to evaluate the frequency of chromosomal variations in azoospermic men and to confirm the cytogenetic exploration of infertile men for diagnosis, treatment and prognosis.

Material and methods

The research presents a retrospective descriptive study of a selected group of 128 infertile men with azoospermia, from the population of the Republic of Moldova during the years 2013-2018. Patients come from infertile couples who are referred to the National Center for Reproductive Health and Medical Genetics. The spermiogram was performed after a period of 2–7 days of sexual abstinence, according to the reference criteria of the 2010 sperm analysis of the World Health Organization (WHO). All patients were cytogenetically investigated by the classic G-banding technique, on 15 peripheral blood lymphocytes being analyzed 15 metaphases of which 5 karyotyped. Nomenclature according to 2016 ISCN (International System of Cytogenetic Nomenclature) was used for reporting the results.

Results

128 men with azoospermia were cytogenetically investigated in 2013–2018 at the department of the National Center for Reproductive Health and Medical Genetics (tab. 1). The number of azoospermic men investigated cytogenetically was distributed by years as follows: 22 patients in 2013, 23 patients in 2014, 22 patients in 2015, 21 patients in 2016, 22 patients in 2017 and 18 patients in 2018, which shows that the number of cytogenetic investigations is relatively constant. The same homogeneity is observed at the age at which patients were referred for consultation: in 2013–2014 the average age was 35 years, in 2015–2016 – 33 years, in 2017 – 26 years, in 2018 – 32 years.

Of the total number of 128 infertile men with azoospermia, 80 (62%) showed normal karyotype 46,XY (tab. 1, fig. 1) and 48 (38%) showed variations in the number or structure of chromosomes. 38 patients (30%) showed variations in the X or Y sex chromosomes, and 10 patients (8%) had variations in the autosomal chromosomes (fig. 1, tab. 2, tab. 3).

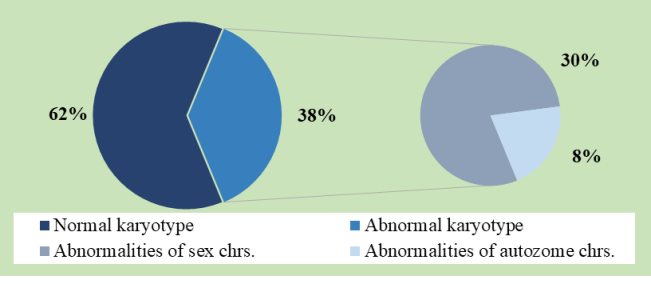

Fig. 1. Frequency of azoospermic men with chromosomal abnormalities in 2013-2018.

In the 38 patients identified with sex chromosomes abnormalities (tab. 2), in 28 cases numerical abnormalities were detected and in 10 cases – structural variations. Among the numerical chromosomal abnormalities, in 25 cases was identified aneuploidy X (Klinefelter Syndrome), in 2 cases – mosaic 45,X/46,XY and in one case – male 46,XX. The structural variations of sex chromosomes detected were in 7 cases duplications of the distal arm of the Y chromosome and in 3 cases deletions of the distal arm of the Y chromosome.

Table 2

Distribution of infertile men with azoospermia by sex chromosomal abnormalities

Karyotype	Abs. No. (n=38)	%, 95$_{CI}$
47,XXY	21	55.3%, 95CI 47.23-63.37
47,XXY/46,XY	3	7.9%, 95CI 3.53-12.27
47,XXY/46,XX(80%/20%)	1	2.6%, 95CI 0-5.2
45,X/46,XY	2	5.3%, 95CI 1.68-8.92
46,XX	1	2.6%, 95CI 0-5.2
46,XYqh+	2	5.3%, 95CI 1.68-8.92
46,XYqh+ (Yqh≤18q)	3	7.9%, 95CI 3.53-12.27
46,XYqh+ (Yqh<18q)	1	2.6%, 95CI 0-5.2
46,XYqh+, 22 ps+ (Yqh=18q)	1	2.6%, 95CI 0-5.2
46,Xdel(Y)(q11.23→qter)	2	5.3%, 95CI 1.68-8.92
46,Xdel(Y)(q11.22→qter)	1	2.6%, 95CI 0-5.2

The most common cytogenetic variant of Klinefelter Syndrome identified was the classical form 47,XXY in 21 cases (84%) (Figure 3), followed by the forms: mosaic

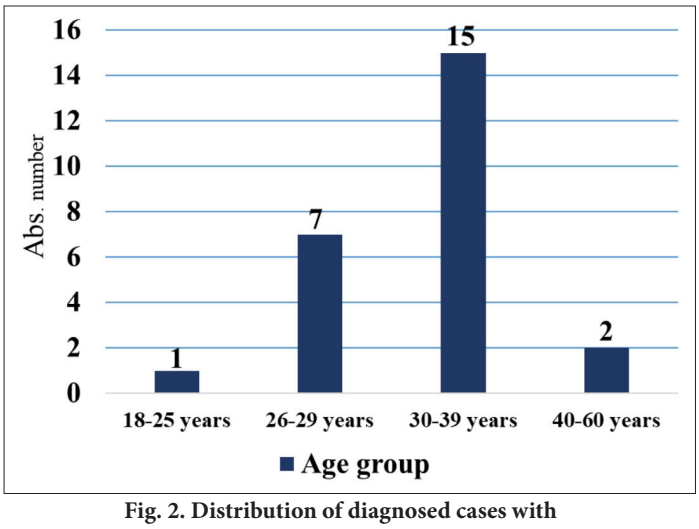

Fig. 2. Distribution of diagnosed cases with Klinefelter Syndrome by age groups

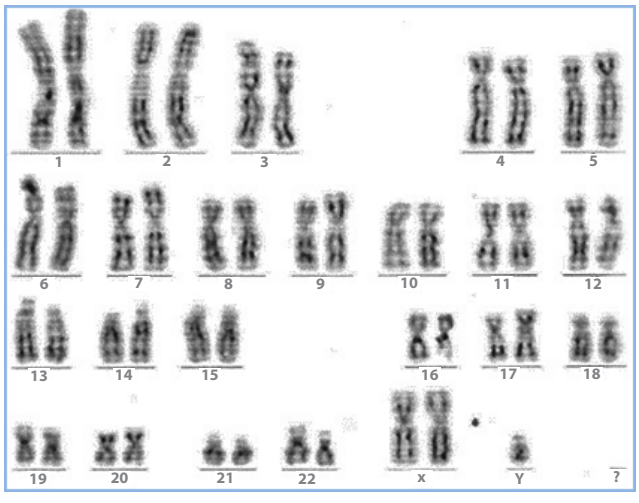

Fig. 3. Karyotype with 47,XXY Klinefelter Syndrome, in a 31-year-old patient

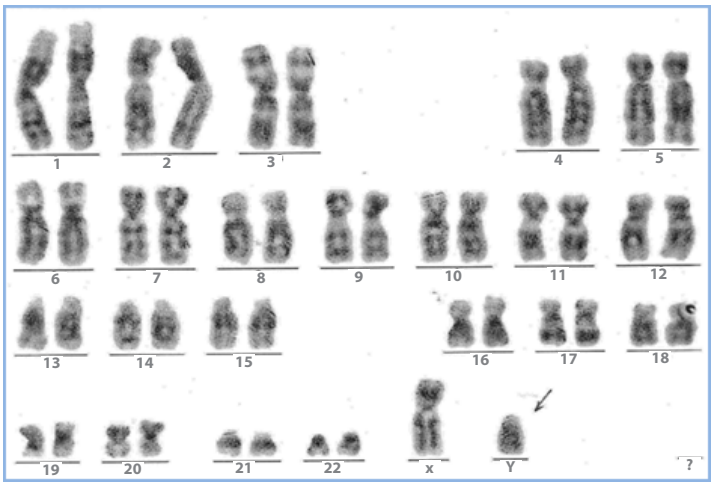

Fig. 4. Karyotype 46,XYqh+ in azoospemic male, 36 years.

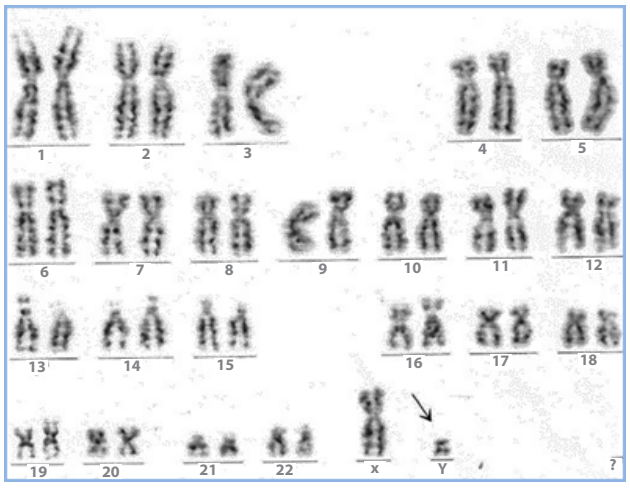

Fig. 5. Karyotype 46,Xdel(Y)(q11.23→qter) in male with azoospermia, 45 years.

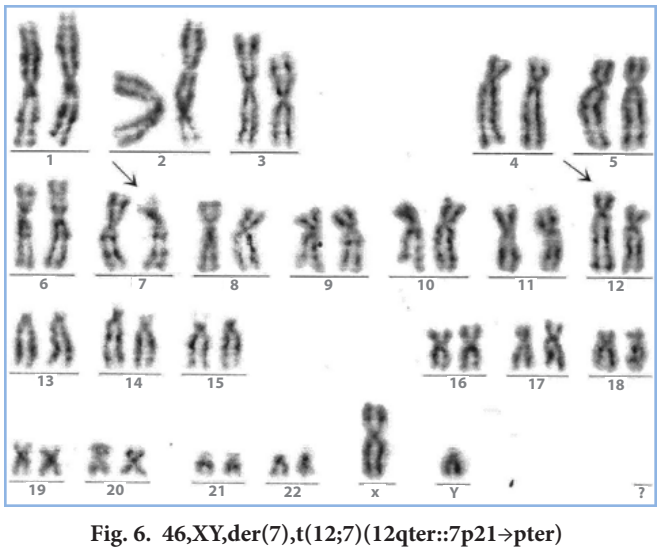

Fig. 6. 46,XY,der(7),t(12;7)(12qter::7p21→pter) karyotype in male, 30 years.

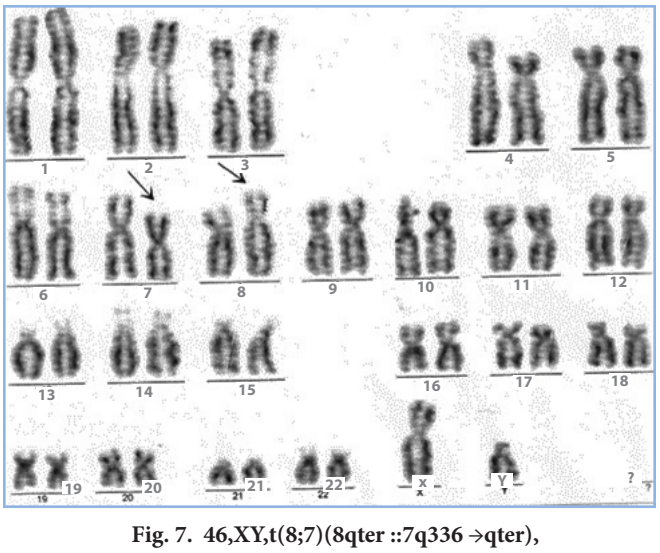

Fig. 7. 46,XY,t(8;7)(8qter ::7q336 →qter), karyotype in male, 31 years.

47,XXY/46,XY in 3 cases (12%) and in 1 case 47,XXY/46,XX (4%). Most cases – 15 patients – were diagnosed at the post-pubertal age of 30–39 years, 7 cases at the age of 26–29 years, 2 cases 40 and more and one case at 24 years.

Table 2

Distribution of autosomal chromosome variations in men with azoospermia

Karyotype	Abs. No (n=10)
46,XY,der(7),t(12;7)(12qter::7p21→pter)	1
46,XY,der(15), t(13;15) (13qter::15q23→qter)	1
46,XY,t(8;7)(8qter ::7q336→qter)	1
46,XY-15-12,+der(15),+rec(12;15),t(13;12)7p+	1
46,XY,der(5),t(9;5)(9pter::5q23.3→qter)	1
46,XY,15ps+	1
46,XY,14 ps+	1
46,XY,13 ps+	1
46,XYinv(9)(p;q)	1
46,XY,1q+	1

In 10 cases, variations of autosomal chromosomes were detected, including duplicate satellites of acrocentric chromosomes 13, 14, 15, and 22 (tab. 3), and in one patient it was accompanied by changes in the sex chromosome.

Discussion

The frequency of chromosomal abnormalities identified in the selected group with azoospermic infertile men in 2013–2018 was (38%), higher than that cited in other bibliographic sources [6, 11]. The results of our study are probably due to thorough clinical selection prior to cytogenetic investigation and, of course, due to the selective group of men with azoospermia. The most common chromosomal abnormalities were identified by sex chromosome abnormalities in 30%. Autosomal chromosome abnormalities were detected in 8% (fig. 1, tab. 2, tab. 3).

Of the 128 cytogenetically investigated azoospermic men, 25 had X disomy with a frequency of 32%. This high frequency of Klinefelter Syndrome among infertile men is also reported in bibliographic sources [11]. The results obtained in our study are similar to the data in the literature which reports the same high incidence of 80–90% for the classic form 47,XXY of Klinefelter Syndrome and in about 20% the mosaic forms are described [12].

Klinefelter syndrome is characterized by both cytogenetic and phenotypic diversity, with age the clinical picture worsens, so the diagnosis of patients at an early age can be failed. The same phenomenon is observed in our study (fig. 2, fig. 3), most cases were diagnosed post-pubertal, which is an unfavorable factor for the success rate of sperm recovery.

The genetic cause of Klinefelter Syndrome is the presence of one or more additional X chromosomes obtained by non-disjunction during maternal or paternal gametogenesis. The severity of the clinical picture is directly proportional to the number of additional X chromosomes. The genes on the additional X chromosomes are inactivated, but in more than 15% they escape the inactivation process, including genes from the pseudoautosomal regions PAR1 and PAR2. A gene imbalance is determined by a higher level of gene expression that can compromise testicular function or influence the meiotic process playing an important role in the pathogenesis of this syndrome [13].

At the same time, 7–8% of individuals with 47, XXY can produce sepermatozoa, in 30–50% micro-TESE allows the recovery of testicular sperm in young people, which helps patients with Klinefelter to conceive their own genetic children; these can be explained by: 1) testicular mosaicism – some spermatogonia lose the supernumerary X chromosome becoming 46,XY ensuring a normal spermatogenesis; 2) selective and variable inactivation of linked X genes that are expressed in the testicles; 3) polymorphisms in the AR gene – a number of trinucleotide repeats CAG from 9 to 37 times – determine normal testosterone levels and, implicitly, normal gonadotropin concentrations that will support the normal functioning of germ cells 47,XXY including spermatogenesis. With age, the chance of sperm recovery in people with Klinefelter syndrome decreases, but studies show that the average age of detection of people with this syndrome is around 25 years, indicating the importance of an early diagnosis that would allow preventive cryopreservation of sperm ejaculated or obtained by micro-TESE to maintain fertility [14].

In 2 male patients, the rare type of the 45,X/46,XY mosaic was identified. The significance of mosaic 45, X/46,XY in bibliographic sources is controversial and presents a great clinical challenge, because it can affect growth, hormonal balance, gonadal development, but also in some cases may have a normal phenotype [15]. Therefore, the detection of these cases without clinical changes can be quite late, as in our study, the first case was detected at the age of 30 and the second at the age of 35. Both patients were investigated cytogenetically due to azoospermia.

A karyotype 46,XX was identified in a 23-year-old azoospermic man. The male phenotype can be explained by the translocation of the masculinization SRY region (Yp11.32) to the X chromosome or one of the other chromosomes, but due to the lack of Yq and AZF genes involved in spermatogenesis – men 46,XX are infertile. The frequency of men with XX in the general population being very rare (1 in 10000, from Chapelle et al. 1990), it is identified only in the case of azoospermic men.

The polymorphic variant of the Y chromosome (Yqh+) was diagnosed in 7 patients. Y chromosomal polymorphisms are mentioned in several studies on male infertility mainly in azoospermia and severe oligozoospermia. This topic is becoming increasingly controversial due to the role of heterochromatin, without having a fully elucidated clinical relevance. The Yqh+ chromosome was associated with an increased risk of pregnancy loss, while in another study this relationship was not found [16, 17]. These patients probably need additional molecular investigations to investigate the involvement of the AZF region.

The prevalence of Y chromosome deletions is estimated at approximately 1: 2000 to 1: 3000 in men [18]. It is the second most common genetic cause of spermatogenesis failure in infertile men after Klinefelter syndrome. In this study, 3 out of 128 azoospermic men are found with a frequency of 2.3% (tab. 2, fig. 5). The association between long arm deletions of the Y chromosome and azoospermia was initially suggested by Tiepolo and Zuffardi in 1976 [18]. In two cases (fig. 5) deletions were detected in the Yq11.23 region. In this locus are located the genes of spermatogenesis of the Y chromosome and designated as Azoospermia Factor (AZF).

Numerous variations of autosomal chromosomes are identified in patients with azoospermia, which are often not expressed by detectable phenotypic changes. Azoospermia in these men can be explained by the involvement of thousands of autosomal genes in the direct or indirect control of testicular formation, their functioning and spermatogenesis. The most common autosomal chromosomal abnormalities detected were balanced chromosomal rearrangements in 9 cases and 1 case being unbalanced. Reciprocal translocations are the most common balanced chromosomal abnormalities, being reported in 0.9 out of 1000 newborns and in about 1% of infertile men [19]. In our study of 128 men with azoospermia balanced simple translocations were detected in 5 cases (3.9%) – t (13; 15), t (12; 7), t (8; 7), t (9; 5), t (13; 12). Balanced chromosomal translocations involve breaking points in two chromosomes and abnormal rearrangement of chromosomal fragments that lead to the transposition of genetic material from one chromosome to another without loss of genetic material, which explains that in most cases carriers with translocations had a normal phenotype [20]. Azoospermia in these cases can be explained by: 1) one of the breaking points interrupts a gene that controls spermatogenesis and leads to blockade of spermatogenesis or incomplete spermatogenesis; 2) chromosomes with translocations conjugate abnormally in prophase I of meiosis which makes chromosome disjunction difficult and gametogenesis is blocked [19, 20]. As with chromosomal translocations, inversions can cause infertility in men. The consequences of this are not to be neglected because there are associated risks: pregnancy loss, children with genetic abnormalities, offspring with fertility problems.

In 3 cases, duplications were detected in the satellites of the acrocentric chromosomes 13, 14, 15, and 22, and in one patient it was accompanied by changes in the sex chromosome. The involvement of these polymorphisms of the listed chromosomes in male infertility is also reported in other specialized studies, such as in the study of S. Penna Videaú et al. 2001 [17]. Although satellites are component parts of heterochromatin, in some studies a positive correlation has been shown between the frequency of acrocentric chromosome variants with satellites and sterility, due to their association with the risk of nondisjunction leading to gametes with aneuploidy [21].

Conclusions

The incidence of chromosomal abnormalities as a cause of male infertility was 38%. Chromosomal rearrangements affect both autosomal chromosomes and X and Y chromosomes. Therefore, the negative prognostic effects of chromosomal abnormalities/variations on spermatogenesis should be clearly explained to individuals with azoospermia during counseling for assisted reproduction. Future studies are certainly needed to identify any new genetic abnormalities and to help a deeper understanding of the causes of male infertility. Cases of infertility with normal karyotype (62% – 46,XY) can be explained by other genetic causes, such as point gene mutations, deletions and nucleotide duplications, which are below the threshold of detection by karyotyping, but are currently identified by various molecular genetic tests.

Given the high frequency of chromosomal abnormalities in infertile men as well as the genetic risks for future generations, the importance of a thorough cytogenetic assessment of them before resorting to assisted reproduction techniques, such as ICSI is mandatory.

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Authors' contribution
SR – designed the research, did statistics and interpreted the data, drafted the manuscript; VM – conceptualized the project and designed the research; SC – collected and interpreted the data; AM – conducted/performed the laboratory work; MS – conducted the laboratory work, revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Preliminary data from the retrospective and prospective observational studies on NSTEMI patient management in Moldova

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Abstract

Background: The outcomes of NSTEMI patients are known to be worse than those of STEMI patients and depend on guideline-directed management. The higher the rate of adherence to guideline recommendations the better the prognosis and outcomes of usually high-risk NSTEMI patients. The best efforts should be made to rapidly and correctly diagnose and manage this condition in order to achieve the best results.
Material and methods: An observational retrospective study was conducted in 3 PCI centers in Chisinau, Moldova, that included all patients hospitalized with NSTEMI through 2019. Another observational prospective study was conducted in the same 3 PCI centers with the consecutive inclusion of all NSTEMI patients in 2020-2021 and established follow-up dates.
Results: Extensive preliminary data from both studies based on 215 patients is presented and compared to that of the FAST-MI registry in France, as an example of care in a developed country.
Conclusions: Preliminary data has contoured a picture of NSTEMI patients management in Chisinau and has already detected major drawbacks to be corrected. The follow-up data will provide more insights on patient outcomes and correlations between management, short-term and long-term outcomes, whereas different biomarkers will be tested for diagnostic and prognostic values. Similar studies are needed in regional hospitals in order to assess the situation in these areas and search for improvement solutions.
Key words: myocardial infarction, NSTEMI, ACS, observational study, STEMI.

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Introduction

The recent decline in cardiovascular mortality in developed countries has been attributed to aggressive primary prevention as well as to the progress made in the treatment of established CVD. A large number of registries has been conducted over time to assess whether clinical practice guidelines have translated in better outcomes for patients with myocardial infarction in daily practice [1]. Observational studies are meant to illuminate the medical reality in a situation where clinicians are usually quite inert at integrating changes proposed by new guidelines into their daily routine. Often, it is somewhat complicated for them to recognize in the sophisticated design of clinical randomized trials that are held in ideally controlled conditions and have strict inclusion criteria, their own population of patients in a setting where all that matters are not the theoretically correct treatment but the one that has already been administered and the results of this treatment. Observational registries in the area of myocardial infarction have over a 100-year history [2]. Among the most well-known in Europe are the SWEDEHEART registry in Sweden and the FAST-MI in France. Currently the NSTEMI Registry of the EORP, a multicenter prospective observational study that started in 2019 is still enrolling patients. The Republic of Moldova is also participating with 4 centers having already included NSTEMI patients. The aim of the study is to elucidate the gaps in the management of NSTEMI patients in different countries compared to the standard of care stated in the current clinical practice guidelines and to report its results to the ESC [3]. At the same time the department of Interventional Cardiology of the Institute of Cardiology in Chisinau has initiated two observational studies (one retrospective and one prospective) with the participation of 3 hospitals that have catheterization labs on-site as a part of the scientific project "Evaluation of instrumental and biochemical markers in management of patients with acute myocardial infarction without ST segment elevation, and assessment of coronary microvascular dysfunction degree".

The data of the patients hospitalized in these hospitals (Institute of Cardiology, Novamed polyvalent hospital and Sfanta Treime municipal hospital) during 2019 with a final diagnosis of NSTEMI has already been analyzed, while ongoing is the prospective study that started in 2020. The study's aim is similar to that of the European registry – to locally assess the gaps in the management of NSTEMI patients in the PCI centers in Chisinau compared to the standard of care. After the analysis of the obtained data and also in accordance with the new ESC guidelines for the management of patients with ACS presenting without persistent ST elevation a local national guideline will be elaborated adapted to local health care conditions. It is universally known that the higher the level of adherence to guideline recommended care in NSTEMI patients the better the short- and long-term outcomes. Therefore, raising the level of this adherence will result in a better prognosis for these patients [4]. In theory we all know what we are supposed to do and how we should treat specific conditions, but in real life-settings different obstacles and clinical challenges make the choices we make for the patient's benefit much more complicated and harder to comply with what the guideline tells us to do.

General information

A retrospective observational study was conducted including all patients diagnosed with NSTEMI in 2019 in 3 participating hospitals with 24/7 on-site PCI. At the same time a prospective observational study that included consecutively all patients with NSTEMI started in the same hospitals in 2020 and is still ongoing, being considered a sequel of the retrospective one. We present the preliminary data from both studies, the fulfillment of objectives 3 to 7 is expected to happen in 2021-2022.

Study objectives

1. Collection of extensive descriptive data on patients with NSTEMI hospitalized in PCI centers of Chisinau.

2. Assessment of management strategy tendencies in everyday practice in these centers.

3. Assessment of gaps in diagnosing and management of NSTEMI patients as well as the level of adherence to the ESC guidelines for the management of NSTE-ACS.

4. Evaluation of MACE rate and structure (mortality, non-fatal-MI, stroke, unstable angina) in patients with NSTEMI compared to STEMI patients after a 12-th month follow-up period.

5. Determination of any correlation and its type between management strategies and outcomes, both short-term and long-term.

6. Determination of the diagnostic and prognostic role of diverse biomarkers in addition to those mentioned in the guidelines for NSTEMI patients.

7. Comparison of the obtained results with the data from the NSTEMI Registry of the EURObservational Research Programme 2019 when published.

Material and methods

Study design

1. An observational retrospective study was conducted in 3 PCI centers in Chisinau, Moldova, that included all patients hospitalized with NSTEMI through 2019. All the data has been analyzed, with a partial patient follow-up.

2. An observational prospective study was conducted in the same 3 PCI centers with the consecutive inclusion of all NSTEMI patients in 2020-2021, follow-up dates established for 30 days, 6 months and 12 months.

Inclusion criteria

1 Patients of 18 years of age and older.

2. Patients with an established NSTEMI diagnosis according to the Universal definition of Myocardial Infarction, without persistent elevation of ST segment, type 1 MI [3].

Organizational moments

For the retrospective study all charts of patients diagnosed with NSTEMI during 2019 in the above mentioned hospitals were selected, excluding those that did not correspond to the inclusion criteria. The prospective study started in 2020 and is currently enrolling consecutively all patients with NSTEMI, that are daily selected from all acute coronary syndromes patients in every hospital.

Data collection

A special questionnaire was elaborated based upon the one from NSTEMI Registry of the EURObservational Research Programme 2019 and adapted to local conditions, consisting of 178 questions. Aside from demographic information, cardiovascular and non-cardiovascular disease history was collected (neurological, renal, pulmonary and oncological), patients' risk factors (smoking status, hypercholesterolemia, diabetes mellitus, obesity, family history of cardiovascular disease, arterial hypertension). Clinical data while in hospital included symptoms at admission and complications during the hospital stay. Investigations data included LVEF, a set of biochemical parameters and other laboratory findings. The therapeutic and interventional management of patients and their timing were also a part of the questionnaire. The established patient follow-up dates were 30 days, 6 months and 12 months. It is important to mention that the management of patients did not include any mandatory actions, being 100% at the physician's discretion. During statistical analysis the mean values and their errors have been calculated, as well as the frequencies of descriptive parameters in percent, valid percent and absolute values.

Patient follow-up

Patients are contacted through telephone at 1, 6 and 12 months, being interrogated about hospital admissions to a cardiology or neurology ward, causes of these admissions and disease complications.

Results

At the moment of this report being written a total of 215 patients that met the inclusion criteria were enrolled into both stu-dies, 83 out them in the Institute of Cardiology,

76 in Sfanta Treime hospital and 64 in Novamed Polivalent hospital. 30 patient charts from the Institute of Cardiology and 2 charts from Novamed Polivalent hospital with a diagnosis of I214 (non-Q myocardial infarction) did not fit into the inclusion criteria, therefore were excluded from the study.

Baseline patient characteristics

The mean age of patients was 66 years (s=10.406), 40.5% (n=87) being females and 59.5% (n= 128) – males. The most common risk factor was arterial hypertension present in 93.9% (n=200) of cases, followed by hypercholesterolemia in 68.1% (n=141) of patients, diabetes mellitus in 37.4% (n=79) and obesity in 27.3% (n=48). The least common risk factor was current smoking found in only 22.7% (n=40) of cases. These data resemble the French cohort of patients from the FAST-MI registry, although arterial hypertension being found only in 63% of cases with NSTEMI. A little less NSTEMI patients in France have hypercholesterolemia – 50% vs. 68.1% in our studies. The current MI episode was the first CV event for 74% (n=159) of patients in Moldova vs. 68% of patients with NSTEMI in France. Cardiovascular disease history included chronic heart failure in 60.8% (n=129) of patients, old MI in 23.7% (n=51) and atrial fibrillation in 18.7% (n=40). 10.7% (n=23) had a previous stroke and 7.9% (n=17) had PAD. Only 4.2% (n=9) of patients had a CABG performed at some point of their lives. The most frequent non-cardiac comorbidity was chronic kidney disease found in 17.8% (n=38) of patients. 13.1% (n=28) were diagnosed with COPD in the past and only 1.6% (n=3) had active cancer [1].

The most frequent class of medication taken by patients before the current episode was ACE-inhibitors and AR-blockers, reported in 66.4% (n=140) of cases, followed by aspirin administered by 61.1% (n=129) of patients, beta-blockers in 49.2% (n=104) and diuretics in 25.8% (n=54) of cases. Unfortunately, statins were only taken by 10.8% (n=23) of patients before the episode, most of which – 7.5% - in small doses. In contrast, statins were on the list of 37% of patients before the NSTEMI episode in France, whilst the rate of aspirin intake is substantially lower than in Moldova – 33% – in accordance with a quite reserved recent aspirin prescription tactics in primary prevention [1, 5].

Table 1

Baseline, demographic data, risk factors, cardiovascular and non-cardiac history

Population with NSTEMI studied (n=215)	
Demographic data	
Institute of Cardiology	83
Novamed Polivalent Hospital	64
Sfânta Treime Hospital	76
Age, years	66.09, S=10.406
Women	40.5% (87)
Men	59.5% (128)
Body mass index	27.9731 S=4.63648
Risk factors	
Arterial hypertension	93.9% (200)
Hypercholesterolemia	68.1% (141)
Diabetes mellitus	37.4% (79)
Current smoking	22.7% (40)
Family history of CVD	17.7% (38)
Obesity	27.3% (48)
CVD history	
Fist CV event	74%, (159)
Old MI	23.7% (51)
History of CABG	4.2% (9)
Chronic heart failure	60.8% (129)
Atrial fibrillation	18.7% (40)
Previous stroke	10.7% (23)
PAD	7.9% (17)
Non-cardiac comorbidities	
Chronic kidney disease	17.8% (38)
COPD	13.1% (28)
Active cancer	1.6% (3)

Table 2

Pre-episode medication

Medication	Population with NSTEMI studied (n=215)
ASA	61.1% (129)
P2Y12 Inhibitor	11% (23)
Statins	10.8% (23)
Statins: low doses	7.5% (16)
Statins: standard doses	3.3% (7)
Beta-blockers	49.2% (104)
ACEI and ARB	66.4% (140)
Aldosterone antagonists	15.6% (33)
Diuretics	25.8% (54)
Oral anticoagulants	5.6% (12)
Antidiabetic drugs	30.9% (66)
DAPT	11% (23)
ARNI	0
PPI	1.4% (3)
Ivabradine	0
H2 receptor antagonists	0.9% (2)

The most characteristic complaint of NSTEMI patients upon admission was typical chest pain present in 94.4% (n=203) of cases, frequently accompanied by shortness of breath in 82.8% (n=178) of patients, as well as increased fatigability in 78.6% (n=169). Fewer patients presented with palpitations – 40.9% (n=88), nausea and vomiting in 16.3% (n=35) of cases. Most of the patients were brought with a Killip class of I and II – 40.9% (n=88) and 40.5% (n=87) of cases respectively. The mean heart rate at admission was 82.51 (s=20.923) per minute. The mean systolic blood pressure upon admission was 140.64 (s=28.455) mm Hg, whereas the mean GRACE score was 84.689 (s=55.95) points. It is quite important to mention that no scores were calculated initially in any of the charts presented, all of the calculations being made after the charts were analyzed during data

collection for the study. The quite low GRACE scores compared to those reported in the FAST-MI study for NSTEMI patients (139 ± 37) probably need to be revaluated. In only 61% (n=130) of cases the diagnosis on admission was actually NSTEMI, followed by unstable/aggravated angina in 17.4% (n=37). Unfortunately, high sensitivity troponin was used on admission in only 21 % (n=47), the mean value for it being 347.5289 (s=687.40176) [1]. The most common ECG presentations are listed in the table below.

Table 3

Current episode, clinical and laboratory findings

Symptoms upon admission	Population with NSTEMI studied (n=215)
Typical angina	94.4% (203)
Dyspnea	82.8% (178)
Fatigability	78.6% (169)
Palpitations	40.9% (88)
Asymptomatic	1.9% (4)
Nausea/vomiting	16.3% (35)
Atypical chest pain	2.8% (6)
Cardiac arrest/syncope	2.8% (6)
Killip class on admission	
I	40.9% (88)
II	40.5% (87)
III	14% (30)
IV	4.7% (10)
Localization of the infarction	
Anterior	26.5% (57)
Inferior	15.8% (34)
Other	46.1% (99)
Diagnosis on admission	
STEMI	6.6% (14)
NSTEMI	61% (130)
Unstable/de novo angina	7% (15)
Unstable/aggravated angina	17.4 % (37)
Unstable/peri-infarct angina	2.3% (5)
Other	5.6 % (12)
ECG at presentation	
Sinus rhythm	86.5% (186)
Atrial fibrillation	13.5% (29)
LBBB	5.1% (11)
RBBB	6.5% (14)
Heart rate on admission	82.51 (s=20.923)
Systolic blood pressure	140.65 (s=28.455)
Diastolic blood pressure	83.13 (s=14.542)
GRACE score	84.689 (s=55.95)
TIMI score	5.72 (s=6.703)
CRUSADE score	35.34 (s=16.221)
Creatinine	112.7507 (s=85.3008)
Hemoglobin	130.877 (s=20.4493)
LDL-c	3.2963 (s=1.0902)
HDL-c	1.2626 (s=0.35145)
Triglycerides	1.8306 (s=0.95976)
High sensitivity troponin	347.5289 (s=687.40176)

Sadly, the time-lapse from the first symptoms to hospital admission is extremely high – the mean of it being almost 12 hours, whereas in France it is of only 155 minutes in NSTEMI patients and 90 minutes for STEMI. Most of the patients were admitted to an intensive care unit – 61.4% (n=132), another 38.6% (n=83) ended up in a cardiology ward. 84.7% (n=182) of the patients got a coronary angiography at some point during their hospital stay, in France this indicator being at 95% of NSTEMI patients. 96.6% (n=172) of patients had a radial puncture, surprisingly almost 10% more than in the FAST-MI registry. The mean time from admission to cath lab in Chisinau was about 26 hours, the majority – 73.1% (n=133) being in the first 24 hours of hospital stay. Percutaneous angioplasties were performed in 63.7% (n=137) of those who got catheterized, most of which happened in the same session as the initial coronary angiography. Drug eluting stents were implanted in 89.6% (n=121) of cases, demonstrating almost the same results as in France [1]. After admission DAPT was given to 82.4% (n=169) of patients, to 85.7% (n=144) of which in the first 24 hours. Among the 3 participating centers only the Institute of Cardiology has cardiac surgery on-site, therefore just two patients from the whole study had a CABG performed during their hospital stay, representing 0.9% of all cases.

Table 4

Management on admission and during hospital stay

Population with NSTEMI studied (n=215)	
Time from pain start to admission (hours)	11.9884 (s=24.8055)
Time from admission to coronary angiography (hours)	25.7115 (s=46.17695)
DAPT on admission	82.4% (169)
DAPT<24 hours from admission	85.7% (144)
DAPT 24-48 hours from admission	6.5% (11)
DAPT >48 hours from admission	7.7% (13)
Place of admission	
Intensive care unit	61.4% (132)
Cardiology ward	38.6% (83)
Admission method	
Ambulance	75.8% (163)
Outpatient clinic transfer	8.4% (18)
Transfer from another hospital	14.4% (31)
Other	1.4% (3)
Interventional treatment	
Coronary angiography	84.7% (182)
Coronary angiography <24 hours	73.1% (133)
Coronary angiography >24 hours	26.9% (49)
Conservative management	15.3% (33)
PCI	63.7% (137)
PCI in the same session of CA	95.5% (131)
PCI <2 hours from admission	26.7% (36)
PCI <24 hours from admission	51.1% (69)
PCI <48 hours from admission	6.7% (9)
PCI >48 hours from admission	15.6% (21)

Type of stent	
DES	89.6% (121)
BMS	0.7% (1)
Balloon angioplasty	8.9% (12)
Vascular access	
Radial	96.6% (172)
Femoral	2.8% (5)
Other	0.6% (1)
Planned CABG in another hospitalization	2.8 % (6)
CABG during hospital stay	0.9% (2)

The treatment while in the hospital included statin administration, however in just 79% (n=169) of cases, close to the 78% in France [1]. Standard statin doses were given to 59.8% (n=126) of patients. 81.3% (n=148) received LMWH, unfractionated heparin being administered in only 4.4% (n=8) of cases.

Table 5

Medication during hospital stay

	Population with NSTEMI studied (n=215)
DAPT	82.4% (169)
Unfractionated heparin	4.4% (8)
LMWH	81.3% (148)
Fondaparinux	1.6% (3)
Statins	
Low dose	8.4% (18)
Standard dose	59.8% (126)
High intensity	11.7% (25)

The most frequent complication during hospital stay in NSTEMI patients was pulmonary edema reported in 16.3% (n=35) of cases, followed by cardiogenic shock in 13% (n=28) of patients. 9.3% (n=20) suffered from acute heart failure and 7.9% (n=17) had a cardiac arrest. In-stent thrombosis occurred in only 0.9% (n=2) out of those who received a stent. Hemorrhages emerged in 2.5% (n=5) of cases. The mean length of hospital stay was 7.55 (s=5.419) days, being similar to that in the FAST-MI registry (7.33 ± 9.0 days) [1].

Table 6

Complications during hospital stay, in-hospital mortality, mean length of hospital stay, place of discharge

Complications during hospital stay	**Population with NSTEMI studied (n=215)**
LVEF %	
>=50%	51.6% (111)
40-49%	29.8% (64)
<40%	7.4% (16)
<=35%	10.7% (23)
Cardiogenic shock	13% (28)
Recurrent MI	3.7% (8)
In-stent thrombosis	0.9% (2)
Cardiac arrest	7.9% (17)
Stroke	1.4% (3)
Hemorrhage	2.3% (5)
Death	8.4% (18)
Acute heart failure	9.3% (20)
Pulmonary edema	16.3% (35)
PE	1.9% (4)
VT/VF	9.3% (20)
Mechanical complication (myocardial rupture)	0.5% (1)
Mean length of hospital stay	7.55 (s=5.419)
Discharge	
Home	73.5% (158)
Rehab	15.8% (34)

At discharge among the prescribed medication ASA was in the top with 90.5% (n=180), beta-blockers prescribed to 89.9% (n=179) of patients, ACEI/ARB in 85.9% (n=170), clopidogrel in 82.3% (n=163) and statins in 61.2% (n=120), more than a half of those receiving standard statin doses. 10.7% (n=21) were given oral anticoagulation, PPI being given with DAPT in 37.8% (n=74) of cases [1].

Table 6

Medication at discharge

	Population with NSTEMI studied (n=215)
ASA	90.5% (180)
Clopidogrel	82.3% (163)
Statins	61.2% (120)
Statins low doses	13.8% (27)
Statins standard doses	35.7% (70)
High intensity statin doses	11.7% (23)
Beta-blockers	89.9% (179)
ACEI/ARB	85.9% (170)
Oral anticoagulation	10.7% (21)
Metformin	19.5% (39)
Insulin	9% (18)
PPI	37.8% (74)
Diuretics	39.3% (77)
H2-receptor antagonists	6.1% (12)

Discussion

The level of participation in the studies of the three centers is relatively high, the overall picture that has started to contour being quite representative, however we must not forget that the given situation only refers to the population from the capital and suburbia that have 4 PCI 24/7 hospitals available at their service. Respectively, the situation concerning ACS management, especially those without ST elevation, outside this area remains on the dark side and is probably substantially different i.e. a lot worse than the one we have studied.

The baseline NSTEMI patient characteristics are close to those reported in the FAST-MI study, with some minor differences mentioned above. The enormous time from symptom onset to admission is very alarming, being almost

4 times greater than the same indicator in France. We could probably speculate that in the COVID-19 era the fear makes the uninstructed about chest pain danger and need to seek urgent medical care population wait at home without soliciting an ambulance until the pain is unbearable or some complications arise. The rate of actual NSTEMI diagnosis at hospital admission is 61%, it being probably a lot higher had the high sensitivity troponin been applied. The causes of its seldom use in the emergency department could be different. Often (historically here), this kind of lab work is not available due to test lack, the first contact physicians actually having to work with either a qualitative troponin or a simple quantitative test, that does not provide high sensitivity testing. In our study we tested the first blood collected from patients with a diagnosis of unstable angina in the emergency department with a high sensitivity troponin test and had many sufficiently positive results bringing the number of patients that could be early diagnosed with NSTEMI. Therefore, initial testin of all unstable anginas in the emergency department or even in the ambulance with a high sensitivity test would raise the number of early diagnosed NSTEMI [6-9]. The access to coronary angiography is surprisingly in a good way high, once the patient is admitted and diagnosed with NSTEMI, being almost 85%, which is still by 10% lower than in France [1]. The vascular access site is a radial access in 9 out of 10 patients, being in accordance with the current guidelines [10-11]. The vast majority of patients that undergo coronary angiography, most of them in the first 24 hours from admission, will also receive a stent in 95% of cases, a drug eluting stent in almost 90% of those [12-14]. There is still a lot of work to be done concerning the use and dosage of statins during hospital stay and after discharge. Clopidogrel remains the only $P2Y_{12}$ inhibitor available on the market, waiting for other recommended $P2Y_{12}$ inhibitors [15] to become available, DAPT being administered in more than 80% of cases. A large number of patients treated during COVID-19's charts are still in the process of analysis. We are awaiting a rise in mechanical MI complications and in-hospital mortality rate as a result of extremely late admission of NSTEMI (and even STEMI) patients. However, apart from in-stent thrombosis rates, the rest of in-hospital complications are still a lot more common than reported by French investigators [1]. The mean length of hospital stay is similar in both countries but we expect it to fall in the context of the pandemic, in the attempt to reduce the time of patient contact with the medical care system. The weak point of all hospitals is unfortunately the access to cardiac surgery that has only become available since 2020 in the Institute of Cardiology, all of the emergency surgeries performed before 2020 had to be arranged via a transfer to another hospital.

At this moment the first three objectives of the study are already contouring, ongoing is the prospective study of patient enrollment and the follow-up of patients from the retrospective part of the study, in order to determine the possible correlations between the management of patients and the short- and long-term outcomes. The collection of biologic material from the patients is aimed at multiple biomarker panels testing additional to those mentioned in the guidelines, exploring their role in patient diagnosis and their prognostic values. We are awaiting the results of the NSTEMI Registry of the EURObservational Research Programme 2019, which is still enrolling patients, but is going to provide extended data about NSTEMI patient management in different countries, not just developed ones, describing lacunas and creating possibilities of their correction on a way of a better health care and prognosis for NSTEMI patients.

Conclusions

Observational studies are a relatively simple and cheap tool for assessing real life situations in everyday medical practice and comparing them with the guideline recommended standard of care [16]. Preliminary data from the observational studies, one retrospective and one prospective have already revealed several drawbacks that need to be corrected while the prospective study continues. Some major drawbacks as the underuse of high-sensitivity troponin and the time lapse from symptom onset to admission need to be addressed as soon as possible, taking into account the possible aggravation of situation due to COVID-19 in 2020 where people with chest pain hesitate even more before soliciting an ambulance. While there are rather small differences in patient profile between Chisinau and data from a developed country like France, the differences in in-hospital complications might suggest we are going to see a similar trend in short- and long-term outcomes. As the next results become available the search for clinical mishandling and its possible solutions continues [1]. Hopefully, enough data is gathered and its analysis will allow us to publish a local clinical practice guideline for the management of patients presenting with ACS without ST segment elevation that will be based on the ESC guideline, but will take into account local possibilities. In order to provide extended information on the situation outside the capital and the suburbia, similar assessment is needed in the regional hospitals aiming at their integration into one system of acute coronary syndrome care in a small country like Moldova, eliminating time and distance discrimination and providing equal access with an adequate timing to PCI centers for patients from any small village. This means focusing on a serious update of the current available STEMI programme and transforming it into acute coronary syndrome programme.

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AP and MP conceptualized the project and drafted the first manuscript. MI and OD interpreted the data. VI, LC, and IP critically revised the manuscript. All authors revised and approved the final version of the manuscript.

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REVIEW ARTICLES

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Stem-cell therapies in critical limb ischemia

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Abstract

Background: Due to stimulation of muscular regeneration in ischemic extremities and increasing blood flow, stem cells are considered a promising new strategy for patients with critical limb ischemia (CLI). So, it is demonstrated that mesenchymal stem cells (MSCs), mononuclear cells derived from bone marrow, peripheral and umbilical blood, adipose tissue encourage genesis of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). By application of stem cell-based therapy, the following results are obtained: increased rate of ulcer healing, increased ankle-brachial index (ABI) and transdermal oxygen pressure ($TcPO_2$), improved revascularization, and reduced rate of amputation surgery. So, stem cell-based therapy demonstrates good clinical outcomes. However, some adverse events related to cell sampling and mobilizations are reported. In addition, because of poor cell survival in condition of ischemia their therapeutic efficacy remains low that indicates further researchers are necessary in this field.
Conclusions: Cell-based therapy is a promising approach in CLI treatment. Its promising results have been already shown in smaller studies; however, large-scale studies are entailed to ascertain their definitive role in anti-ischemic therapy.
Kew words: hypoxia, critical limb ischemia, peripheral arterial disease, transplantation, stem-cell therapy, tissue repair, angiogenesis.

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Introduction

Critical limb ischemia (CLI) represents the most severe manifestation of peripheral arterial disease (PAD). According to European Conciliation Committee, the incidence of CLI is about 500-1000 cases per 1.000.000 patients and constitutes a considerable social and economic burden. The prevalence of this disease in general population aged between 60–90 years is about 1% (0.5%–1.2%), however, there is a great difference when we compare the data from general populational studies and registers of vascular surgery. Gender differences in the prevalence of CLI varies in different studies, the Men:Women ratio is about 3:1. Even so, in older persons aged 50 or over it is equal to 1:1. It is associated with high mortality [1, 2]; about 25% of patients with this diagnostic die, and major amputations are required in 10–40% of cases when revascularization failed or in patients with "no-option" [3-5].

Due to the lack of a standard treatment guideline, CLI is still considered an orphan disease. The treatment of CLI and its symptoms includes pharmocological therapy as the first option, and invasive procedures for long-term revascularization [6]. Even the improvement of the available curative options are undoubtedly necessary, invalidity rate because of amputation in this category of patients is still high.

The main goal of the proposed treatment strategies is focused on recovery of damaged by ischemia tissues. This goal may be achieved by applications of cell-based therapies. Its beneficial effect is determined by induction vascular regeneration and angiogenesis stimulation [7]. So, cellular therapy has come into view as a new frontier in this regard.

A lot of studies have demonstrated yet that stem-cell therapy (SCT) can ensure tissue vascularization after established ischemic modification in the limbs [8-12]. Functional endothelial cells can be obtained from different cellular sources and they can be used as potential therapy in the treatment of different cardiovascular diseases. However, their therapeutical efficacy remains unclear. In this literature review different therapeutical approaces about stem cell aplication in CLI treatment are demonstrated.

In order to realize the goal of the study contemporary scientific literature containing the following key words "hypoxia", "critical limb ischemia", "peripheral arterial disease", "transplantation", "stem-cell therapy", "tissue repair", and

"angiogenesis" were selected from PubMed database. For advanced selection of literature sources, the following filters were applied: articles published in English within the time period 2002-2019. Only original research articles (preclinical, clinical and experimental studies), meta-analysis and systematized literature reviews were selected.

After the primary examination of articles' titles and exclusion the papers which did not correspond to overall goal and were not accessible for the full text review, the reference lists of relevant publications were also examined in order to find additional useful bibliography. The information was systematized and presented in the form of systematic review.

Results

According to the search strategy 872 articles on SCT and their role in CLI treatment were found. After processing 125 articles were selected as relevant. The final bibliography contains 29 articles in the area. The data about stem cells efficacy in the treatment of CLI were collected.

Study 1: Eriko Tateishi-Yuyama et al. 2002 [13]. **Study characteristics:** Prospective clinic randomized study (n=47). **Results:** The efficacy and safety of autologous implantation of bone marrow-mononuclear cells (BM-MNCs) in patients with ischaemic limbs because of peripheral arterial disease were evaluated. All the patients enrolled in this study were assigned in two groups: group A (n=25) and group B (n=22). Patients with unilateral limb ischemia from the first group were treated by injection of BM-MNCs into the gastrocnemius of the ischemic limb and saline solution into the opposite limb. Patients from group B presented bilateral leg ischemia. They also were treated by transplantation of (BM-MNCs) in one leg. However, injection of peripheral blood-mononuclear cells was used as control. The safety and feasibility of the treatment were evaluated according to the following criteria: ABI and rest pain. Follow-up period consisted of 24 weeks with an intermediate evaluation at 4 weeks. Significant improvement of ABI, transcutaneous oxygen pressure ($TcPO_2$), rest pain, and pain-free walking time were recorded in legs injected with BM-MNCs. So, the beneficial effect of BM-MNCs transplantation in achievement of therapeutic angiogenesis was clinically demonstrated. It is determined by the cells ability to supply endothelial progenitor cells and to secrete various angiogenic factors or cytokines.

Study 2: Takashi Iwase et al. 2005 [14]. **Study characteristics:** Animal study. **Results:** In this study efficacy of stem cells transplantation of mesenchymal stem cells (MSCs) and BM-MNCs in CLI was evaluated; also, their therapeutic potential was compared. For this purpose, a rat model of hindlimb ischemia was developed. Analysis of 3 weeks follow-up period was done and the following criteria were studied: laser Doppler perfusion index, blood perfusion, and capillary density. The data analysis demonstrated a greater improvement of ischemia in MSC-group.

Study 3: Ivana Rosova et al. 2007 [12]. **Study characteristics:** Animal study. **Results:** The MSCs properties and their impact in injured tissue regeneration were studied. It was demonstrated that intra-arterial administration of BM-MSCs in 24 hours may induce revascularization enhancement in ischemic limb. Also, the study data analysis clearly suggested that preculturing MSC under hypoxic condition improves their regenerative potential.

Study 4: Nihan Ranjan Dash et al. 2009 [10]. **Study characteristics:** Randomized experimental study (n=24). **Results:** The efficacy and feasibility of autologous BM-MSCs in combination with standard wound dressing regimen in the treatment of lower extremities chronic non-healing ulcers were evaluated. Researches demonstrated quicker regeneration of trophic ulcers with improvement of pain-free walking distance in the experimental group with no significant alteration in the biochemical parameters.

Study 5: Gabriel P Lasala et al. 2010 [15]. **Study characteristics:** Phase I clinical trial. **Results:** The researchers demonstrated the efficacy of autologous BM-MNSc and MSCs infusion in ischemia treatment. After a ten months follow-up the improvements of life quality, walking time and ABI were achieved. In addition, increased perfusion in ischemic limbs was confirmed by angiographic and 99mTc-TF perfusion scintigraphy scores.

Study 6: J Hoffmann et al. 2010 [11]. **Study characteristics:** Animal study. **Results:** The effectiveness of BM-MSCs in the treatment of occlusive arterial diseases was studied. In addition, the cells properties in hypoxic conditions and normoxia were examined. It was demonstrated that due to better survival in hypoxic conditions and improved production of vascular endothelial growth factor (VEGF) BM-MSCs transplantation leads to an increase in vessel density when comparing to other groups.

Study 7: R Kolvenbach et al. 2010 [16]. **Study characteristics:** Prospective, clinic randomized study (n=8). **Results:** The study included 8 patients with CLI, in all cases surgical treatment was indicated. Adjunctive intraoperative SCT with BM-derived stem cells was performed in combination with intervention. For cells' processing a point-of-care system was used. A discreet increasing of ABI was determined in five patients, and high recovery rates were obtained. However, two major amputations and one minor amputation were needed postoperatively.

Study 8: Debin Lu et al. 2011 [17]. **Study characteristics:** Randomized, double-blind, controlled study (n=20). **Results:** The therapeutic effects of intramuscular administration of BM-MSCs and BM-MNCs in patients with diabetic CLI and foot ulcers (Fontaine class IV) were studied. The follow-up period was 24 months. The researchers demonstrated better clinical results in MSCs-treated group (complete ulcer healing, improved limb perfusion, pain-free walking time, ABI, $TcPO_2$, and magnetic resonance angiography). Of note, neither cell type resulted in any adverse effects.

Study 9: Dirk H Walter et al. 2011 [18]. **Study characteristics:** Multicenter, phase II, double-blind, randomized study (n=40). **Results:** Feasibility and benefits of intraarterial injection of BM-MNC in patients with CLI were evaluated. The increase of ABI was not observed in the experimental group; however, cell therapy was associated with

accelerated ulcer healing and reduced rest pain.

Study 10: Naomi Idei et al. 2011 [19]. **Study characteristics:** Prospective, clinic randomized study (n=51). **Results:** The long-term results of BM-MSCs administration in patient with CLI were evaluated (the study included 51 patients: 25 with PAD and 26 with Buerger disease). In both groups, ABI and $TcPO_2$ significantly increased after 1 month. In addition, the COX model revealed that BM-MNC implantation correlated with prevention of major amputation. However, in patients with PAD, ABI and TcPO2 gradually decreased during 3-year follow-up and returned to baseline levels.

Study 11: Aaron Liew et al. 2012 [9]. **Study characteristics:** Literature review. **Results:** The authors provided an overview of the potential role of MSC-based therapies for CLI, put into discussion the proposed mechanism of stem cells' actions in the improvement of ischemic tissue regeneration (such as paracrine, immunomodulatory, and myogenic/endothelial differentiation effects) and analyzed certain factors: cell dose, timing, and appropriate route of administration – critical to the success. Also, data obtained from preclinical studies and current early-phase human trial were discussed. Animal researches demonstrated that by administration of MSCs and modified MSCs derived from various sources (bone marrow, umbilical cord blood, fetal membrane, and adipose tissue) significant improvement in mouse/rat models of ischemia can be obtained. In almost all cases intramuscular route was chosen. The effect is determined by enhancement of blood perfusion and capillary density. Clinical human studies also confirmed the angiogenic effect of MSC therapy. Finally, the authors highlighted the main directions in the field development.

Study 12: Gabriel P Lasala et al. 2012 [20]. **Study characteristics:** Phase II clinical trial (n=26). **Results:** The efficacy of a combined cell product (mesenchymal stem cells in conjunction with endothelial progenitor cells) given intramuscularly (gastrocnemius infusion) was evaluated and compared with a placebo product. Improvements in walking time, ABI, and life quality without any adverse effects were established only in the cell-treated limb with no modifications in the contralateral leg, where the placebo was introduced. Also, scintigraphic examinations (technetium-99m-tetrofosmin scintigraphy) were realized. It demonstrated increased perfusion exclusively in the cell-treated limbs.

Study 13: Han Cheol Lee et al. 2012 [21]. **Study characteristics:** Prospective, clinic randomized study (n=15). **Results:** The stem-cell therapy effectiveness in ischemia treatment was examined. It was demonstrated that intramuscular injections of autologous MSCs derived from adipose tissue in patients with Buerger's disease and diabetic foot are feasible and safe, clinical improvement being recorded in 66.7% of cases. During the folow-up period minor amputations were required only in five cases. Walking time, collateral blood vessels formation, ulcers recovery and clinical symptoms recovery were improved; but, ABI was unchanged.

Study 14: Pawan K Gupta et al. 2013 [22]. **Study characteristics:** Prospective, double blind randomized placebo controlled multi-center study (n=12). **Results:** In the study the patients with CLI (PAD) as per Rutherford classification in category II-4, III-5, or III-6 with infra-inguinal arterial occlusive disease were included, in all of them revascularization treatment being impossible or failed. Intramuscular route of administration (gastrocnemius of the ischemic limb) was used for cells delivery. The efficacy and safety of intramuscular administration of allogeneic BM-MSCs in patients with CLI were determined (improvement in the rest pain score, ABI, ankle pressure). The regenerative effects were determined by stimulation of angiogenesis and inducing immunomodulatory environment *in situ*. In addition, adverse effect incidence in experimental group was lower than in placebo one.

Study 15: Hendrik Gremmels et al. 2013 [23]. **Study characteristics:** Study review, namely Gupta and colleagues report [14]. **Results:** The authors mentioned the importance of Gupta et al. study, as a welcome addition in the field of investigating STC in PAD. It was practically demonstrated that SCT is a promising avenue in the treatment of patients with very few other options. However, the mechanism of MSC-mediated improvements is still unclear; it indicates that additional investigations are necessary.

Study 16: Jae Choon Ryu et al. 2013 [24]. **Study characteristics:** Animal study. **Results:** The main study goal was to test hypothesis that treatment of limb ischemia with multipotential adult progenitor cells (MAPCs) promotes recovery of blood flow. The limb ischemia in mice was induced by ligation of iliac artery. MAPCs were injected intramuscularly on day 1. Optical imaging demonstrated cells' survival for 1 week. Contrast-enhanced ultrasound showed a more complete blood flow recovery in the experimental group. Fluorescent microangiography demonstrated more complete distribution of flow to microvascular units in the MAPC-treated mice. So, MAPCs efficacy in promoting flow recovery in ischemic tissue was demonstrated.

Study 17: Wing-Hon Lai et al. 2013 [8]. **Study characteristics:** Prospective, preclinical randomized, experimental study (n=15). **Results:** The researchers studied the therapeutic efficacy of endothelial-like cells (EC) in the treatment of cardiovascular diseases. Functional EC were derived from BM-MNCs (BM-EC), human embryonic stem cells (hESC-EC), and human induced pluripotent stem cells (hiPSC-E). *In vitro* (tube formation, migration and cytokine expression profiles) and *in vivo* testings (attenuation of hind-limb ischemia in mice) were performed. It was demonstrated that hESC-EC and hiPSC-EC are useful in the treatment of tissue ischemia.

Study 17: Juan Jose Parcero et al. 2014 [25]. **Study characteristics**: Case report. **Results:** The angiogenic properties of autologous adipose-derived stromal cell (ASC) were studied, as well as their safety and feasibility of clinical application. The patient, a 80-year-old female from the USA enrolled in the study, received radiation therapy due to the presence of a squamous cell carcinoma and as a result developed a non-healing lesion. Because the traditional treatment was non-effective, the patient received cellular therapy. Approximately $35.1*10^6$ autologous cells were ad-

ministered intravenously, and another $81.2*10^6$ cells were implanted directly at the edges of lesions and throughout the ulcers. Complete healing, closure, and disappearance of ulcers with symptoms reduction were achieved. So, it was demonstrated that ASC can help improve or eliminate non-healing lesions and seems to be a treatment alternative in advanced ischemic disorders.

Study 18: Rita Compagna et al. 2015 [6]. **Study characteristics:** Literature review. **Results:** The aim of the study was to perform a systematic analysis of the most recent scientific literature on the application of STC in the treatment CLI of different etiologies. In the study 1031 eligible full text articles on stem cells biology, physiology, and differentiation into vascular cells were included. In addition, the data about actual indications for SCT, methodology of stem cells sampling, optimal route of administration (intramuscular *vs* intra-arterial), and the clinical and adverse effects was reported.

Study 19: Yanyi Xu et al. 2015 [7]. **Study characteristics:** Animal study. **Results:** The researchers demonstrated that stem cell survival can be increased even under the low oxygen and nutrient environment. The effect may be obtained by introducing a prosurvival environment into the delivery system.

Study 20: Venkatesh Ponemone et al. 2017 [26]. **Study characteristics:** Prospective, clinic randomized study (n=17). **Results:** The safety and therapeutic effectiveness of autologous bone marrow cell concentrate in revascularization of CLI patients were described. The study included 17 patients. The cellular concentrate was prepared utilizing an intraoperative point-of-care device and injected intramuscularly. Significant improvements in ABI, TcPO2, mean rest pain and intermittent claudication pain scores, wound/ulcer healing, and 6-minute walking distance were observed. Adverse effects were reported just in seven (41.2%) patients, three (17.6%) patients underwent major limb amputation (above the ankle), two (11.8%) patients underwent minor amputation (digit/s), and two unrelated deaths. So, cells' injection was found to be safe, easy, and inexpensive procedure for definite ameliorating of limb ischemia.

Study 21: Arun Sharma et al. 2019 [27]. **Study characteristics:** Literature review. **Results:** The authors presented a comprehensive review of the contemporary scientific literature about therapeutic angiogenesis with stem cells. The benefic aspects of SCT, such as improvement in ABI, $TcPO_2$, reduction of pain, and reduced rates of limb amputation were described. Challenges and limitations of the SCT (anti-ischemic mechanism, ideal cell type, therapeutic dosage, and optimal route of administration) and future study directions were put into discussion.

Study 22: Stempeutics Research Pvt. Ltd. (Bangalore, India) [28]. **Study characteristics:** Phase ½ clinical trial. **Results:** The study was performed from commercial perspective. The efficacy of off-the-shelf allogeneic BM-MSCs administered intramuscularly into the patients with CLI was estimated. No adverse reaction or rejection were found. In addition, improvement in ABI and a reduction in the number of ulcers were reported.

Study 23: Pluristem Therapeutics Inc. (Haifa, Israel) [29]. **Study characteristics:** Two phase 1 trials (n=27). **Results:** The effectiveness of allogeneic placenta-derived MSCs injected intramuscular was demonstrated. Within six-month follow-up, major amputation was necessary only in one case. Significant improvement in blood flow and quality of life was found. In addition, the total pain score reduced.

Discussion

By systematization of collected data, we determined that the cellular grafts are efficient in the treatment of CLI of different etiologies. For this purpose different cell types may be used, such as BM-MSCs, BM-MNCs, mesenchymal cells derived from adipose tissue, stem cells derived from umbilical cord blood, and their combinations. The appropriate routs of cells administration are intramuscular and intra-arterial [5, 7, 17]. For appreciation of SCT efficacy the following criteria are proposed [5-21]: ABI, $TcPO_2$, ankle pressure, capillary density, pain-free walking distance, rest pain, perfusion index, magnetic resonance angiography, wound/ulcer recovery, adverse effects, amputation rate, and life quality.

Conclusions

SCT is evolving as a promising newer tool in the management of severe peripheral vascular diseases; its application in practice may significantly improve the ischemia treatment with good clinical outcomes and reduced amputation rate. Initial animal and clinical studies are supportive of its safety and feasibility. However, the acceptance of this mode of therapy as a standard of care is still a matter of debate and supplementary studies are necessary for effectiveness evaluation and establishing its definite survival benefit.

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Authors' contribution
SV conceptualized the project and performed data collection; TM drafted the manuscript; AC interpreted the data and contributed to the final version of the manuscript; VN took the lead in writing the manuscript. All the authors revised the manuscript critically and approved the final version of the manuscript.

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Predictive molecular markers of resistance to chemotherapy in breast cancer

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Abstract

Backgraund: Breast cancer is one of the three most common cancers along with lung and colon cancer. It is a leading cause of cancer deaths in both developing and developed countries. Within 2 decades, neoadjuvant chemotherapy (NAC) has become a standard treatment option in breast cancer. Relevant articles were identified by means of PubMed, Embase, Web of Science, Cochrane Library and Springer Link databases published during the years 2010-2019, describing the role of molecular biomarkers in the assessment of NAC for breast cancer.

Conclusions: The size of the breast primary tumor, the affection of the regional lymph nodes, the degree of tumor differentiation, the expression of hormone receptors, HER2neu, ki67 serve as main criteria for predicting the response to NAC. Preoperative core needle biopsy is the gold standard procedure in cancer diagnostics, in the analysis of predictive biomarkers, particularly utilizing histomorphological characteristics. Carrying out a larger number of cycles of NAC as well as correlating the schemes in relation to the immunohistochemical types have a direct influence on obtaining a good response to treatment. Patients with a pathologic complete response had superior survival outcomes compared with patients who had residual disease.

Key words: breast cancer, neoadjuvant chemotherapy, biomarker, predictor.

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Introduction

Breast cancer (BC) is the most common malignancy among women, with a growing incidence. Every tenth primary cancer patient is diagnosed with breast cancer. It is also the leading cause of cancer death in women [1].

Breast cancer is a group of heterogeneous diseases with numerous genetic alterations and relatively uniform histological phenotypes. Therefore, identification of the histological characteristics that can help to predict the therapeutic response or the clinical prognosis in breast core needle biopsy (CNB) specimens can prove valuable [2].

Neoadjuvant chemotherapy (NAC) of BC improves outcomes, especially in patients with locally advanced and inflammatory cancer. Further insight into clinic-pathological factors influencing outcomes is essential to define the optimal therapeutic strategy for each category of patients and to predict the response to the treatment [3].

Presently, preoperative core needle biopsy (CNB) is the gold standard procedure in cancer diagnostics. In addition to its diagnostic role, recent data have suggested another role for CNB in the analysis of predictive biomarkers, particularly utilizing histomorphological characteristics [4].

In addition, significant volume reduction in tumors after neoadjuvant chemotherapy may permit subsequent, successful breast-conserving surgical treatment [5].

There is significant variability in the histopathologic response of tumors to neoadjuvant chemotherapy, with approximately 15% of patients achieving a complete response, whereas, at the other end of the spectrum, 15% of patients display minimal change or progressive disease. Currently, the underlying mechanism for this variability is unknown. Contributing factors may include the diverse genetic background and hormonal environment of the tumor. Previous studies have focused on the correlation between the response of tumors to chemotherapy and various factors, such as histologic grade, DNA ploidy, cell kinetics, and hormonal receptor status of the primary tumor. However, those studies yielded inconsistent results [6, 7, 8].

Therefore, despite the cumulation of more information about biomarker impact in breast cancer chemotherapy, mostly treatment regimens are standard, so the 5-year survival rate did not serve to make significant changes.

It was searched what the PubMed, Embase, Web of Science, Cochrane Library and Springer Link databases published during the years 2010-2019. The author identified relevant articles describing the role of molecular biomarkers in the assessment of neoadjuvant chemotherapy for breast cancer.

Discussion

The purpose of this review is to present the role of assessing predictive molecular markers in selecting the chemotherapeutic treatment needed for breast cancer patients.

NAC that is designed to be used before surgical removal of a tumor has attracted special attention in oncology [9].

The application of neoadjuvant chemotherapy in locally advanced breast cancers has demonstrated high efficacy by transforming inoperable tumors into operable, avoiding radical mastectomies in ~ 25% [9].

The indications for NAC at present are quite broad: BC in

the early stages in preparation for organ-threatening operations, locally advanced BC, edema-inflammatory form of the disease, regional lymph nodes affection, and big size of the primary tumor [10].

There are several benefits of using neoadjuvant chemotherapy. It provides a unique opportunity to evaluate the response to treatment with a complete pathological response that acts as a surrogate marker of survival and for a faster assessment of the efficacy of new therapeutic agents and early cessation of ineffective treatment. In addition, in case of resistance to treatment, dose adjustment and / or switching to another drug relieves patients of the burden of toxicity and side effects. NAC provides an opportunity for individualized therapy and allows the collection of tumor samples before, during, and after treatment for translational research [11].

A number of data have been published in the literature on the importance of applying long-term neoadjuvant chemotherapy in cases of chemoresistant breast cancer [12, 13, 14].

The appearance of chemoresistance of primary breast tumors is of primordial importance in the modern treatment of BC. The theoretical-practical aspects that clarify the acquisition of cancer cell resistance to chemotherapeutic drugs are insufficiently studied in the literature. Various theories are assumed by which the gene encoding the transport protein of chemotherapeutic drugs is disrupted, the genetic modification of the receptors of the cancer cell membrane, the changes of intracellular transport, etc. Thus, the study of the predictive factors of the appearance of chemotherapeutic resistance is of great importance in the evaluation of individualized drug treatment schemes [15].

The response rate of the tumor to NAC can be evaluated by several methods: clinical examination (assessment of tumor size, skin changes and peritumoral regions), breast imaging (ultrasound, mammography, MRI), postoperative morphopathological examination. Particular attention is paid to the assessment of the degree of pathomorphosis in the postoperative histopathological examination, the assessment of morphological changes of the tumor and peritumoral region, the assessment of tumor cellularity [16].

The response to NAC is assessed by changing the size of the primary tumor and the affected lymph nodes in the pre- and post-treatment phase. There are 3 types of response to NAC in the literature: pathologic complete response (PCR), near complete response (NCR) defined by the presence of residual primary tumor < 1 cm^3, partial pathologic response (PPR) defined by the presence of residual primary tumor measuring > 1 cm^3 [8].

In cases with PCR, the authors mention a better prognosis [17].

Studies have shown a response rate to NAC with a variation between 20-30% depending on the immunogenetic profile and the chemotherapeutic scheme used [18].

Achieving complete and partial remission of NAC has better long-term results, with better overall survival compared to cases where tumors do not respond to therapy [19].

Several studies have shown that the immuno-genetic profile of the tumor can serve as a primary criterion in assessing the rate of subsequent response to treatment. Triple-negative and HER2neu-positive tumors (with hormone-negative receptors) are more aggressive and serve as a criterion for performing NAC. The best response to NAC is found in tumors with small size, high degree of differentiation, the presence of tumor necrosis, hormone-negative receptors, the presence of HER2neu receptor positivity [20, 21].

Luminal type A, compared to other immunohistochemical types, has a better prognosis and in most cases does not require neoadjuvant treatment. The rate of PCR after NAC in the case of Luminal A type is 6%, compared to Luminal B – 10%, Her2neu – 47%, Basal-type – 37% [18, 22, 23].

Total and breast PCR rates were higher in HR negative (HR–) patients (26% and 32%, respectively) than in HR positive (HR+) patients (4% and 7%, respectively). Compared to HR+ patients, HR– patients had higher recurrence rates (38% versus 22%). Human epidermal growth factor receptor 2 positive patients treated with neoadjuvant trastuzumab (NAT) demonstrated higher total PCR (34% versus 13%), breast PCR (37% versus 17%), and nodal PCR rates (47% versus 23%) compared to HER2+ patients not treated with NAT. Furthermore, HER2+ patients who received NAT had lower recurrence rates (5% versus 42%) and increased overall survival (97% versus 68%) [18].

Zhang and co-authors noticed that HER2+ patients have poor response to neoadjuvant chemotherapy with 5-fluorouracil, doxorubicin, cyclophosphamide (FAC) [17].

So, Transtuzumab, humanized anti-HER2 monoclonal antibody, is considered to be first-line treatment for the patients with HER positive Breast Cancer [2, 17].

The Ki67 index also plays an important role in assessing the need for NAC performance. Some authors have evaluated the higher efficacy of NAC in cases with high Ki67 [15].

NAC cannot modify the molecular subtype of the tumor. Changing the status of receptors after neoadjuvant chemotherapy does not show any change in the cellular origin of the tumor [24].

The histological grade of the CNB specimen represents the significant predictors of chemotherapeutic response using the percentage of the area occupied by the tumor infiltrating lymphocytes (TILs), retraction artifact status, small cell-like feature status, level of tumor necrosis, and clear cytoplasm status [4].

The authors mention a directly proportional correlation. The higher grade of differentiation has the better response rate to NAC. Histologically low differentiated tumors have a lower response rate to NAC. The degree of pathomorphosis is the main indicator of the response to NAC. Usually the absence of response correlates directly with the first grade of pathomorphosis, while the fourth grade of pathomorphosis correlates directly with PCR [4, 15, 23].

The assessment of the prognosis depending on the changes of the tumor biomarkers serves an important criterion in establishing the subsequent medical conduct of this patient. The change in the status of hormone receptors after performing NAC, from negative to positive, is interpreted as a favorable indicator of disease prognosis. The change in Her2 status from positive to negative confirms the efficacy of NAC and good prognosis of the disease. The absence of response to NAC in cases of Her2-positive and triple-negative tumors serves as an unfavorable prognostic factor [20, 25].

Randomized prospective studies are needed to select a more balanced choice of patient characteristics and treatment schemes at the beginning and to evaluate the treatment response more appropriately.

Conclusions

1. The size of the breast primary tumor, the affection of the regional lymph nodes, the degree of tumor differentiation, the expression of hormone receptors, HER2neu, ki67 serve as main criteria for predicting the response to neoadjuvant chemotherapy.

2. Preoperative core needle biopsy (CNB) is the gold standard procedure in cancer diagnostics, in the analysis of predictive biomarkers, particularly utilizing histomorphological characteristics.

3. Carrying out a larger number of cycles of neoadjuvant chemotherapy as well as correlating the schemes in relation to the immunohistochemical types have a direct influence on obtaining a good response to treatment.

4. Patients with a PCR had superior survival outcomes compared with patients who had residual disease.

5. The standardization and improvement of methods to assess the response to induction chemotherapy are sorely needed.

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Diagnostic markers of urinary bladder tumors

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Abstract

Background: The perfect method for laboratory diagnosis of bladder cancer should have high sensitivity and specificity, should be easily reproducible, inexpensive, be suitable for primary diagnosis, screening, and follow-up of patients, for timely detection of recurrence. In clinical practice, for bladder cancer diagnostics have been used the following markers: UBC, BTA, "ImmunoCyt", NMP22, "UroVision», and others. Each method has relative advantages and disadvantages. The study has demonstrated an influence on the test result of the histological structure and grade of the tumor, presence of hematuria, urolithiasis, chronic inflammatory malignancies, recent surgical procedures on the urinary tract. Apparently, the use of a palette of markers in connection with imaging techniques will increase the diagnostic capabilities, but it is still not clear which elements should be present in such palette.
Conclusions: At present, basic diagnostic methods for bladder cancer remain: USG, MRI, CT, and endoscopic methods. The laboratory methods that exist are not informative enough. Each marker has serious restrictions, but possibly the complex application will allow increasing the diagnostic value in the future, therefore it is necessary to develop new markers of bladder cancer or to study the results of the complex application of several known markers to increase the value of the laboratory diagnosis of primary bladder cancer and recurrent.
Key words: bladder tumors, tumor markers.

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Introduction

Bladder cancer is an actual problem of modern oncourology, because of high rates of prevalence, recurrence, progression, and a major burden on the medical healthcare system and the economy. In 2015 incidence of bladder cancer ranked ninth and mortality thirteenth worldwide. It ranked the highest in high-sociodemographic index countries at position eighth for incidence and eleventh for cancer deaths [1]. Bladder cancer is divided into two main categories: non-muscle-invasive bladder cancer (NMIBC) which constitutes 75% and muscle-invasive bladder cancer (MIBC) which makes up 25% [2]. The perfect method of laboratory diagnosis of bladder cancer must be extremely sensitive and specific, easily reproducible, inexpensive, suitable for primary diagnosis, screening, and monitoring of patients, to detect recurrence on time [3].

Currently, the main diagnostic tool for the detection of bladder cancer remains cystoscopy, which is an effective but invasive method of diagnostics. Even after a flexible cystoscopy, we have pain during urination in 50% of cases, urinary frequency in 37%, visible hematuria at 19% of patients, and infection in 3% of cases [4]. Sensitivity and specificity of cystoscopy ranged from 62 to 84% and 43 to 98%, respectively, depending on the type, stage, and grading of the tumor, it has a low sensitivity for carcinoma in situ (CIS) [5]. Long-term observation thereby remains the keystone of long-term management, and cystoscopy for over 80 years remains the gold standard. However, cystoscopic access is economically expensive for the medical healthcare system and burdensome for patients, therefore, for decades, there has been a search for non-invasive urine biomarkers that can match or even improve the specificity and sensitivity of cystoscopy. However, current guidelines do not recommend the use of urinary biomarkers in the management of bladder cancer patients [6].

The use of urine-based biomarkers to detect bladder cancer seems to be an attractive alternative. Urinary biomarkers are in direct contact with the bladder and can come in a variety of forms, such as proteins, metabolites, deoxyribonucleic acid (DNA), different types of ribonucleic acid (RNA), and single nucleotide polymorphisms. The existence of variations in the expression of those molecules may be related to bladder cancer [7].

For the diagnosis of bladder tumors, cytological methods and different tumor markers are used. There are several classifications of tumor markers. According to the purpose of the research, markers are divided: which are used in the primary diagnosis and for the prognosis of recurrences, progression, and tumor metastasis [8]. Depending on the type

of material studied, the following markers are distinguished: urinary, serum, and tissue. The evaluation of markers in urine is of major clinical interest, because the given method is non-invasive, reduces the number of cystoscopes, and allows obtaining sufficient material for investigation [9].

However, in a lot of biomarker studies, we see displacement due to the severe and advanced cases of disease which probably rise apparent sensitivity (the percent of correctly identified cases) and inclusion of healthful volunteers which is probably to rise evident specificity (the percent of correctly identified controls), or the use of patients with big primary tumors when the aim is to discover slight recurrent tumors [10]. Another high pitfall in the measuring of urinary biomarkers for bladder cancer is haematuria: haematuria is a symptom and sign of bladder cancer but is not the biological reason for bladder cancer. Thereby, each protein represented in the blood can appear to act as a biomarker in case-control studies where haematuria is not matched, but will not be bladder cancer-specific [11].

Urinary cytology

Urine cytological investigation is the standard laboratory method for diagnosing bladder tumors with which other methods are compared. Cytology is used in clinical practice, it is a non-invasive method where voided or obtained with special instrument urine is examined for exfoliated cancer cells. We can mention as summary data that the diagnostics of cytology is not significant and constitutes in medium: specificity 40 – 44% and sensitivity 30 – 35%. There is a correlation between sensitivity and the degree of tumor differentiation: G1 – 13 – 16%; G2 – 31 – 36%; G3 – 70 – 84%, Tis – 92 – 94%, i.e. the more aggressive is the tumor, the greater is the possibility of detection, but to establish the diagnosis it is necessary to have a well-trained cytologist [12, 13].

Ajit D. et al. reported results of cytological investigations in 951 patients with bladder cancer, 1831 samples were performed. The histopathological examination of the bladder bioptate was performed as a control method. There were 173 false-negative and 6 false-positive results. The general specificity was 82% and the sensitivity – 96%. The main cause of false-negative results was related to high tumor differentiation, when the sensitivity of the method is lower. False-positive results can be explained by changes related to chronic inflammation of the urothelium [14]. Another example: Lokeshwar V. et al. studied 690 patients with the single episode of macrohematuria. All patients underwent urethrocystoscopy, ultrasound scan (USG), urine insemination, blood analysis, and urine cytology. Results: general sensitivity was – 40.2%, specificity – 98.7%, positive predictive value – 81.4%. The authors signed that with the help of the cytological examination it was not possible to highlight formations that would not be diagnosed with routine methods [15].

In 2016 the Paris Working Group published the standardized system for reporting by category the diagnosis of urinary cytology, which was validated in several retrospective studies [16, 17]. The Paris system includes the following groups [18]:

- Adequacy of urine specimens (Adequacy);
- Negative for high-grade urothelial carcinoma (Negative);
- Atypical urothelial cells (AUC);
- Suspicious for high-grade urothelial carcinoma (Suspicious);
- High-grade urothelial carcinoma (HGUC);
- Low-grade urothelial neoplasia (LGUN).

Tumor markers

There are several markers that are used in the diagnosis of bladder cancer (Table 1). In clinical practice, the following testing systems have received the most widespread: UBC cancer antigen, BTA, NMP-22, UroVision, ImmunoCyt, CYFRA 21-1, CK 20, and others.

Bladder cancer antigen (UBC) is a soluble fragment of cytokeratins 8 and 18 (intermediate microfilaments of epithelial cells). With the active proliferation and malignant cell transformation, cytokeratin expression increases [19]. The discriminant level is 32 μg/L. The sensitivity of the method is 60-78% for primary patients, the specificity can reach 95%. The correlation between the stage of the tumor

Table 1

Sensitivity and specificity of diagnostic tests for bladder cancer

Name of the test	Marker	Sensitivity %	Specificity %	Comments
Urinary cytology	Cytological examination of urine	40 - 44	30 - 35	Control method
UBC	Cytokeratin levels 8 and 18	54	97	Low sensitivity
BTA	Antigen, linked to urinary bladder cancer	50 - 80	50 - 75	Diagnostic significance decreases in the presence of urinary tract diseases
NMP-22	Nuclear matrix protein	50 - 90	70 - 85	Low sensitivity in invasive non-muscular tumors (50%) to invasive (90%), the high negative predictive value
ImmunoCyt	High molecular weight carcinoembryonic antigens and mucins	50 - 95	60 - 85	High sensitivity to well-differentiated tumors
UroVision	In situ fluorescence hybridization	70 - 100	66 - 93	Costly and time-consuming method
CYFRA 21.1	Cytokeratin levels 19	73	41	Low specificity
CK 20	Cytokeratin levels 20	85	76	
Survivin	Survivin levels	82	90	The costly and time-consuming analysis process

process and the proliferative activity of the tumor cells was observed. According to Todenhofer T. et al., who analyzed the results of the diagnosis of bladder cancer in 177 patients, at a discriminatory level of 12.3 ng/ml, the sensitivity of the method was 57.8% and the specificity – 66.7% [20]. For the bladder cancer antigen rapid test (UBC-rapid test), Ecke et al. in 2017 reported: sensitivity of 87% for detecting carcinoma in situ, 71% for high-grade non-muscle invasive bladder cancer, 60% for high-grade muscle invasive bladder cancer, and 30% for low-grade non-muscle invasive bladder cancer [21].

The bladder tumor antigen (BTA) is a single-chain protein, which is associated with human complement factor H (hCFHrg), with the property of a germ factor. BTA is determined in urine, discriminatory level 14 Un/ml. Leyh H. et al. studied 414 patients with invasive non-muscular tumors of the bladder. The sensitivity of the BTA test was 70%, specificity – 90%. A correlation was established between sensitivity and tumor degree of differentiation: an increase was marked in sensitivity from 17% in G1 to 64% in G2 and up to 92% in G3. The sensitivity of the method in recurrences was 67%. The sensitivity of the method also increases with increasing stage of the pathology: from 50% to 90%. For example, in stage Ta, the sensitivity of the BTA test was 53.8%, but in T1 – already 76% [22, 23].

The quantitative BTA (BTA TRAK®) test is performed in a specialized laboratory, whereas the qualitative BTA (BTA stat®) is a point-of-care test with an immediate result (Polymedco Inc., Cortlandt Manor, New York, USA). They have a sensitivity of 65% versus 64%, and a specificity of 74% versus 77%, respectively [24, 25]. However, the specificity of both of these tests is significantly decreased since false positives have been noted to occur due to the presence of human complement factor H-related protein in blood. Hematuria can be presented in different urological malignancies, such as urolithiasis, inflammation, recent use of instrumentation, other genitourinary malignancies, and intravesical Bacillus Calmette Guérin (BCG) therapy which causes local inflammation [24-27].

The European Association of Urology examines the diagnostic values of each of the proposed test systems. By combining the most preferred properties are considered: "ImmunoCyt", NMP-22, and "UroVision" [28].

Nuclear matrix protein 22 (NMP22) may be identified in urine as a biomarker of the death of the urothelial cells. This marker is often elevated in the urine of patients with bladder cancer and can thus be used in the finding of this disease. The NMP22®BladderChek® and NMP22®BC test kit is qualitative and quantitative enzyme immunoassay tests, respectively (originally Matritech Inc., Newton, MA, USA). The sensitivity of 69% and specificity of 77% quantitative NMP22 BC test kit is compared to a sensitivity of 58% and a specificity of 88% for the qualitative NMP22 test [25, 29]. However, are common false-positive results, because NMP22 is emitted from apoptotic cells which also occur in case of hematuria, infection, or inflammation [25, 30-32]. Its discriminatory level is 10 Un/ml. One of the benefits of the given test is a high negative predictive value. This marker is not widespread due to insufficient diagnostic value, but it is considered that its diagnostic role may be more significant when used in the palette of bladder cancer markers [33].

ImmunoCyt™/uCyt+™ is an immunocytochemical test that utilizes fluorescently marked antibodies that are guided against three antigens: two mucins which are specifically detected on malignant exfoliated urothelial cells and a glycosylated form of carcinoembryonic antigen [34]. This method has a high sensitivity in well-differentiated tumors and is less affected by concomitant inflammatory changes of the urinary tract, more preferably to be used in the primary diagnosis. Sensitivity is 50-95%, specificity – 60-85% [35]. The sensitivity of this test is higher than cytology, but the specificity is lower [36]. False positives are seen during infection or inflammation and there is poor sensitivity in T2 bladder cancers. Moreover, interobserver variability exists; trained cytopathologists are therefore necessary [37]. It is only approved for the surveillance of bladder cancer patients [38].

Widespread received the method for detecting chromosomal rearrangements using in situ fluorescence hybridization (FISH). FISH is a technique that uses fluorescently labeled deoxyribonucleic acid (DNA) probes to assess cells for genetic alterations [39]. Exfoliated urothelial cells are detected in voided urine, are hybridized on a slide. They are further examined for chromosomal aberrations which are found in bladder cancer: aneuploidy of chromosomes 3, 7, and 17, and a loss of locus 9p21 [38, 40]. In a meta-analysis, the specificity of the test was stated to be 83%, and the sensitivity to be 72% in the context of equivocal cytology [41]. Another recent meta-analysis of studies of UroVysion™ has calculated its sensitivity and specificity in detecting bladder cancer at 63% and 87%, respectively [39]. The lack of sensitivity for low-grade bladder cancers remains [42].

Jeong S. et al. analyzed the results of the CYFRA 21-1, NMP22, UBC and FDP tests in 250 patients. Of these, 54 were diagnosed with bladder cancer. The control group consisted of 196 patients with inflammatory diseases of the urinary tract, benign prostatic hyperplasia, hematuria of non-tumor etiology. The level of the studied markers was significantly higher in the study group than in the control group. The best results were observed with CYFRA 21-1 and NMP 22 [43].

Ludecke G. et al. investigated the influence of hematuria intensity on the level of UBC, NMP22, and BTA markers. As study material, they used freshly heparinized blood titrated in the urine of conventionally healthy people at different concentrations. The level of UBC and NMP22 did not increase at different intensities of macrohematuria. The BTA test showed the worst results: false-positive results were recorded in the presence of over 150 red blood cells in the visual field [44].

The combination of markers increases the diagnostic value compared to using each separate marker. Todenhöfer T. et al. studied the results of the application: urine cytological examination, FISH, ImmunoCyt, and NMP22. Diagnostic data from 808 primary care patients and 505 patients with non-invasive muscle bladder cancer recurrence

were analyzed. The complex application of these markers has demonstrated a high negative predictive value, which potentially makes it possible to use them as an additional control method between programmed cystoscopes [45].

Table 1 presents generalized data on sensitivity and specificity of different markers compared to cytological examination of urine.

If the main goal is to avoid unnecessary cystoscopies, rather than looking for markers with high sensitivity and specificity, the focus should be on identifying a marker with a very high negative predictive value. A test capable of predicting the absence of the tumor will be of great use in daily clinical practice [46]. Promising new urinary biomarkers, which evaluate several targets, have been tested in multicenter prospective studies with a very high negative predictive value [47, 48]. More studies are needed to obtain truthful information about diagnostic markers of bladder tumors for their implementation and use in daily practice.

Conclusions

It should be noted that currently the basic methods, routine for primary diagnosis and clarification of bladder cancer, remain: ultrasound scan (USG), magnetic resonance imaging (MRI), computed tomography (CT), and endoscopic methods (cystoscopy in white light, fluorescence, narrowband imaging, and others). The laboratory methods that exist are not informative enough, each method has relative advantages and disadvantages. Each marker has serious restrictions, but possibly the complex application will allow increasing the diagnostic value in the future. Apparently, the use of a palette of markers in connection with imaging techniques will increase the diagnostic capabilities, but it is still not clear which elements should be present in such palette.

Improving the diagnosis of bladder cancer is possible by combining the efforts of oncologists, urologists, morphologists, geneticists, and molecular biologists. Thus, the present study confirmed that it is necessary to develop new markers of bladder cancer or to study the results of the complex application of several known markers to increase the value of the laboratory diagnosis of primary bladder cancer and recurrent.

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IV, AP, AC designed the trial and drafted the first manuscript; VG interpreted the data and revised the manuscript critically. The authors revised and approved the final version of the manuscript.

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Angioarchitecture of the major duodenal papilla and its relevance for endoscopic sphincterotomy

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Abstract

Background: The advantages of endoscopic retrograde cholangiopancreatography (ERCP) over traditional surgery for correction of various biliary and pancreatic pathologies became apparent immediately after its introduction into large clinical practice and today are also not in doubt. ERCP and endoscopic sphincterotomy (EST) are characterized by efficacy similar to open surgery, but significantly less traumatic, relatively easy, a decrease in the degree of perioperative surgical and anesthetic risk, and a reduction in the time of in-hospital treatment and postoperative recovery. However, therapeutic ERCP with EST can be complicated by gastrointestinal bleeding, the degree of which can range from mild to very severe and even life-threatening. Although the greatest risk for the development of bleeding after EST is caused by preexisting coagulopathy, the anatomical features of the arterial blood supply to the pancreaticoduodenal region and major duodenal papilla should also be taken into account during the endoscopic procedure.
Conclusions: The communicating artery, directly vascularizing the area of the major duodenal papilla, usually originates from the posterior superior pancreaticoduodenal artery, and entering in the anterior pancreaticoduodenal arcade. The smallest number of papillary arteries, distributed in potential accessibility to the sphincterotomy incision, are located in the zone between 10 and 11 o'clock of the papilla Vater circumference. Hence, the preferred performance of EST in this area can be accompanied by a significant reduction in the risk of arterial bleeding after ERCP.
Key words: major duodenal papilla, bleeding, arterial supply, endoscopic retrograde cholangiopancreatography, endoscopic sphincterotomy.

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Introduction

The advantages of endoscopic retrograde cholangiopancreatography (ERCP) over traditional surgery for correction of various biliary and pancreatic pathologies became apparent immediately after its introduction into large clinical practice and today are also not in doubt. ERCP and endoscopic sphincterotomy (EST) are characterized by efficacy similar to open surgery, but significantly less traumatic, relatively easy, a decrease in the degree of perioperative surgical and anesthetic risk, and a reduction in the time of in-hospital treatment and postoperative recovery. Unfortunately, both therapeutic and diagnostic ERCP can be accompanied by various and sometimes very serious complications, such as acute pancreatitis, bleeding, duodenal perforation, cholangitis, cholecystitis, and sepsis. However, the majority of gastrointestinal intraluminal hemorrhages are associated primarily with the performance of EST, and not diagnostic ERCP [1].

There are several classifications of bleeding after ERCP / EST based on time of the onset (immediate or delayed), endoscopic and clinical significance, and severity [2]. Immediate or intraprocedural bleeding is defined by most authors as an episode of hemorrhage that occurs at the time of EST, i.e. during or just after the electrosurgical tissue incision is performed [2, 3, 4]. Delayed bleeding after EST is considered any bleeding that occurs after completion of ERCP, manifested as melena, hematemesis or hematochezia, with a decrease in hemoglobin level from the baseline [2]. Delayed bleeding can become evident from few hours to several days [1, 2, 4] and even 2-3 weeks [3, 5] after the initial EST.

The incidence of EST-related bleeding ranges from 2% to 3% [5], but in some studies it reaches up to 48% [4]. A certain degree of immediate active bleeding (from oozing to pulsating) is observed during EST in 10-30% of cases [3, 4]. The overall prevalence of delayed bleeding after EST is between 4% and 10% [4].

Risk factors for the post-EST bleeding have been divided traditionally into patient-related, anatomy-related, and technical-related to endoscopic therapeutic procedure [5]. Although the greatest danger for the development of bleed-

ing after EST are factors associated with the patient's comorbidities, primarily coagulopathy (liver cirrhosis, thrombocytopenia, and the use of anticoagulants), anatomical factors also play a significant role.

In case of the correctly positioned duodenoscope in the second part of the duodenum and just below the papilla, biliary EST is performed by stepwise incisions oriented between 11 and 1 o'clock [6, 7]. The length of the sphincterotomy is variable, but usually between 1 and 1.5 cm and not extending beyond the intramural segment of the bile duct. This direction and length of incision correspond to the anatomical location of biliary sphincter and the lumen of overlying common bile duct. The segment of the papilla circumference between 11 and 1 o'clock has even been designated by some authors as a "safety zone" referring to duodenal perforation [8]. However, patients with anatomical variants of the papillary vessels may be at increased risk of post-EST bleeding [9].

Arterial blood supply to the pancreaticoduodenal zone

It is well known that the main blood supply to the pancreaticoduodenal region is provided by anterior and posterior arterial pancreaticoduodenal arcades. Further vascular configuration of the pancreaticoduodenal region is comprised of a series of the marginal vessels, and their *vasa recta* [10]. In an anatomical study, W. Kimura found that the artery in the direction of the papilla Vater branches from the posterior superior pancreaticoduodenal artery and then passes along the right side of the common bile duct [11]. He did not find any other such large artery going in the direction of the major duodenal papilla. Therefore, the author considered this artery to be the main and extremely important for the blood supply to the papilla Vater and the distal part of the common bile duct (fig. 1).

Later, the communicating arteries that directly vascularize the region of major duodenal papilla were thoroughly studied and classified by H. Yamaguchi into two types [12]. The first type, or "typical" communicating artery usually originates from the posterior superior pancreaticoduodenal artery, runs posterior to the common bile duct, then passes anteriorly between the main and accessory pancreatic ducts before terminating in the anterior pancreaticoduodenal arcade. The communicant artery of the second type runs in a similar postero-anterior direction, but inferior to the main pancreatic duct.

A total of 70 of the nearly 100 papillary arteries studied by the author arose directly or indirectly from communicating arteries, with 50 directly from a "typical" artery. The posterior pancreaticoduodenal arcade was the source of 26 papillary arteries, and the anterior pancreaticoduodenal arcade – of only two. The average external diameter of the papillary arteries ranged from 0.75 mm to 0.6 mm [12]. Thus, dorsal-ventral blood flow along the communicating arteries is dominant. In other words, the major duodenal papilla is located on the "dorsal vascular territory" of the pancreatic head [12].

The assumption about predominantly "posterior" direction of blood supply to the papilla region was confirmed in the studies of W. Kimura [11] and H. Furukawa [13]. The latter established an angiographic border in the pancreatic head between the arterial areas of the celiac trunk and the superior mesenteric artery. As expected, the border between two sources of arterial blood supply, on the whole, corresponded to the embryonic line of junction of the ventral and dorsal pancreas buds. The main pancreatic duct runs along the border of two vascular areas, while the common bile duct with a major duodenal papilla belonged to the celiac arterial territory [13].

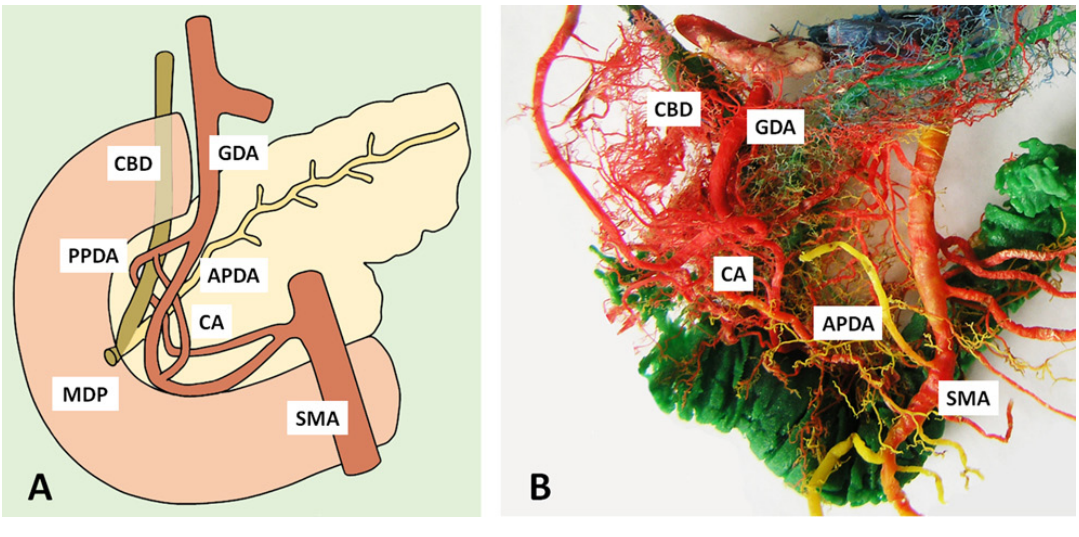

Fig. 1. Arterial supply of the pancreaticoduodenal region and the zone of the major duodenal papilla

(A) The communicating artery, which directly supplies with blood the major duodenal papilla, as well as other main arteries of the pancreaticoduodenal zone (GDA – gastroduodenal artery, SMA – superior mesenteric artery, APDA – anterior pancreaticoduodenal arcade, PPDA – posterior pancreaticoduodenal arcade, CA – communicating artery, CBD – common bile duct, MDP – major duodenal papilla).

(B) Polychromatic vascular corrosion cast of a fresh cadaver pancreaticoduodenal specimen demonstrating the pancreaticoduodenal arterial arcades and a typical communicating artery from which papillary arteries originate.

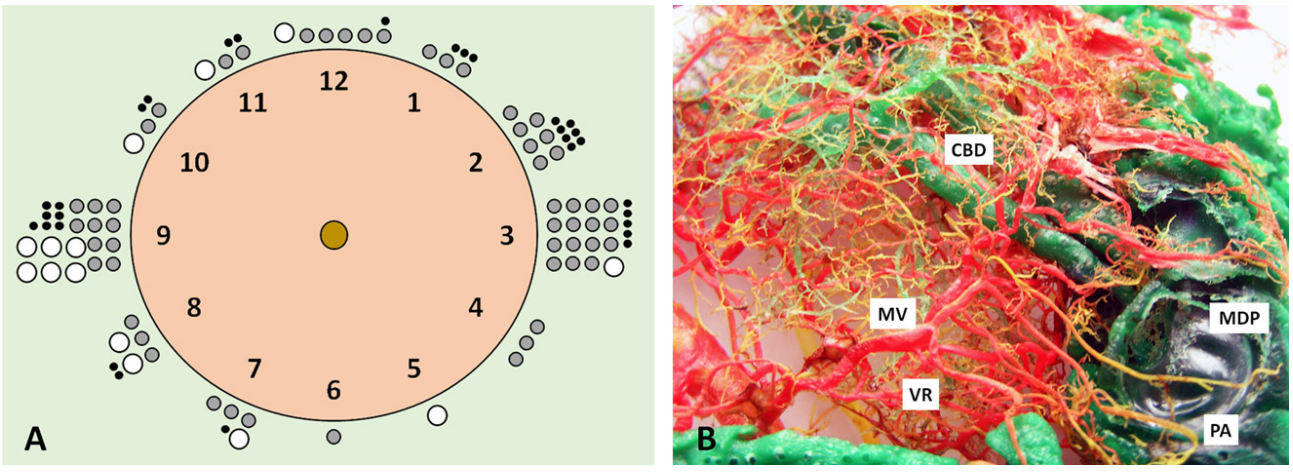

Fig. 2. Distribution of the papillary arteries

(A) Distribution of papillary arteries entering within 5 mm of the major duodenal papilla in typical endoscopic orientation, seen from the duodenal lumen during ERCP. Arterial diameters: <0.50 mm (small black dots); 0.51-0.75mm (medium gray dots); and 0.76-1.0 mm (large white dots) (Adapted with permission from Mirjalili SA, Stringer MD. The arterial supply of the major duodenal papilla and its relevance to endoscopic sphincterotomy. Endoscopy. 2011 Apr;43(4):307-311 [16]).

(B) Polychromatic vascular corrosion cast of the peripapillary zone including the terminal portion of common bile duct (CBD) and major duodenal papilla (MDP), showing the marginal vessels (MV), their *vasa recta* (VR), and a number of papillary arteries (PA).

The origin of arterial branches supplying the papilla Vater zone, mainly from the posterior pancreaticoduodenal artery, was also noted in clinical studies described selective endovascular embolization to arrest gastroduodenal bleeding [14, 15].

Papillary arteries

A noteworthy original study by S. A. Mirjalili and M. D. Stringer was addressed to arterial blood supply directly to the major duodenal papilla [16]. It should be noted right away that the study has two limitations. First, received by the authors findings are related only to arterial bleeding after EST. Although arterial bleeding is potentially more serious, damage to the veins surrounding papilla during intervention with the further venous bleeding is also possible. Second, specimens of the pancreaticoduodenal complex were harvested *en bloc* from 26 cadavers, none of which had known pancreaticobiliary or duodenal diseases. At the same time, inflammatory or neoplastic pathological conditions can change the normal anatomical relationships, including the topography of arteries in the papilla Vater region.

The study of S. A. Mirjalili and M. D. Stringer documents for the first time the distribution of papillary arteries around the circumference of the major duodenal papilla. Generally, there is no such anatomical definition as a "papillary artery". However, this study meant papillary arteries that could be damaged during therapeutic ERCP. They included two options: (1) An artery that penetrates the duodenal wall within 5 mm from the circumference of the entrance of the common bile duct and main pancreatic duct; or (2) an artery that penetrates the wall of the common bile duct or main pancreatic duct within 5 mm of their insertion into the duodenal wall [16].

The results of the study suggest that most of the papillary arteries are located in the region at 3 and 9 o'clock (fig. 2). The data that the area between 10 to 12 o'clock contained only about 15% of the arteries are of the greatest theoretical and practical importance, and if the segments of 10 and 11 o'clock are considered together, then only 10% of the papillary arteries were located here. Moreover, in almost half of observations, there were no arteries in this area at all [16].

Thus, a decreasing risk of arterial bleeding after ERCP can be achieved by performing EST preferentially in the area corresponding to 10-11 o'clock of the papilla circumference [1, 6, 16].

Conclusions

Therapeutic ERCP with EST can be complicated by gastrointestinal bleeding of various degree, which can range from mild to very severe and even life-threatening. Although the greatest risk for the EST-related bleeding have patients with preexisting coagulopathy, the anatomical features of the arterial blood supply to the pancreaticoduodenal region and major duodenal papilla should also be considered during the endoscopic procedure. The communicating artery, directly vascularizing the region of the major duodenal papilla, usually originates from the posterior superior pancreaticoduodenal artery and ends in the anterior pancreaticoduodenal arcade. The smallest number of papillary arteries distributed in the potential accessibility of a sphincterotomy incision are located in the zone corresponding to 10-11 o'clock of the papilla circumference. As a result, the preferred performance of EST in described area can be associated with decreasing risk of arterial bleeding after ERCP.

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EP drafted the first version of the manuscript; SS collected data and examined the specimens; EG conceptualized the project and contributed to the final version of the manuscript. All the authors revised the manuscript critically and approved the final version of the manuscript.

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