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EARLY COMPLICATIONS AFTER RADICAL OPERATIONS IN BREAST CANCER PATIENTS

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Abstract

Breast cancer is one of the most common malignancies in women. In many cases, a major component of complex treatment for breast cancer is surgery – radical mastectomy or radical breast resection.

The aim of the work – to investigate the frequency and structure of complications after radical surgery with dissection of axillary lymph nodes in breast cancer patients.

Material and methods. The baseline and surgical results of 147 women with breast cancer who underwent radical mastectomy or radical breast resection with lymph node dissection were analysed.

Results. Complications in the early period after surgery for breast cancer were found in 76 (51.7 %), including postoperative extremity edema in 60 (40.8 %); lymphorrhea – in 37 (25.2 %), seroma – in 33 (22.4 %); wound infection in 18 (12.2 %), necrosis of the wound edges – in 15 (10.2 %) patients. Correlation of postoperative edema with almost all other complications was found, lymphorrhea and seroma were most associated with swelling and with each other; necrosis of edges with postoperative edema. Wound infection was significantly associated with lymphorrhea. Patients' age, stage of disease, and immunohistochemical type of tumour did not affect the development of complications. With increasing BMI, the incidence of complications increased significantly (χ^2=9.530; p=0.009). The tendency to decrease the frequency of complications during reconstructive surgery was revealed (42.6 % versus 58.1 %, p=0.064), and adjuvant radiotherapy, on the contrary, contributed to the increase of complications (57.8 % versus 43.8 %, p=0.090).

Conclusion. Radical operations with lymph node dissection in patients with breast cancer are characterized by a high frequency of early postoperative complications, mainly associated with disorders of lymphatic outflow, which indicates the need for a set of measures of preoperative preparation, improvement of surgical technique.

Keywords: breast cancer, radical surgery, lymph node dissection, complications.

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1. Introduction

Breast cancer is one of the most common malignancies in women [1, 2]. In many cases, a major component of complex treatment for breast cancer is surgery – radical mastectomy (RME) or radical breast resection (RBR) [3]. In addition to extensive tissue removal of the mammary gland, radical surgery is facilitated by lymph node dissection (LND) in the areas of potential metastasis, which leads to damage to the elements of lymphatic outflow and is one of the leading causes of complications of early and late postoperative period [4, 5]. In the early period after surgery, the most common complications are the formation of lymphocele (seroma), lymphorrhea, postoperative edema, wound infections and impaired wound healing [6, 7], whose frequency increases during reconstructive operations especially using autoplastic methods [8]. The most common complication is seroma, the frequency of which reaches 85 %. Their development is associated both with the features of surgical interventions, and with the individual characteristics of patients [9]. No less relevant are wound complications, which not only worsen the cosmetic results of the operation, but also increase the cost of treatment [10].

These complications lead to an increase in the duration and cost of treatment, may cause other post-mastectomy complications and impairment of quality of life [11, 12]. Therefore, it is very important to study the factors contributing to the development of postoperative complications and to develop measures for their prevention.

The aim of the work – to investigate the frequency and structure of complications after radical surgery with axillary lymph nodes dissection in breast cancer patients.

2. Material and methods

We analysed the baseline condition and results of surgical treatment of women with breast cancer who underwent radical surgery (RME or RBR with LND) from 2010 to 2019 at the Kharkiv Regional Oncology Center or at Medical Center "Molecule" (Kharkiv) and met the following criteria: histologically confirmed breast cancer, operable tumour without distant metastases (M0), presence of results of clinical, instrumental and immunohistochemical (IHC) research, in prospective study - consent of the patient to participate in the study and processing of personal data.

The study design was considered by Ethics Committee of the Kharkiv Medical Academy of Postgraduate Education at the planning stage of the study and found to be in conformity with the principles of the Helsinki Declaration of General Assembly of the World Medical Association (1964–2000), the Council of Europe Convention on Human Rights and Biomedicine (1997), the relevant provisions of WHO, the International Council of Medical Scientific Societies, the International Code medical ethics (1983) and the laws of Ukraine.

The final analysis included 147 women, middle-aged (49.1±11.6) years (from 26 years to 82 years), including those aged up to 39 years – 32 (21.8 %), 40–49 years – 46 (31.3 %), 50–59 years – 42 (28.6 %), more than 59 years – 27 (18.4 %). Overweight (body mass index (BMI) – 25–39 kg/m^2) was found in 49 (33.3 %) patients, obesity (BMI from 30 kg/m^2) in 10 (6.8 %) cases.

I stage of breast cancer was diagnosed in 31 (21.1 %) patients; IIA – in 46 (31.1 %); IIB – in 23 (15.6 %); IIIA – 21 (14.3 %); IIIB – in 20 (13.6 %); IIIC – in 6 (4.1 %). In 81 (55.1 %) patients the left breast was affected, in most cases the process was localized in the central part of the breast – 54 (36.7 %) and in the upper-outer quadrant – 53 (36.1 %). Less frequently in the upper-inner quadrant – 19 (12.9 %), lower-outer – 10 (6.8 %), lower-internal – 9 (6.1 %), in two or more quadrants – 2 (1.4 %). By IHC type: luminal A – 53 (36.1 %), luminal B – 19 (12.9 %), HER2+ – 9 (6.1 %), three-negative BC (TNBC) – 66 (44.9 %). The degree of histological differentiation: G1 – 21 (14.3 %), G2 – 57 (38.8 %), G3 – 69 (46.9 %).

All patients received treatment according to current clinical guidelines [3]. In 113 (76.9 %) patients performed RME, in 34 (23.1 %) RBR. In all patients performed LND 2–3 order. Reconstructive surgery was performed in 61 (41.5 %) patients, including 3 (2.0 %) primary allograft using a silicone implant, 52 (35.4 %) implanting a silicone prosthesis after dermotension using expander, in 6 (4.1 %) cases – two-stage prosthesis of the breast a year or more after RME.

Chemotherapy (CT) was received 139 (94.6 %) patients, while in the neoadjuvant mode (NCT) – 49 (33.3 %) patients; hormone therapy – 17 (11.6 %) women (12 of them after the course of CT). 83 (56.5 %) women received adjuvant radiotherapy.

The frequency and pattern of postoperative complications in the entire sample of patients were analyzed. The main focus is on the complications most characteristic of the surgery for the BC: seroma (fluid accumulation in the surgical area after removal of drainage), lymphorrhea (serous discharge from the drainage more than 100 ml in the first day after surgery, then more than 50 ml for more than 3 days), postoperative edema (the difference of the circumference of the shoulder above the elbow joint between the upper extremities up to 2 cm – 1 st., 2–6 cm – II st., more than 6 cm – III st.) and wound complications (infection and necrosis of the wound edges).

The obtained results were processed with statistical programs PSSP (open program, which does not require a license) with the use of methods of descriptive statistics, criterion χ^2, correlation analysis according to Spearman criterion.

3. Results

Complications in the early period after breast surgery were detected in 76 (51.7±5.2 %). The most common complication was postoperative extremity edema, which was observed in 60 (40.8±4.6 %) patients, of whom I st. – 23 (38.3±7.1 %), II st. – 33 (55.0±8.5 %) and III st. – 4 (6.7±2.9 %). Intensive and/or prolonged lymphorrhea was observed in 37 (25.2±3.7 %) patients, seroma (lymphocele) in 33 (22.4±3.5 %). Wound infection was detected in 18 (12.2±2.5 %) patients, wound necrosis (WN) in 15 (10.2±2.3 %).

Most complications of the early postoperative period were associated: in 24 (16.3±2.9 %) patients there were one complication, in 53 (36.1±4.4 %) patients a combination of two or more complications was found.

The correlation of complications is illustrated by the results of correlation analysis (**Table 1**).

It was revealed strong correlation of postoperative edema with almost all other complications, first of all, with the manifestations of lympho-venous outflow disorders: lymphorrhea (r_S=0.546; p<0.001); seroma (r_S=0.525; p<0.001). In a lesser extent, but also significantly, this complication was correlated with the development of wound necrosis (r_S=0.311; p<0.001). Lymphorrhea and seroma were most associated with edema (r_S=0.546; p<0.001 and r_S=0.525; p<0.001) and with each other (r_S=0.239; p =0.004); wound necrosis with postoperative extremity edema (r_S=0.311; p<0.001). Wound infection was significantly associated with lymphorrhea only (r_S=0.167; p=0.043).

Table 1

Correlation analysis of surgery complications for breast cancer

Complications	Lymphorrhea	Postoperative extremity edema	Seroma	Wound infection	Wound necrosis
Lymphorrhea	–	0.546** <0.001	0.239** 0.004	0.167* 0.043	0.115 0.165
Postoperative extremity edema	0.546** <0.001	–	0.525** <0.001	0.122 0.140	0.311** <0.001
Seroma	0.239** 0.004	0.525** <0.001	–	0.152 =0.067	0.043 0.605
Wound infection	0.167* 0.043	0.122 0.140	0.152 =0.067	–	0.109 0.188
Wound necrosis	0.115 0.165	0.311** <0.001	0.043 0.605	0.109 0.188	–

*Note: * – Spearman correlation coefficient (r_S), p<0.05; ** – Spearman correlation coefficient (r_S), p<0.001*

The foregoing testifies to the common pathogenesis of postoperative complications, which are mainly associated with disorders of lymphatic outflow due to extensive LND. However, the development of postoperative edema can also be a manifestation of inflammatory-infectious processes in the wound area and a consequence of the inflammatory reaction in the necrosis of the wound edges.

A very important issue is the general background that contributes to the development of complications, i.e. the initial risk factors. The most available indicators for a long observation period were epidemiological data, features of the underlying disease and treatment modalities (**Tables 2, 3**).

Number of complications not depended from patients' age (χ^2=2.169; p=0.538), stage of the disease (χ^2=1.886; p=0.868), and IHC type of the tumour (χ^2=2.446; p=0.485). Nevertheless, there is some increase in the incidence of complications with Her2 + (66.7 %) and TNBC (56.1 %) compared with luminal A (47.2 %) and luminal B (42.1 %) types. Significant differences were found in the analysis of the frequency of complications depending on BMI: with increasing BMI, the frequency of complications significantly increased (χ^2=9.530; p=0.009) (**Table 2**).

In addition, the frequency of complications was affected by treatment modalities (**Table 3**).

Table 2

Number of patients with complications after breast cancer depending of baseline data

Indicator	Number – n (%)		χ^2	p
	No complications (n=71)	With complications (n=76)		
Age groups:				
30–39 y. (n=32)	19 (59.4 %)	13 (40.6 %)		
40–49 y. (n=46)	20 (43.5 %)	26 (56.5 %)	2.169	0.538
50–59 y. (n=42)	20 (47.6 %)	22 (52.4 %)		
>59 y. (n=27)	12 (44.4 %)	15 (55.6 %)		
BMI:				
<25 kg/m^2	50 (56.8 %)	38 (43.2 %)	9.530	0.009
25–29.9 kg/m^2	20 (40.8 %)	29 (59.2 %)		
≥30 kg/m^2	1 (10.0 %)	9 (9 (90.0 %)		
IHC type:				
Luminal A	28 (52.8 %)	25 (47.2 %)		
Luminal B	11 (57.9 %)	8 (42.1 %)	2.446	0.485
Her2+	3 (33.3 %)	6 (66.7 %)		
TNBC	29 (43.9 %)	37 (56.1 %)		
Stage:				
I	16 (22.5 %)	15 (19.7 %)		
IIA	24 (33.8 %)	22 (28.9 %)		
IIB	9 (12.7 %)	14 (18.4 %)	1.886	0.865
IIIA	11 (15.5 %)	10 (13.2 %)		
IIIB	8 (11.3 %)	12 (15.8 %)		
IIIC	3 (4.2 %)	3 (3.9 %)		

Note: BMI – body mass index; IHC – immunohistochemistry; TNBC – three-negative breast cancer; p – by the criterion χ^2

Table 3

Dependence of the incidence of postoperative complications depending on the method of treatment

Method of treatment	Number – n (%)		χ^2	p
	No complications (n=71)	With complications (n=76)		
Type of operation:				
RME (n=113)	59 (52.2 %)	54 (47.8 %)	2.996	0.083
RBR (n=34)	12 (35.3 %)	22 (64.7 %)		
Reconstruction:				
No (n=86)	36 (41.9 %)	50 (58.1 %)	3.441	0.064
Yes (n=61)	35 (57.4 %)	26 (42.6 %)		
Reconstruction method:				
primary implant (n=3)	2 (66.7 %)	1 (33.3 %)	5.423	0.143
Expander implant (n=52)	28 (53.8 %)	24 (46.2 %)		
delayed implant (n=6)	5 (83.3 %)	1 (16.7 %)		
Adjuvant radiotherapy:				
No (n=64)	36 (56.3 %)	28 (43.8 %)	2.869	0.090
Yes (n=83)	35 (42.2 %)	48 (57.8 %)		
NCT:				
No (n=98)	44 (44.9 %)	54 (55.1 %)	1.362	0.243
Yes (n=49)	27 (55.1 %)	22 (44.9 %)		

Note: RME – radical mastectomy; RBR – radical breast resection; NCT – neo-adjuvant chemotherapy

No significant differences were found depending on the treatment modality, although there were some patterns. First of all, increase of complications rate in RBR compared to RME was found (64.7 % vs. 47.8 %, p=0.083), but it should be noted that, regardless of the volume of surgery, all patients had LND in similar volume. Performing reconstructive surgery reduced the incidence of complications (42.6 % versus 58.1 %, p=0.064), with the smallest number of complications observed during the breast reconstruction a year or more after the initial operation. In patients with NCT, there was a slight decrease of complications rate (44.9 % versus 55.1 %, p=0.243), and adjuvant radiotherapy, on the contrary, contributed to increase of complications rate (57.8 % versus 43.8 %, p=0.090).

4. Discussion

In our study, which combines retrospective and prospective analysis of the results of surgical interventions for breast cancer, complications were found in 51.7 % of patients. The high frequency of complications in our observations can be explained by the lymphatic node dissection, which was performed in all cases. In similar observations. A. Lucci et al. (2007) after RME with LND, adverse surgical events (wound infection, seroma, paresthesia) were reported in 70 %. [13]. M. O. Abass et al (2018) reported about complications in 42 % of women after similar surgery [6].

In the structure of complications in our study, the leading place is occupied by postoperative extremity edema, which was found in 40.8 % of patients. The occurrence of edema of the ipsilateral limb within a month after surgery is more often regarded not as a complication but as a manifestation of other complications or as a normal course of the postoperative period. In our study, postoperative edema was most commonly associated with other complications and was observed in 60.0 % of patients with the presence of wound infection, in 80.0 % of patients with necrosis of the wound edges, in 86.5 % of patients with lymphorrhea, in 84.8 % of patients with seroma. However, in 10.8 % of patients postoperative edema was observed in the absence of other complications, indicating other pathogenetic mechanisms of its development.

Quite often in the postoperative period, lymphorrhea (25.2 %) and seroma (22.4 %) were detected. Most authors attribute these complications to LND, and the frequency reported by other researchers is very variable. M.O. Abass et al (2018) reported what seroma after RME with LND was detected in 15.6 % of cases [6]. In another study, the rate of seroma was dependent from the use of a hemostasis device: 37.5 % of patients were using standard electrosurgical device, while using a new electrosurgical device (PEAK PlasmaBlade) – in 10 % [14]. Srivastava V. et al. reported that the seroma rate come to 85 % and can be considered not a complication but a side effect of the operation [9].

The incidence of wound complications (wound infections and necrosis of the wound edges) was almost indistinguishable from similar studies in other studies [6]. Their development depends on many factors, so the statistics are very variable. In particular, according to a recent review of clinical studies, wound infections after mastectomy were detected in 3–15 % of cases [15]. Researchers from Poland report that infectious complications were detected in 6.2 % of patients, and after alloplastic reconstruction in 14.6 % of cases. [16]. Risk factors for surgical site infections was BMI greater than 25, American Society of Anesthesiology classification of 3 or higher, diabetes mellitus, surgical time 2 hours and greater, and current smoking status [17].

Another line of research to this problem is to study the effectiveness of prevention methods. Patient-specific complication risk factors are not modifiable, therefore a variety of methods for improving surgery techniques are proposed, including advanced hemostasis devices [14.18], optimization of LND technique, ligation of lymph vessels, methods of wound drainage [19]. However, the evidence base for the effectiveness of these methods is limited [20]. Thus, the problem of prevention of complications after radical surgery needs further research.

Study limitations. This study combines the results of a retrospective analysis and a prospective study. The main emphasis is on the clinical and pathological features of patients and methods of surgical treatment. The features of preoperative preparation, the experience of the surgeon, and postoperative treatment were not analysed. However, these indicators may affect the immediate results of surgical treatment.

Perspective of further research. Further studies of the problem of postoperative complications are needed to identify modifiable risk factors and the development of methods to reduce the risk of these complications.

5. Conclusion

Thus, radical breast surgery with LND in patients with breast cancer is characterized by high incidence of early postoperative complications, mainly associated with disorders of lymphatic outflow. Analysis of the incidence of complications based on baseline demographic and clinical data revealed a reliable association with BMI only. There were no reliable associations with therapies, but there was a marked increase in the incidence of complications after RBR, adjuvant radiotherapy, and their decrease after reconstructive surgery and NCT. Although LND leads to the development of these disorders, it remains a necessary element of radical surgery, in particular, with N-positive status, T3-4, TNBC, which indicates the need for a complex preoperative preparation, improvement of surgical equipment and postoperative management of patients.

Conflict of interest

No conflict of interest.

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LIVER ABSCESSES: A 10-YEAR VINNYTSYA UNIVERSITY STUDY

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Abstract

According to MEDLINE database there were about 1278 papers on liver abscess published in a period from 2001 to 2015.

The aim of the study is to improve liver abscess treatment results comparing minimally invasive and traditional operative techniques.

Materials and methods. 137 patients were included in the study and divided on two comparison groups. Traditional methods were used for the treatment of 66 participants of the control group (48.2 %). For 71 patients (51.8 %) of the general group the mini-invasive drainages were predominating.

Results. Cholangiogenic causes of liver abscesses were found in 41 patients (29.93±3.91 %), cryptogenic ones – in 37 (27.01± ±3.79 %), haematogenous causes – in 29 (21.17±3.49 %), contact ones – in 16 (11.68±2.75 %), posttraumatic ones – in 11 (8.03±2.32 %) and purulent distraction of metastases – in 3 (2.19±1.25 %).

Single abscesses occurred more often – in 117 (85.40±3.02 %), multiple once – in 20 (14.60±3.02 %). Mostly 3, 6 and 7 liver segments were damaged – 19 (13.88±2.95 %), 35 (25.55±3.73 %), 44 (32.12±3.99 %).

In control group, the abscess drainage via laparotomy was performed on 58 patients (87.88±4.02 % of 66 ones) versus 21 (29.58±5.42 % of 71 ones) in general group. Percutaneous drainage was used in 8 (12.12±4.02 %) and in 44 (61.97±5.76 %) cases respectively. 6 or 8.45±3.30 % laparoscopic interventions were used only in the general group. Finally, mini-invasive drainages were applied in the greater part of general group - 50 (70.42±5.42 %) versus 8 ones (12.12±4.02 %) in control group.

Conclusions. Minimally invasive liver abscess drainages showed a significant reduction of postoperative complications from 24.24±5.27 % in the control group to 12.66±3.95 % in the general group, shortening of hospital terms from 14.6±1 in control to 5.2± ±0.8 days and decreasing of mortality from 7.58±3.26 % to 2.82±1.96 %.

Keywords: liver abscess, mini-invasive surgery, laparoscopic liver surgery, drainage of liver abscess, puncture of liver abscess.

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1. Introduction

In recent years, the amount of liver abscesses (LA) has been significantly increasing [1, 2]. It has obviously been demonstrated in numerous studies.

According to MEDLINE database in a 2001–2015 period there were 1278 documents published on LA. Scopus search has shown the similar results – 1303 records [3].

LA is the most frequent abdominal organs suppuration with an incidence from 5 to 20 in 100000 hospitalizations [3, 4]. Increasing of LA is connected with innovative diagnostic capabilities in medicine, such as ultrasound, computerized tomography and magnetic resonance imaging [5, 6]. The incidence of gallstone disease and its complications have also been growing [7, 8]. The LA reasons include portal vein and biliary retrograde infection, sepsis and other cryptogenious suppurations [9]. At the same time, authors explain the increasing of LA because of constantly increase of hepatobiliary mini-invasive surgical procedures [10, 11]. Nowadays, at the same time

there are changes in the sensitivity of LA microflora to antibacterial and antiseptic drugs and presence of many multiresistant germs [1, 10].

Mortality rate among the patients with LA is between 4.5 % to 31 %, but it is decreasing thanks to modern and timely diagnostic efficacy methods, effective antimicrobial agents and mini-invasive surgical procedures with ultrasound or computerized tomography guidance [12, 13]. In this article, we are presenting our 10-year treatment experience of LA.

The aim of the work. To improve treatment results of patients with liver abscesses admitted to Vinnytsya regional hospital comparing mini-invasive methods and traditional operative techniques.

2. Materials and methods

This study was conducted at National Pirogov Memorial Medical University and Regional Clinical Hospital named after N. I. Pirogov, Vinnytsia, Ukraine from January 2008 to December 2018.

All 137 hospitalized patients with a confirmed LA diagnosis (based on clinical, laboratory-biochemical, radiological and instrumental presentations) were divided into two groups (general – 71 persons, operated from 2014 to 2018, and control – 66 ones, operated from 2008 to 2013). 66 patients of control group (48.2 %) were treated using traditional methods such as external drainage, fenestration, pericystectomy, segmental liver resection, hemigepatectomy. Among 71 patients (51.8 %) of general group, preference was given to mini-invasive surgical interventions. Invasive procedures included aspiration, percutaneous drainage and surgical interventions. Both groups were representative by gender, age, causes, localization of LA and women were dominating. The age of the patients ranged from 26 to 83 years, with the median age 59 years old.

Before entering the patient in the study protocol, a written voluntary agreement to participate in this study was obtained in accordance with the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. The study protocol was approved by the Local Ethics Committee (LEC) of the Vinnytsya National Pirogov Memorial Medical University, protocol №8, dated 05.10.2017).

Most often, the disease occurred in middle-aged people and elderly (81.6 %) ones. Females accounted for 1.39 times more than males. Moreover, in the age over 60 years the incidence of LA among women was 1.2 times more frequent than among men. After 75 years, the LA incidence was approximately the same in both genders.

The duration of the disease, ranging from the appearance of the first clinical signs to the admission at a surgical hospital lasted from 4 days to 7 months. Patients with a disease period from 7 days to 1 month were predominant (62.1 % and 63.4 % in both groups). The 7-month period was established in 1 patient whose disease was recurrent.

Concomitant pathology was equally common in both groups and was presented in 117 (85.4 %) patients. Almost a half of patients had a combination of several diseases. Undoubtedly, comorbidities had a negative impact on the course of the essential disease.

3. Results

The study of the LA causes showed that cholangiogenic causes of liver abscesses were found in 41 patients (29.93±3.91 %), cryptogenic ones – in 37 (27.01±3.79 %), hematogenous causes – in 29 (21.17±3.49 %), contact ones – in 16 (11.68±2.75 %), posttraumatic ones – in 11 (8.03±2.32 %) and purulent distraction of metastases – in 3 (2.19±1.25 %). The majority of patients with LA presented fever – 100 %, abdominal pain under the right rib arch 92.70±2.22 %, chills – 31.39±3.96 %, decrease appetite – 46.72±4.26 %, weight loss – 62.77±4.13 %, jaundice – 16.79±3.19 % cases.

Laboratory tests showed leukocytosis with shifting to the left, moderate anemia, increasing of SRI, hypoproteinemia, hyperbilirubinemia, increasing of liver enzymes as well as hypercoagulation.

The general condition of 87 (63.5 %) patients was severe – 54.74±4.25 % or extremely severe – 8.76±2.42 %. The rest 50 patients (36.5 %) had the medium degree of severity – 29.20±3.88 % or satisfactory condition – 7.30±2.22 %.

LA were grouped as small ones (≤5 cm, n=57 or 41.61±4.21 %), large abscesses (5 cm to 10 cm, n=72 or 52.55±4.27 %) and giant abscesses (>10 cm, n=8 or 5.84±2.00 %). The bigger size was taken into account in case of irregular LA border.

As a rule, LA affected almost all liver segments, though, the 3rd (13.88±2.95 %), 6th (25.55± ±3.73 %) and 7th segments (32.12±3.99 %) were more often affected. 20 patients (14.60±3.02 %) suffered from multiple LA in both liver lobes.

In both groups the volume of LA was from 10 to 200 ml. All patients were operated on as it is presented in **Table 1**.

Table 1

The kinds of interventions in patients with LA

No.	Laparotomy open approaches			Percutaneous drainage			Laparoscopic approaches		
	Kind of intervention	Control group	General group	Kind of intervention	Control group	General group	Kind of intervention	Control group	General group
1	External drainage	48	7	Punction needle aspiration	–	6	Punction needle aspiration	–	1
2	Fenestration	5	5	Percutaneous drainage	8	38	External drainage	–	3
3	Pericystectomy	2	4		–	–	Fenestration	–	2
4	Resection of multiple segments with abscesses	2	3		–	–		–	–
5	Hemigepatectomy	1	2		–	–		–	–
	N=137	58	21		8	44		–	6

In control group, the operation of liver abscess drainage via laparotomy was performed in 58 cases (87.88±4.02 % of 66 patients) versus 21 (29.58±5.42 % of 71 patients) in the general group. Percutaneous drainage of abscesses – 8 (12.12±4.02 %) and 44 (61.97±5.76 %) respectively. Laparoscopic operations 6 or 8.45±3.30 % took place only in the general group and included external percutaneous drainage in 3 or 4.23±2.39 % cases, fenestration – in 2 or 2.82±1.41 %, puncture-needle aspiration in 1 or 1.41±1.40 %.

The complications were presented with suppuration of postoperative wound – 4 (6.06± ±2.94 %) in control group versus 1 (1,41±1.40 %) in general group, exudative pleuritis on the right – 4 (6.06±2.94 %) versus 3 (4,23±2.39 %), bile leakage – 2 (3.03±2.11 %) versus 2 (2.82± ±1.41 %), pneumonia – 4 (6.06±2.94 %) versus 2 (2.82±1.41 %), early adhesive intestinal obstruction – 2 (3.03±2.11 %) versus 1 (1,41±1.40 %) respectively. The length of hospital stay was also reduced from 14.6±1 in control to 5.2±0.8 days in general group ($p<0.05$).

7 (5.1 %) patients died – 5 or 7.58±3.26 % in control and 2 or 2.82±1.96 % in general group ($p<0.05$).

4. Discussion

Nowadays there are no well established guidelines for treatment of patients with LA. According to recent LA studies, different authors present different treatment approaches. Recent articles have offered puncture needle aspiration as a first-line approach because it is simple, comfortable and cheap [8, 14]. Ultrasound-guided needle aspiration of LA followed by pipe drainage is safe and shows good treatment results [15, 16]. In our opinion, percutaneous drainage is a more effective and curable method for LA, particularly when its sizes exceed 5 cm in diameter. It has been proved by some authors who reported that being larger than 5 cm LA had a higher rate of curative affec-

tion if it is treated with antibiotics only [17]. Other scientists wrote that bigger part of their patients with LA (47 %) were subjected to a percutaneous drainage or were treated using open drainage (34 %) [18]. Recent research (2016) has reported about conservative antibiotics treatment of patients with LA in 28 % cases, 39.3 % patients received percutaneous drainage and 32.7 % underwent surgical incision and drainage [9]. Other publication also said about antibiotic therapy alone for the treatment of multiple hepatic abscesses [19]. Instead, our results have shown no one patient with only antibiotics, the amount of LA percutaneous drainage reached 62 % with decreasing of open surgical operations. Our mind is divided by many authors who supported the fact that only aggressive operative techniques should be received as more effective ones [12, 20]. We want to emphasize that using of laparoscopic approaches for LA in our study showed good results without postoperative complications or mortality.

The limitations of the research. The study included all patients older than 18 years old with the diagnosis of LA from January 2008 to December 2018. The diagnosis of LA confirmed through ultrasound investigation and clinical picture.

The further research plan includes the study of widening the indications for percutaneous drainage with ultrasound and CT support to develop effective guidelines for LA.

5. Conclusions

The minimally invasive surgical drainage techniques in patients with LA significantly reduced ($p<0.05$) the number of postoperative complications from 24.24±5.27 % in the control group to 12.66±3.95 % in the general group, shortening of inpatient terms from 14.6±1 in the control group to 5.2±0.8 days in the general group and decreasing of mortality rate from 7.58±3.26 % to 2.82±1.96 % respectively.

The using of laparoscopic approaches for LA showed good results without postoperative complications or mortality.

Conflict of interest

There is no conflict of interest.

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PHENOTYPICAL CHARACTERISTICS OF THE BIOLOGICAL PROPERTIES OF STAPHYLOCOCCI WITHDRAWN FROM PATIENTS WITH ALLERGIC DERMATITIS

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Abstract

Atopic dermatitis, eczema, allergic dermatitis occupy the main place among dermatoses, where the allergic component is leading in the onset and development of the disease. The most common complication of allergic dermatitis is the attachment of a secondary pyococcus infection, which is associated with a decrease in the antimicrobial resistance of the skin surface. Therapy of infectious lesions is complicated by the increasing resistance of the main pathogens of pyoderma – Staphylococcus aureus and Staphylococcus epidermidis – to widely used antibiotics.

The aim of the research: to determine the phenotypic features of staphylococci extracted from patients with allergic dermatitis to assess their pathogenic potential.

Materials and methods. The object of the study was 369 staphylococcus isolates removed from affected and intact skin sections of patients with allergic dermatitis, as well as from representative skin sections of healthy individuals undergoing inpatient treatment at the Department of Dermatology of "Institute of Dermatology and Venereology of NAMS of Ukraine". Biochemical identification and biological properties of staphylococci were determined using methods of classical bacteriology.

Results. As a result of the conducted researches, it is established that the complex of phenotypic traits of the removed staphylococcus cultures indicates the presence in the pathogen of factors related to the resistance of the host protection mechanisms and determines the intensity of the alterative action of the infectant in relation to the host organism, the phenotypic manifestation of the studied factors was higher in the staphylococcus isolates removed from the affected skin areas of patients with allergic dermatitis.

Conclusions. The level and frequency of phenotypic expression of pathogenicity factors are more pronounced in microorganisms obtained from patients from affected and intact areas compared to controls, which confirms their pathogenetic role in the burden of the disease, which in turn can be used as an auxiliary differential diagnosis criterion.

Keywords: clinical strains of staphylococci, allergic dermatitis, infectious complications, pathogenicity factors.

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1. Introduction

Atopic dermatitis, eczema, allergic dermatitis occupy the main place among dermatoses, where the allergic component is leading in the onset and development of the disease. Attention to these diseases is due to the high incidence; constantly recurrent course; increasing the number of trigger factors; insufficient effectiveness of traditional therapy [1].

According to a number of authors, about a tenth of the world's population is ill with eczema. In industrialized countries, this figure reaches 32–45 %, accounting for 51 % of all allergic dermatitis in some regions. According to various authors, the incidence of eczema in Ukraine is from 8 to 30 % of all skin diseases and is the most common pathology in the practice of a dermato-

venereologist [2]. According to the World Health Organization and national literature, the number of eczema patients has increased significantly in the last decade [1]. The eczema is characterized by the presence of acute inflammatory symptoms: intense itching of the skin, recurrent course, and increased sensitivity of patients to various exogenous and endogenous stimuli, tendency to spread and deepen the process. The most common complication of the eczematous process is the attachment of secondary urinary and fungal infections, which is associated with a decrease in antimicrobial resistance of the skin surface [3]. Treatment of infectious lesions is complicated by the increasing resistance of the main pathogens of pyoderma – *Staphylococcus aureus* and *Staphylococcus epidermidis*. Uncontrolled use of external antimicrobials, the sensitivity of which is lost, delays the process of rehabilitation of the infection and promotes the subsequent selection of resistant flora [4].

In atopic dermatitis (AD) also the course of the disease is often complicated by the adherence to a secondary infection. This feature reflects the anti-infective protection disorders inherent in patients with hypertension. In the case of *S. aureus* infection, the adverse effect of the microorganism is provided by T-lymphocyte-activating superantigens, stimulating the secretion of pro-inflammatory cytokines, and activating IgE antibody formation, which leads to the degranulation of caudal cells and the release of biologically active substances. In general, in addition to the classical scheme of appearance of IgE in the case of IgE-mediated AD, there is another mechanism of stimulation of its formation. This mechanism is associated with the action of superantigens, high molecular weight proteins, which include some antigens of bacterial or viral origin [5, 6]. Some studies have shown that in patients with AD whose skin was colonized with *S. aureus* containing the superantigen genes, the mean score on the AD severity scale – SCORAD – was significantly higher than in patients whose skin was colonized with *S. aureus* isolates without superantigen genes [7].

Taking into account the above data on the negative effect of *S. aureus* on the course of blood pressure, it should be noted that although it is not a commensal skin, but it is known that the place of its vegetation may be nasal passages, from which шт 20 % of the population the microorganism is extracted, and its translocation to the skin may occur [8, 9].

In addition, in recent years, a great deal of attention has been paid to the study of microbiocenosis and penetration of microorganisms into the skin in patients with chronic allergic skin diseases. The skin microbiota is considered as part of a meta-organism that includes a macro-organism and a set of all microorganisms - symbionts. This implies the existence of complex relationships between the microbiota and the human body, and, first, with its immune system, which not only regulates the interaction of the organism with the microbiota, but also itself formed under its influence [10, 11]. At the same time, when investigating the microbiome of the skin of patients with allergic dermatitis, saprophytic species are often removed: *S. epidermidis, S. haemolyticus, S. saprophyticus, S. capitis, S. warneri, S. hominis, S. simulans* and *S. cohnii* harmful effects on the human body, but these properties are inherent in these microorganisms provided vegetation on healthy skin. However, it is often not possible to draw a clear line between saprophytes and pathogens that inhabit healthy human skin. Normal microflora play an important role in protecting the body against pathogenic microorganisms, although it may itself be a contributor to infectious disease [12, 13]. However, it is known that pathogens of coagulase-negative staphylococci such as proteases, hemolysins, lipases, etc. provide storage of these microorganisms on the damaged skin [7, 13]. That is why the study of the biological properties of coagulase-negative staphylococci with a definition of their pathogenic potential is a necessary diagnostic measure for the rational treatment of infectious complications of allergic dermatitis.

The aim of the research: to determine the phenotypic features of staphylococci extracted from patients with allergic dermatitis to assess their pathogenic potential.

2. Materials and methods

The object of the study were 369 isolates of staphylococci, removed from affected and intact areas of patients' skin for allergic dermatitis, as well as from representative areas of skin of healthy individuals undergoing inpatient treatment at the Department of Dermatology of "Institute of Dermatology and Venereology of NAMS of Ukraine" in 2017–2019 years. Biochemical identification

and biological properties of staphylococci were determined using bacterioscopic and bacteriological methods of investigation [14–16].

Statistical processing of the results was performed using the software package for Microsoft Excel 2003. The arithmetic mean values for a series of data (M) and the error of averages (m) were calculated. The reliability of the data obtained was evaluated by pairwise comparison and determination of the confidence interval based on the Student's t-factor calculation (t). The differences were considered statistically significant at $p<0.05$. The degree of correlation was estimated using Pearson correlation analysis (R) to determine statistical significance (p). If the value of the correlation coefficient R per module is closer to 1, then it means that there is a strong connection, and if closer to 0 – the connection is weak or missing.

3. Results of the research

As a result of the analysis of bacteriological studies, comparative data were obtained regarding the staphylococcal component of the skin microbiocenosis of 114 patients with allergic dermatitis and 20 practically healthy persons. From these groups, 386 strains of microorganisms (346 strains from patients and 40 strains from practically healthy persons) were isolated, which were assigned to 4 genera: *Staphylococcus, Streptococcus, Micrococcus and Corynebacterium spp.*, with predominance of varieties of staphylococci – 369 strains (95.6 %), which were removed both individually and in interspecies and intraspecific associations (predominantly).

In the beginning, the composition of the skin microbiota of patients with atopic dermatitis, eczema, and a group of practically healthy individuals was analyzed. In **Fig. 1, 2** presents the ratio of the most common types of staphylococci in the skin biotopes of patients with allergic dermatitis.

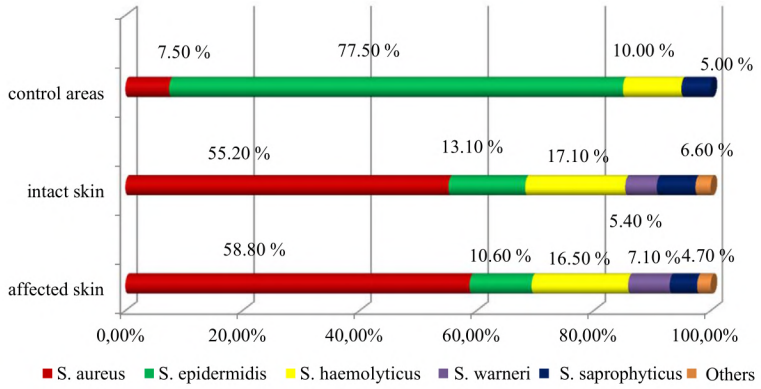

Fig. 1. Determination of the composition of staphylococcal component of the skin biotope of patients with AD and healthy individuals

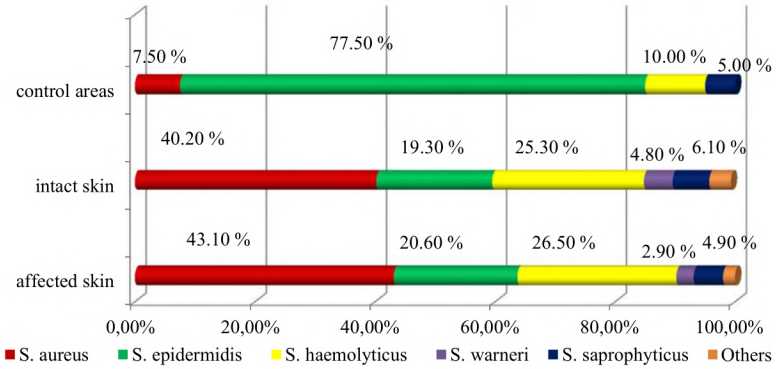

Fig. 2. Determination of the composition of staphylococcal component of the skin biotope of eczema patients and healthy individuals

In the next phase of the study, the obtained data from pathogenic staphylococcal cultures were analyzed to determine the enzymatic activity of staphylococcus cultures (**Table 1**).

Table 1

Enzymatic activity of laboratory strains of staphylococci extracted from patients with allergic dermatitis

Staphylococcus type	Presence of activity			
	haemolytic	lipolytic	proteolytic	
			Fermentation of milk	Gelatin fermentation
	n, abs. (M±m, %)	n, abs. (M±m, %)	n, abs. (M±m, %)	n, abs. (M±m, %)
S. haemolyticus	75 (100 %)	62 (82.7±4.4 %)	37 (49.3 ±5.8 %)	18 (24.0±4.9 %)
S. epidermidis	31 (55.46.6± %)	43 (76.8±5.6 %)	41 (73.2 ±5.9 %)	11 (19.6±5.3 %)
S. saprophyticus	9 (47.411.5± %)	7 (36.8 %)	3 (15.8 %)	1 (5.2 %)
S. warneri	6 (35.3 %)	8 (47.1 %)	4 (23.5 %)	2 (11.8 %)
Total	121 (72.5±3.5 %)	120 (71.9±3.5 %)	85 (50.9±3.9 %)	32 (19.2±3.0 %)
S. aureus	170 (100 %)	127 (74.7±3.3 %)	162 (95.3±1.6 %)	21 (12.3±2.5 %)

The next stage of the study was to investigate the antibiotic resistance of the extracted laboratory strains of *S. aureus* to antibacterial preparations of different chemical groups, as the most pathogenic representative of the genus, to identify such antibacterial agents that led to the most complete elimination of the pathogen. The obtained data are shown in **Fig. 3.**

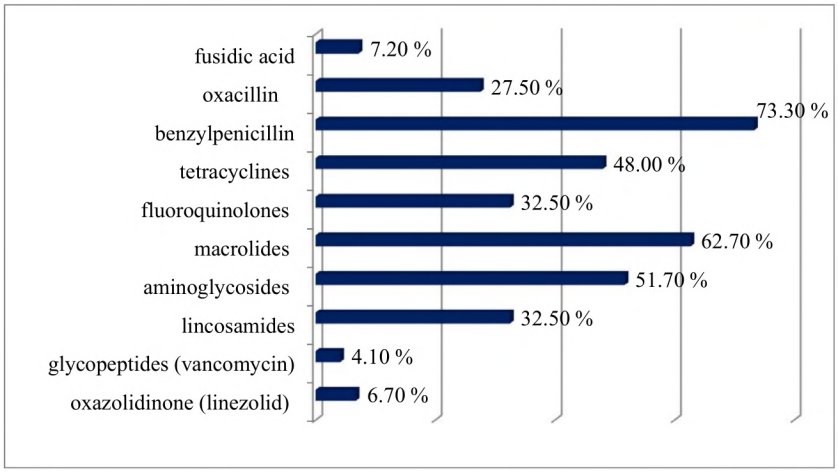

Fig. 3. Resistance rates of *S. aureus* strains isolated from patients with allergic dermatitis

4. Discussion

Staphylococci – representatives of normal microflora of the skin, mucous membranes of the respiratory and digestive tract, but they can cause severe infectious complications in patients with allergic dermatitis. The phenomenon of competitive relationships between *S. aureus* and *S. epidermidis* is also described in the literature. Substances that produce *S. epidermidis* (autoinducers) are known to block the formation of toxins in many strains of *S. aureus*, whereas enzymes synthesized by *S. aureus* do not inhibit the proliferation of *S. epidermidis* [13, 17].

According to the research results, the bacterial flora in persons with allergic dermatitis was significantly different from the microflora of healthy people and had its own characteristics, due to the formation of new microbial associations, which changed the habitat and the relationship between the associates. The study of microbial constituents of biotopes showed the dominance of microorganisms of the genus *Staphylococcus* in the skin cells of both patients and healthy individ-

uals. The difference was observed in the species composition of staphylococci and the degree of insemination of individual lesions. The appearance of non-resident staphylococcus species with higher pathogenic potential in affected and intact skin areas was a distinctive feature for most patients (**Fig. 1, 2**). As can be seen from **Fig. 1**, lesions of *S. aureus* strains (58.8 %) were observed in the lesions of the affected skin with a gradual decrease in the areas of intact skin and skin of healthy individuals (55.2 % and 7.5 %, respectively). The number of *S. epidermidis* strains found was inversely related to *S. aureus* strains (10.6 %, 13.1 %, and 77.5 %, respectively). No correlation relationships were found for *S. haemolyticus* strains depending on the extraction site.

As can be seen from **Fig. 2**, the prevalence of *S. aureus* strains (43.1 %) was observed in the lesions of the affected skin of eczema patients with a gradual decrease in the areas of intact and healthy skin (40.2 % and 7.5 %, respectively). The number of found *S. epidermidis* strains was inversely related to *S. aureus* strains (20.6 %, 19.3 %, and 77.5 %, respectively). The density of colonization in the centers of damaged skin of patients with allergic dermatitis averaged 10^7–10^8 CFU/ml, in intact areas and skin of healthy individuals – 10^3–10^5 CFU/ml.

The study of the biological properties of staphylococci showed that all isolates had typical morphology (gram-positive cocci) and biochemistry, in the catalase test – catalase-positive, all the extracted *S. aureus* strains had coagulase activity.

Haemolytic activity (β-type) was possessed by 72.5±3.5 % of coagulase-negative staphylococci, of which 55.4±6.6 % of *S. epidermidis* cultures (**Table 1**). Lipolytic activity was found in 71.9±3.5 % of coagulase-negative staphylococci, more frequently in *S. haemolyticus* (82.7±4.4 %) and *S. epidermidis* (76.8±5.6 %). When determining this trait in *S. aureus* cultures, it was found that 74.7±3.3 % of the strains were positive in this test. It is important to note that lipolytic activity was more commonly observed in strains of *staphylococci* extracted from affected lesions of patients with allergic dermatitis than from intact or control areas.

Staphylococci with proteolytic activity are more aggressive and, in the body, cause toxic tissue damage [11]. In the study of proteolytic activity, it was noted that about half of the coagulase-negative staphylococcus cultures fermented milk more often (50.9±3.9 %), with *S. epidermidis* strains making up 73.2±5.9 % and less frequently diluting gelatine (19.2±3.0 %), regardless of the locus of crop selection. A similar trend was observed among laboratory isolates of *S. aureus*: most cultures fermented milk (95.3±1.6 %) and infrequently diluted gelatine (12.3±2.5 %).

A direct statistically significant relationship was established in *S. haemolyticus* and *S. aureus* between haemolytic activity and lipolytic activity (R=0.99, p=0.0001 and R=0.9, p=0.0001, respectively), and between haemolytic activity and fermentation of milk of designated varieties of staphylococci – R=0.95, p=0 and R=0.99, p=0.0001, respectively. For *S. epidermidis* cultures, these values were slightly lower – between haemolytic activity and lipolytic activity (R=0.46, p=0.01) and between hemolytic and fermentation of milk – R=0.49, p=0.04. The obtained data indicate the importance of individual pathogens of these microorganisms both in the development and maintenance of the inflammatory process of infectious genesis, and of the combined influence of these factors at a certain stage of the infectious process.

In determining the resistance of *S. aureus* to antibacterial preparations of different chemical groups (**Fig. 3**), the detection of 73.3 % of penicillin-resistant strains draws attention, with 27.5 % of them being so-called MRSA strains, which makes it impossible prescribing to the patient any β-lactam antibiotics. Isolated strains showed moderate sensitivity to aminoglycosides, lincosamides and tetracyclines – ranging from 49 % to 52 %, and quite low to macrolides – up to 37.3 %. Also noteworthy is the appearance in the structure of antibiotic resistance of vancomycin-resistant strains – 4.1 %, which indicates an increase in the aggressive potential of the pathogens obtained. The sensitivity of the extracted pathogens was highest for the preparations of fusidic acid, oxazolidinone and fluoroquinolones – 92.8 %, 93.3 % and 67.5 % of the strains, respectively. It is known that the use of one class of antibiotics may increase the risk of developing resistance to another class, so at the next stage of the study, a comprehensive assessment of the resistance of the extracted strains of *S. aureus* was made, taking into account the prevalence of polyresistant strains. Based on the monitoring, 54 % of MDR strains and 4 % of XDR strains were detected. Significant is the complete absence of PDR strains, i.e. microorganisms resistant to all classes of known antibiotics.

Study limitations. The study included adult patients with allergic dermatitis diagnosed with atopic dermatitis (L 20.0) and eczema (L 30.0 – other dermatitis) according to the International Classification of Diseases 10.

Prospects for further research. Further studies include the study of correlations between phenotypic and genotypic traits of extracted pathogen isolates in order to develop algorithms for predicting the severity of marked allergic dermatitis.

5. Conclusions

Thus, the complex of phenotypic features of the removed staphylococcus cultures indicates the presence in the pathogen of factors associated with the resistance of host protection mechanisms, on the one hand, and high pathogenic potential, on the other, which promotes active colonization of both affected and intact areas of the skin, which provides the conditions for long-term persistence and determines the intensity of the alterative action of the infectious agent against the host organism.

The level and frequency of phenotypic expression of pathogenicity factors are more pronounced in microorganisms obtained from patients from affected and intact areas compared to controls, which confirms their pathogenetic role in the burden of the disease, which in turn can be used as an auxiliary differential diagnosis criterion.

Conflict of interests

There is no conflict of interest.

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DYNAMICS OF FUNCTIONAL CONDITION AND QUALITY OF LIFE IN PATIENTS WITH ASTHMA-COPD OVERLAP AND CONCOMITANT ARTERIAL HYPERTENSION AGAINST THE BACKGROUND OF COMPLEX THERAPY

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Abstract

Patients with ACO have significant poorer health-related quality of life and more severe functional limitations compared to asthma and COPD alone. Most commonly, chronic respiratory disease is associated with cardiovascular disease, such as arterial hypertension. However, the impact of concomitant cardiac diseases on the quality of life and functional status of patients with ACO remains poorly understood.

The aim of the work was to study dynamics of functional condition and quality of life in with ACO and concomitant AH against the background of complex therapy.

Materials and methods. We selected for participating in the study 100 patients with ACO and concomitant AH. Examination of the patients included: clinical methods, spirometry, and questinaries – mMRS, CAT, SGRQ, performing 6MWT.

Results. After 16 weeks of treatment there were no changes in lung functional status in patients on standard treatment, at the same time, in group of patients who had an active rehabilitation program, there was a significant improvement in the bronchial response to the action of bronchodilators, although other indicators of the functional status of the lungs didn't show significant changes. Patients

who additionally used an active rehabilitation program had a significant improvement in clinical symptoms, shortness of breath, and quality of life according to CAT, mMRC, and SGRQ scores, respectively. There was also a significant increase in distance during the 6MWT in this group of patients.

Conclusions. Conducting an active rehabilitation program (physical rehabilitation in combination with an educational program and self-management) in group of patients with ACO and concomitant AH, who are on standard medical treatment, significantly improves the bronchial response to the action of bronchodilators, decreases clinical manifestations, shortness of breath and improve quality of life and exercise tolerance, according to CAT, mMRC, SGRQ and 6MWT questionnaires, respectively.

Keywords: asthma-COPD overlap, arterial hypertension, functional status, quality of life, rahabilitation.

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1. Introduction

Chronic respiratory diseases are a serious problem of modern medicine, due to their widespread prevalence, high rates of mortality; also, it is a leading cause of disability and medical costs [1]. The most common respiratory diseases are chronic obstructive pulmonary disease (COPD) and asthma [2].

However, a combination of symptoms of asthma and COPD is observed in many of patients, especially those over 40 years of age with a history of concomitant smoking. According to various authors, the proportion of such patients ranges from 15 to 55 % [3]. For clinical practice, in 2014 Global Initiative for Asthma (GINA) and Global Initiative for COPD (GOLD) guideline [4] suggested the syndrome approach and the term asthma-COPD overlap syndrome (ACOS), later changed to Asthma-COPD Overlap (ACO) in GINA 2017 Recommendations [3].

Most commonly, chronic respiratory disease is associated with cardiovascular disease, such as arterial hypertension (AH). According to the literature, the incidence of combination of AH with chronic respiratory disease ranges from 4 to 27 % [5].

ACO patients have significant respiratory symptoms, poor quality of life, frequent exacerbations that lead to more frequent medical visits, comorbidities, and increasing doses of medication compared to asthma and COPD [6, 7]. Studies have shown that patients with ACO have poorer health-related quality of life than patients with asthma or COPD alone [8].

Some studies have shown that patients with ACO have functional limitations based on such functional tests as the 6-minute walk test (6MWT) and BODE index, which in turn have an important prognostic relationship with mortality and exacerbation rates [9].

However, these studies excluded patients with comorbid conditions, so the impact of concomitant cardiac diseases on the quality of life and functional status of patients with ACO remains poorly understood.

Aim of the research – to investigate dynamics of functional condition and quality of life in with ACO and concomitant AH against the background of complex therapy.

2. Materials and methods

The study was performed on the basis of the pulmonology department of the Municipal Non-commercial Enterprise «City Clinical Hospital No. 13» of Kharkiv City Council (Kharkiv, Ukraine) during 2018–2019. We selected for participating in the study 100 patients (48 men and 52 women) with ACO and concomitant AH.

Inclusion criteria for the study: age 45–65 years (54.38±4.57 years; the presence of ACO according to the criteria recommended by the joint GINA and GOLD document on diagnosis of bronchial obstructive diseases from 2017 [3], the criteria of the American Thorax Society (ATS) [10] and the criteria of the Spanish recommendations for bronchopulmonary diseases [11].

The diagnosis was based on the presence of all 3 major criteria and at least 1 minor criterion. The major criteria included: permanent airflow obstruction, age of the patient ≥40 years, smoking>10 years or the equivalent of air pollution, as well as documented history of asthma up to 40 years or bronchial obstruction reversibility (BOR)>400 ml; minor criteria: a documented history of atopy or allergic rhinitis, 2 separate BOR tests >12 % or 200 ml, and a blood eosinophil count

of ≥300/μl. We selected 2 degree by GOLD (50–80 %) of airflow obstruction. Patients with stage II controlled AH were included in the study, whose diagnosis was determined according to the commonly accepted classification of blood pressure (BP) levels (as recommended by ESH/ESC) [12].

Exclusion criteria were obesity, diabetes mellitus, chronic infectious, systemic, oncological and psychiatric diseases, chronic heart failure with a left ventricular ejection fraction of less than 55 %, the presence of a history of myocardial infarction, stroke and signs of ischemic heart disease, heart valve regurgitation more than 2 degree.

Examination of the patients included: clinical methods – analysis of complaints and medical history of patients, standard clinical examination with measurement of BP and heart rate (HR), anthropometric study with determination of body mass index (BMI), spirometry with BOR with inhalation of 400 mcg of salbutamol using the computer system "SPIROLAB" (manufactured by KhAI-Medika Research Institute in Kharkiv) according to the recommendations of ATS/ERS [13]. Shortness of breath was assessed by the Modified Medical Research Council Dyspnoea Scale (mMRS) [14], a comprehensive assessment of the severity of clinical symptoms was performed with COPD Assessment Test (CAT) [15], to assess the quality of life was used the questionnaire St. George's Respiratory Questionnaire (SGRQ) [16]. Functional status analysis included performing the 6MWT according to ATS recommendations [17].

The study was conducted in accordance with the requirements of the GCP, the Council of Europe Convention on Human Rights and Biomedicine, the Helsinki Declaration of the World Medical Association and was approved by a local ethics committee of Kharkov Medical Academy of Postgraduate Education (protocol No. 5, from 12 Nov 2019). All patients signed informed consent for participating in the clinical trial.

Patients were randomly divided into 2 groups of 50 patients who did not differ significantly in age, gender, smoking status, and BMI. All patients received triple therapy in stable dosage: inhaled corticosteroid, prolonged β2 agonist and prolonged anticholinergic drug according to GINA / GOLD recommendations [3]. In addition to basic therapy, patients in the first group were provided with a rehabilitation program, recommended by GOLD, which included: an educational program (smoking cessation, correct use of inhaler devices, early recognition of exacerbation, decision-making and taking action, and when to seek help), the formation of self-management skills (goals of motivating, engaging and supporting the patients to positively adapt their health behavior(s) and develop skills to better manage their disease), a program of complex physical rehabilitation (strength training and aerobic training, upper extremities exercise training, inspiratory muscle training and metered walking) [18]. At the baseline all patients were assessed for spirometry with BOR test, BP and HR measurment, exercise tolerance by using 6MWT. This program was monitored on a weekly visit. The re-evaluation was performed after 16 weeks of comprehensive treatment. The examination was carried out during a period of remission characterized by stable clinical symptoms and indicators of respiratory function.

The obtained data were processed using the Statistic application. Quantitative data are presented as mean values (M) and standard deviations (SD). To describe qualitative variation, we used the frequency at which the traits were assessed in the group. The critical significance level of statistical significance at the null hypothesis test was assumed to be 0.05. Parametric and non-parametric methods were used to compare the central parameters of the groups: Student's t-test, Wilcoxon tests (W) and Mann-Whitney (MU). The pairwise comparison of groups used the criterion U – Mann-Whitney (MU), to compare the occurrence of a feature in the frequency analysis in groups used the Pearson test χ-square (χ^2).

3. Results

The initial clinical and functional characteristics of the patients are presented in **Table 1**. The average age of the examined patients was 54.38±4.57 years. Patients had extensive smoking experience (29.82±6.22 packs) and were active smokers (70 patients) or smokers in the past (30 patients). All examined patients had a normal body weight (BMI of 25.15±2.12 kg/m^2). Patients in both groups did not differ significantly in sex, smoking status, BP, HR, and BMI.

Table 1
Clinical and functional characteristics of patients

Indicators	ACO with AH, n=100		P>0,05
	Group 1	Group 2	
	n=50	n=50	
Age, years	53.62±4.18	55.14±ᵒ.01	0.103
Gender, women/men	26(52 %)/24 (48 %)	25(50 %)/25 (50 %)	0.842
Smoking status, smokers/smokers in the past	34 (68 %)/16 (32 %)	36 (72 %)/14 (28 %)	0.663
Smoking experience, pack/years	30.42±6.08	28.86±4.98	0.163
BMI, kg/m^2	25.92±7.19	24.42±8.08	0.329
Systolic BP, mm Hg	136.16±9.15	134.84±10.12	0.496
Diastolic BP, mm Hg	82.43±8.48	85.36±10.95	0.138
HR, beat/min	73.22±9.73	75.46±7.57	0.202

There was also no discrepancy between the baseline condition of patients in both groups in spirometry results and quality of life.

A re-evaluation of spirometry after 16 weeks of treatment there were no changes in lung functional status in patients on standard treatment (group 2). At the same time, in the context of complex treatment in patients who had an active rehabilitation program (group 1), there was a significant improvement in the bronchial response to the action of bronchodilators (p=0.033), although other indicators of the functional status of the lungs did not show significant changes (**Tables 2, 3**).

Table 2
Assessment of spirometry, quality of life and functional status of patients against standard treatment and rehabilitation programs

Indicators	Group 1, n=50		p
	Baseline	After treatment	
FEV 1, % of predicted	64.12±13.82	68.38±14.83	0.140
FEV 1/ FVC, %	64.27±5.68	66.21±6.94	0.129
FEF 25, % of predicted	25.16±16.91	27.35±17.46	0.526
FEF 50, % of predicted	23.91±9.67	28.57±8.83	0.014
FEF 75, % of predicted	28.92±12.82	30.26 ±14.42	0.625
FVC, % of predicted	77.45±14.94	81.46±16.29	0.203
Reversibility after bronchodilator inhalation, %	18.05±5.63	20.12 ± 3.78	0.033
CAT, points	18.86±7.26	15.36±6.38	0.012
mMRC, points	2.23±0.62	1.84±0.86	0.018
SGRC, points	43.86±17.24	34.56±16.87	0.010
6MWT, meters	435.61±78.63	476.45±66.56	0.010

The quality of life scores varied significantly between groups. There were no significant changes in quality of life in standard-treated patients (Table 3). At the same time, patients who additionally used an active rehabilitation program had a significant improvement in clinical symptoms, shortness of breath, and quality of life according to CAT, mMRC, and SGRQ scores, respectively (**Table 2**). There was also a significant increase in distance during the 6MWT in Group 1.

Table 3

Assessment of spirometry, quality of life and functional status of patients against standard treatment in patients

Indicators	Group 2, n=50		p
	Baseline	After treatment	
FEV 1, % of predicted	63.96±12.62	64.21±14.55	0.927
FEV 1/ FVC, %	63.72±5.48	62.87±6.28	0.473
FEF 25, % of predicted	25.51±9.94	25.82±8.86	0.870
FEF 50, % of predicted	24.56±6.34	24.94±7.45	0.784
FEF 75, % of predicted	28.76±11.22	29.02±13.81	0.918
FVC, % of predicted	77.03±16.97	75.84±15.27	0.622
Reversibility after bronchodilator inhalation, %	17.56±6.23	17.64±5.27	0.945
CAT, points	19.06±8.04	18.89±7.25	0.912
mMRC, points	2.18±0.58	2.06±0.72	0.361
SGRC, points	40.68±14.83	39.74±11.28	0.722
6MWT, meters	427.87±68.45	435.84±70.26	0.567

4. Discussion

Patients with ACO have severe manifestations. The use of all medicines included in the standard of treatment of these group patients, unfortunately, have limited potential to influence the organic changes in the lungs, which determine the functional parameters of spirometry.

AH may have an additional negative effect on functional status and quality of life on this group of patients. The B. Ding and M. Small study showed a significantly higher rate of concomitant cardiac comorbidity, including AH in COPD and ACO patients, compared to asthma alone [19], the same results were obtained by DJ Maselli et al [20], but these studies did not investigate the influence of concomitant AH on the course of ACO regarding quality of life, functional status.

A number of studies have found significantly higher levels of symptoms and exacerbations, decreased quality of life [8, 20] and functional status of patients with ACO [9, 21, 22], however, studies have not analyzed the association of complaints, clinical, and functional status with concomitant pathology of patients.

Some studies have aimed to find ways that can improve the quality of life and functionality in patients with ACO on the background of basic drug therapy. A. Rodrigues et al, when investigating the effectiveness of a 12-week training program in patients with ACO compared patients with COPD, received improvements in pulmonary function, 6MWT, peripheral and inspiratory muscle strength, SGRQ, however, no significant difference was found in the results of the two groups, as well as differences in 6MWT and mMRC in the dynamics of treatment [23, 24]. The fatigue of our study showed that conducting an active rehabilitation program (physical rehabilitation in combination with an educational program) in patients with ACO with concomitant AH significantly improves the bronchial response to the action of bronchodilators by spirometry test, reduces the clinical manifestation and exercise tolerance, according to CAT, mMRC, SGRQ, and 6MWT questionnaires, respectively.

The limitations of the research. The present study was intended to investigate only the functional status and quality of life ACO patients with concomitant AH and did not include analysis of clinical and laboratory parameters for these patient groups, as well as the study of patients with other chronic respiratory diseases and variants of comorbidity.

The further research plan includes the study of clinical and laboratory status in patients with ACO with concomitant AH, analysis BP, HR, saturation rates in dynamics of complex therapy in ACO with AH group of patients.

5. Conclusions

Conducting an active rehabilitation program (physical rehabilitation in combination with an educational program and self-management) in group of patients with ACO and concomitant AH, who are on standard medical treatment, significantly improves the bronchial response to the action of bronchodilators, decreases clinical manifestations, shortness of breath and improve quality of life and exercise tolerance, according to CAT, mMRC, SGRQ and 6MWT questionnaires, respectively.

Conflicts of interest

No conflict of interest.

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PECULIARITIES OF STATE OF PROTECTION AND AGGRESSION FACTORS IN PATIENTS WITH DIABETES MELLITUS TYPE II AND GASTROESOPHAGEAL REFLUX DISEASE

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Abstract

Diabetes mellitus is one of the most serious problems of the clinical medicine. This is determined by the fact that it is followed by multisystemic affects, as well as complications on the side of other organs and systems, among which a special place is occupied by gastroesophageal reflux disease. As for the combination and mutual influence of diabetes mellitus and gastroesophageal reflux disease, this issue has not been studied yet, the data of modern literature are not complete and quite contradictory.

The aim of the study: to investigate the state of the factors of aggression and protection of the oesophageal mucosa in patients with diabetes mellitus type II with concomitant gastroesophageal reflux disease without associated pathology.

Method. There were two groups of patients under observation. The first group included 45 patients with diabetes mellitus type II with concomitant gastroesophageal reflux disease (26 men and 19 women). The second group included 38 patients with gastroesoph-

ageal reflux disease without associated pathology – 20 men and 18 women. By sex, age, body weight, Helicobacter pylori infection, smoking and alcohol consumption, both groups were comparable. The surveillance program included determining the compensation ratio of carbohydrate metabolism and the state of the factor. The antioxidant protection factor was assessed by the level of catalase activity in the blood serum, as well as by the diameter of the celiac trunk and the blood flow velocity in it. Statistical processing of the obtained data was carried out with the aid of the program WINDOWS STATISTIKA 6.0. For all types of analysis, differences were considered statistically significant with $p<0.05$.

Results. During the study, we found that in patients with diabetes mellitus type II with concomitant gastroesophageal reflux disease, as well as in patients with gastroesophageal reflux disease without associated pathology, the level of pH-metry was reduced, but with varying measures of confidence. At the same time, we found that patients with GERD without associated pathology had a decrease in the blood flow velocity in the celiac trunk. Concurrently, we ascertained that the decrease in the blood flow velocity in patients of both groups reduced the diameter of the celiac trunk.

Conclusions. In patients with diabetes mellitus type II, concomitant gastroesophageal reflux disease has a subtle clinical presentation that is affected by a significant decline in mucosal sealing protection factors. In patients with GERD without associated pathology, typical clinical manifestations, accompanied by inflammation, acid regurgitation and dyspepsia, are more vivid.

Keywords: diabetes mellitus type II, gastroesophageal reflux disease, level of pH-metry, factors of aggression and protection, oesophageal mucosa.

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1. Introduction

Diabetes mellitus (DM) remains one of the most serious problems of clinical medicine, which is determined by its high incidence, severity of complications, disability and heavy mortality [1, 2].

Every year the mortality rate of diabetic patients steadily increases, the incidence prognosis is about 5–7 % per year [3, 4].

In 2011, according to the European Diabetes Association, there were 266 million people in the world who suffered from diabetes mellitus, in addition, 4.6 million people died of this disease every year.

In Ukraine, more than 1.3 million people with diabetes mellitus are registered, which makes about 2 % of the total population of the country. At the same time, epidemiological studies have shown that the true incidence is 2-3 times higher [5, 6].

Already there are more than 260 million patients with diabetes mellitus in the world, which is 2.8 % of the world's population, and by 2025 their number will reach 300 million. In Russia, according to the World Health Organization, the number of people suffering from diabetes mellitus is also projected to rise from 4.6 million in 2000 to 5.3 million by 2030 [7, 8].

Such dynamics is caused by the fact that diabetes mellitus is accompanied by multisystemic affects, as well as early and frequent complications on the side of other organs and systems, including upper gastrointestinal, among which a special place is occupied by gastroesophageal reflux disease (GERD).

These concomitant diseases largely predetermine the course of diabetes mellitus and its complications and affect the life expectancy of the patient.

For today, the pathology of cardiovascular and renal systems in patients with diabetes mellitus has been studied fairly well, which is sufficiently reflected in numerous publications. However, the gastroenterological aspects of diabetes mellitus are much less studied.

As for the combination and mutual influence of diabetes mellitus and gastroesophageal reflux disease, this issue has not been studied much so far, the data of modern literature are not complete and are quite contradictory.

At the same time, clinical researches devoted to the study of the state of the oesophagus in patients with a combination of GERD and DM are few and do not give a complete answer to many questions on the formation and development of this comorbid pathology. In particular, there is no precise data on the incidence, peculiarities of the GERD course with various types of diabetes mellitus depending on the duration of its course and the nature of the hypoglycemic therapy. In addition, there has been no profound comparative analysis of changes in the secretory (acid-pro-

ducing) function of the gaster, the peculiarities of the state of metabolic processes with underlying gastroesophageal reflux disease, depending on the type of diabetes mellitus, which affect the pathogenetic mechanisms of GERD formation with underlying DM [12, 13].

All these facts of a few studies devoted to the investigation of the GERD formation mechanisms with underlying DM have not been fully clarified, and therefore require further investigation, which conditions this study.

The aim of the study: to investigate the state of the factors of aggression and protection of the oesophageal mucosa in patients with diabetes mellitus type II with concomitant GERD without associated pathology.

2. Materials and Methods

The study was conducted from 2016 to 2019 based on the Department of Therapy, Rheumatology and Clinical Pharmacology, the Gastroenterology Department of the Kharkiv City Student Hospital, as well as on the basis of the Endocrinology Department of the Kharkiv Regional Clinical Hospital.

There were two groups of patients under observation. The first group included 45 patients with diabetes mellitus type II with concomitant GERD (26 men and 19 women). The mean age of patients was 46.4±3.1 years; the body weight was 63.2±2.8 kg.

Patients of the first group in the clinical setting had in equal measure, along with the symptoms characteristic of DM, moderate acid regurgitation (in 25 patients, 55.5 %), dysphagia (in 22 patients, 48.8 %), and constantly appearing inflammation (in 23 patients, 51.1 %).

The studies were carried out in accordance with the Declaration of Helsinki; the procedures were approved by the local ethics committee of Kharkiv medical academy of postgraduate education, protocol No. 5, 12.11.2019. Informed consent for the participation was obtained from all the patients.

As a result of fibroadastroduodenoscopy, there were revealed hyperemia, oedema of the oesophageal mucosa and muscular fractures in the patients, in addition 33 patients had gastroesophageal reflux, and 12 (26.7 %) patients had multiple erosions of the oesophageal mucosa.

The second group (experimental) included 38 patients suffering from GERD without concomitant pathology – 20 men and 18 women, whose mean age was 45.6±4.1 years, and the body weight was 62.7±3.1 kg.

In patients with GERD without associated pathology, in the clinical setting the main symptoms in most patients were inflammation (in 33 patients, 86.9 %), acid regurgitation and dysphagia (in 26 patients, 78.9 %). Hyperaemia, muscular fractures and mucosal oedema of the lined lower oesophagus, revealed as a result of fibroadastroduodenoscopy, were less evident than in the patients of the first group, and gastroduodenal reflux was present in the majority of patients (in 24 patients, 73.6 %).

The level of infection of Helicobacter pylory in both groups was insignificant and reached I degree of dissemination in 67 patients (87 %) and II degree in 11 patients (13 %).

Thus, by gender, age, body weight, Helicobacter pylori infection, smoking and alcohol consumption, both groups were comparable.

Patients without additional concomitant pathology were taken for the study. All patients had no need for insulin; there were no complications of diabetes mellitus. The arterial pressure of patients of both groups did not exceed 135/85 mm. gt; the body mass index of all patients was within the normal range and made to 21.3±1.9 kg/m^2.

The duration of the gastroesophageal reflux disease in both groups did not exceed 5 years, the severity of the symptoms was moderate, signs of Barrett's oesophagus and atrophy of the mucous membrane of the oesophagus and gaster were not revealed in fibroadastroduodenoscopy.

The diagnosis of GERD was determined according to the Montreal Consensus (2006), taking into account the clinical presentation and the data of the fibroadastroduodenoscopy.

The diagnosis of DM was confirmed in accordance with the criteria proposed by the WHO experts, including the presence of glycaemia more than 6.1 mmol/l in the fasted state.

The exclusionary criteria were presence of other organic diseases, including diseases of the digestive and endocrine systems, as well as the patient's refusal to participate in the study.

The examination program included determining the degree of compensation of carbohydrate metabolism by the level of glycosylated haemoglobin HbA1c by chromatographic method; the state of the aggression factor – according to the level of pH-metry in the body and antrum of the gaster.

To assess the gastric secretion we used a 24-hour pH-metry, carried out with the help of the Gastroscan-24 (Russia) by the standard procedure, since this procedure allows to measure the pH directly in the oesophagus and gaster, as well as the intragastric pH-metry, using special biolive pH-probes with antimony-calomel electrodes (antral and gastric) on the apparatus of IKJ-2 (gaster acid indicator) (production Ukraine) by a standard procedure, since this intragastric pH-metry allows to measure the pH directly in the gaster in the basal period, with continuous record of pH changes in different parts of the gaster and oesophagus.

The antioxidant protection factor (AOP) was assessed according to the level of the serum catalase activity determined with the aid of the standard set of IBL reagents (production Germany), as well as according to the diameter of the celiac trunk and the blood flow velocity in it, since the protective function of the oesophageal mucosa is provided by normal regeneration of the epithelium and adequate state of local blood flow [14, 15].

The local blood flow was assessed by the diameter of the celiac trunk and the blood flow velocity in it with the determination of PSV – peak systolic velocity and EDV – end-diastolic velocity by the pulsed wave Doppler sonography with colour mapping performed on the ALOKA SSD-750 (Japan) and ULTIMA pro-30 (production Ukraine).

15 practically healthy individuals of the same sex and age were used for the comparison.

Statistical processing of the data was carried out using the WINDOWS STATISTIKA 6.0 program. Comparison of the indices in the groups was carried out by the method of parametric statistics (t – Student's test). The interrelation between the indices in the groups was estimated using the Pearson correlation analysis (r is the correlation coefficient). Differences were rated with $p<0.05$ for all types of analysis of statistical significance.

3. Results

In the course of the study, we found that among patients with DM with concomitant GERD, as well as among patients with GERD without associated pathology, the level of pH-metry was reduced, although with varying degrees of reliability.

In particular, in patients with GERD without concomitant pathology, the pH-metry in the gaster was in the range from 0.5 to 1.0, in the antrum from 4 to 5.8, and on average its level (0.83±0.09 and 5.2±0.1) in comparison with the group of healthy individuals for which the average level of pH-metry in the antrum was equal to 1.62±0.05 and in the gaster 7.1±0.1, made statistically significant ($p<0.001$) difference.

In the group of patients with DM with concomitant GERD, the acidity indices both in the gaster (from 0.8 to 1.5) and in the antrum (5.8–7.0) were higher, and their average level (1.40±0.1 and 6.05±0.1) with a lesser degree of reliability ($p_2<0.05$) differed ($p_1<0.05$) not only from the norm, but also from the group of patients with GERD without associated pathology ($p_3<0$, 05). During daily pH monitoring, it was noted that the highest acidity values were recorded in morning hours 6.00–7.00 am (**Table 1**).

At the same time, we found that patients with GERD without associated pathology had a decrease in the blood flow velocity in the celiac trunk and amounted from 8 to 12 cm/sec. On average, the blood flow velocity decreased to 10.7±1.06 cm/sec, and which, in comparison with the control group, whose blood flow velocity in the celiac trunk was 13.5±0.92 cm/sec, the difference was statistically ($p<0.05$) reliable.

Among the patients with DM with concomitant GERD, the blood flow velocity in the celiac trunk decreased even more from 8 to 5 cm/sec, and on average dropped to 6.9±0.82 cm/sec, making a statistically significant difference not only when compared with the norm ($p <0.001$), but also with the average blood flow velocity of patients with GERD without associated pathology ($p<0.05$).

At the same time, there were no significant changes between PSV and EDV in both treatment groups (**Table 2**).

Table 1

Average parameters (table of gastric acidity) of pH-metry in patients with DM with concomitant GERD, in patients with GERD without associated pathology and in healthy individuals of the control group

pH-metry indices	Patients with DM with concomitant GERD n=45	Patients with GERD without associated pathology n=38	Control group n=20	p
In gaster	1.40±0.1	0.83±0.09	1.62±0.05	$p_1<0.05$ $p_2<0.001$ $p_3<0.01$
In antrum	6.05±0.5	5.0±0.1	7.2±0.1	$p_1<0.05$ $p_2<0.001$ $p_3<0.05$

Note: p_1 – degree of reliability of the difference between the group of patients with DM with concomitant GERD and the norm; p_2 – between the group of patients with GERD without associated pathology and the norm; p_3 – between the group of patients with DM with concomitant GERD and the group of patients without associated pathology

Table 2

Mean indices of the blood flow in the celiac trunk and the catalase activity in patients with DM with concomitant GERD and patients with GERD without associated pathology

Indices	Patients with DM with concomitant GERD, n=45	Patients with GERD without associated pathology, n=38	Control group, n=20	p
Diameter of the celiac trunk (cm)	0.59±0.05	0.76±0.05	0.93±0.07	$p_1<0.001$ $p_2<0.05$ $p_3<0.05$
Blood flow velocity (cm/sec)	6.9±0.82	10.7±1.06	13.5±0.92	$p_1<0.001$ $p_2<0.05$ $p_3<0.001$
Catalase (U/ml)	0.27±0.025	2.7±0.029	3.8±0.017	$p_1<0.001$ $p_2<0.05$ $p_3<0.001$

Note: p_1 – the degree of reliability of the difference between the group of patients with DM with concomitant GERD and the norm; p_2 – between the group of patients with GERD without associated pathology and the norm; p_3 – between the group of patients with DM with concomitant GERD and the group of patients without associated pathology

Concurrently, we ascertained that the decrease in the blood flow velocity in patients of both groups reduced the diameter of the celiac trunk. Moreover, in patients with GERD without associated pathology (experimental group), the diameter of the celiac trunk was determined in the range from 0.4 to 0.8 cm, and on average its size (0.76±0.05 cm) was statistically significantly ($p<0.05$) smaller than in healthy individuals of the control group (0.93±0.07 cm).

In most patients with DM with concomitant GERD, the diameter of the celiac trunk was even smaller and on average its size (0.59±0.05 cm) was smaller not only in comparison with the norm ($p<0.001$), but also in comparison with the average data of the patients with GERD without associated pathology.

At the same time, we found that the greater microcirculatory disorders were in correlation with the decrease in the level of catalase activity.

In particular, in patients with GERD without associated pathology, the level of catalase activity was determined in the range from 2.12 U/ml to 3.23 U/ml, and on average its level was (2.7±0.029) statistically significantly (p_2<0.05) below the norm (3.8±0.017).

At the same time, in the patients with concomitant GERD, the catalase activity dropped below 0.23 to 0.29 U/ml, and on average its level (0.268±0.01) was statistically significantly not only lower than the norm (p_1<0.001), but also lower than the average indices of the group of patients with GERD without associated pathology.

4. Discussion

The questions of the comorbid course of diabetes mellitus type II and gastroesophageal reflux disease represent, on the one hand, great scientific and practical interest, and on the other hand, they remain open on several positions.

This way, some authors believe that there is no interrelation between diabetes mellitus and gastroesophageal reflux disease [16, 17], while other researchers note that diabetes mellitus type II and gastroesophageal reflux disease often accompany each other [18, 19], and that the prevalence of GERD symptoms among patients with diabetes mellitus type II is higher than in the general population [20].

In this regard, the obtained data show that there is a pathogenetic dependence between diabetes mellitus type II and gastroesophageal reflux disease, which is determined by the fact that diabetes mellitus has a direct effect on the reduction of regional blood flow and antioxidant activity indices, which are one of the basic factors of the oesophageal mucosa protection. At the same time, the pathogenetic effects of diabetes mellitus have less effect on the increase of the aggression mechanisms of the gastric juice.

A number of researchers concludes that gastroesophageal reflux disease can be considered as a regular complication of diabetes mellitus [21].

In this connection, our study shows that these statements do not always take place, as in the vast majority of our patients gastroesophageal reflux disease has been diagnosed before the onset of diabetes mellitus.

Some authors state that the degree of manifestation of GERD rises with the increase of duration and severity of the diabetes mellitus [15, 19].

In the context of our studies, this position is confirmed, as a connection, although not reliable, was established between the duration of the course of diabetes mellitus type II, the degree of its severity, and the severity of clinical manifestations of gastroesophageal reflux disease.

At the same time, the suggestion that one of the pathogenetic mechanisms of primary development of GERD against the background of diabetes mellitus may be neuropathy, since it is capable of causing motor dysfunction of the upper digestive tract, including disorders of the lower oesophageal sphincter regulation [13, 18], and needs further research. However, the interest in these processes of disorders of the regional blood flow, antioxidant protection processes, taking an active part in the regulation of motor function of the digestive tract, make it possible to talk about its legitimacy.

Thus, the changes we detected in the spectrum of the studied parameters and the presence of a close correlation between them undoubtedly indicate that diabetes mellitus has a direct effect on reducing the protective properties of the oesophageal mucosa and, to a lesser extent, on increasing the aggression of gastric juice, thereby creating favourable conditions for the initiation and formation of GERD with underlying diabetes mellitus.

Due to the fact that in patients with diabetes mellitus type II, which is characterized by inveterate course and disorders of all metabolic forms, affection of the oesophagus and accordingly formation of GERD with various forms of its manifestation (erosive or non-erosive) is a regular event. Therefore, in case of diabetes mellitus type II, even with the slightest suspicion of GERD, it is necessary to perform fibrogastroduodenoscopy for its early detection and timely treatment prescription.

5. Conclusions

1. In patients with diabetes mellitus type II, concomitant gastroesophageal reflux disease has a mild clinical presentation due to a decrease in the secretion of the gastric mucosa and an

increase in acidity in it that occurs against the background of a significant decrease in mucosal barrier protection factors, which is indicated with a high degree of reliability by decrease in the catalase enzyme activity, deceleration of the blood flow velocity in the celiac trunk and narrowing of its diameter.

2. In patients with GERD without associated pathology, the typical clinical manifestations accompanied by inflammation, acid regurgitation and dyspepsia are more vivid and accompanied, with a greater degree of certainty, by increased gastric aggression (acidity).

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A MULTI-MARKER MODEL FOR PREDICTING DECOMPENSATED HEART FAILURE IN PATIENTS WITH PRIOR ACUTE MYOCARDIAL INFARCTION

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Abstract

The aim of the study was to assess the prognostic value of determining the plasma concentration of NT-proBNP and ST2 in the patients with decompensated HF and prior acute myocardial infarction and their combination in this category of patients.

Materials and methods. There were examined 120 patients with acute myocardial infarction and stage II A-B decompensated chronic HF according to the classification proposed by Vasylenko V. Kh. and Strazhesko M.D., NYHA functional class (FC) III–IV. The patients with Q-QS wave MI (60 individuals) and non Q MI (60 individuals) were divided into 4 groups depending on the treatment methods.

Study groups were homogenous by age, gender, disease severity, duration of the post-infarction period, clinical signs of decompensation, which served as a basis for inclusion of the patients in the study.

All the patients underwent the six-minute walk test in a quiet 30–50-m long hospital corridor in the morning. N-terminal pro-B-type brain natriuretic peptide (NT-proBNP) and ST-2 were analyzed in all patients.

Results. Promising biomarkers of HF decompensation in the post-infarction period were studied. In the patients with prior Q-QS MI and decompensated HF, NT-proBNP level was (950.38±3.15) pmol/l ($p<0.05$); in the patients with prior MI without signs of decompensated HF, it was (580.15±3.03) pmol/l ($p<0.05$); in apparently healthy individuals, the level of NT-proBNP was found to be (111.20±3.47) pmol/l.

ST2 level was (14.80±1.61) ng/ml, (36.00±1.43) ng/ml and (49.22±1.40) ng/ml in the patients of Group 1, Group 2 and Group 3, respectively ($p<0.05$).

Similar changes were found in patients with decompensated HF in postinfarction period after non Q MI.

Conclusions. The increase in plasma concentration of sST2 is associated with the activation of both neurohumoral and fibrous pathways and can help in detecting the patients with decompensated HF in the post-infarction period and predicting the risk of its development.

Our results confirmed the results of other multiple studies reporting ST2 in combination with NT-proBNP to be valuable tools for prognosing the development of decompensated HF in the patients with prior MI. ST2, alongside with NT-proBNP, is a promising biomarker to be included in the diagnostic panel for detecting acute HF and can provide additional information on risk stratification for such patients during hospitalization and at the time of discharge from the hospital.

Keywords: acute myocardial infarction, decompensated heart failure, biomarkers, NT-proBNP, ST2.

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1. Introduction

Heart failure (HF) is a syndrome affecting more than 5.7 million people annually and is the most frequent cause of hospitalization worldwide [1]. It is a major public health challenge. Both rapid and quality identification of the individuals at high risk of HF development in the post-infarction period and the determination of new therapeutic strategies should be considered [2]. However, the main recommendations for the prevention of cardiovascular diseases have not paid enough attention to HF prediction; the main mechanisms of its development in the post-infarction period have not been fully known [3]. Biomarker model for diagnosing HF has become an integral part of modern medicine; however, it needs further study and continuous improvement [4].

B-type brain natriuretic peptide (BNP) is a hormone synthesized and secreted by cardiomyocytes in response to ventricular pressure and volume overload [5]. These peptides are known to play a crucial role in maintaining homeostasis in the cardiovascular system and serve as counter-regulatory hormones for pressure and volume overload [6]. The Breathing not properly multinational study, conducted in 2002, was the first large study that evaluated the effectiveness of BNP as a cardiac marker [7]. Several factors can increase serum levels of natriuretic peptides. They include age, female sex and renal insufficiency [8]. Low levels of these peptides during the observation period indicated the reduction in the effects of HF, while elevated levels were associated with unfavorable prognosis for patients [9, 10].

ST2 is a member of the interleukin-1 (IL-1) superfamily. It is defined as a ligand for interleukin-33 (IL-33). ST2 has two main isoforms: transmembrane or cellular (ST2L) and soluble (ST2s) forms [11, 12]. ST2 is a member of the Toll-like/IL-1 receptor superfamily. The ST2 gene, found on human chromosome 2q12, is expresses in 4 isoforms; two of them contain transmembrane receptor (ST2 ligand or ST2L) and soluble serum circulating receptor (sST2) which can be detected in blood plasma [13–15]. All the forms are able to bind to IL-33; however, they have different effects. IL-33 is secreted in response to overstretching of certain cells that act as a barrier (e.g. endothelial cells, lung and intestinal epithelial cells, keratinocytes, fibroblasts and smooth muscle cells) and are regulated in case of inflammation [16, 17]. If it binds to ST2L, it functions as an alarm signal for the processes of protection against fibrosis and hypertrophy [18]. If sST2 binds to IL-33, it serves as a decoy receptor that prevents IL-33 signaling thereby inhibiting fibrosis and hypertrophy that occurs in case of elevated sST2 levels [19] by neutralizing the beneficial activity of circulating IL-33 [20, 21].

According to the latest research, ST2s is considered as a potential marker of inflammation and cardiac remodeling. In 2003, Weinberg et al. found that short-term changes in plasma sST2 levels were prognostically valuable for diagnosing mortality and heart transplantation in the patients with HF regardless of natriuretic peptide levels [22, 23].

According to the reasons mentioned above, novel biomarkers of HF prediction can improve the risk identification, as well as to provide an understanding of the pathophysiological mechanisms involved in this process [24]. New recommendations, being developed because of evidence-based clinical guidelines, are undoubtedly waiting for more data obtained from randomized multi-centered clinical trials to develop novel marker-associated system for detection and treatment of HF [25].

Two biomarkers, assessed in this study, are the parts of separate biological processes; therefore, they are able to provide independent and additional prognostic information on the occurrence of decompensated HF in the post-infarction period.

2. Materials and methods

There were examined 120 patients with Q-QS wave and non-Q wave myocardial infarction (MI), stage II A-B decompensated chronic HF according to the classification proposed by Vasylenko V. Kh. and Strazhesko M. D., NYHA functional class (FC) III-IV. The patients with Q-QS wave MI (60 individuals) were divided into 4 groups depending on the treatment methods: Group I comprised 15 patients who received basic therapy in accordance with the protocols of the Ministry of Health of Ukraine (lisinopril – 10 mg once a day; bisoprolol fumarate – 10 mg once a day; eplerenone – 50 mg once a day; valsartan – 40 mg twice a day; ivabradine – 5 mg twice a

day); Group 2 included 15 patients who, on the background of basic therapy, received a preparation of succinic acid according to the proposed scheme; Group 3 included 15 patients who, on the background of basic therapy, received arginine preparations according to the proposed scheme; Group 4 comprised 15 patients who, on the background of basic therapy, received succinic acid and arginine preparations according to the proposed scheme. The patients with non-Q wave MI (60 individuals) were divided into 4 analogous groups. All patients were examined and observed based on the Department of myocardial infarction No 2 of the Ivano-Frankivsk regional clinical cardiology center from 2016 to 2018.

Study groups were homogenous by age, gender, disease severity, duration of the post-infarction period, clinical signs of decompensation, which served as a basis for inclusion of the patients in the study. The mean age of patients in all groups averaged (56.67±5.72) years, for men – (56.76±5.71), women – (56.4±5.75). The largest group was with patients aged 45–59, respectively 65.83 % (79).

The members of the Ethics Commission (extract from protocol No. 5 dated January 13, 2016) at the Ivano-Frankivsk National Medical University, decided that this study would not contradict the main provisions of the GCP, Convention Council of Europe on human rights and biomedicine, the Helsinki Declaration of the World Medical Association on ethical principles for the conduct of scientific medical research with the participation of man and the Law of Ukraine «On Medicines». All patients signed an informed consent to participate in a clinical trial.

All the patients underwent the six-minute walk test (6 MWT) in a quiet 30–50-m long hospital corridor in the morning. Blood samples were taken on an empty stomach after the patients had rested for at least 20 minutes. In 5 minutes, the samples were centrifuged for 10 minutes at 4° C and then, they were frozen at –70° C until analysed. N-terminal pro-B-type brain natriuretic peptide (NT-proBNP) was analyzed in the laboratory using clinically available enzyme-linked immunoassay (Roche Elecys, Roche Diagnostics). ST2 was analysed using ST2 enzyme-linked immunosorbent assay (ELISA) (critical diagnostics) with the lower detection limit of 2 ng/ml, the upper detection limit of 200 ng/ml, the intra-assay coefficient of variation <2.5 % and the inter-assay coefficient of variation <4.6 %. The results obtained were statistically processed on the personal computer by means of software package STATISTICA-7. During the work, the arithmetic mean M, the mean quadratic deviation δ, the mean error of the arithmetic mean m, the number of variant (n), the probability of the difference of the two arithmetic "p" were calculated, the values of $p<0.05$ were estimated as probable.

3. Research results

During this study, promising biomarkers of HF decompensation in the post-infarction period were studied. For this purpose, there was analyzed the mean value of serum NT-proBNP and ST2 levels of the studied patients. The levels of biomarkers in the patients with prior MI depending on the presence of decompensated HF are presented in **Table 1.**

In the patients with prior Q-QS and non-Q MI with decompensated HF, NT-proBNP level was (950.38±3.15) pmol/l ($p<0.05$); in the patients with prior MI without signs of decompensated HF, it was (580.15±3.03) pmol/l ($p<0.05$); in apparently healthy individuals, the level of NT-proBNP was found to be (111.20±3.47) pmol/l.

ST2 level was (14.80±1.61) ng/ml, (36.00±1.43) ng/ml and (49.22±1.40) ng/ml in the patients of Group 1, Group 2 and Group 3, respectively ($p<0.05$).

In the patients with decompensated HF signs after non Q and Q-QS MI, significantly higher serum NT-proBNP levels were observed. Moreover, in the patients of Group 3 with inadequate response to physical activity, the level of NT-proBNP increased significantly to (1048.06±4.83) pg/ml; in the patients of Group 2 and healthy individuals, it was (619.03±4.70) pg/ml and (116.20±4.83) pg/ml, respectively ($p<0.05$).

Considering the results of the 6 MWT and enzyme-linked immunoassay of serum NT-proBNP level in the patients with decompensated HF secondary to prior MI, there was conducted correlation analysis between the indicator of exercise tolerance, namely the distance walked over a span of 6 minute, and the concentration of this marker.

Table 1

Levels of biomarkers in the patients with prior myocardial infarction depending on the presence of decompensated heart failure

Indicator, units of measurement	Apparently healthy individuals (n=20)	Patients with prior MI without decompensated HF (n=40)	Patients with prior MI and decompensated HF (n=120)
NT-proBNP before physical activity, pg/ml	111.20±3.47	580.15±3.03 p_1<0.05	950.38±3.15 p_1<0.05 p_2<0.05
NT-proBNP after physical activity, pg/ml	116.20±4.83 Δ49.ε+	619.03±4.70 p_1<0.05	1048.06±4.83 p_1<0.05 p_2<0.05
ST2	14.80±1.61	36.00±1.43 p_1<0.05	49.22±1.40 p_1<0.05 p_2<0.05

Note: p_1 – statistically significant difference in the indicators as compared to apparently healthy individuals; p_2 – statistically significant difference in the indicators as compared to the patients with prior MI without decompensated HF

4. Discussion

The study allowed us to confirm that the patient's response to graded exercises and serum levels of NT-proBNP and ST2 play the most significant role in clinical and prognostic assessing the post-infarction period complicated by decompensated HF. It was earlier reported, that ST2 is an efficient marker of HF. The IL/ST2 pathway is correlated with the severity of HF clinical course and is a factor in the decline of cardiac function in HF patients [26]. It is thought to be a cardio-protective signalling pathway that is activated by cell damage and mechanical stress, through the release of IL-33 from cardiac cells. Activation of IL-33 prevents myocardial hypertrophy and fibrosis through interaction of IL-33 and transmembrane bound ST2L [27]. As noted, sST2 is a circulating form, which lacks the transmembrane and cytoplasmic domains [28].

For disease monitoring, serial test results proved to be specifically useful. Higher levels of sST2 are associated with more severe clinical symptoms and with other objective measures of HF severity, such as higher natriuretic peptide levels [29]. Although, levels of this enzymes have been used widely for the earlier diagnosis or exclusion of chronic HF in the outpatient setting, their use in the acute care settings has only partially been adopted, because their role has remained uncertain [30].

6 MWT is the simple walk test and particularly is applicable in the HF population due to the risk of acute cardiac events associated with the gold standard maximal exercise testing for assessing functional capacity [31] and determine functional capacity, which refers the ability of a person to perform activities of daily living and reflects the ability of the cardiac system to sustain aerobic metabolism.

Therefore, 6 MWT is used to determine the extent of myocardial contractility changes. High levels of NUP and ST2 indicate a poor prognosis. Therefore, an inadequate response to measured exercise and increased immunological parameters as quantitative HF markers may be useful not only for diagnosis but also for risk stratification, decision making on optimization of treatment of such contingent of patients and decision on discharge.

Study limitations. A limitation of the study is the fact, that in a course, that in most patients with HF, exercise tolerance reduces and the 6 MWT is used to determine the degree of changes in myocardial contractility, it was found high levels of natriuretic peptide and ST2 indicate an unfavorable prognosis. Therefore, an inadequate response to graded exercises and increase in the concentration of immunological indicators as quantitative markers of HF can be useful for diagnosis, as well as risk stratification, making decisions on optimizing the treatment of such patients and discharging them.

Prospective for the further research. Since the main limits of changes in NT-pro BNP and ST2 depending on the signs of decompensated HF secondary to acute MI have been deter-

mined, we plan to develop an algorithm for predicting the development of this syndrome in the post-infarction period under conditions of lower FC formation and assessing the quality of therapy, as well as determining the frequency and time periods of using these peptides for making therapeutic decisions.

5. Conclusions

Thus, we can conclude that the increase in plasma concentration of sST2 is associated with the activation of both neurohumoral and fibrous pathways, and can help in detecting the patients with decompensated HF in the post-infarction period and predicting the risk of its development. Our results confirmed the results of other multiple studies reporting ST2 in combination with NT-pro BNP to be valuable tools for prognosing the development of decompensated HF in the patients with prior MI. ST2, alongside with NT-pro BNP, is a promising biomarker to be included in the diagnostic panel for detecting acute HF and can provide additional information on risk stratification for such patients during hospitalization and at the time of discharge from the hospital.

Conflicts of interest

No conflict of interests.

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MORPHOLOGICAL AND STRUCTURAL CHANGES IN MYOCARDIUM, LIPID AND CARBOHYDRATE METABOLISM DURING DIFFERENT OUTCOMES OF CHRONIC HEART FAILURE IN PATIENTS WITH ISCHEMIC HEART DISEASE AND DIABETES MELLITUS TYPE II

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Abstract

Diabetes mellitus (DM) type II is an independent risk factor for cardiovascular system injury. To avoid progression of ischemic heart failure (IHF) it is important to find early signs of myocardial injury also as carbohydrate and lipid metabolism alterations.

The objective of the study: to establish features of structural and functional changes in myocardium, carbohydrate and lipid metabolism, in patients with different outcomes of chronic heart failure (CHF), caused by IHD and DM type II.

Material and methods. Examination of 100 patients who have CHF with IHF and DM type II was performed. Patients were divided in two groups, according to outcome: group I (n=66) – patients with favorable outcome, mean age 60.0 [55.8; 63.3] years, group II (n=34) – unfavorable outcome of CHF, mean age 58.0 [55.0; 60.3] years.

We analysed complaints, cardiologycal anamnesis, cardiovascular risk factors, and physical examination data. Transthoracic echocardiography (TTE), carbohydrate and lipid panel were assessed to find out early specific signs of myocardial injury.

Results. We find out statistically significant associations between TTE results, lipid panel and CHF progression in study population.

Conclusions. Comparative analysis showed that degree of CHF in patients with IHD and DM type II that have preserved LV EF is associated with: duration of DM and CHF, arterial hypertension (AH) level and degree of carbohydrate and lipid metabolism disturbances. Early TTE signs of unfavorable outcome are: increase of transmitral deceleration time (Dt), increase of mean PA pressure (PA MP) even in range lower the 20 mmHg.

Keywords: carbohydrate metabolism, lipid metabolism, myocardium morpho-functional state.

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1. Introduction

There are 415 millions of patients around the world with diabetes mellitus (DM), 90 % of them have DM type II [1]. Despite available scientific data about risk factors and DM-screening

programs running, incidence of disease still increases. According to experts of International Diabetic Federation (IDF), in 2035 amount of people with DM type II will reach 592 million, this is approximately every tenth. This shows significance of ongoing study on genotype and phenotype of DM to individualise treatment for this kind of patients [2].

DM type II leads to micro- and macrovascular complications, and after that ischemic heart disease (IHD). Epidemiological data shows that cardiovascular complications are the main reason of morbidity and mortality among patients with DM (80 % of patients with DM type II mortality is connected to cardiovascular complications) [2]. Almost in 50 % patients that have IHD, diagnosis of the DM type II, impaired glucose tolerance or impaired fasting glucose is found first time. According to this, American Heart Association (AHA) established presence of DM type II as equivalent of high risk of vascular complications, which is comparable with present cardiovascular diseases [3].

Nowadays there is a big interest in pathophysiological mechanisms, responsible for changes in cardiomyocites during DM type II [1]. Hyperglycemia leads to decrease in ability to resist oxidative stress by increase of glucose utilisation in citric acid pathway, increase in inflammatory response, that have influence on lipid metabolism and immune response, and leads to chronic inflammation in arterial vessel wall, endomyocardial fibrosis, necrosis and apoptosis of endothelial cells and cardyomiocytes [4, 5]. This mechanisms combined with disorders of mineral metabolism, impairment of Ca^{2+} regulation of myofilament function, increase of active oxygen forms level, increased lipotoxycity, autonomic nervous system dysfunction, which one, according to progressive autonomic neuropathy, decrease vasodilative effect of sympathetic stimulation to coronary vessels resistance – thus accelerates coronary vessels calcification and appearance of CHF with DM type II [6, 7].

Despite present scientific data of DM type II influence on cardiovascular pathology, pathophysiology of myocardial ischemia during DM type II needs further investigation. One group of patients with DM type II have coronary artery disease that prevents normal blood flow to myocardium, another have microvascular changes without plaques, and absence or presence of endothelial dysfunction [8]. There is lack of data about main aspects of diabetic coronary microvascular dysfunction in structure of cardiovascular diseases, role of genetic types of ATP-dependent potassium channels that determine course of IHD by balancing coronary blood flow and cardiac performance [9, 10].

In modern era, it is important to study mechanisms of development and specific clinical signs of CHF on the early stage. Investigations should lead to development of new methods and materials of prophylactic and treatment.

The objective of the study: to highlight patterns of cardiac morphology and function, lipid and carbohydrate metabolism during different courses of heart failure in patients with IHF and DM type II.

2. Material and methods

We performed complex examination of 100 male patients with ischemic CHF with IHD and DM type II after in-hospital treatment. All patients signed an informed consent to participate in the study.

Examination was performed in therapy department of community-owned non-profit organization “City clinic of urgent and emergency medicine named after prof. A. I. Meschaninov” Kharkiv`s City Council, Ukraine, between February 2015 and January 2017.

Inclusion criteria: age between 50 and 70 years, CHF II functional class (FC) NYHA classification, LV EF≥50 % (according to criteria of European Society of Cardiology, 2016) [11], postinfraction cardiosclerosis with DM type II [12], glomerular filtration rate (≥50 ml/min/1.73 m^2), NT-proBNP≥125 pg/ml.

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study. No funding for this study.

Exclusion criteria: age more then 70 years, resistive arterial hypertension (AH), secondary AH, LV EF<50 %, NT-proBNP<125 pg/ml, pulmonary artery hypertension, congenital and

acquired heart diseases, IHD that requires administration of short-acting nitrates more than two times a week, non-coronary myocardial diseases, chronic kidney disease with glomerular filtration rate GFR<50 ml/min/1,73 m^2, comorbidities: chronic obstructive pulmonary disease, bronchial asthma, anemia Hb≤90 g/l, acute and chronic inflammatory myocardial diseases, postoperative state, oncological diseases, inflammatory gastro-intestinal diseases, haemopoetic diseases, traumatic injuries, alcohol abuse.

Patients were divided in two groups according to outcome: group I (n=66) – favourable outcome, mean age 60.0 [55.8; 63.3] years, group II (n=34) – unfavourable outcome, mean age 58.0 [55.0; 60.3] years.

Complaints, cardiological anamnesis, cardiovascular risk factors, physical examination data, laboratory and instrumental examination, including 12-leads ECG, were analysed. AP was measured three times on both brachial arteries in sitting position, not earlier than 30 minutes after physical exertion, with mean AP (MAP) calculated. Pulse AP (PAP) was measured as difference between systolic AP (sAP) and diastolic AP (dAP). Mean AP (MAP) calculations are: MAP=0.42*(sAP–dAP)+dAP.

Insulin plasma level was measured with Insulin Rapid AccuBind ELISA kits (Monobind Insulin, USA) agents. Serum glucose level was measured with biochemical analyser Flexor E («Vital Scientific N.V.», Netherlands), kit «Glucose SPL» colorimetric method GOD-POD. The homeostatic model assessment index (HOMA-ir) was calculated according to the formula:

$$\text{fasting insulin (microU/L)} \times \text{fasting glucose (nmol/L)}/22.5.$$

Homa-ir more than 2.5 was pointed as insulin resistence

Serum glycated haemoglobin (HbA1c) was measured with Liquidirect kit (Human GmbH, Germany).

We measured levels of total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides (TG) by fermentative method with automated biochemical photometer Prestige 24 (Japan) with kits PZ CORMAY S.A. (LQ CHOL; LQ TG; HDL DIRECT; LDL DIRECT, Poland) Also we calculate atherogenic index (AI) by the A.M. Klimov formula (1977) [13]: AI=(TC–HDL)/LDL. NT-proBNP was measured with «ELISA kit».

Transthoracic echocardiography (TTE) was performed with SiemensACUSONIC 2000 (SiemensMedicalSolution, Mountain View, USA), phased-array probe 3,5–7 MHz. Were measured: left ventricle end-diastolic volume (LV EDV), left ventricle end-systolic volume (LV ESV), left atrium linear dimension (LALD), left ventricle ejection fraction (LV EF), transmitral deceleration time (Dt), left ventricle isovolumic relaxation time (IVRT), the ratio of peak velocity blood flow in early diastole to peak velocity flow in late diastole E/A ratio, the ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity (E/e′), mean pulmonary artery pressure (PA MP).

We measured endothelium dependent dilation in the brachial arteries (EDD). Degree of EDD was measured by reactive hyperemia, observed by wide-broad linear probe 5–12 MHz in colour doppler mode three times in left and right brachial arteries with 15 minutes interval, method by Celermajer D. S. [14] modified after Ivanova A. V. [15].

Observational period was 12 month after in-hospital treatment.

Standard medication was provided: β-blocker bisoprolol – 2.5–5 mg at morning, ACE inhibitor ramipril – 5–10 mg once at evening, amlodipin – 2.5–10 mg at evening, ASA – 75 mg once after day meal, atorvastatin 20 mg once after day meal, if additional hypotensive therapy needed – indapamid – 2.5 mg once at morning, metformin 100-1500 mg per day.

Statistical analysis was performed with SPSS 19 software for Windows. Quantitative variables are described as median (M), 25 and 75 quartiles (M [Q1; Q3]) qualitative data – as frequency of the events (% from normal observations). In order to identify differences between independent samples was used Mann-Whitney U-criteria. Frequency of sign occurrence in groups was compared by χ^2 criteria.

3. Results

After 12 months of study, enrolled patients were divided in two groups depending on outcomes. Group I (n=66) – favourable outcome of IHF, age 60.0 [55.8; 63.3] years, group II (n=34) – unfavourable outcome, mean age 58.0 [55.0; 60.3] years. There was no statistically significant difference in age (p>0.05) (Table 1). In group II CHF progressed, that was characterized by LV EF decrease in 13 patients (38 %), diastolic dysfunction in 14 (41 %) patient, death in 7 (20 %) patients.

Duration of IHD varies from 5 to 10 years. Duration of DM type II – from 5 to 9 years. Family history of DM type II had 36.4 % of patients in group I and 58.8 % in group II (p=0.03). Every patient with DM type was in subcompensated state (fasting glucose <7.6 mmol/l, glycated haemoglobin <8.0 %).

During analysis of family and clinical history in both groups, CHF was significantly associated duration of IHD (p=0.014), and DM type II (p=0.001) Statistically significant difference in IHD family history appearance was not found in both groups (p>0.05), also as in BMI (p>0.05) (**Table 1**).

Table 1

Demography and anamnestic data of patients with IHD in both groups (Me [Q_1; Q_3])

Indicator, unit	Group I, (n=66)	Group II, (n=34)	p
Age, years	60.0 [55.8; 63.3]	58.0 [55.0; 60.3]	>0.05
IHD present, years	6.0 [5.0; 7.0]	7.00 [5.0; 10.0]	0.014
DM type II present, years	5.0 [5.0; 6.0]	6.0 [5.0; 9.0]	0.001
BMI, kg/m²	26.9 [26.1; 27.9]	25.6 [24.8; 29.5]	>0.05
IHD family history, %	40.9	58.8	>0.05
DM type II family history, %	36.4	58.8	0.03

During the analysis of AH level was found that AP from 160/100 to 179/99 mmHg statistically significant more frequent was found in group I – 71.2 % vs 47.1 % (p=0.03), at the same time AP 180/110 mmHg and higher was found more frequent in group II – 52.9 % vs 28.8 % (p=0.02). Physical exertion tolerance, smoking did not differ (p>0.05) (**Table 2**).

Table 2

Clinical examination data of patients with IHD and DM type II in both groups

Indicator, unit	Group I, (n=66)	Group II, (n=34)	p
Physical exertion: impaired, %	80.3	91.2	>0.05
Smoking, %	72.7	82.4	>0.05
AH level:			
– AP from 160/100 to 179/99 mmHg, %	71.2	47.1	0.02
– AP 180/110 mmHg and more, %	28.8	52.9	0.02

In group II values of sAP (p=0.001), pAP (p=0.001) and HR (p=0.001) were statistically sig-nificant higher than in group I (**Table 3**). 6 min walk test had similar results in both groups (p>0.05) (**Table 3**).

Main factor, responsible for atherosclerosis in patient with DM type II is hyperglycaemia. Comparing data in both groups, was found statistically significant higher HbA1c (p=0,001), almost twice higher HOMA-ir (p=0.001), fasting glucose (p=0.004), postprandial glucose (p=0.002), insulin level (p=0.001) in group II (**Table 4**).

Table 3

Hemodynamic values, 6 min walk test in patients with IHD and DM type II (Me [Q_1; Q_3])

Indicator, unit	Group I, (n=66)	Group II, (n=34)	p
sAP, mmHg	135.0 [130.0;145.0]	145.0 [140.0;151.25]	0.001
pAP, mmHg	50.0 [45.0;60.0]	65.0 [50.0;75.0]	0.001
HR, min^{-1}	68.5 [65.75;74.0]	75.0 [69.5;78.0]	0.001
6 min walk test, m	366.5 [338.75;389.0]	371.0 [355.0;382.5]	>0.05

Table 4

Carbohydrate metabolism characteristics in patients with IHD and DM type II (Me [Q_1; Q_3])

Indicator, unit	Group I, (n=66)	Group II, (n=34)	p
HbA1c, %	7.0 [6.6; 7.2]	7.4 [7.8; 7.6]	0.001
Fasting glucose, mmol/l	6.05 [5.78; 6.3]	6.35 [5.9; 6.9]	0.004
Postprandial glucose, mmol/l	8.4 [7.8; 8.8]	8.9 [8.4; 9.2]	0.002
Insulin, mcME/ml	19.2 [15.3; 22.6]	29.3 [26.8; 33.3]	0.001
HOMA-ir	4.9 [4.0; 6.4]	8.2 [7.3; 9.4]	0.001

Comparing lipid metabolism values in both groups, statistically significant higher values of TC (p=0.001), LDL (p=0.001), atherogenic index (p=0.001) was found in group II. No difference was found between TG and HDL (p>0.05) (**Table 5**).

Table 5

Lipid metabolism characteristics in patients with IHD and DM type II (Me [Q_1; Q_3])

Indicator, unit	Group I, (n=66)	Group II, (n=34)	p
TC, (mg/dl)	233.5 [224.5;245.3]	261.0 [249.8;277.0]	0.001
TG, (mg/dl)	167.5 [156.0;183.3]	169.0 [148.0;184.3]	>0.05
HDL, (mg/dl)	42.0 [41.0;43.0]	42.0 [41.0;43.0]	>0.05
LDL, (mg/dl)	159.0 [148.6;171.5]	184.2 [172.8;197.7]	0.001
AI	4.6 [4.3;4.8]	5.1 [4.9;5.7]	0.001

In group II (n=34) compared to group I (n=66) was found significant larger LALD (p=0.001). LV EF was preserved in both groups, but it was statistically significant lower in group II (p=0.001) (**Table 6**).

Table 6

Cardio-vascular ultrasound measurement in patients with IHD and DM type II (Me [Q_1; Q_3]).

Indicator	Group I, (n=66)	Group II, (n=34)	P
LALD, mm	35.5 [34.8; 36.6]	37.2 [36.0; 38.2]	0.001
EdV, ml	113.9 [106.4; 123.8]	115.5 [107.3; 118.9]	>0.05
EsV, ml	39.6 [35.0; 44.1]	41.9 [37.9; 45.0]	>0.05
LV EF, %	66.3 [64.4; 67.6]	62.8 [60.8; 66.4]	0.001
Dt, sec	0.24 [0.22; 0.25]	0.25 [0.24; 0.26]	0.001
IVRT, sec	0.13 [0.12; 0.14]	0.14 [0.13; 0.14]	>0.05
E/A ratio	0.76 [0.72; 0.78]	0.69 [0.64; 0.74]	0.001
E/e`, ratio.	7.29 [6.95; 7.67]	7.38 [6.61; 7.90]	>0.05
PA MP, mmHg.	16.1 [14.4; 17.7]	17.0 [16.0; 18.3]	0.003
Brachial arteries EDD, %	9.00 [8.50; 9.60]	6.85 [5.48; 9.25]	0.001

No difference in EsV and EdV was found (p>0.05). Significantly higher was PA MP (p=0.003) and Dt (p=0.001) in group II. E/A ratio was higher in group I (p=0.001), IVRT and E/e` ratio doesn`t differ in two groups (p>0.05).

Brachial arteries EDD was higher in group I (p=0.001) (**Table 6**).

Significantly higher level of NT-proBNP was in patients group II – 308.5 [210; 351.2] pg/ml compared to the group I – 190 [161.5; 225.3] pg/ml (p=0.001).

4. Discussion

HFpEF is a complex clinical condition, both in terms of diagnosis and treatment of this syndrome. In the actual practice of the physician, it is found that patients with HFpEF are receiving the same treatment as patients with HFrEF [16, 17]. However, if prognosis for RAAS blockers, beta-blockers, or mineralocorticoid receptor antagonists has been shown to improve prognosis for patients with HFpEF, there is no evidence for the efficacy of these drugs in patients with HFpEF [18].

In our study, unfavourable outcome of CHF in patients with CHD and DM type II was associated with higher incidence of CHD and DM duration; also, there was tendency of higher age. In group II rates of DM type II family history, AP level was significantly high, also there was a tendency to higher incidence of CHD family history and lower physical exertion tolerance.

Unfavourable outcome of CHF was associated with more severe lipid and carbohydrate disturbances. Lipid metabolism disturbance leads to severe forms of DM type II that is also demonstrated in another study. A study of the state of lipid metabolism in patients with coronary heart disease and CHF showed in 45.9 % of cases of hypercholesterolemia, in 62 % – increase in low-density lipoprotein, in 15.3 % – decrease in high-density lipoprotein [19].

To date, the possibility of identifying patients who are prone to the progression of LV diastolic dysfunction in CHF is widely discussed in the literature. Early detection of these patients and more careful monitoring can help improve treatment outcomes and develop new treatment options [20, 21].

Even if patients with CHD and DM type II have LV EF preserved (HFpEF), in case of unfavourable outcome EF was significantly lower and was a strong predictor of one. All patients had left ventricle diastolic dysfunction 1 degree, despite normal LALD, but median was higher in group II. DT was higher in group II, that shows more severe diastolic dysfunction degree.

Group II is characterized by higher rate of endothelial dysfunction, which shows severe remodelling processes in vessels.

The coexistence of HFpEF and type 2 diabetes predicts an increased risk of morbidity and mortality. Therefore, there is an increasing need to find new diagnostic tools and treatments to improve clinical outcomes in patients with CHF and type 2 diabetes. Accordingly, it is important for both states to optimize drug therapy and lifestyle while balancing the potential for side effects of medication. Although there are no specific guidelines for the management of patients with HFpEF suffering from diabetes [22].

Limitations of the study. Our study is limited by the number of patients with strict inclusion criteria. This failed to analyse the association of CHD and DM type II in patients with borderline LV EF.

Prospects for further research. Detection of simple predictors of adverse CH course in patients with CHD and DM type II is an urgent task of modern medicine. Achieving this goal makes it possible to stratify a group of patients with high-risk CH complications for closer observation. The increase in the number and groups of patients with CH, the duration of follow-up, will help to identify more sensitive predictors of adverse course of pathology.

5. Conclusions

Severity of CHF with preserved LV EF in patients with IHD and DM type II is associated with disease duration, AH level and severity of lipid and carbohydrate metabolism disturbances.

Higher blood level of total low-density lipoproteins, HbAc1, glycemia, index HOMA and atherogenic index was in patients with unfavourable outcome.

In patients with unfavourable outcome was more low LV EF but in range not lower than 50 % and EDD.

Early markers of unfavourable outcome are increase of dT and increases of PA MP even in range not higher than 20 mmHg.

Conflict of interest

There is no conflict of interest.

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ST2 PLASMA LEVEL IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITHOUT ST ELEVATION AND DIFFERENT CLINICAL CHARACTERISTICS

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Abstract

The aim. Estimation of ST2 plasma level in patients with acute myocardial infarction without ST elevation (NSTEMI) and its relationship with different clinical characteristics.

Materials and methods. 165 patients aged from 35 to 79 (average of 60.7±0.8 years) with various forms of coronary artery disease (CAD) with and without arterial hypertension were examined. The variability of plasma ST2 level in different forms of CAD and in NSTEMI group was analyzed depending on gender-age and clinical characteristics and features of the disease course.

Results:

The results of the present investigation were that the ST2 level in the main cohort was in range from 5.5 to 233.9 (in the middle – 49.8±3.5 ng/ml (median indicator – 34.7 and the interquartile range – 21.9 and 59.1 respectively).

Significantly higher ST2 levels were found in patients with NSTEMI, unlike the comparison group, in the median analysis (35.9 vs. 27.7 ng/ml, p=0.047) and no statistical differences were observed in the mean values.

In patients with NSTEMI, a certain association of ST2 level in plasma with the MI course was detected. A higher level of neurohormone is registered with anterior unlike posterior ECG localization of MI; at high unlike moderate risk on the GRACE scale; when complicated unlike the uncomplicated course of MI; in the case of acute HF and cardiac arrhythmias unlike patients with the absence of these manifestations in the acute period of MI.

Conclusions. High variability of ST2 level in plasma was demonstrated in patients with NSTEMI on the first day after destabilization (minimum and maximum values – 12.7 and 233.9 respectively, median – 35.9 and interquartile range – 25.9 and 55.7 ng/ml).

It is shown that significantly higher ST2 level in plasma is determined in patients with acute MI regardless of its variant among different clinical forms of CAD.

It is found that significantly higher level of ST2 in patients with NSTEMI is recorded in the case of concomitant HTN and type 2 diabetes, with smoking and heavy cardiovascular heredity. Proved influence of the character of MI course on the level of ST2

in plasma, significantly higher level of neurohormone was determined with anterior localization of MI, high risk on the GRACE scale (≥140 points), complicated course of MI, development of cardiac arrhythmias and HF in the acute period of MI.

Keywords: NSTEMI, biomarkers, ST2, GRACE scale.

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1. Introduction

The analysis of the health dynamics status among Ukrainian population in recent years shows a negative trend, namely the deterioration of life quality and patients life expectancy [1, 2]. An indisputable leader in this process is coronary artery disease (CAD) and especially its acute forms, which are associated with high mortality [3, 4]. On this basis, the study of different aspects of this problem is of great interest for the practical health care of Ukraine.

Among the acute forms of CAD, NSTEMI is associated with extremely unanticipated course and unfavorable prognosis in the near and distant period [4]. Therefore, the development of NSTEMI stratification and prognosis is in the focus of scientific society. In this sense, various non-invasive biomarkers, such as troponin I (TnI), natriuretic peptide (BNP and NT-proBNP), galectin-3, growth factor-expressing gene 2 (ST2) and others, have been recently studied in detail [5]. Among the latter, a great deal of attention is paid to ST2 [6].

ST2 as a neurohumoral factor attracted attention due to already available scientific data on its unique properties and a fairly wide range of pathophysiological effects and a potential role in the diagnosis and prognosis of asymptomatic and symptomatic structural remodelling and fibrosis of myocardium and myocardial dysfunction [7, 8]. The diagnostic value of the biomarker has been demonstrated in both acute coronary syndromes and chronic stable heart failure [9, 10]. ST2 has proven to be a highly informative marker in predicting left ventricular remodeling and monitoring positive treatment response in patients with systolic heart failure (HF), exceeding the sensitivity of factors such as NT-proBNP, highly sensitive cardiac troponin T and galectin-3 [11, 12].

Thus, the modern scientific sources analysis demonstrates the prospect of using ST2 level in plasma as a diagnostic marker that reflects the presence and severity of structural remodelling of myocardium in various cardiovascular pathologies, including MI [13, 14].

The aim of the study was to evaluate ST2 level in plasma among the patients with NSTEMI and its relationship according to different clinical characteristics.

2. Materials and methods

All studies conform to the principles of the Declaration of Helsinki of the World Medical Association. The study protocol, the form of informed consent of patients and other documents related to the study were approved at the meeting of the Academic Council of the National Pirogov Memorial Medical University, Vinnytsya (excerpt from the protocol No. 2 from 28.11.2017). Informed consent to participate in the study was discussed and signed by all study participants.

The study is based on the results of a comprehensive examination of 165 patients aged 35 to 79 (average 60.7±0.8) years with various forms of CAD with and without arterial hypertension (HTN). Among the surveyed 114 (69.1 %) patients were male and 51 (30.9 %) were female respectively. The ratio of men to women was 2.2 to 1.0, indicating a significant predominance in the study of male patients ($p<0.0001$).

At the time of their inclusion in the study, all patients were hospitalized in the cardiology department for infarction patients of the Vinnytsya Regional Clinical Medical Diagnostic Center for Cardiovascular Pathology for 2016–2018 years.

The criteria for patient inclusion in the study were: different forms of CAD (NSTEMI, STEMI, NSTE-ACS, chronic coronary syndromes) and patients age from 30 to 80 years. The exclusion criteria were:

1) past and repeated acute myocardial infarction;
2) the age of patients under 30 and over 80;
3) HF NYHA III–IV at the time of the study inclusion;

4) diseases of the respiratory system, kidneys and liver, which were accompanied by signs of pulmonary, renal and hepatic failure; anemic conditions with haemoglobin levels below 110 g/l;

5) presence of rheumatic and congenital heart defects, idiopathic and inflammatory myocardial lesions;

6) malignancies, severe neuropsychiatric disorders, alcohol abuse.

The main clinical trial was presented to 90 patients with NSTEMI aged 38 to 79 (mean 61.3±1.1) years. Among them, 60 (66.7 %) patients were male and 30 (33.3 %) were female respectively. The ratio of men to women was 2.0 to 1.0 ($p<0.0001$). It was noted that in the main clinical group, as well as in the general cohort of patients, men predominated significantly. Time from the moment of destabilization to hospitalization in the hospital ranged from 1 to 24 and averaged 10.6±1.0 hours. In the vast majority (87.8 %) of patients with NSTEMI according to the ECG recorded anterior localization of MI (in 87.8 % – depression of ST-segment from 2 to 5 mm and in 12.2 % – T-wave inversion from 2 to 6 mm in two or more leads) and an increase in plasma troponin levels compared to the reference rate of 2.5 to 18.4 ng/ml (7.5±0.5 ng/ml on average).

As groups of comparison were formed 3 groups with other different clinical forms of CAD. Thus, group 1 consisted of 25 patients with verified chronic coronary syndromes (CCS) (mean age – 60.6±2.4 years, men – 68.0 % and women – 32.0 % respectively); group 2 – 25 patients with NSTE-ACS (mean age – 61.4±2.4 years, males – 68.0 % and females – 32.0 %, respectively) and 3rd – 25 patients with STEMI (mean age 58.3±2.3 years, males 80.0 % and females 20.0 %, respectively). In patients with STEMI, unlike the main group, there was a significantly shorter mean period from the destabilization onset to hospitalization (6.3 vs. 10.6 h, $p<0.001$) and the incidence of anterior MI localization (64.9 % vs. 87.8 %, $p=0.006$) and a higher mean troponin level (10.4 vs. 7.5 ng/ml, $p=0.003$) which was logical.

The majority of the surveyed had concomitant HTN, the incidence of which was 84.4 % (n=76) in the main group and 85.3 % (n=64) in the comparison groups. Most patients had observed Crade II (32.9 % and 46.1 %, n=30 and 36) and Crade III of HTN (40.6 % and 35.9 %, n=37 and 27 respectively). Such risk factor as smoking was determined in almost half of the surveyed – in 45.6 % (n=41) of patients in the primary and in 58.7 % (n=44) – comparison groups respectively. In almost one third of patients, abdominal obesity of Grade I-II (BMI > 30 kg / m2) was determined in 32.2 % (n=29) of the primary and 42.7 % (n=32) of the comparison groups respectively. 11.1 % (n=10) and 14.7 % (n=11) of patients were diagnosed with type 2 diabetes mellitus and 50.0 % (n=45) and 42.7 % (n=32), respectively with impaired heredity in cardiovascular disease.

Laboratory study of the plasma ST2 levels was performed by enzyme-linked immunosorbent assay in all patients on the first day of hospitalization before hospital coronary and ventriculography (CVG). Presage® ST2 Assay EIA Test Kits No. BC-1065E and BC-1066E manufactured by CRITICAL DIAGNOSTICS 3030 Bunker Hill St. Suite 117A San Diego, CA 92109 were used to determine ST2.

The difference between the main clinical group and the comparison group, the gender difference and the difference of ST2 level depending on different clinical characteristics were calculated using nonparametric statistical methods (Mann-Whitney test). The statistical significance of the difference between the measured parameters among the CAD groups was calculated by Kruskal-Wallis ANOVA & Median test.

3. Results

The results of the present investigation were that the ST2 level in the main cohort was in range from 5.5 to 233.9 (in the middle – 49.8±3.5 ng/ml (median indicator – 34.7 and the inter-quartile range – 21.9 and 59.1 respectively). **Tables 1-2** show indicators of statistics, which characterize the variability of ST2 level in small groups of illnesses.

Noteworthy significantly higher ST2 level in patients with NSTEMI, unlike the comparison group, in the analysis of medians (35.9 vs. 27.7 ng/ml, $p=0.047$) and no statistical differences in the mean values. The latter index clearly argues, in the case of high variability of indicators in groups, the need to use nonparametric methods of comparison (the table compares the median of the indicator according to Mann-Whitney U test).

Table 1

ST2 variation in plasma in the main clinical and comparison groups

Groups	Patients number	Variational statistics indicators		
		Min–Max (ng/ml)	Mean±Standard error (ng/ml)	Median (25 and 75 Percentile) (ng/ml)
Patients with NSTEMI	90	12.7–233.9	50.3±4.5	35.9 (25.9; 55.7)
Patients with other forms of CAD	75	5.5–200.4	46.2±5.3	27.7 (18.4; 68.9)
Mann-Whitney U test: **p=0.047**				

Note: NSTEMI – non-ST elevation myocardial infarction

Table 2

ST2 variations in patients with various forms of CAD

Groups	Patients number	Variational statistics indicators		
		Min–Max (ng/ml)	Mean±Standard error (ng/ml)	Median (25 and 75 Percentile) (ng/ml)
1. NSTEMI	90	12.7–233.9	50.3±4.5	35.9 (25.9; 55.7)
2. NSTE-ACS	25	10.9–180.2	41.4±8.9	24.1 (17.1; 31.2)
3. STEMI	25	12.5–200.4	56.3±10.1	36.1 (21.6; 77.3)
4. CCS	25	5.5–158.4	39.8±8.8	23.4 (15.1; 48.9)
Intergroup analysis of ST2 level by Kruskal-Wallis ANOVA & Median test				
Group	**1**	**2**	**3**	**4**
1		0.02	1.00	0.01
2	0.02		0.04	0.73
3	1.00	0.04		0.03
4	0.01	0.73	0.03	

In turn, the comparison of ST2 levels in patients with various forms of CAD (**Table 2**) showed significant differences between groups with acute MI (1 and 3 groups) and patients with NSTE-ACS and CCS (2 and 4 groups) – 35.9 and 36.1 against 24.1 and 23.4 ng/ml, $p<0.05$ in the absence of significant differences between patients with different types of MI (35.9 and 36.1 ng/ml, $p=1.00$). Therefore, it should be thought that a significantly higher level of ST2 is present in patients with acute MI, regardless of its variant compared with patients with CCS and NSTE-ACS.

The results of ST2 analysis in the main clinical group in gender and age (**Table 3**) did not show significant differences in biomarker's levels in women and men ($p=0.38$), as well as patients of different age criteria ($p=0.94$).

Table 3

Plasma ST2 levels in patients with NSTEMI by sex and age

Group	Patients number	Mean±Standard error (ng/ml)	Median (25 and 75 Percentile) (ng/ml)
Male	60	50.1±5.0	36.9 (28.6; 56.6)
Female	30	50.7±9.3	34.1 (22.2; 53.9)
Mann-Whitney U test: p=0,38			
up to 60 years	39	56.1±8.9	35.4 (25.9; 59.1)
60 years and older	51	46.1±4.5	36.9 (27.4; 55.3)
Mann-Whitney U test: p=0,94			

ST2 level analysis in plasma depending on different clinical characteristics (**Table 4**) showed significant higher hormone levels in patients with concomitant HTN (39.9 vs. 31.2 ng/ml, p=0.04) and type 2 diabetes (68.5 vs. 35.5 ng/ml, p=0.04), in the presence of such a risk factor as smoking (39.6 vs. 32.7 ng/ml, p=0.03) and impaired cardiovascular heredity (41.8 vs. 32.6 ng/ml, p=0.01). Therefore, based on the data obtained, it should be noted that in patients with NSTEMI, the ST2 level in plasma, in a certain way, depends on the presence of concomitant diseases such as HTN and type 2 diabetes, risk factors such as smoking and impaired hereditary cardiovascular pathology. The highest ST2 level was observed in patients with concomitant type 2 diabetes mellitus (68.5 ng/ml). The latter confirms the already known fact that type 2 diabetes is considered to be the most powerful factor in the development of myocardial dysfunction and HF in patients with MI.

Table 4

Plasma ST2 levels in patients with NSTEMI depending on different clinical characteristics

Group	Patients number	Mean±Standard error (ng/ml)	Median (25 and 75 Percentile) (ng/ml)
MI	52	55.8±7.2	36.7 (28.2; 57.9)
Absent	38	43.2±4.5	35.0 (25.9; 52.1)
Mann-Whitney U test: p=0.47			
Concomitant HTN	76	50.8±5.2	39.9 (25.9; 56.1)
Absent	14	47.7±8.4	31.2 (20.8; 41.4)
Mann-Whitney U test: **p=0.04**			
Smoking	41	53.2±6.1	39.6 (28.6; 59.1)
Absent	49	48.1±6.6	32.7 (23.6; 50.8)
Mann-Whitney U test: **p=0.03**			
Burdened heredity in cardiovascular morbidity	45	60.6±7.6	41.8 (30.6; 73.3)
Absent	45	40.5±4.8	32.6 (22.4; 46.1)
Mann-Whitney U test: **p=0.01**			
Abdominal obesity (BMI> 30 kg/m^2)	29	46.3±8.3	34.6 (28.6; 41.8)
Absent	61	52.3±5.5	37.1 (25.9; 59.1)
Mann-Whitney U test: p=0.29			
Diabetes	10	85.3±23.0	68.5 (28.2; 100.5)
Absent	80	45.9±4.0	35.5 (25.9; 52.1)
Mann-Whitney U test: **p=0.003**			

In turn, analysis of ST2 level depending on MI nature (**Table 5**) showed that a significantly higher level of neurohormone was determined at anterior localization of MI according to the ECG (37.9 vs. 29.4 ng/ml, p=0.03), in the case of high risk on the GRACE scale (≥140 points) (42.0 vs. 30.3 ng/ml, p=0.01) and complicated course of MI (40.7 vs. 31.9 ng/ml, p=0.03). The complicated course of NSTEMI was registered by us in 22 (24.4 %) patients. Of these, 15 (16.7 %) observed acute cardiac arrhythmias, 3 (3.3 %) had cardiac conduction abnormalities, and 4 (4.4 %) had acute HF, which was consistent with Killip III. Of the acute cardiac arrhythmias, almost half (53.3 %, n=48) of patients reported frequent ventricular extrasystole with episodes of unstable ventricular tachycardia, 20.0 % (n=18) had attacks of persistent ventricular monomorphic tachycardia and 26.7 % (n=24) had paroxysmal atrial fibrillation. From 3 (3.3 %) of patients with acute conductivity disorders, in 2 (66.7 %) cases reported intermittent atrio-ventricular block of II-III degree and in 1 case (33.3 %) – sino-atrial block of II degree.

Table 5

ST2 levels in patients with NSTEMI depending on MI course

MI course characteristics	Patients number	Mean±Standard error (ng/ml)	Median (25 and 75 Percentile) (ng/ml)
Frontal localization of MI	79	53.5±5.4	37.9 (29.6; 58.9)
Rear localization of MI	11	38.1±5.0	29.4 (23.2; 47.9)
Mann-Whitney U test: **p=0,03**			
High risk (≥140 points) by Grace	36	46.1±5.7	42.0 (30.8; 64.1)
Low and moderate (<140 points) by Grace	54	33.3±6.7	30.3 (21.8; 55.2)
Mann-Whitney U test: **p=0,01**			
Uncomplicated course	68	42.8±5.5	31.9 (25.2; 46.4)
Complicated course	22	49.2±8.0	40.7 (32.9; 61.5)
Mann-Whitney U test: **p=0,03**			
Acute heart rate disorders	15	62.7±15.1	47.1 (31.2; 87.5)
Absent	75	47.8±4.5	35.6 (25.7; 55.3)
Mann-Whitney U test: **p=0,004**			
Conduction abnormalities	3	72.3±28.3	43.7 (38.2; 57.9)
Absent	87	49.0±4.5	35.2 (25.7; 55.3)
Mann-Whitney U test: p=0,13			
Acute HF (Killip III)	4	57.0±7.4	56.3 (35.4; 103.9)
Absent	86	40.7±5.0	35.2 (25.9; 56.1)
Mann-Whitney U test: **p=0,02**			

In addition, the analysis showed a significantly higher ST2 level in patients with NSTEMI in the presence of acute cardiac arrhythmias (47.1 vs 35.6 ng/ml, p=0.004) and acute HF (Killip III) (56.3 vs 35,2 ng/ml, p=0.02) during hospitalization of patients.

Thus, in patients with NSTEMI, a certain association of ST2 level in plasma with the MI course was detected. A higher level of neurohormone is registered with anterior unlike posterior ECG localization of MI; at high unlike moderate risk on the GRACE scale; when complicated unlike the uncomplicated course of MI; in the case of acute HF and cardiac arrhythmias unlike patients with the absence of these manifestations in the acute period of MI.

4. Discussion

The results obtained in the course of our study indicated a correlation of ST2 level with different clinical and instrumental characteristics. Thus, an increased level of biomarker was determined in patients with myocardial infarction compared with the control group, which is confirmed by the literature [15, 16]. The rate was higher in the group of patients with concomitant hypertension, hereditary history, smoking, diabetes and obesity. Most of the literature is centred around STEMI, while ST2 is not well understood in NSTEMI [9, 17].

We investigated ST2 levels depending on the localization of myocardial infarction and its complications, which obtained conclusive data regarding the increase of the level of biomarker in the main group compared with the control group.

Given that ST2 levels are correlated with the risk of an unfavourable course of myocardial infarction, its determination in the early stages of the disease allows for appropriate correction of treatment, such as dose modification of drugs or changes in their combinations. If relevant clinical data are available with regard to STEMI, there is very little data regarding NSTEMI [18].

Study limitations. This study examines the therapeutic aspects of the ratio of ST2 levels and the clinical-instrumental characteristics of NSTEMI, while the surgical aspects are

forced to be neglected. However, the data obtained during the study confirm the indisputable relationship between the level of biomarker and the character of the course of CAD and concomitant HF [19, 20].

Prospects for further research. Of great interest are the associations of ST2 level with the character of the anatomic lesions of the coronary arteries, in particular, the correlative connections according to the SYNTAX score, but we attribute this research to further and prospective studies. The following data will help to optimize the tactics of treating patients in the early period of myocardial infarction immediately after emergency coronary angiography and angioplasty.

5. Conclusions

1. High variability of ST2 level in plasma was demonstrated in patients with NSTEMI on the first day after destabilization (minimum and maximum values – 12.7 and 233.9 respectively, median – 35.9 and interquartile range – 25.9 and 55.7 ng/ml).

2. It is shown that significantly higher ST2 level in plasma is determined in patients with acute MI regardless of its variant among different clinical forms of CAD.

3. It is found that significantly higher level of ST2 in patients with NSTEMI is recorded in the case of concomitant HTN and type 2 diabetes, with smoking and heavy cardiovascular heredity. Proved influence of the character of MI course on the level of ST2 in plasma, significantly higher level of neurohormone was determined with anterior localization of MI, high risk on the GRACE scale (≥ 140 points), complicated course of MI, development of cardiac arrhythmias and HF in the acute period of MI.

Conflict of interest

There is no conflict of interest.

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RESISTANCE OF *S. AUREUS* ATCC 25923, *E. COLI* 055K59 No. 3912/41 AND *P. AERUGINOSA* 27/99 TO THE WASH-DISINFECTANT «MILKODEZ»

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Abstract

The aim of the work – the article presents the results of determining of the resistance of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/4 and *P. aeruginosa* 27/99 test cultures in planktonic form and in biofilm to our developed «Milkodez» acid detergent.

Materials and methods. Microbial biofilms were grown on MPB in 5 cm disposable plastic Petri dishes. To determine the effect of disinfectants on microbial biofilms, 3 Petri dishes with biofilms of each of the test cultures were used. One of the Petri dishes served as control and she had for 15 minutes made 5 cm^3 of saline NaCl solution, in the second – 5 cm^3 of hot water (t=70±5 °C), and in the third – 5 cm^3 of acidic detergent «Milkodez». Microbial biofilms were fixed for 10 min. 96º with ethyl alcohol for 10 min. were stained with a 0.1 % solution of crystalline violet, and the remnants of the unabsorbed paint were removed with phosphate buffer. The biofilm dye was extracted with 96º of ethyl alcohol, which was photocolometrically investigated at 570 nm to establish the density of the formed biofilms. The density of the formed microbial biofilms was considered low in optical density of the extract up to 0.5 units, average – from 0.5 to 1.0 units; and high – over 1.0 units

The resistance of planktonic forms of test cultures of microorganisms to disinfectants was determined in sterile tubes, which made 10 cm^3 (t=70±5 °C) of 0.5 % of their working solutions and 0.1 cm^3 (1 billion microbial bodies) of the standard test – cultures. The culture was maintained for 15 min. and made ten – fold plantings on IPA in Petri dishes.

Incubation of mesophilic microorganisms was carried out in a thermostat at a temperature of 30 °C, and psychrophilic – 20 °C. After 48 hours the calculation of the growing colonies were carried out. The results were expressed in colony forming units (CFU).

Results. Due to the impact on microbial biofilms formed by the test cultures of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 for 15 min. 0.5 % solution of acid detergent «Milkodez» the optical density of the solutions was respectively 0.64, 0.72, 0.45 units. The results obtained indicate that the melkodez caused a decrease in the biofilm-forming ability of *S. aureus* ATCC 25923 3.2 times, in *E. coli* 055K59 No. 3912/41 – 1,7 times and in *P. aeruginosa* 27/99 – 2.8 times, compared to control. However, the density of one – day microbial biofilms formed by *S. aureus* ATCC 25923 and *E. coli* 055K59 No. 3912/41 was medium, and *P. aeruginosa* 27/99 was low. It has been proven that the «Milkodez» acid detergent developed is more effective than the prototype «Hypracid», since it caused the death of 100 % of planktonic test cultures and the number of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 formed in the biofilm that survived after its application was 2.7, 3.2 and 1.4 times lower, respectively.

Conclusions. It was found that the test cultures were able to form high – density biofilms, since the optical density of the extract in the control was in the range from 1.28 to 2.05 units, which is greater than 1.0 units. Acid wash detergent «Milkodez» for 15 minutes of exposure causes the formation of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 biofilms of low and medium density and reduces their biofilm capacity by 3.2, 1.7 and 2.8 times, respectively. Its use provides the death of 100 % of the planktonic forms of the test cultures under study and reduces their number in the biofilm by 2.7, 3.2 and 1,4 times more, respectively, compared to «Hypracid» detergent.

Keywords: *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/4, *P. aeruginosa* 27/99, biofilms, «Milkodez».

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1. Introduction

In modern conditions of milk production, the decisive factor affecting its quality is the sanitary condition of milking equipment [1, 2]. The main share of mechanical and bacterial con-

tamination of milk is formed due to insufficiently washed milking equipment and milk equipment [3]. Due to its exploitation, various composition and properties of deposits are formed on the inner surfaces of pipelines, which are an excellent medium for the development of microorganisms [4, 5]. Most of these surfaces are closed and have limited access to cleaning and disinfection. In case of insufficiently effective sanitary treatment, the development of microflora occurs, which enters the next batch of milk, which significantly degrades its safety and quality [6]. As a result, the grade and, consequently, the sale price decrease [7, 8].

It is known that microorganisms are able to survive on abiotic surfaces of dairy equipment due to their extremely important property – the ability to form biofilms [9, 10]. A biofilm is a living collection of several types of bacteria that is constantly updated, attached to a biogenic or abiogenic surface and surrounded by a polysaccharide matrix, which is a stable structure and is one of the important factors for bacterial protection [11, 12].

The formation of the biofilm adversely affects the quality of the milk obtained and the safety of the finished product since, in addition to saprophytes, pathogens can also be involved in them [13, 14]. This complex process involves the formation of micro–colonies, structuring and their maturation, which significantly increases the survival of bacteria in the body and the environment [15, 16]. Microbial resistance in a biofilm is determined by different gene expression in a multicellular population, which causes some cells to transition to a persistent state, which in turn causes inherited resistance or tolerance to different antimicrobials [17, 18].

According to Verran J. (2010), bacterial resistance in biofilms to disinfectants is about 100 times greater than that of planktonic microorganisms. This is due to the fact that the bacteria in the biofilms are in a metabolically inert state, causing them to have poor antimicrobial agents, and because the pores and channels of the biofilms do not allow large molecules of disinfectants to enter the biofilm [19].

That is why, in the development of detergents for sanitary processing of milking equipment, it is necessary to select such active substances that would destroy the intercellular polysaccharide – peptide matrix of the biofilm and adversely affect not only planktonic cultures of microorganisms, but also on bacteria.

The aim of our work was to investigate the resistance of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/4 and *P. aeruginosa* 27/99 test cultures in planktonic form and in biofilm to our developed «Milkodez» acid detergent.

2. Materials and Methods

The researches were conducted at the department of microbiology and virology, Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, and Ternopil Research Station of the Institute of Veterinary Medicine of NAAS of Ukraine during 2018-2019.

Using in vitro experiments, we studied the effect of acidic detergent «Milkodez» on planktonic forms of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/4 and *P. aeruginosa* 27/99 and daily microbial biofilms formed by these test cultures. As a prototype used registered in Ukraine acidic agent «Hypracid». To obtain microbial biofilms, 5 cm^3 of meat-peptone broth and 1 cm^3 (10 thousand CFU in 1 cm^3) of daily bacterial test culture were introduced into disposable 5 cm plastic Petri dishes. After incubation for 24 hours the contents of the cups were poured into the solution. To remove planktonic (unattached) bacteria, the cups were washed three times with sterile phosphate buffer and kept at room temperature until complete drying of the contents.

The effect of «Milkodez» on microbial biofilms was investigated on three Petri dishes with biofilms formed by the test cultures. In the first cup with the biofilm of the corresponding test culture, which served as a control, 5 cm^3 of saline NaCl was introduced, in the second – 5 cm^3 of hot water (t=70±5 °C), and in the third – 5 cm^3 of acid detergent «Milkodez» (t=70±5 °C). The exposure was 15 min. The contents of the cups were drained, the cup was washed with sterile phosphate buffer and kept at room temperature until complete drying. The microbial biofilms were then fixed for 10 minutes with 96º ethyl alcohol. After decanting, they were again dried for 10 minutes; were stained with a 0.1 % solution of crystalline violet. Removal of the residues of the non–adsorbed paint was carried out with phosphate buffer and dried again in air. The biofilm paint was extracted

by application for 20 minutes 3 cm^3 of 96° ethyl alcohol. To determine the density of the formed biofilms photocolometrically at a wavelength of 570 nm, determine the optical density of ethyl alcohol, which was carried out the extraction of paint [20]. The density of the formed microbial biofilms was considered low in optical density of the extract up to 0.5 units, average – from 0.5 to 1.0 units; and high – over 1.0 units [21].

To determine the resistance of microorganisms in daily biofilms to «Milkodez» and «Hypracid» in Petri dishes with pre-formed biofilms for 15 minutes 5 cm^3 (t=70±5 °C) of their 0.5 % solutions were added. The disinfectants were drained and the contents of the cup washed three times with phosphate buffer solution and 5 cm^3 of sterile NaCl solution was added. The sterile swab was carefully washed from the walls and bottom of the cup microbial biofilm, selected 1 cm^3 suspension from which did a number of ten fold dilutions. The determination of the stability of planktonic forms of test cultures of microorganisms to disinfectants was carried out in sterile tubes, which made 10 cm^3 (t=70±5 °C) of 0.5 % working solutions and 0.1 cm^3 (1 billion microbial bodies) of the standard test cultures. The culture was maintained for 15 minutes and from the resulting slurry a number of ten fold dilutions were prepared. Crops from each dilution were carried out in Petri dishes, in which 1 cm^3 of the suspension was introduced and filled with molten and cooled to 45±1 °C MPB [22].

Incubation of mesophilic microorganisms was carried out in a thermostat at a temperature of 30 °C, and psychrophilic – 20 °C. After 48 hours the calculation of the growing colonies were carried out. The results were expressed in colony forming units (CFU).

The obtained numerical material was subjected to statistical processing by the method of variational statistics with determination of mean values and mean error. The probability of differences between the mean values during the analysis was evaluated using Student's t test (t). The difference between the values was considered probable when the probability of the difference was $p \leq 0.05$.

3. Results

In **Fig. 1** is shown the optical density of ethyl alcohol, which carried out the extraction of paint from bacterial biofilms formed *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99, indicating that these test cultures are capable to form high-density biofilms, since the optical density of the extract in the control ranged from 1.28 to 2.05 units, which is greater than 1.0 units.

Processing of microbial biofilms for 15 minutes with hot water (t=70±5 °C) caused a significant ($p \leq 0.01$) decrease in the optical density of the solutions. Thus, the optical density of the solution, by which biofilms were washed formed by *S. aureus* ATCC 25923 was 1,11 units, biofilms *E. coli* 055K59 No. 3912/41 – 1.04 units and *P. aeruginosa* 27/99 – 0.83 units, which was 1.8, 1.6 and 1.5 times lower, respectively, compared to the control.

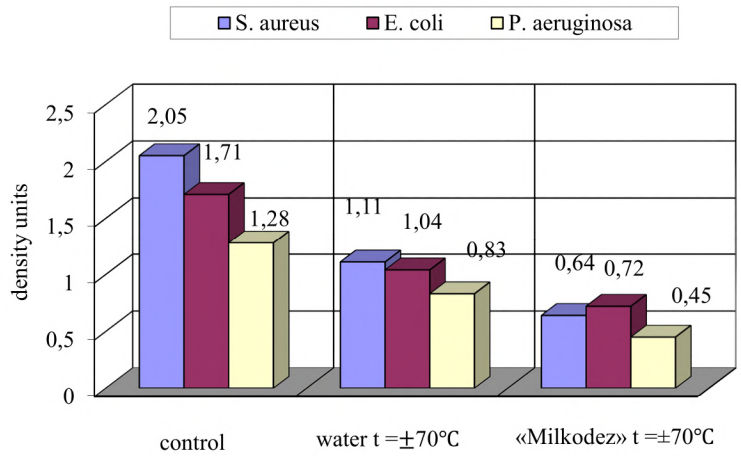

Fig. 1. The optical density of ethyl alcohol, which was carried out the extraction of paint from bacterial biofilms formed by the test cultures of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99

Due to the impact on microbial biofilms formed by the test cultures of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 for 15 min. 0.5 % solution of acid detergent «Milkodez» the optical density of the solutions was respectively 0.64, 0.72, 0.45 units. The results obtained indicate that the «Milkodez»caused a decrease in the biofilm–forming ability of *S. aureus* ATCC 25923 3.2 times, in *E. coli* 055K59 No. 3912/41 – 1.7 times and in *P. aeruginosa* 27/99 – 2.8 times, compared to control. However, the density of one – day microbial biofilms formed by *S. aureus* ATCC 25923 and *E. coli* 055K59 No. 3912/41 was medium, and *P. aeruginosa* 27/99 was low.

Important in the study of new disinfectants is the study of the resistance to them of microorganisms, which are in planktonic form and formed into biofilms (**Table 1**).

Table 1

The resistance of the test cultures of microorganisms to working solutions of acidic agents «Milkodez» and «Hypracid», lg CFU/cm^3, M±m, n=5

Microorganisms		The number of microorganisms in 1 cm^3 depends		
		Control	«Hypracid»	«Milkodez»
S. aureus ATCC 25923	planktonic	7.08±0.351	2.08±0.087*	0
	biofilm	8.38±0.327	4.97±0.192*	1.86±0.079* ^{o}
E. coli 055K59 No. 3912/41	planktonic	7.08±0.351	0	0
	biofilm	8.52±0.357	4.87±0.162*	1.23±0.046* ^{o}
P. aeruginosa 27/99	planktonic	7.04±0.274	0	0
	biofilm	5.73±0.234	3.15±0.122 *	2.20±0.083* ^{o}

*Note: ^{o} – p≤0.001– to the «Hypracid»; * – p≤0.001 – to control*

It is established that the developed acidic detergent «Milkodez» in working concentration of 0.5 %, for exposures of 15 minutes caused the death of all test cultures of microorganisms that were in planktonic form. At the same exposure, the prototype agent «Hypracid» caused the death of planktonic forms *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 and showed a bactericidal effect against the *S. aureus* ATCC 25923 test culture, since their number decreased by 1 cm^3, compared to the control, 3.4 times (p≤0.001) and amounted to 2.08±0.087 lg CFU.

Biofilm to the acidic agent «Milkodez» indicate that for 15 min the exposure of its working solution did not ensure the death of all microorganisms. Thus, after the destruction of the biofilm formed *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 and sowing 1 cm^3 of the resulting suspension on a nutrient medium, after 48 hours of incubation on it increased respectively 1.86±0.079, 1.23±0.046 and 2.20±0.083 lg CFU. Compared to controls, the number of *S. aureus* ATCC 25923 was 4.5 times lower (p≤0.001), *E. coli* 055K59 No. 3912/41 – 6.9 times, and *P. aeruginosa* 27/99 – 2.6. times.

P. aeruginosa 27/99 and the least resistant *S. aureus* ATCC 25923 were the least resistant of the tested test cultures formed into the biofilm to the «Hypracid» prototype preparation. *S. aureus* 25923 was 4.97±0.192 lg CFU, *E. coli* 055K59 No. 3912/41 – 4.87±0.162 and *P. aeruginosa* 27/99 – 3.15±0.122 lg CFU, which, compared to the control, was smaller respectively 40.7, 42.8 and 45.1 % (p≤0.001).

It has been proven that the «Milkodez» acid detergent developed is more effective than the prototype «Hypracid», since it caused the death of 100 % of planktonic test cultures and the number of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 formed in the biofilm that survived after its application was 2.7, 3.2 and 1.4 times lower, respectively.

4. Conclusion

1. Acid detergent «Milkodez» for 15 minutes of exposure causes a decrease in biofilm–forming capacity of *S. aureus* ATCC 25923 3.2 times, in *E. coli* 055K59 No. 3912/41 – 1.7 times and in *P. aeruginosa* 27/99 – 2.8 times and the formation of low biofilms and medium density.

2. The use of «Milkodez» provided 100 % destruction of planktonic forms of *S. aureus* ATCC 25923, *E. coli* 055K59 No. 3912/41 and *P. aeruginosa* 27/99 and reduced their number in the biofilm by 2.7, 3.2 and 1.4 times more, respectively, compared to «Hypracid» detergent.

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