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Sepsis Course and Outcome Depends on the Genetic Variant in the 3`-Region of Aquaporin 4 Gene *AQP4* and Comorbidities

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Summary

Aquaporins 4 and 5 are proteins that form water channels in the cell membrane, participate in the transfer and migration of immune cells, being expressed on many cell types including CNS astrocytes, kidney cells, lungs, and the immune system. We have previously shown that *AQP*5 genetic polymorphism is associated with different outcomes of abdominal sepsis. Since another common aquaporin protein, *AQP*4, is also expressed on the surface of immunocompetent cells, determining cell motility, it was suggested that *AQP*4 may also be important in the pathogenesis of sepsis, and that *AQP*4 polymorphism may predetermine sepsis severity and outcome. *AQP*4 rs1058427 genetic polymorphism has not been studied earlier.

The aim of the study was to determine the effects of region 3` polymorphism in the *AQP*4 gene on the clinical course and outcome of sepsis.

Materials and methods. The prospective study included 290 ICU patients from three clinical hospitals in Moscow aged 18–75 years with clinical signs of sepsis (SEPSIS-3, 2016).

Results. It was found that the minor T allele of the *AQP*4 rs1058427 gene provides strong protection against septic shock, as among GG genotype carriers septic shock developed in 66%, but in presence of the minor T allele dropped to half of cases (*P*=0.009, Fisher's exact test, OR=1.99, 95% CI: 1.12–3.55, *N*=290). There was a significant association between *AQP*4 rs1058427 genetic polymorphism and 30-day hospital mortality in a subgroup of patients with more severe organ dysfunction and higher comorbidity burden (cardiovascular diseases, type II diabetes mellitus) requiring extracorporeal treatment modalities and ventilator support for 5 or more days (*N*=66). Carriers of the minor T allele showed better survival rates as compared *AQP*4 rs1058427 GG genotype carriers (5 deaths out of 10 and 47 deaths out of 56, respectively, *P*=0.003, Fisher's exact test, *N*=66, OR=5.22, 95% CI: 1.25–21.82, *P*=0.009, log-rank criterion).

Conclusion. The minor *AQP*4 rs1058427 T allele is associated with protection against septic shock and better survival in sepsis in a group of ICU patients with high comorbidity burden requiring extracorporeal life support interventions.

Keywords: sepsis; septic shock; genetic polymorphism; AQP4

Conflict of interest. The authors declare no conflict of interest.

Introduction

Sepsis remains the leading cause of mortality in intensive care units worldwide. The greatest mortality in sepsis is due to the development of septic shock (SS). SS is defined as sepsis (according to SEPSIS-3 criteria, 2016) associated with severe hemodynamic, cellular and metabolic disturbances, with a higher risk of death than in sepsis without shock [1]. The high mortality in sepsis/septic shock prompts the search for biomarkers, including molecular genetic ones, to identify groups of patients at high risk for adverse outcomes of critical illness, in order to better justify and target the early use of high-tech treatment options and improve survival rates. The study of genetic polymorphisms in sepsis may help in the earliest stratification of patients into groups at risk of adverse sepsis progression and outcome.

Meanwhile, the clinical heterogeneity of sepsis may be determined by a variety of its pathophysiological mechanisms and different patterns of specific pathogenetic pathways depending on the environmental factors and genetic characteristics of the patient. Studies of genetic polymorphisms may help us to understand the causes of the diversity of mechanisms of disease progression and poor outcome in sepsis and to determine the relationship of this diversity with allelic variants of polymorphic genes. In the future, the results of such studies may be used to personalize treatment according to predisposition to specific pathogenetic elements of sepsis.

Currently, there is a growing body of research indicating that gene variants, particularly single nucleotide polymorphisms, play a significant role in individual variation in the inflammatory response and determine the adverse or favorable course and outcome of sepsis [2–4]. Attempts have been made to develop approaches to personalize treatment of sepsis [5], including those based on natural genetic variability and its association with a variety of clinical phenotypes and mechanisms of sepsis [6–8].

In this context, genetic polymorphisms of loci controlling key pathophysiological processes leading to sepsis and/or determining its adverse outcome (septic shock) are of greatest interest. Such genes include genes of the innate and adaptive immunity that control immunome. Immunological mechanisms are known to play a key role in the development of sepsis, and many manifestations of sepsis, including septic shock, depend on the recruitment of proinflammatory immune cells that may damage the vascular endothelium, resulting in perfusion defects. Recently, immune cell migration has been associated with genetic polymorphisms of *AQP*4 and *AQP*5, proteins that form water channels in the cell membrane. Both proteins are expressed in various cells, including cells of the brain (astrocytes), kidney, lung and immune system [9, 10]. Both proteins have been implicated in the development of cerebral edema, migration of immune cells, and maintenance of the blood-brain barrier [11]. *AQP*4 is known to control the survival of nervous system cells and T cells [12, 13], and inhibition of this protein in vivo reduces the number of T lymphocytes in lymph nodes with concomitant accumulation in the liver [12, 14]. *AQP*4 is expressed by cardiomyocytes, and *AQP*4 deficiency reduces myocardial tissue damage and the severity of edema in myocardial infarction [15, 16]. *AQP*4 plays a role in the development of regulatory T cells in the thymus; *AQP*4 knockout mice showed reduced levels of CD4+ and CD25+ regulatory T cells [17, 18]. Brain inflammation in septic encephalopathy causes *AQP*4 activation, which is associated with increased brain edema [19, 20]. In addition, *AQP*4 expression is upregulated in astrocytes during sepsis [21].

*AQP*4 genetic variants have been identified as potential prognostic biomarkers in brain injury (rs3763043, rs3875089) [22], perihematomal edema in patients with hemorrhagic stroke (rs1058427) [23], and in patients with hemorrhagic stroke (rs3875089, rs3763043, rs11661256) [24].

Abnormal migration of different populations of immunocompetent cells, primarily myeloid cells and lymphocytes, determines endothelial cell damage, which is crucial for the development of organ failure, and immunosuppression that indirectly affects the bacterial load. Since *AQP*4 gene product is involved in the recruitment and migration of immune cells, which are directly related to the pathophysiology of sepsis, we hypothesized that the *AQP*4 gene polymorphism may contribute to the sepsis pathogenesis. Indeed, The *AQP*4 rs1058427 genetic polymorphism has only been studied in relation to the progression of hemorrhagic stroke. The relationship between the *AQP*4 rs1058427 genetic polymorphism and the course and outcome of sepsis (including cohorts with multiple comorbidities) has not been studied. As a result, the aim of our study was to examine the impact of the *AQP*4 3' region polymorphism on the course and outcome of sepsis in ICU patients with various comorbidities.

Materials and methods

A close-label, uncontrolled, noncomparative, randomized trial was conducted. The primary endpoint was the incidence of septic shock and the secondary endpoint was mortality in groups of patients with different comorbidities.

According to available data, the incidence of septic shock in groups of patients with sepsis and significant comorbidity is at least 75 percent. Based on this, the sample size was calculated. According to the formula for calculating the sample size,

$$\mathbf{N=(t^2\times P\times Q)/\triangle^2}$$ **[23, 24],**

where t is the critical value of the Student's t-test (at the significance level of 0.05) of 1.96, $\triangle$ is the maximum allowable error (5%), P is the proportion of cases in which the studied characteristic occurred (75), Q is the proportion of cases in which the studied characteristic did not occur (25), the estimated total number of patients *(N)* was 288 [25, 26].

Patients from three ICUs (N=290) participated in the study. No sex differences and age were found between patients from the three ICUs (Table). In ICUs 2 and 3, extracorporeal treatment methods were widely used, so the SOFA score of patients in these ICUs was significantly higher, and more patients with significant comorbidity were included in the sample. The number of patients with diabetes mellitus was significantly higher in ICU 2 and 3 than in ICU 1, and the number of patients with cardiovascular disease was higher in ICU 2 than in ICU 1. Extracorporeal treatments (ECT) (hemodialysis, hemodiafiltration, hemofiltration, LPS adsorption, or their combinations) were used in 51% of patients in ICUs 2 and 3. Indications for ECT were conventionally divided into «renal» (acute kidney injury, including underlying chronic renal disease, decompensated chronic renal failure, need for renal replacement therapy) and «extrarenal» (severe intoxication, hyperkalemia, metabolic acidosis and other disorders of water-electrolyte balance, need for endotoxin adsorption, and others). The higher number of patients with renal diseases in ICU 2 was due to the presence of a tertiary nephrology center in the hospital where ICU 2 was located (Table).

Table. Demographics and morbidity of ICU patients with sepsis.

Parameter	Value in patients of various ICUs			P_{1-2}	P_{1-3}	P_{2-3}	Total
	ICU 1	ICU 2	ICU 3				
Men, *N* (%)	76 (53)	29 (50)	55 (62)	0.76	0.22	0.17	160 (55)
Women, *N* (%)	67 (47)	29 (50)	34 (38)				130 (45)
Age, years *M* (IQR)	61 (50–70)	60 (46–68)	60 (50–68)	0.25	0.27	0.8	60 (49–69)
SOFA score on admission, *M* (IQR)	3 (2–5)	6 (5–7)	6 (3–9)	**<0.001**	**<0.001**	0.97	5 (3–7)
Peritonitis, *N* (%)	42 (30)	3 (5)	11 (12)	**0.001**	**0.004**	0.25	56 (19)
Community acquired pneumonia, *N* (%)	15 (10)	2 (3)	17 (19)	0.16	0.07	**0.005**	34 (12)
Cardiovascular disease, *N* (%)	16 (11)	15 (26)	19 (22)	**0.016**	0.04	0.56	50 (17)
Pancreatitis or pancreatic necrosis, *N* (%)	11 (8)	5 (9)	7 (8)	0.78	1	1	23 (8)
Renal failure, pyelonephritis, renal stone disease, atypical hemolytic-uremic syndrome, *N* (%)	3 (2)	24 (42)	11 (12)	**<0.001**	**0.003**	**0.001**	38 (13)
Trauma, *N* (%)	4 (3)	3 (5)	11(12)	0.4	**0.006**	0.25	18 (6)
Phlegmon, *N* (%)	—	—	4 (5)	—	0.02	0.023	4 (1)
Neoplasms, *N* (%)	15 (10)	3 (5)	2 (2)	0.29	0.02	0.38	20 (7)
Hepatitis or cholecystitis, *N* (%)	12 (9)	—	1 (1)	0.02	0.02	1	13 (5)
Peptic ulcer, *N* (%)	4 (3)	—	1 (1)	0.3	0.65	1	5 (1)
Mesenteric thrombosis, *N* (%)	6 (4)	1 (2)	—	1	0.08	0.39	7 (2)
Appendicitis, *N* (%)	6 (4)	—	1 (1)	0.18	0.2	1	7 (2)
Other*, *N* (%)	9 (6)	2 (3)	4 (5)	0.5	0.7	1	15 (5)
Underwent surgery, *N* (%)	57 (39)	35 (60)	53 (59)	0.01	0.005	1	145 (50)
Diabetes mellitus, *N* (%)	18 (13)	26 (45)	38 (43)	**<0.001**	**<0.001**	0.74	82 (28)
Coronary heart disease, *N* (%)	37 (26)	24 (41)	24 (27)	0.041	0.08	0.75	85 (29)
Septic shock, *N* (%)	87 (61)	35 (60)	59 (66)	1	0.5	0.86	181 (62)
Total, *N*	**143**	**58**	**89**				**290**

Примечание. * — osteoporosis due to hormone imbalance, diverticulosis, metachromatic leukodystrophy, necrotizing fasciitis, abscess, cyst, spontaneous esophageal rupture, hernia, gastritis. *N* — number of patients; *M* — median value; IQR — interquartile range. *P*-values calculated by Mann–Whitney test or Fisher's exact test.

Allelic variants of *AQP*4 rs1058427 were determined by tetraprimer polymerase chain reaction followed by electrophoretic separation and gel identification of stained products. Using the Primer-BLAST software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), the following primers were selected and synthesized at Eurogen LLC:

*AQP*4 1 for. 5`-TATTGGCAAAACTGGGGATT-3`

*AQP*4 2 for. 5`-CCCAATCTCTGCTCTCTCAA-3`

*AQP*4 2 rev. 5`-GATTATCAACAAATGTCACGAGAAG-3`

*AQP*4 1 rev. 5`-TGCAACCATGTTGTACCTTG-3`

The significance of differences between groups was assessed using the χ^2 criterion with Yeats' correction for sampling continuity and Fisher's exact test (FET). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to estimate the risk of fatal sepsis in patients with different genotypes. Normality of the distribution of variables was determined using the Shapiro–Wilk test. Qualitative variables were presented as absolute numbers with percentage fractions. For non-normal distribution, the Mann–Whitney *U*-criterion was used to assess differences between groups, and medians and interquartile ranges (IQRs) were calculated. The Kaplan–Meier method and the log-rank test were used to determine differences in survival. Differences were considered significant at $P<0.05$. Bonferroni correction was used to compare demographics and morbidity among patients in the three ICUs, and differences were considered significant at $P<0.0166$. Statistical analysis was performed with MedCalc version 11.6 and SigmaStat version 3.5.

Results

The distributions of *AQP*4 rs1058427 genotype frequencies were as follows: GG, 80%; GT, 18%; TT, 2% (*N*=290), which followed the Hardy–Weinberg law (χ^2=0.772, *P*=0.38) and did not differ significantly from the distribution in the group of apparently healthy volunteers (GG, 80%; GT, 18%; TT, 2%; χ^2=0.85, *P*=0.36, *N*=154, Fig. 1) and from the genotype frequencies in the North American population [23].

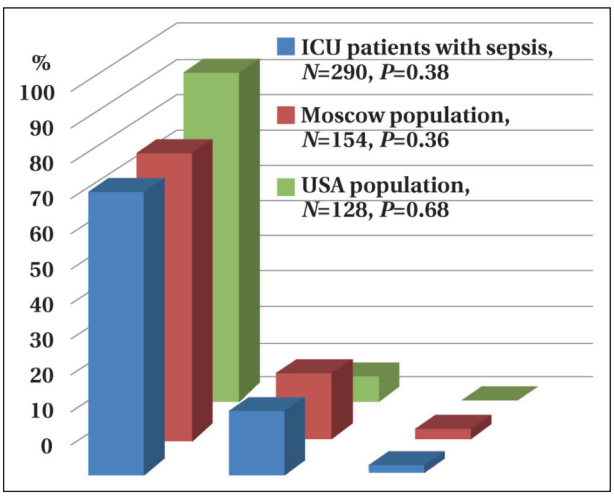

Fig. 1. *AQP*4 rs1058427 genotype frequencies among ICU patients, apparently healthy donors, and in USA population [23].

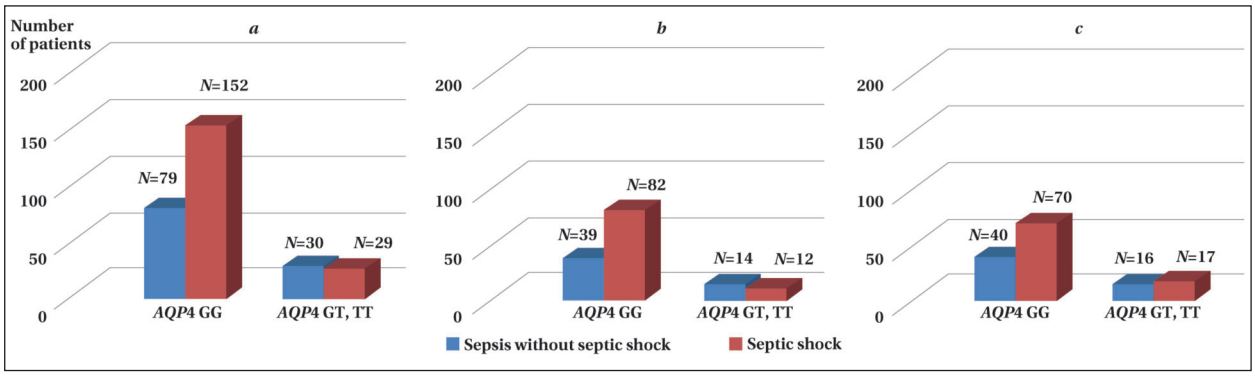

Fig.2. The incidence of septic shock in ICU patients with different *AQP*4 rs1058427 genotypes.
Note. *a* — all patients, *N*=290, *P*=0.009, Fisher`s exact test (FET), OR=1.99, 95% CI: 1.12–3.55. *b* — patients from ICU 2 and ICU 3, *N*=147, *P*=0.045, FET, OR=2.45, 95% CI: 1.04–5.79. *c* — patients from ICU 1, *N*=143, *P*=0.295, FET.

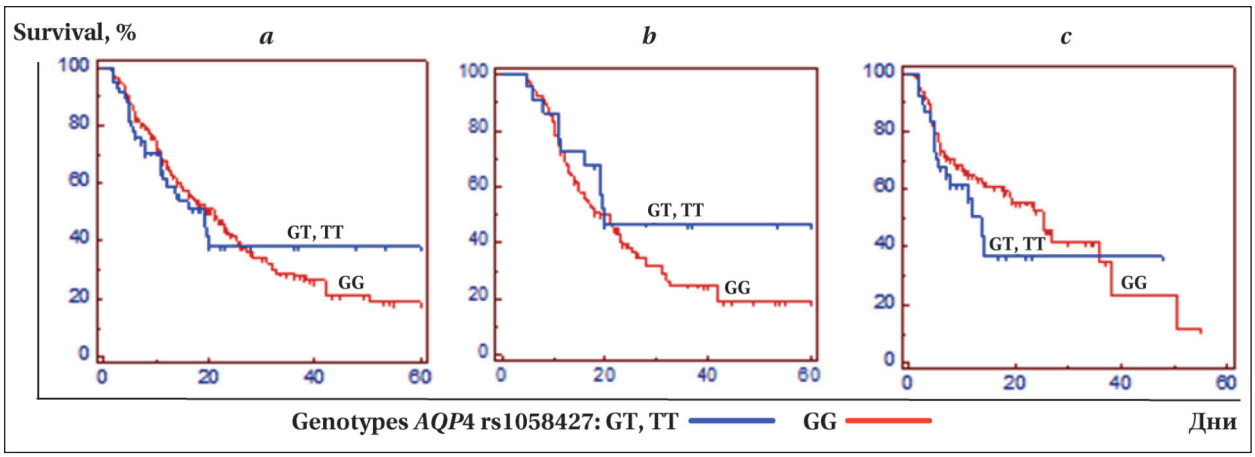

Fig. 3. Survival rate of sepsis patients with different *AQP*4 rs1058427 genotypes.
Note. *a* — all patients, *N*=290, *P*=0.995, log-rank test. *b* — patients who were on mechanical ventilation for 5 days or more, *N*=125, *P*=0.176, log-rank test. *c* — patients who were on mechanical ventilation less than 5 days, *N*=165, *P*=0.238, log-rank test.

When investigating the possible association of the variant genotypes of *AQP*4 rs1058427 with the incidence of septic shock, we found that the incidence of septic shock was significantly lower in carriers of the minor allele of *AQP*4 rs1058427 T with sepsis (Fig. 2). As shown in the figure, septic shock developed in 66% of the patients carrying the GG genotype, in contrast to only half of the patients with the T minor allele (Fig. 2, *a*, *P*=0.009). Thus, the *AQP*4 rs1058427 T allele protects against the development of septic shock in sepsis.

Further analysis of the incidence of septic shock in patients from different ICUs showed that this association was significant (*P*=0.045) only in patients from ICUs 2 and 3 (Fig. 2, *b*), who differed from patients from ICU 1 in having a higher SOFA score on admission, a higher proportion of patients with comorbidities such as type 2 diabetes mellitus, and renal disease (Table), indicating a more frequent need for ECT. In the subgroup of ICU 1 patients (Fig. 2, *c*, patients with fewer comorbidities, no ECT), the protective effect of the minor allele was not significant (*P*=0.295), although the trend remained the same.

Thus, in the group of patients with increased comorbidities and more severe multiorgan failure (median SOFA 6.0), the presence of the T minor allele in the 3' region of the *AQP*4 gene in patients (genotypes GT and TT) is associated with a more favorable course of sepsis, i.e. a reduced likelihood of life-threatening septic shock compared to patients carrying the major G homozygous allele (genotype GG).

Examination of the association between *AQP*4 genotype and mortality using the log-rank criterion revealed no significant differences in mortality among patients with different *AQP*4 rs1058427 genotypes (Fig. 3). However, in the subgroup of patients requiring prolonged (more than 5 days) mechanical ventilation support, there was a trend toward an association between reduced mortality and the presence of the minor T allele of *AQP*4 (Fig. 3, *b*).

In contrast to patients from ICU 1, patients from ICU 2 and 3 with sepsis were characterized by high comorbidity (significantly increased frequency of renal disease and diabetes), increased need for mechanical ventilation (for 5 or more days), and increased SOFA scores of organ failure on admission.

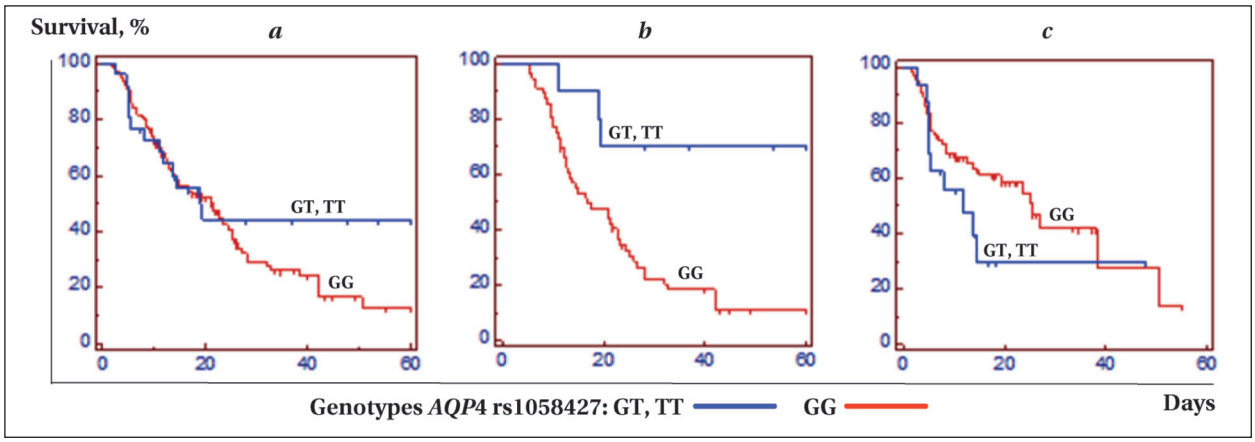

Fig. 4. Survival rate of ICU 2 and ICU 3 sepsis patients with multiple comorbidities with different *AQP*4 rs1058427 genotypes and duration of ventilation.
Note. *a* — all patients, *N*=147, *P*=0.32, log-rank test. *b* — patients who were on mechanical ventilation for 5 days or more, *N*=66, *P*=0.003, Fisher`s exact test; *P*=0.009, log-rank test. *c* — patients who were on mechanical ventilation less than 5 days, *N*=81, *P*=0.14, log-rank test.

Analysis of the genotype distribution revealed that patients with multiple comorbidities were characterized by a significant association of the major *AQP*4 rs1058427 GG genotype with increased mortality (Fig. 4, *b*). Among carriers of the *AQP*4 rs1058427 GG major genotype, 47 out of 56 patients died, and among carriers of the T minor allele, 5 out of 10 patients died (*P*=0.003, FET, *N*=66, OR=5.22, 95% CI: 1.25–21.82, *P*=0.009, log-rank test). No association between mortality and *AQP*4 rs1058427 genotype was found for all patients in ICU 2 and 3 (Fig. 4, *a*) and for those on mechanical ventilation for less than 5 days (Fig. 4, *c*).

Individual analysis of data from ICU 1 patients with less severe organ damage, shorter duration of ventilatory support and need for ECT did not reveal an association between mortality, duration of ventilation and *AQP*4 rs1058427 genotype (Fig. 5, *a*, *b*, *c*).

Discussion

The present data demonstrate for the first time the protective value of the T *AQP*4 rs1058427 allele in septic shock. Presumably, this fact may explain the effect of allele T on mortality in patients with multiple comorbidities requiring prolonged ventilatory support. Previously, it was only known that the minor allele T *AQP*4 rs1058427 was significantly associated with increased perihematomal edema after intracerebral hemorrhage [23]. The severity of the edema may be due to the upregulation of *AQP*4. The same reason may also explain the association between the presence of the T variant and the potential «protection» against septic shock as well as the reduction of mortality in sepsis. We suggest that the presence of the T allele, which presumably may determine more intensive immune cell responses in antibacterial immunity, reduces the risk of septic

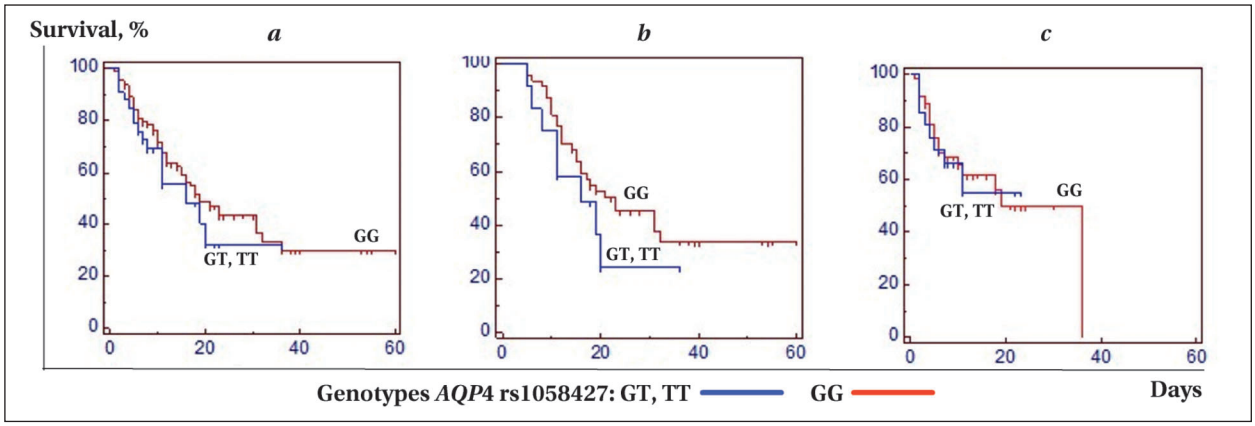

Fig. 5. Survival rate of ICU 1 sepsis patients without comorbidities with different *AQP*4 rs1058427 genotypes and duration of ventilation.
Note. *a* — all patients, *N*=143, *P*=0.330, log-rank test. *b* — patients who were on mechanical ventilation for 5 days or more, *N*=59, *P*=0.246, log-rank test. *c* — patients who were on mechanical ventilation less than 5 days, *N*=84, *P*=0.837, log-rank test.

shock by reducing bacterial load and toxigenic bacterial endotoxins.

Data from other authors also support this hypothesis. For example, *AQP*4 is known to be expressed in circulating CD4+ and CD8+ T lymphocytes, whereas inhibition of *AQP*4 molecules transiently reduces T lymphocyte counts in murine blood [12]. In addition, *AQP*4 inhibition significantly reduced T cell proliferation and cytokine production in vitro [10].

CD4+ and CD8+ T lymphocytes are critical for protection against sepsis. This has been demonstrated by several studies showing that (a) increased expression of the anti-apoptotic gene Bcl-2 in T cells prevented apoptotic loss of T cells in sepsis and increased survival [27]; (b) transfer of T lymphocytes to mice lacking them provided protection against sepsis [28]; (c) sepsis-associated apoptosis of CD4+ and CD8+ T lymphocytes resulted in lymphopenia and immunosuppression in patients with advanced sepsis [29]. This suggests that the sepsis-induced decrease in the number and activity of CD4+ and CD8+ T lymphocytes may significantly increase the risk of secondary infections. Post-sepsis immunologic disorders, probably associated with genetic polymorphism, may contribute significantly to increased mortality in sepsis survivors in the next few years.

The single nucleotide substitution *AQP*4 rs1058427 is located in the region of the *AQP*4-AS1 (aquaporin 4 antisense RNA 1) gene (ENSG00000260372), which transcribes long non-coding RNAs (lncRNAs). The sequences of four transcripts located at the substitution site are ENST00000579964.6 *AQP*4-AS1-203, 1645 bp, ENST000000628174.2 *AQP*4-AS1-206, 919 bp, ENST00000582605.5 *AQP*4-AS1-204, 525 bp, ENST00000627963.2 *AQP*4-AS1-205, 381 bp.

*AQP*4-AS1 is known to downregulate *AQP*4 expression [30]. Logically, some SNPs in the RNA gene region may cause changes in the activity of lncRNAs that regulate *AQP*4. And if the guanine to thymine substitution in the *AQP*4 gene variant rs1058427 leads to a decrease in *AQP*4-AS1, this will result in increased *AQP*4 transcription and upregulation of aquaporin protein, which controls the initial stages of immune cell recruitment and migration.

There is evidence for an association between lncRNAs mapped to the *AQP*4 gene region, *AQP*4 expression and the development of retinal dysfunction in diabetes mellitus. *AQP*4-AS1 is a long non-coding RNA transcribed from the antisense strand of the *AQP*4 gene. A recent study showed an increase in *AQP*4-AS1 in response to high glucose levels or oxidative stress. Inhibition of *AQP*4-AS1 protected against diabetes-induced retinal vascular dysfunction and resulted in increased production of *AQP*4 RNA [30]. This may be a mechanism for the protective effect of the T allele of *AQP*4 rs1058427 in the group of patients, almost half of whom had diabetes. Since there is evidence for the effect of long non-coding RNA on *AQP*4 mRNA levels, the mutant variant of *AQP*4-AS1 ENSG00000260372 transcripts could possibly alter the ability of the lncRNA to affect the *AQP*4 gene expression.

More than 17,000 genes encoding lncRNAs have been described in the human genome. The *AQP*4-AS1 lncRNA SNP rs527616 is associated with age in breast cancer. *AQP*4-AS1 levels have also been shown to be lower in breast tumor tissue compared to healthy tissue. In addition, *AQP*4-AS1 expression was higher in patients with stage I disease and small tumor size, suggesting its association with a better prognosis [31].

Long non-coding RNAs are a diverse group of RNA molecules that are often expressed in a tissue-specific manner. They are molecules containing more than 200 nucleotides that are not translated. Five groups of lncRNAs have been identified: sense, antisense, double-stranded, intronic and intergenic lncRNAs, depending on their position relative to the protein-coding gene. In the cytoplasm, lncRNAs act in a variety of ways. They can alter the stability of mRNA transcripts, either by blocking translation through double-stranded binding to the mRNA or by promoting cap-independent translation. The lncRNA genes contain microRNA sequences and can be expressed in association with them. In addition, lncRNAs prevent microRNAs and proteins from binding to their normal targets [32].

The lncRNAs play an important role in the regulation of gene expression. Depending on the presence of regulatory patterns, lncRNAs can be divided into those that act in cis position, affecting the expression and/or chromatin status of nearby genes, and those that perform multiple functions in trans position [33]. There is evidence that signaling pathways of the proinflammatory transcription factor NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) and toll-like receptors increase lncRNA level in pancreatic beta cells during inflammation [32].

Recent studies have demonstrated the potential of the lncRNAs NEAT1, MALAT1, ITSN1-2, MEG3 and ANRIL as biomarkers of sepsis. Several lncRNAs are involved in hyperinflammation in sepsis through the TLR4 signaling pathway [33]. However, the specific mechanism of action of lncRNA *AQP*4-AS1 ENSG00000260372 is still unknown.

Recently, the role of *AQP*4 in the activation of the antigen-specific receptor of T cells has been identified [34], and there is evidence for increased *AQP*4 expression in activated T cells and decreased levels in cells undergoing apoptotic cell death [35]. Given the data on the involvement of *AQP*4 in antigen recognition [34] and subsequent cell migration [12], a relatively high expression of *AQP*4 in

T cells could lead to more intense migration of antigen-stimulated T cells and the involvement of a greater number of interacting B cells in the adaptive immune response to bacterial antigens. In this case, the *AQP*4-dependent increase in T lymphocyte activity will provide a stronger antibacterial defense capable of preventing life-threatening severe endotoxin-mediated septic shock. Therefore, the presence of an alternative genotype, *AQP*4 rs1058427 GG, may be associated with the development of septic shock.

In contrast, the minor T allele of *AQP*4 rs1058427 is associated with protection against septic shock. However, its effect is seen only in a specific group of ICU patients, half of whom required high-tech ECT. This suggests that genetic variability at the rs1058427 site in the 3' region of the *AQP*4 gene may be associated with an unfavorable course of sepsis only in a specific clinical phenotype characterized by increased comorbidity, which contributes significantly to the clinical heterogeneity of septic patients. On the other hand, an association or causal relationship between the minor variant T rs1058427 in the 3' region of the *AQP*4 gene and the clinically significant outcome of ECT is possible, accounting for the predominance of patients with this genotype among highly comorbid survivors.

Thus, *AQP*4 rs1058427 allelic variants may be candidate prognostic markers for alternative course and outcome of sepsis especially in ICU patients with severe comorbidities, whose T allele of *AQP*4 rs1058427 has prognostic significance for both development of septic shock and mortality.

Conclusion

The *AQP*4 rs1058427 GG genetic variant predisposes to a more severe course of sepsis in ICU patients with the development of septic shock, whereas the minor *AQP*4 rs1058427 T allele is associated with protection against septic shock and fatal outcome in a subgroup of ICU patients receiving ECT and ventilator support for more than 5 days. Severe comorbidity associated with the need for extracorporeal treatments is the environmental factor revealing the protective role of a single nucleotide mutation in the 3' region of the *AQP*4 gene

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The Role of Endothelinergic and Nitroxidergic Reactions in Predicting the Functional Outcome in Patients with Ischemic Stroke of Different Severity

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Summary

The aim of this study was to assess the value of nitric oxide (NO) and endothelin-1 (ET-1) serum concentrations as potential biomarkers for predicting the functional outcome in patients with acute ischemic stroke.

Material and methods. A total of 37 patients diagnosed with ischemic stroke and admitted to a multidisciplinary vascular center were included in the study. The patients were divided into two groups based on the severity of neurological deficits as determined by the National Institutes of Health Stroke Scale (NIHSS): Group 1 consisted of 20 patients with NIHSS scores <15, and Group 2 consisted of 17 patients with NIHSS scores ⩾15. The functional outcome was assessed using the NIHSS absolute values and the degree of disability measured by the Modified Rankin Scale (mRS) by comparing the values before and after baseline treatment. Lab evaluation included quantitative assessment of stable NO and ET-1 metabolites in patient's serum at admission and on day 10 of hospital stay. The SPSS Statistics V23.0 for Windows software package, Python programming language, and Pandas and SciPy libraries were used for statistical data processing.

Results. Group 1 patients demonstrated a statistically significant decrease in NIHSS (P=0.0013) and mRS (P<0.0001) scores, which was indicative of a favorable functional outcome. Group 2 patients showed some recovery of only neurological deficit measured by NIHSS scale (P=0.0012), changes in degree of disability by mRS were statistically insignificant. On Day10 of hospital stay, both groups showed a clinically significant increase in ET-1 content, and slight change in NO concentration. NIHSS score demonstrated a significant negative correlation with baseline ET-1 concentrations: R=–0.82, P=0.00023 — in Group 1; R=–0.55, P=0.00075 — in Group 2. Modified RS scores showed negative correlation with NO (R=–0.50, P=0.00044) and ET-1 (R=–1.0, P=0.0074) concentrations in Group 1, and positive correlation with NO (R=0.55, P=0.0023) and ET-1 (R=0.33, P=0.04) concentrations in Group 2.

Conclusion. Monitoring of NO and ET-1 serum concentrations provides valuable insights for personalized assessment of the anticipated functional outcome in patients with cerebral ischemia. Further research and the development of prognostic mathematical models are needed to validate the use of endothelial function markers as predictive indicators of patients' recovery potential during the acute phase of ischemic stroke

Keywords: ischemic stroke, nitric oxide, endotelein-1, biomarker

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, accounting for 25% of all deaths [1]. Ischemic stroke (IS) is a major cause of morbidity and mortality as well as a significant socioeconomic problem [2]. The search for predictive biomarkers of stroke progression and functional outcome is ongoing. As such biomarkers, nitric oxide (NO) and endothelin-1 (ET-1) are promising candidates [3]. ET-1 is a multifunctional peptide with cytokine-like activity produced by almost all endothelial cell types [4]. Endothelial dysfunction is characterized by increased ET-1 production in response to a variety of events, including hyperglycemia, hypercholesterolemia, hypertension, estrogen deficiency, and biochemical and mechanical abnormalities [5, 6]. Despite numerous studies in recent years demonstrating the long-term and potent vasoconstrictor effect of ET-1 on cerebral vessels, its role in the pathophysiological mechanisms of cerebral ischemia is still under active investigation [7, 8]. NO is a signaling molecule that is also produced by endothelial cells and has potent vasodilator and anti-inflammatory effects which are necessary to maintain vascular homeostasis [9]. Endothelial dysfunction is directly related to both

a decrease in the production of active NO metabolites and changes in the sensitivity of endothelial cells to NO. Increased NO production occurs in many brain conditions, including acute cerebrovascular accidents and neurodegenerative diseases [10]. The opposing effects of NO and ET-1 on the regulation of local vascular tone are balanced in healthy tissues and dysregulated in cerebral ischemia [11]. Thus, these markers of endothelial dysfunction are of particular interest and can be used as early predictors of blood flow and coagulation disturbances associated with vasoconstriction, leukocyte adhesion, and platelet activation [12]. The role of NO- and ET-driven mechanisms in the regulation of vascular endothelial motor function in acute IS remains poorly understood [13], which provides the rationale for studying changes in the levels of stable NO and ET-1 metabolites in patients with different severity of IS. Mathematical models for prediction and assessment of severity and outcome of stroke are also promising.

The aim of the study was to evaluate the feasibility of using serum levels of nitric oxide and endothelin-1 as prognostic biomarkers of functional outcome in acute ischemic stroke.

Materials and methods

The prospective cohort study was approved by the Independent Ethical Committee of the Center for Clinical Research of the Immanuel Kant Baltic Federal University (protocol 34, dated 29.09.2022). The study included 37 patients admitted to the primary vascular center of an emergency hospital with the diagnosis of ischemic stroke. The sample size of the study was not predetermined. All subjects signed 2 copies of the informed consent prior to all study procedures. To verify the IS subtype according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [14], clinical and diagnostic examinations were performed according to the officially approved standards of medical care for stroke patients. At the time of admission, all patients underwent a neurological examination and other routine tests, including transcranial Doppler ultrasound of extra- and intracranial vessels, 12-lead electrocardiography, complete blood count and blood biochemistry, and pulse oximetry. Neuroimaging parameters were assessed by computed tomography (CT) and magnetic resonance imaging (MRI). If necessary, additional tests included MR/CT angiography, echocardiography, ECG Holter monitoring, detailed coagulation study, examination for systemic diseases, and lumbar puncture. All patients were assessed on admission for level of consciousness according to the Glasgow Coma Scale (GCS) and stroke severity according to the National Institutes of Health Stroke Scale (NIHSS). Patients were divided into two groups according to the severity of neurological deficit on the NIHSS scale, which allowed more accurate stratification of the patients studied and use of the clinical data. Group 1 included 20 patients with NIHSS neurological deficit of less than 15 points, of whom 13 (65%) were males and 7 (35%) were females. The mean age of the patients was 68.3±5.6 years. The initial NIHSS score in the group was 6 [2; 9] points, which corresponded to moderate severity.

Group 2 included 17 patients with neurological impairment on the NIHSS scale >15 points, of whom 9 (52.9%) were men and 8 (47.1%) were women. The mean age of the patients was 67.9±4.9 years. The patients' baseline NIHSS score was 18 [15; 28] points, which corresponded to a severe ischemic stroke.

Inclusion criteria were clinical signs and symptoms consistent with the diagnosis of ischemic stroke and age between 60 and 82 years. Exclusion criteria were hemorrhagic stroke and transient ischemic attack.

The functional outcome criteria for acute ischemic stroke were selected from major clinical scales such as the NIHSS Stroke Severity Scale and Modified Rankin Scale (mRS) disability. The change in patient status was expressed in absolute values, and the difference between NIHSS and mRS scores before and after initial treatment was calculated.

The laboratory study included measurement of serum stable metabolites of NO and ET-1. Blood samples were collected on admission and on day 10 of hospitalization.

BD Vacutainer® Plus Serum tubes were used to collect 6 ml of venous blood. CEA482Hu Cloud-Clone Corp. kits, Nitric Oxide Assay Kit A013-2-1 Cloud-Clone Corp. kits were used to determine serum NO and ET-1 levels by enzyme-linked immunoassay.

Statistical data analysis was performed using SPSS Statistics V23.0 for Windows software with Pandas and SciPy libraries, following the appropriate guidelines [15].

The distribution of quantitative variables was evaluated using the Shapiro–Wilk criterion. Quantitative variables with normal distribution were described by arithmetic mean *(M)* and standard deviation *(SD)*. For non-normal distribution, quantitative data were described by median *(Me)*, lower and upper quartiles *(Q1–Q3)*.

Data with normal distribution were compared using ANOVA analysis of variance for dependent and independent samples. For non-normal distribution, the non-parametric Wilcoxon test was used. Differences in the frequencies of parameters in two independent groups were analyzed using Fisher's exact criterion with two-sided confidence limits and the χ^2 criterion with Yates correction. The significance level was set at $P<0.05$. For multiple comparisons of variables, the Bonferroni correction ($P<0.0125$) was used to reject false positives.

The correlation coefficient r was calculated to assess the relationship between functional outcome parameters and laboratory diagnostic parameters. The value of r was [–1; 1], where –1 was complete inverse dependence, 0 was the absence of any dependence, and 1 was complete direct dependence. To evaluate the correlation of continuous values, including parameters measured in points, the Fechner's method for calculating correlation coefficients in small samples was selected:

$$r_f = \frac{n_a - n_b}{n_a + n_b},$$

where n_a is the number of matched signs for differences, n_b is the number of unmatched signs.

The presence of correlation between the values was confirmed if the coefficient exceeded the threshold module value of 0.2. The significance of r was confirmed by calculating the *P*-value [16]:

$$t = \frac{r \times \sqrt{n-2}}{\sqrt{1-r^2}},$$
$$p\text{–}value = 2 \times P(T > t),$$

where *r* is the correlation coefficient, *n* is the sample size, and *P(T>t)* is the probability of obtaining the value of *t* in the distribution *T* with (n-2) degrees of freedom.

The standard value of 0.05 was chosen as the threshold value. If the *P*-value was less than 0.05, the correlation coefficient was considered significant. Correlation coefficients with a *P*-value greater than 0.05 were excluded from consideration.

Results

Patients were treated according to standard stroke care protocols. Thrombolytic therapy was not administered because of contraindications or because patients were hospitalized outside the therapeutic window. Subtypes of ischemic stroke (TOAST criteria), comorbidities, and clinical scores were identified based on clinical and instrumental examinations (Table 1).

Diabetes mellitus was more frequently diagnosed in group 1 than in group 2, whereas the main comorbidity in group 2 was brachiocephalic atherosclerosis according to transcranial Doppler ultrasound. The severity of disability according to mRS was 2 [0; 4] points in group 1 patients, which corresponded to a moderate impairment of performance, and 4 [2; 5] points in group 2 patients, which corresponded to a severe impairment of performance and inability to manage their physical needs without assistance. Patients in group 2 had a significant decrease in mRS and NIHSS scores ($P<0.0001$) compared to group 1. No significant differences were found in basic demographic and laboratory parameters.

Changes in NO and ET-1 concentrations, NIHSS and mRS at the time of admission and on day 10 of hospitalization are shown in Table 2.

Patients in the first group showed a significant decrease in NIHSS score ($P=0.0013$) and mRS disability level ($P<0.0001$), indicating a favorable functional outcome. Patients in the second group showed

Table 1. Main clinical and statistical characteristics of brain ischemic stroke in patients with different severity of disease according to NIHSS.

Parameter	Values in groups		*P*-value
	Group 1, *N*=20	Group 2, *N*=17	
Demographic characteristics			
Men, *N* (%)	13 (65.0)	9 (52.9)	0.455
Women, *N* (%)	7 (35.0)	8 (47.1)	0.455
Mean age, yeas	68.30±5.63	67.90±4.91	0.820
Subtypes of ischemic stroke (TOAST criteria, % of patients)			
IS due to atherosclerosis of large arteries (atherothrombotic)	30.0	29.4	0.968
IS due to cardiogenic embolism (cardioembolic)	40.0	29.4	0.500
IS due to occlusion of small arteries (lacunar)	30.0	35.3	0.731
IS of unknown etiology	0	5.9	0.271
Comorbidity (% of patients)			
Atherosclerosis (>50%)	35.0	64.7	0.0365
Diabetes mellitus	30.0	17.6	0.0228
Atrial fibrillation	25.0	35.3	0.494
Hypertension	75.0	58.8	0.294
Recurrent stroke	25.0	17.6	0.586
Clinical scales (points)			
Glasgow coma scale	15±0.01	14.47±1.2	0.0617
mRS	2 [0; 4]	4 [2; 5]	<0,001*
NIHSS	6 [2; 9]	18 [15; 28]	<0,001*
Laboratory parameters			
NO (mmol/L)	0.001589±0.001	0.0016601±0.002	0.889
ET-1 (pg/mL)	25.02±8.36	24.90±8.62	0.966
ET-1 (pg/mL)	25,02±8,36	24,90±8,62	0,966

Note. * — significant differences between groups.

Table 2. Comparative characteristics of laboratory and clinical parameters at admission and on day 10 of hospital stay.

Parameter	Values in groups				*P*-value
	Group 1, *N*=20		Group 2, *N*=17		
	Day 1	Day 10	Day 1	Day 10	
NIHSS (points)	6 [2; 9]	2.5 [0; 7]	18 [15; 28]	12 [4; 20]	P_1=0.0013* P_2=0.0012* P_3<0.001*
mRS (points)	2 [0; 4]	0.75 [0; 2]	4 [2; 5]	3.5 [2; 5]	P_1⩽0.0001* P_2=0.214 P_3<0.001*
NO (mmol/L)	0.001589±0.001	0.001628±0.001	0.0016601±0.002	0.001330±0.001	P_1=0.875 P_2=0.547 P_3=0.373
ET-1 (pg/mL)	25.02±8.36	31.62±9.14	24.90±8.62	31.24±8.93	P_1=0.018 P_2=0.0431 P_3=0.903

Note. P_1 — difference between the parameters on days 1 and 10 of hospital stay in group1; P_2 — difference between the parameters on days 1 and 10 of hospital stay in group 2; P_3 — difference of parameters between groups on day 10 of hospital stay; * — significant differences.

only an improvement of neurological deficit according to NIHSS (*P*=0.0012). Changes in mRS disability score were insignificant in this group. Analysis of changes in NO and ET-1 showed an increase in ET-1 levels on day 10 of hospitalization in patients in both groups.

No significant changes in NO levels were found in patients of groups 1 and 2 (Fig. 1).

When examining the correlations between baseline levels of NO, ET-1 and functional outcome parameters according to the mRS and NIHSS scales, relationships of different strength and direction were found.

The most significant correlations were found for the NIHSS score with baseline ET-1 levels in group 1 (*r*=–0.82, *P*=0.00023) and group 2 (*r*=–0.55, *P*=0.00075) patients and for NO (*r*=0.50, *P*=0.0036) in group 2 patients (Fig. 2, *a*).

When assessing the association of patient disability at admission based on mRS with NO and ET-1 levels, a negative correlation was found with both NO (*r*=–0.50, *P*=0.00044) and ET-1 (*r*=–1.0, *P*=0.0074) concentrations in group 1 patients. Group 2 patients showed a positive correlation of NO (*r*=0.55, *P*=0.0023) and ET-1 (*r*=0.33, *P*=0.04) with mRS scores (Fig. 2, *b*).

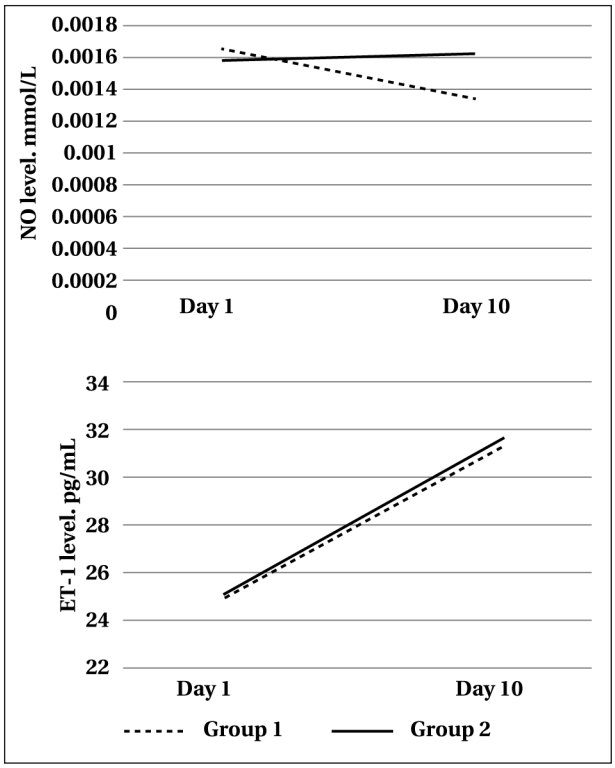

Fig. 1. Changes in serum concentrations of NO and ET-1 in patients with acute ischemic stroke.

Discussion

Prognostication of functional outcome of acute ischemic stroke using various predictors is an essential component of personalized medicine [17]. Evaluation of biochemical markers of endothelial dysfunction together with stroke severity score (NIHSS) and patient independence score (mRS) is a useful clinical decision support tool in the management of acute ischemic stroke [18].

In this study, a greater reduction in mRS independence score was found in patients with severe ischemic stroke compared to patients with moderate neurological deficits according to NIHSS. The results are consistent with other studies reflecting the close relationship between NIHSS and mRS scores and the importance of IS severity in predicting functional disability outcomes after stroke [19–21]. Changes in NIHSS and mRS parameters suggest a favorable functional outcome for patients with moderate IS severity. In patients with severe IS, a significant decrease in NIHSS parameters was also found, but the severity of neurological deficit at day 10 remained high (12 [2; 20] points) and corresponded to a moderate impairment on mRS (3.5 [2; 5] points), which indicates insignificant functional

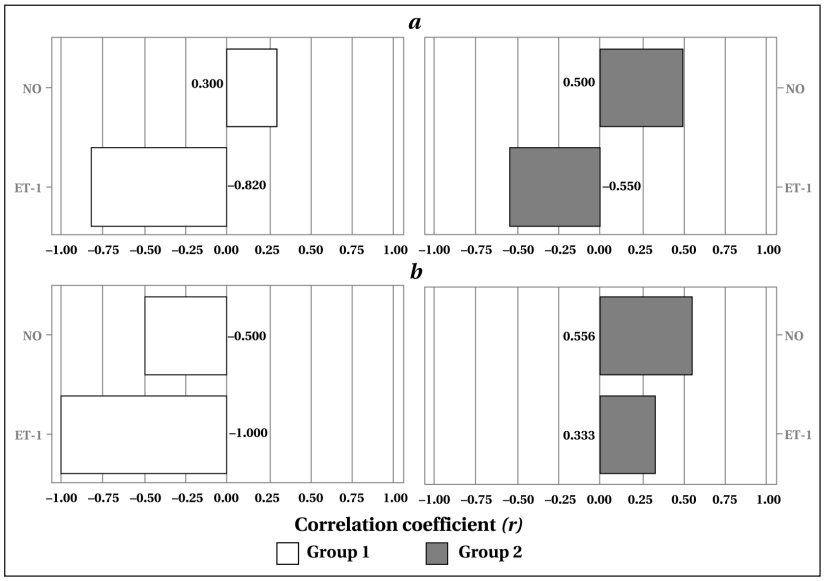

Fig. 2. Correlation between the levels of ET-1 (pg/mL) and NO (mmol/L) and the NIHSS *(a)* and mRS *(b)* scores in patients with acute ischemic stroke.

improvement and cannot be considered as a marker of favorable prognosis.

In patients with IS, circulating endothelin levels are elevated compared to baseline and normal levels [22]. Analysis of changes in laboratory parameters showed an increase in ET-1 levels on day 10 of hospitalization compared to baseline in patients of both groups. This increase may be due to several mechanisms underlying the pathogenesis of IS, such as hypoxia, neuroimmune processes, hypercoagulation, and platelet activation in ischemic areas, which are directly related to impaired vascular endothelial secretion [23]. On the other hand, atherosclerotic vascular changes also predict endothelial dysfunction and, accordingly, increased ET-1 production by damaged endothelial cells [24–26].

In patients with moderate severity of IS, a slight increase in stable NO metabolites has been observed, indicating preserved microcirculatory compensatory mechanisms and a good prognosis in terms of functional outcome [27]. Increased deficiency of endogenous NO in patients with severe neurological deficit may be due to insufficient deactivation of lipid peroxidation and decreased antioxidant defense, which play a critical role in the regulation of cerebral microvascular tone, leading to worsened hypoxia [28, 29]. High ET-1 concentration together with NO deficiency contributes to the persistence and worsening of vasoconstrictor responses, progression of neurological deficits, and predicts an unfavorable functional outcome in patients with a high NIHSS score [30].

Regardless of stroke severity, a negative correlation was found between baseline ET-1 levels and NIHSS neurological deficit scores. Despite the well-established vasoconstrictive effect of ET-1, which negatively affects recovery, the results obtained show an inverse correlation and regression of neurological deficit associated with a significant increase in ET-1.

One of the possible explanations for this is the autocrine-paracrine effect of endotelin-1 at low concentrations, leading to the release of vascular relaxing factors from the endothelium [31–33].

Another neuroprotective mechanism contributing to the regression of neurological signs is also determined by the ET-1-mediated effect and is associated with the inhibition of endothelial cell apoptosis [34]. The positive correlation of NIHSS scores with NO levels has been shown in several studies demonstrating the positive effect of free NO metabolites on the reduction of neurological signs through vasodilation and mediated neuroprotection [35, 36]. The increase in NO and ET-1 levels in patients with moderate ischemic stroke corresponds to the reduction of disability on the mRS scale and indicates a positive functional outcome according to this criterion.

The role of NO and ET-1 expression in the increase in mRS scores in patients with severe ischemic stroke, which reflects a trend toward poor functional recovery and patient disability, is still debated. Limitations of our study include the small number of patients and the lack of a control group.

Conclusion

The study of NO- and ET-1-driven mechanisms of regulation of endothelial vascular function and their role in the pathogenesis of ischemic stroke is a promising direction. Evaluation of NO and ET-1 expression and changes may be used for personalized assessment of predicted functional outcome in patients with acute ischemic stroke.

For a more accurate evaluation of predictors of functional disability in stroke patients, further studies with larger sample size and longer follow-up are warranted, as well as the development of prognostic mathematical models to validate the use of endothelial function markers for predicting patient recovery in acute ischemic stroke.

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The Effect of ACE Inhibitors/ARBs Withdrawal on the Risk of Postoperative Complications in Abdominal Surgery

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Summary

A significant proportion of patients undergoing non-cardiac surgery receive therapy with angiotensin converting enzyme (ACE) inhibitors/angiotensin II receptor blockers (ARBs), which are usually prescribed for treatment of arterial hypertension and CHF. Current guidelines fail to provide clear consensus on whether it is worth discontinuing ACEi/ARBs before non-cardiac surgery.

The aim of this research was to assess the contribution of pre-op ACEi/ARBs withdrawal to the development of postoperative complications in patients after abdominal surgery using data from STOPRISK database.

Materials and methods. Data of 1945 patients from of the STOPRISK database was used for the analysis. Patients were retrospectively divided into two groups: first group (*N*=471, 24.2%) included patients subjected to ACEi/ARBs withdrawal 24 h before surgery, second group (*N*=1474, 75.8%) included patients continuing on ACEi/ARBs therapy. The 30-day outcomes were analyzed — postoperative complications (acute kidney injury, acute respiratory distress syndrome, anastomosis failure, arrhythmias, circulatory arrest, cardiogenic pulmonary edema, postoperative delirium, myocardial infarction, pneumonia, ileus, postoperative bleeding, pulmonary embolism, acute cerebrovascular accident, wound infection) and mortality. We were not evaluating intraoperative and postoperative arterial hypotension and hypertension, we analyzed the use of vasopressors as a surrogate marker. ACEi/ARBs re-initiation after surgery was not evaluated.

Results. One or more post-operative complications were documented in 113 patients (5.8%). Only postoperative delirium was more common in patients (1.06% vs. 0.27%, *P*=0.027) after ACEi/ARBs withdrawal 24 h before surgery, the difference reached statistical significance.

Sub-analysis in the group of patients with arterial hypertension as the only comorbidity showed no statistically significant differences in the outcomes. Sub-analysis in the group of patients with CFH showed higher incidence of postoperative delirium after ACEi/ARBs withdrawal (2.68% vs. 0.6%, *P*=0.023). The logistic regression analysis showed that the risk of developing postoperative delirium is influenced by age, vasopressor support, and ACEi/ARBs withdrawal (the area under the curve for the model was 0.92 (0.90–0.93).

Conclusion. Rates of pre-op ACEi/ARBs withdrawal (24.2%) are consistent with published data. In the entire cohort, ACEi/ARBs withdrawal resulted in higher incidence of postoperative delirium, as well as in the subgroup of patients with CHF, while ACEi/ARBs withdrawal in the subgroup of patients with arterial hypertension had no influence on postop complications.

ACEi/ARBs withdrawal, along with hemodynamic instability and older age, contributes to the development of postoperative delirium, which is the subject of future research.

Keywords: angiotensin converting enzyme inhibitors; angiotensin II receptor blockers; postoperative complications; abdominal surgery

Conflict of interest. The article presents the interim results of a multicenter study of the All-Russian public organization «Federation of Anesthesiologists and Reanimatologists (FAR) «Concomitant diseases and stratification of risks of postoperative complications in abdominal surgery — STOPRISK». Some authors of this paper head organizations conducting research: K. M. Lebedinsky — President of the FAR; I. B. Zabolotskikh — First Vice-President of the FAR; A. I. Gritsan — Vice-President of the FAR; A. N. Kuzovlev — Director of the V.A. Negovsky Research Institute of General Reanimatology, Federal Scientific and Clinical Center of Reanimatology and Rehabilitation (FSCC RR). The remaining authors declare no conflict of interest.

Author contribution. All authors meet all four ICMJE authorship criteria, and contributed to the conception of the article, acquisition and analysis of factual data, writing and editing the text of the article, revisiting and approving the final version for publication.

Registration of the study. The study was registered in the international database https://clinicaltrials.gov under the auspices of the All-Russian Public Organization «Federation of Anesthesiologists and Reanimatologists» (principal investigator I. B. Zabolotskikh), study number NCT03945968.

Introduction

The challenge of safe anesthesia in abdominal surgery remains relevant despite continuous improvements in anesthetic techniques. The incidence of postoperative complications is 18–24% [1, 2].

Many patients undergoing non-cardiac surgery are treated with angiotensin converting enzyme inhibitors (ACEIs)/angiotensin II receptor blockers (ARBs), which are usually prescribed as first-line antihypertensive agents [3–5]. In addition, ACEIs/ARBs are also used to treat patients with chronic left ventricular dysfunction and patients with diabetic nephropathy and a very high risk of postoperative complications [6]. However, suppression of the renin-angiotensin-aldosterone system may cause hypotension during induction of anesthesia, as shown by meta-analyses [7, 8]. Data on the role of ACEIs/ARBs in lowering blood pressure after induction of anesthesia are contradictory, and some studies have failed to find this effect [9]. There are also conflicting data on the effect of ACEI/ARB administration on the development of postoperative complications, ranging from a significant increase in mortality and cardiac complications when ACEIs/ARBs are taken before surgery [3] to a lack of effect [8] and even a negative effect of drug withdrawal on the incidence of complications due to the development of postoperative hypertension [10]. The RAAS is also known to modulate the coagulation system, influence capillary leakage associated with inflammation, and be involved in the pathophysiology of coronary atherothrombosis [11–13], therefore its preoperative inhibition may have an unpredictable effect on the postoperative period. All this has led to a lack of consensus in national European and North American guidelines on the need to discontinue ACEIs/ARBs before non-cardiac surgery.

The aim of this study was to investigate the role of ACEI/ARB withdrawal in the development of postoperative complications in patients undergoing abdominal surgery according to the STOPRISK database.

Materials and methods

Data Collection. By the time of interim analysis, data on perioperative parameters of 6,283 patients who underwent abdominal and pelvic surgery were obtained from 32 centers in 21 cities representing 8 federal districts for the period from July 1, 2019 to March 1, 2022. 6,195 patients were selected; 88 patients were excluded due to missing data required for analysis (Fig. 1).

All centers received local ethics committee approval prior to the study. Patients signed an informed consent form to participate in the study.

According to the study protocol (available on the WIT website at https://goo.su/5vL6oBI) [14], information was collected on all patients who met the eligibility criteria on a selected day.

Inclusion criteria for the subanalysis were presence in the STOPRISK database, long-term history of treatment with ACEIs/ARBs (3 months or more).

Exclusion criteria were ACEI/ARB discontinuation less than 24 h prior to surgery.

The study cohort was retrospectively divided into a group of patients who discontinued ACEIs/ARBs 24 h before surgery and a group of patients who continued ACEIs/ARBs until surgery.

Secondary analysis. A group of patients with hypertension as the only comorbidity and a group of patients with chronic heart failure in combination with other comorbidities were chosen from all patients included in the final analysis. In each of the above groups, subgroups were selected according to the pattern of ACEI/ARB prescription, i.e., discontinuation 24 h before surgery and continuation (Fig. 1).

Study endpoints. 30-day mortality and postoperative complications were assessed according to the classification of the Working Group of the European Society of Anesthesiologists and the European Society of Intensive Care Medicine [15]:

- Acute kidney injury
- Acute Respiratory Distress Syndrome (ARDS)
- Anastomotic failure
- Cardiac arrhythmias
- Circulatory arrest
- Cardiogenic pulmonary edema
- Postoperative delirium (ICU-CAM scale)
- Myocardial infarction
- Pneumonia
- Paralytic ileus
- Postoperative bleeding
- Pulmonary Embolism (PE)
- Acute Cerebrovascular Accident (ACVA)
- Wound infection

Intraoperative vasopressor requirements were also assessed.

Statistical analysis of data was performed using MedCalc software version 19.1.3 (MedCalc Software Ltd).

Data with normal distribution (Kolmogorov–Smirnov test) were presented as mean ± standard deviation, and data with non-normal distribution were presented as median (25–75 percentiles).

Baseline characteristics of patients in different groups and outcomes were compared using the χ^2 criterion for binary variables (or Fisher's exact test when the expected frequency of an event was less than 10) and the independent samples *t*-test for continuous variables with normal distribution and the Mann–Whitney test for variables with non-normal distribution. Repeated measures analysis of variance (RMANOVA) was used to compare the values of a variable at different stages of the study.

Multivariate logistic regression analysis was also performed to assess the contribution of multiple independent variables to the outcome.

Study registration. The study was registered in the international database https://clinicaltrials.gov under the auspices of the Russian Federation of Anesthesiologists and Reanimatologists (principal investigator I. Zabolotskikh), study number NCT03945968.

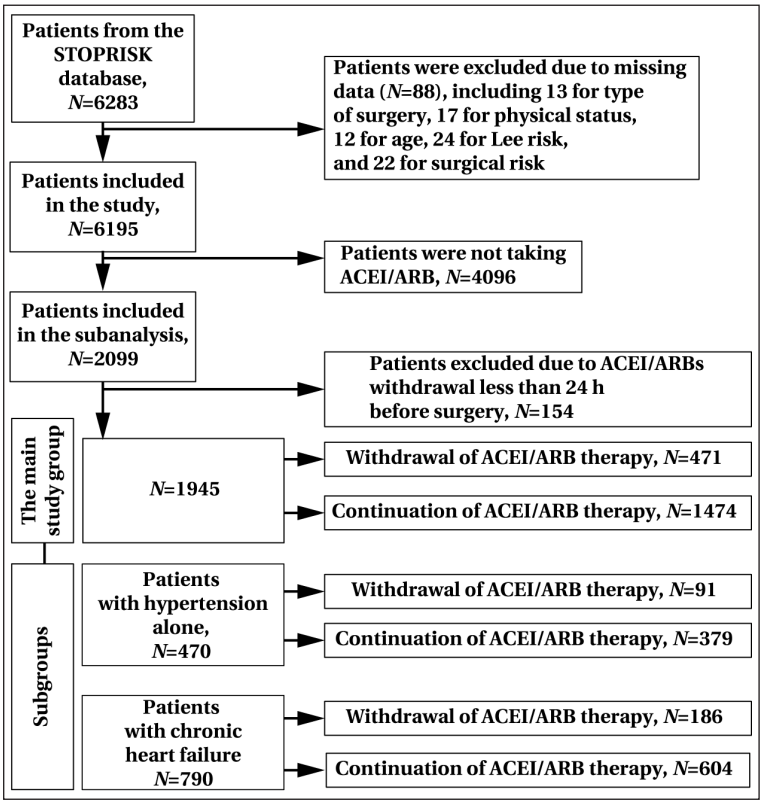

Fig. 1. Study flowchart.

Results

Among all patients in the STOPRISK database (6195 patients), comorbidities were registered in 3492 patients (56.4%), with a single condition observed in 1394 patients (22.5%), a combination of two conditions in 1052 patients (17.0%), three conditions in 606 patients (9.8%), four conditions in 308 patients (5.0%), and more than 4 comorbidities in 132 patients (2.1%) (Fig. 2).

Among patients with one comorbidity, hypertension was the most common (78%), diabetes mellitus (DM) was observed in 6%, CHD in 4%, COPD and arrhythmias in 3%, asthma and CKD in 2%, history of ACVA in 1%, and other diseases represented 6%. In patients with two comorbidities, the combination of hypertension with CHF, CHD, or DM was predominant (more than 80%); in patients with three comorbidities, the combination of hypertension and CHF with CHD or DM was most common (more than 75%); whereas in patients with four or more comorbidities, the combination of hypertension, CHF, and CHD with DM, arrhythmia, COPD, CKD, and ACVA was seen (more than 70%).

The results of 1945 patients were included in the analysis (Fig. 1). 471 patients were taking ARBs and 1474 patients were on ACE inhibitors.

In 471 (24.2%) patients, RAAS-inhibiting drugs were discontinued 24 hours before surgery, and in the remaining patients, the drugs were continued.

Comparison of baseline characteristics in the group of patients with ACEI/ARB withdrawal and in the group with continued administration is shown in Table 1. Patients with ACEI/ARB withdrawal had a lower Lee score.

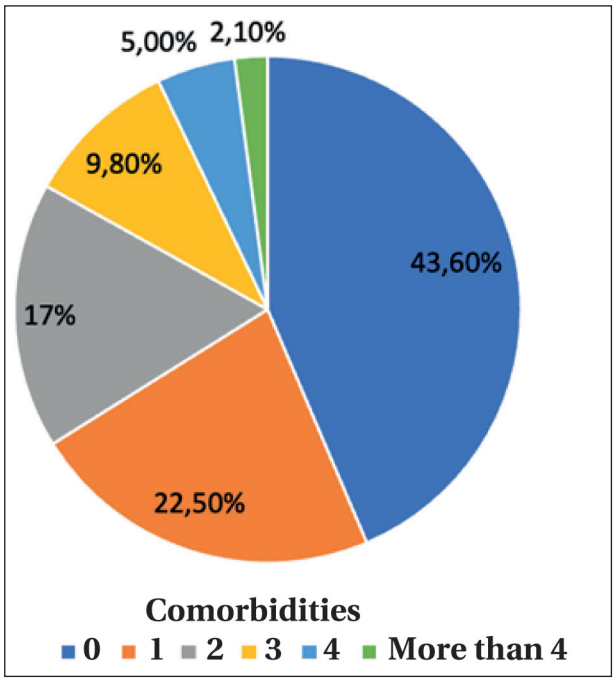

Fig. 2. Number of comorbidities in the studied cohort.

Complications were documented in 113 patients (5.8%). The frequency of complications is shown in Table 2.

Subanalysis in the subgroup of patients with hypertension alone showed that patients who continued to take ACEIs/ARBs were older, had a higher

Table 1. Parameters of the main study group.

Parameter	Values			*P*
	Total (*N*=1945)	Treatment withdrawal, *N*=471	Treatment continuation, *N*=1474	
Age, years	63 (57–70)	64 (57–70)	63 (57–70)	0.94
Surgical risk, %				
Low	26.7	25.9	26.9	0.65
Moderate	56.8	57.9	56.4	0.5
High	13.1	14.6	12.6	0.25
ASA class	2 (2–3)	2 (2–3)	2 (2–3)	0.15
Lee score, points	**1 (0–2)**	**1 (0–1)**	**1 (0–2)**	**0.0141**
Frequency of vasopressor support, %	5.45	7.22	4.88	0.05

Table 2. Complications in the main study group.

Parameter	Values			*P*
	Total (*N*=1945)	Treatment withdrawal, *N*=471	Treatment continuation, *N*=1474	
Complications	5.8	7.4	5.3	0.09
AKI	0.57	0.64	0.54	0.73
ARDS	0.31	0.42	0.27	0.63
Anastomotic failure	1.03	0.64	1.15	0.43
Cardiac arrhythmias	0.62	1.06	0.47	0.17
Circulatory arrest	0.26	0.21	0.27	1.0
Postoperative delirium	**0.46**	**1.06**	**0.27**	**0.042***
Pneumonia	1.39	1.27	1.42	1.0
Paralytic ileus	1.7	2.34	1.49	0.22
Postoperative bleeding	0.57	0.64	0.54	0.73
Pulmonary embolism	0.1	0	0.14	1.0
Cerebrovascular accident	0.21	0.21	0.20	1.0
Wound infection	0.87	1.27	0.75	0.26

Note. Here and in Tables 3, 4, 6: * — significant differences (Fisher's exact test).

Table 3. Parameters of patients with hypertension.

Parameter	Value		*P*
	Treatment withdrawal, *N*=91	Treatment continuation, *N*=379	
Age, years	58 (48–63)	61 (54–66)	0.0027
	Surgical risk, %		
Low	40.6	39.8	0.8
Moderate	57.1	54.6	0.6
High	1.1	0.8	0.7
ASA class	2 (2–2)	2 (2–3)	0.0162
Lee score, points	**0 (0–1)**	**1 (0–1)**	**0.003***
Frequency of vasopressor support, %	3.3	1.8	0.41

Table 4. Complications in the hypertensive patient group.

Parameter	Value		*P*
	Treatment withdrawal, *N*=91	Treatment continuation, *N*=379	
Complications	2.2	1.3	0.62
Anastomotic failure	0	0.5	1.0
Circulatory arrest	0	0.26	1.0
Paralytic ileus	0	0.52	1.0
Wound infection	**2.2**	**0**	**0.007***

Table 5. Parameters of patients with chronic heart failure.

Parameter	Value		*P*
	Treatment withdrawal, *N*=186	Treatment continuation, *N*=604	
Age, years	68 (60–72)	66 (60–72)	0.5
	Surgical risk, %		
Low	23.1	19.2	0.24
Moderate	60.2	55.1	0.22
High	16.1	21.02	0.14
ASA class	3 (2–3)	3 (2–3)	0.9
Lee score, points	1 (1–2)	1 (1–2)	0.57
Frequency of vasopressor support, %	11.3	6.8	0.06

Table 6. Complications in patients with chronic heart failure.

Parameter	Value		*P*
	Treatment withdrawal, *N*=186	Treatment continuation, *N*=604	
Complications	10.2	7.6	0.22
AKI	1.07	1.32	1.0
ARDS	1.07	0.66	0.63
Anastomotic failure	1.07	1.32	1.0
Cardiac arrhythmia	1.07	0.66	0.63
Circulatory arrest	0.53	0.5	1.0
Postoperative delirium	**2.68**	**0.6**	**0.037***
Pneumonia	2.15	1.8	0.76
Paralytic ileus	3.8	2.3	0.29
Postoperative bleeding	0	0.8	0.59
Pulmonary embolism	0	0.3	1.0
Cerebrovascular accident	0	0.5	1.0
Wound infection, %	1.07	1.3	1.0

ASA score, and were at higher cardiovascular risk (Table 3).

Drug discontinuation occurred in one in five patients. Nevertheless, no significant differences in outcomes were observed, except for the incidence of wound infection, which was not documented in the group of patients who continued ACEIs/ARBs (Table 4). Notably, the incidence of postoperative complications in this group was 1.2%.

Subanalysis in patients with comorbid chronic heart failure showed that ACEI/ARB withdrawal occurred in 23.5% of cases. The subgroups with drug withdrawal and with treatment continuation did not differ in their characteristics (Table 5).

As for the outcomes, a higher incidence of postoperative delirium was found in the ACEI/ARB withdrawal group (Table 6). In total, complications were observed in 65 patients (8.2%).

Logistic regression analysis showed that the risk of postoperative delirium was associated with age, vasopressor support, and ACEI/ARB withdrawal. The equation coefficients are shown in Table 7 ($P<0.0001$, $R^2=0.25$, Hosmer-Lemeshow test, $\chi^2=4.39$, $P=0.82$).

Table 7. Parameters of the logistic regression equation.

Variable	Coefficient	Standard error	*P*
Vasopressor support (yes/no)	2.03364	0.71632	0.0045
Age, years	0.075098	0.027042	0.0055
ACEI/ARB withdrawal (yes/no)	1.52839	0.70570	0.0303
Constant	–16.33507	4.08762	0.0001

The odds ratios for the identified factors are shown in Table 8.

The area under the curve for the model was 0.92 (0.90–0.93) (Fig. 3). The cut-off point was >–5.04 (sensitivity 100% (66.4–100%), specificity 73.62% (70.4–76.7%)). The odds ratio was 159.2 (95% CI 9.2–2745.8, *P*=0.0005), and the incidence of postoperative delirium was 4.1% in the high-risk group (according to the identified cut-off point) and 0% in the low-risk group.

Table 8. Odds ratio (OR) of identified risk factors for postoperative delirium.

Variable	OR	95% CI
Use of vasopressors	7.6419	1.8770–31.1128
Age	1.0780	1.0223–1.1367
Withdrawal of ACEIs/ARBs	4.6107	1.1563–18.3856

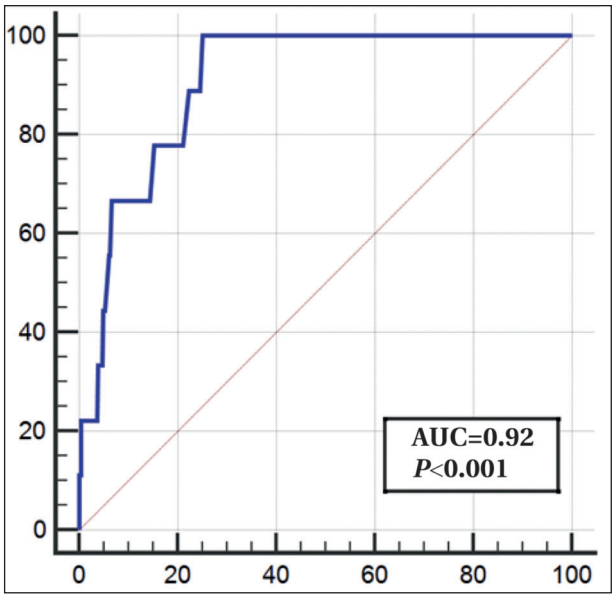

Fig. 3. ROC curve for the logistic regression equation showing its predictive value in assessing the risk of postoperative delirium.

Discussion

Of all patients (615) in the study cohort, ACEIs/ARBs were used in 33.9%, with 24.2% of preoperative 24 h withdrawals. Similar data were obtained in the VISION study, one of the largest studies to evaluate the impact of ACEI/ARB withdrawal on postoperative complications: ACEIs/ARBs were used in 32.6% of patients and withdrawn in 25.9% of cases [3].

We found no significant differences in the incidence of complications in the overall cohort of patients, except for postoperative delirium. Surprisingly, the frequency of vasopressor use was higher when ACEIs/ARBs were discontinued, despite the fact that such patients had a lower cardiovascular risk, whereas literature data suggest that hypotension is more common when drugs are continued [8], although this has not necessarily been confirmed [9]. The subanalysis showed that the findings were due to the patterns obtained in the subgroup of patients with chronic heart failure, since no significant differences were found in the subgroup with hypertension, and the complication rate was very low.

The finding of a lower incidence of postoperative delirium in the group of patients receiving preoperative ACEIs/ARBs (OR 4.6 with 95% CI 1.15–18.38) is interesting. Farag E. et al (2020) compared the incidence of postoperative delirium in non-cardiac surgery and found no effect of withdrawal of ACEIs/ARBs on its development. However, the use of these drugs in the postoperative period was associated with a lower incidence of delirium [16], even after adjustment for baseline and intraoperative factors. In addition, not only was the incidence of delirium higher, but the onset of delirium was earlier (19 h versus 64 h) in the group that did not receive the drug postoperatively. Therefore, the authors suggested that ACEIs/ARBs should be present at biologically relevant concentrations to prevent delirium.

The suggestion of a protective effect of ACEIs/ARBs in the prevention of delirium can be explained by the unique characteristics of the renin-angiotensin system. In particular, angiotensin II has neurotoxic effects mediated by its action on the angiotensin type 1 receptor. On the other hand, there is evidence in the literature for neuroprotective effects of the alternative renin-angiotensin system mediated by angiotensin, angiotensin III, and angiotensin IV [17]. Angiotensin-converting enzyme inhibitors increase brain levels of substance P, which is normally degraded by angiotensin-converting enzyme, which in turn increases the activity of neprilysin [18], an enzyme that breaks down β-amyloid [19]. In addition, ACEIs increase the production of angiotensin, which has neuroprotective and anti-inflammatory effects and leads to cerebral vasodilation [17]. The adverse effects of angiotensin II on the brain are mainly due to its action on the angiotensin 1A subtype receptor and include hypertension, inflammation, increased oxidative stress, blood-brain barrier disruption, and neurotoxicity.

Several publications suggest that angiotensin

II also induces nitric oxide production and promotes axon growth through activation of the angiotensin type 2 receptor and is an important factor in central nervous system development [17, 20]. The use of angiotensin type 1 receptor blockers enhances angiotensin II stimulation of the neuroprotective angiotensin type 2 receptor. In addition to blocking the angiotensin type 1 receptor, angiotensin receptor blockers also induce microglial polarization, which has anti-inflammatory and neuroprotective effects [21–23]. These effects have also been demonstrated in clinical trials. Therapy with losartan compared to atenolol in elderly hypertensive patients significantly improves cognitive functions, especially immediate and delayed memory [24]. In addition, the use of losartan in patients with hypertension improves cognitive functions, including memory, attention/concentration, comprehension, anxiety/depression, and interpersonal relationships [25]. Thus, the available evidence suggests that inhibition of the classical renin-angiotensin system pathway and simultaneous stimulation of alternative renin-angiotensin system pathways by ACEIs/ARBs have neuroprotective and anti-inflammatory effects, which may explain the reduced incidence of postoperative delirium.

Another possible mechanism influencing the risk of postoperative delirium is the potential neuroprotective effect of bradykinin [26], whose levels increase with the use of ACEIs.

The role of age and hemodynamic instability in the development of postoperative delirium has also been reported in the literature [27, 28].

The data on the lower incidence of wound infection in the ACEI/ARB withdrawal group are intriguing. The effects of ACEIs on the immune system and the anti-inflammatory properties of ACEIs are well-known [29] and may contribute to the risk of infectious complications. However, it is not clear how significant this impact is with short-term withdrawal, and this pattern we obtained requires further investigation.

Invasiveness of surgery may be an important factor associated with outcomes after non-cardiac surgery in patients taking ACEIs/ARBs and should be considered before their withdrawal or continuation. Previous prospective studies [30–32] and retrospective reviews [33, 34] have reported outcomes for a wide range of surgical procedures, from minimally invasive to major vascular surgery. Their results indicate that the use of ACEIs/ARBs is associated with an increased incidence of hypotension [30] and AKI in low-risk surgery [33, 34], but no effect on mortality was found [31]. In contrast, researchers found a 5-fold increased risk of mortality in major vascular surgery [32]. In the cited study, the invasiveness of the surgery had no effect on outcome, although preliminary outcome data have shown that this factor may have an impact, but is far from being decisive [35].

Study limitations. The current study is an observational study, which does not allow to exclude the influence of the reasons behind the decision to discontinue ACEIs/ARBs.

The study analyzed the effect of ACEI and ARB withdrawal in the same group because their effects on the risk of hemodynamic events during anesthesia are similar and are most often studied together. Because unexpected patterns were found, the final analysis should also be performed separately in each group.

The study did not evaluate intra- and postoperative hypo- and hypertension, and a surrogate parameter, the frequency of vasopressor use, was used. Re-initiation of ACEIs/ARBs in the postoperative period was not evaluated.

The data presented are preliminary.

The data obtained on the potential impact of ACEI/ARB withdrawal on the risk of postoperative delirium need validation and randomized trials.

Conclusion

The rate of ACEI/ARB withdrawal in patients undergoing abdominal and pelvic surgery was 24.2%, which correlates with literature data.

In the overall cohort, ACEI/ARB withdrawal was associated with a higher incidence of postoperative delirium. Subanalysis in the group of patients with chronic heart failure confirmed this pattern, whereas in the group of patients with hypertension, ACEI/ARB withdrawal did not affect outcome.

Together with hemodynamic instability and advanced age, ACEI/ARB withdrawal contributed to the development of postoperative delirium, which requires further investigation.

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Responsiveness to Infusion Load under Regional Anesthesia after Off-Pump Coronary Artery Bypass Graft Surgery

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Summary

Objective. To evaluate the effect of erector spinae plane block (ESPB) and epidural anesthesia on responsiveness to infusion load after coronary bypass surgery on a beating heart.

Materials and methods. A prospective randomized single-center study included 45 patients who were grouped into 3 equal arms based on anesthesia techniques: general anesthesia in combination with ESPB (GA+ESPB), general anesthesia and epidural anesthesia (GA+EA) and general anesthesia without regional techniques (GA). Patient's response to volume loading was assessed using dynamic and orthostatic tests after transfer from the operating room and at the end of the first postoperative day. Passive leg raise (PLR) and standard bolus injection tests were done at the first stage; changes in hemodynamic parameters during verticalization were additionally evaluated at the second stage. Patients with >10% cardiac index (CI) increase after PLR test and >15% increase after bolus injection test were categorized as responders.

Results. The concordance of obtained results in PLR and bolus injection tests for the GA+ESPB, GA+ EA and GA groups at the first stage was 0.53 (95% CI 0.12–0.94), 0.68 (95% CI 0.30–1.00) and 0.61 (CI 0.24–0.99), at the second stage — 0.70 (0.32–1.00), 0.84 (95% CI 0.55–1.00) and 0.82 (95% CI 0.47–1.00), respectively. There were no differences in distribution of responders between the groups. CI dynamics did not differ between the groups during verticalization, and there were no associations of CI changes during verticalization with the preceding PLR test results. The dynamics of troponin T and NT-proBNP did not differ between the groups.

Conclusion. Methods of regional anesthesia (SPB or EA) do not significantly affect the responsiveness to infusion therapy in the postoperative period after coronary bypass surgery on a beating heart.

Keywords: regional anesthesia; epidural anesthesia; coronary bypass surgery; responsiveness to infusion therapy; orthostatic reactions

Conflict of interest. The authors declare no conflict of interest.

Introduction

Assessment of fluid responsiveness has long been a cornerstone of critical care medicine, as fluid therapy is a key method to optimize hemodynamics and perfusion and should be administered when indicated with appropriate assessment of efficacy [1]. Traditionally, dynamic tests have been used to evaluate the effects of fluid therapy. One of these is the standard bolus challenge (infusion of 7 mL/kg) [2, 3], which is irreversible and increases the risk of hyperhydration and tissue edema [4]. A viable alternative to this test is the passive leg raising (PLR) test, which has a hemodynamic effect equivalent to an infusion of 300–500 mL of crystalloid solution. In addition to reversibility, the PLR test has high sensitivity and specificity, providing a good predictive value for fluid therapy responsiveness [5]. Other dynamic tests, including mini-bolus challenge, end-expiratory occlusion test, and assessment of plethysmogram variability, are not sufficiently accurate, especially during spontaneous breathing [6].

In addition to dynamic tests, static and dynamic preload parameters can be used to assess the effects of fluid therapy. Unlike dynamic parameters, including stroke volume variation, pulse pressure, and plethysmogram, static parameters have not been shown to be reliable for assessing volume status, but central venous pressure (CVP) remains a valuable measure of right ventricular filling [7]. Meanwhile, right ventricular failure is a limiting factor affecting the accuracy of dynamic methods to assess responsiveness to fluid loading and should be considered when performing such methods [8].

The rate of adaptation of the cardiovascular system to changes in body position is mainly determined by the autonomic nervous system. Thus, if the chronotropic or vasomotor response to baroreceptor activation is disturbed, the change in body position will result in orthostatic responses [9]. However, no single method has been proposed to diagnose orthostatic hypotension. For its indirect assessment, a head-up tilt table test or an active standing approach have been proposed [10]. The hemodynamic effects of upright positioning are equivalent to a 500–1000 mL decrease in preload due to blood pooling in the lower extremities,

splanchnic and pulmonary circulation [11]. In cardiac surgery, the use of regional anesthetic techniques, which can affect several hemodynamic parameters, increases the incidence of orthostatic reactions by up to 33% [12]. However, the effect of regional anesthesia techniques, including erector spinae plane block (ESPB), on fluid responsiveness after cardiac surgery remains controversial [13].

The aim of the study was to evaluate the effect of erector spinae plane block (ESPB) and epidural anesthesia on fluid responsiveness after off-pump coronary artery bypass grafting (CABG).

Materials and methods

The study was approved by the local ethics committee of the Northern State Medical University of the Ministry of Health of the Russian Federation (Arkhangelsk) (protocol 03/04-20 of April 29, 2020).

A single-center, prospective, randomized, controlled pilot study of patients undergoing elective off-pump CABG under sevoflurane anesthesia was conducted at the E. Volosevich First Regional City Clinical Hospital (Arkhangelsk). The study was not blinded. Patients were randomized 1:1:1 using the envelope method into the following groups: 1) combination of general anesthesia (GA) with sevoflurane and erector spinae plane block (ESPB) at the Th5 level using 20 mL of 0.5% ropivacaine intraoperatively followed by prolonged infusion of 0.2% ropivacaine after CABG (GA + ESPB group), 2) combination of sevoflurane general anesthesia with epidural anesthesia (EA) with 10–14 mL of 0.75% ropivacaine at the Th2-4 level followed by prolonged infusion of 0.2% ropivacaine (GA+EA group), 3) sevoflurane general anesthesia without regional anesthesia (GA group).

Inclusion criteria were signed voluntary informed consent to participate in the study, age greater than 18 years and not greater than 70 years, elective stand-alone off-pump CABG, ejection fraction greater than 40%, and sustained sinus rhythm.

Exclusion criteria were refusal to participate in the study, refusal of regional anesthesia (EA or ESPB), myocardial infarction within the previous 30 days, severe chronic obstructive pulmonary disease (GOLD stage II and greater, need for continuous therapy with inhaled steroids), chronic kidney disease stage IV and V, poor control of diabetes mellitus (glycated hemoglobin more than 8%), obesity with body mass index more than 40 kg/m². Intraoperative conversion to cardiopulmonary bypass or inadequate regional anesthesia were considered criteria for post-randomization exclusion from the study.

On admission to the operating room, patients in the GA+ESPB group underwent peripheral vein (Vasofix Braunule, BBraun, Germany) and radial artery (Arteriofix, BBraun, Germany) catheterization. In the supine position under ultrasound guidance (Philips CX-50, USA), catheterization of the neurofascial space of the erector spinae muscle at the level of the transverse process of Th5 (Perifix, BBraun, Germany) was performed bilaterally and the catheter was guided cranially at a distance of 4–5 cm from the tip of the needle. A 20 mL bolus of 0.5% ropivacaine was injected through the catheter on each side. After induction of anesthesia (propofol 1–2 mg/kg, fentanyl 2–3 μg/kg, pipecuronium bromide 0.08 μg/kg), tracheal intubation and lung ventilation (Datex Ohmeda Aespire View, GE Carestation 650, GE Healthcare technologies, USA) were performed with a tidal volume of 6 mL/kg and parameters necessary to maintain saturation greater than 96% and normocapnia. Under anesthesia, patients underwent right internal jugular vein catheterization (Intradyn F8, BBraun, Germany) followed by pulmonary artery catheterization (Corodyn TDF7, BBraun, Germany). Anesthesia was maintained with sevoflurane at MAC 0.7–1.5. In the postoperative period, analgesia was provided by continuous infusion of 0.2% ropivacaine at a rate of 5–12 mL/hour until the patient was transferred from the ICU.

In the GA+EA group, epidural catheterization (Perifix, BBraun, Germany) was performed before induction of anesthesia through a midline approach at the level of Th2–Th3 or Th3–Th4. Anesthesia was maintained during the intraoperative period with sevoflurane 0.7–1.5 MAC. The analgesic component of anesthesia included intermittent injection of 10–14 ml of 0.75% ropivacaine. Postoperative analgesia was provided by continuous infusion of ropivacaine 0.2% at a rate of 3–6 mL/h and fentanyl 4–10 μg/h.

In the GA group, induction of anesthesia and tracheal intubation were performed according to the same procedure. Anesthesia was maintained intraoperatively with sevoflurane 0.7–1.5 MAC, analgesia was achieved by fentanyl administration at 2–3 μg/kg/hour.

The intraoperative infusion consisted of 1000 ml of balanced solutions in all patients. The first-line drug for control of perioperative hypotension was norepinephrine 0.2–0.3 μg/kg/min. In case of insufficient hemodynamic effect (mean arterial pressure (MAP) less than 65 mm Hg), dobutamine 5–7 μg/kg/min or change of surgical approach, in particular conversion to cardiopulmonary bypass, were considered. Patients remained in the ICU for the first two days of the postoperative period. Fluid therapy was adjusted by the attending physicians according to the patient's condition.

Forty-eight patients who underwent elective off-pump CABG between May 2020 and February 2023 were included in the study (Fig. 1). After excluding one patient from each group, 45 patients (37 men and 8 women) were included in the analysis.

Mean arterial pressure (MAP), heart rate (HR), CVP, mean pulmonary artery pressure (PAP), pul-

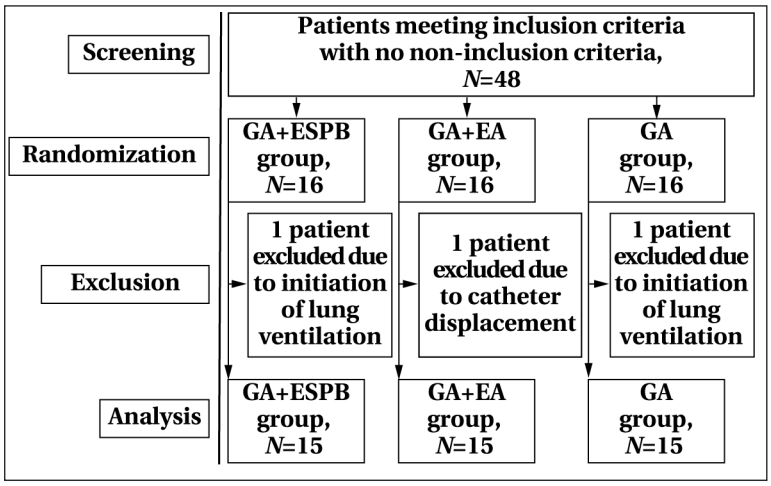

Fig. 1. Flowchart of the trial.
Note. GA — general anesthesia; EA — epidural anesthesia; ESPB — erector spinae plane block.

monary artery wedge pressure (PAWP), cardiac index (CI), stroke volume index (SVI), systemic vascular resistance index (SVRI), pulmonary vascular resistance (PVR) (Nihon Kohden monitors, Japan) were measured immediately after the patient was transferred from the operating room to the ICU and on the next postoperative day when the patient was transferred to the cardiac surgery unit. In both stages of the study, arterial and venous blood gas parameters, as well as changes in troponin T and NT-proBNP on day 1 of the postoperative period were determined compared to preoperative values.

After transport to the ICU with continued propofol sedation at a dose of 1–2 mg/kg/hour to achieve synchronization with the ventilator at the RASS sedation level of 2–3 points, all patients were tested for fluid responsiveness. First, the PLR test was performed, followed 10 minutes later by the standard bolus challenge (500 mL of balanced crystalloid solution over 5 min). At the end of the first postoperative day, before the patient was transferred out of the ICU, these tests were repeated, followed by an assessment of the hemodynamic effects during upright positioning of the patient, for which CI, HR, and SVI were measured in the sitting position on the bed and then in the standing position. Thermodilution measurements were performed after a 5 min period of position stabilization with continuous assessment of subjective comfort and monitoring of vital signs (Fig. 2).

Patients were considered to respond to fluid loading if the CI increased by more than 10% from baseline in the PLR test and by more than 15% in the bolus test (BT).

Statistical analysis was performed using SPSS v 21.0 (SPSS Inc, USA) and Python 3.11.0 with packages Numpy 1.24.1, Pandas 1.5.2, Matplotlib 3.6.2. Data distribution was assessed using the Shapiro-Wilk criterion. For normal distributions, analysis of variance was used for between-group comparisons, and the Kruskal–Wallis test was used for non-normal distributions. Within-group changes were assessed using the Wilcoxon test with Bonferroni correction for multiple comparisons. The relationship between categorical variables was assessed using Pearson's χ^2 test. The kappa-Cohen coefficient was used to determine the consistency between dynamic tests. Two-sided significance level criteria were used. Data were presented as mean (standard deviation) *[M (SD)]* for normal distribution or as median (interquartile range) *[Me (IQR)]* for non-normal distribution. Categorical variables were presented as frequencies. Differences were considered significant at $P<0.05$.

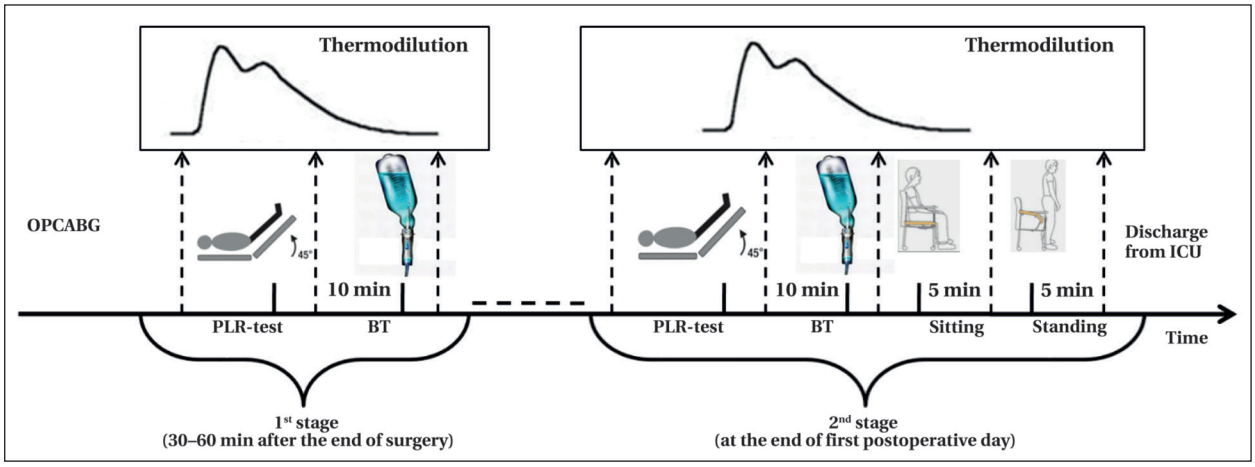

Fig. 2. Sequence of thermodilution test for fluid responsiveness assessment and during upright positioning.
Note. OPCABG — off-pump coronary artery bypass grafting; PLR — passive leg rising; BT — bolus test.

Table 1. Perioperative characteristics of patients.

Parameter	Values in groups			*P*
	GA+ESPB	GA+EA	GA	
Preoperative				
Age, years	60.1 (4.8)	60.7 (8.0)	62.7 (7.3)	0.53
Percentage of men, %	73	80	93	0.35
Body mass index, kg/m²	26.2 (2.8)	28.2 (4.3)	27.1 (3.0)	0.41
Euroscore II, %	1.07 (0.73)	0.85 (0.43)	1.34 (0.77)	0.06
CHF, NYHA class	2.0 (0.2)	2.0 (0.3)	2.0 (0.1)	0.47
Intraoperative				
Duration of surgery, min	174.3 (18.2)	178.3 (31.8)	179.8 (28.8)	0.85
Intraoperative fluid balance, mL	612.0 (206.0)	641.3 (262.1)	648.0 (159.9)	0.89
Postoperative				
Fluid balance during day 1, mL	336.0 (615.6)	599.3 (570.9)	630.7 (382.5)	0.26
Fluid infusion during day 1 of postoperative period, mL	1700 (25)	1700 (200)	1700 (500)	0.72
NT-proBNP, ng/mL	398.9 (275.4)	642.0 (1183.0)	725.4 (1121.8)	0.65
Troponin T, pg/mL	179.6 (161.0)	199.2 (109.6)	243.5 (250.3)	0.62

Table 2. Cardiac index changes during tests of fluid responsiveness and orthostatic tests.

Period	Parameter	Values in groups			*P*
		GA+ESPB	GA+EA	GA	
Admission to ICU	CI_{rest}	2.40 (0.54)	2.22 (0.67)	2.16 (0.58)	0.521
	CI_{PLR}	2.53 (0.58)	2.44 (0.70)	2.44 (0.66)	0.914
	*P-value**	0.048	0.012	0.001	
	CI_{BT}	2.82 (0.70)	2.59 (0.83)	2.35 (0.47)	0.203
	*P-value**	0.005	0.011	0.055	
End of Day 1	CI_{rest}	2.47 (0.34)	2.78 (0.65)	2.58 (0.44)	0.243
	CI_{PLR}	2.61 (0.47)	2.94 (0.71)	2.83 (0.59)	0.291
	*P-value**	0.169	0.026	0.026	
	CI_{BT}	2.73 (0.45)	2.97 (0.62)	2.64 (0.41)	0.193
	*P-value**	0.007	0.064	0.277	
	$CI_{sitting}$	2.75 (0.50)	3.20 (0.83)	2.83 (0.53)	0.152
	*P-value**	0.029	0.172	0.035	
	$CI_{standing}$	2.24 (0.41)	2.59 (0.61)	2.62 (0.65)	0.151
	*P-value**	0.173	0.391	0.934	

Note. CI — cardiac index; PLR — passive leg raising; BT — bolus test. * — when compared to the resting value.

Results and Discussion

The mean age of the patients was 61.2 (6.7) years, the body mass index was (27.3 (0.4) kg/m²), the class of chronic heart failure was 2.0 (0.4), and the preoperative risk according to the Euroscore II scale was 1.1 (0.6) %. All parameters did not differ between the two groups. The average revascularization index was 2.4. Intraoperative fluid administration was prearranged, and no differences in fluid volume and balance were found in the postoperative period (Table 1).

On admission to the ICU, fluid responsiveness tests showed a significant increase in CI: after the PLR test, CI increased in the GA+EA and GA groups, after the bolus infusion — in the GA+ESPB and GA+EA groups (Table 2). At the end of day 1, CI was significantly increased only during the bolus test in the GA+ESPB group due to the increase in SVI (*P*=0.004) (Fig. 3). This can be explained both by the variable severity of the hemodynamic effects of the different regional anesthesia techniques and by differences in the hemodynamic effects of the methods used to assess fluid responsiveness.

When analyzing the parameters that determine CI, the GA+ESPB group showed an increase in HR during upright positioning (*P*=0.001) (Fig. 3). This may indicate the consistency of the baroreceptor reflex in ESPB [21]. Godfrey et al. also found a greater significance of HR increase compared to stroke volume during the PLR test [6].

When analyzing fluid responsiveness, there was no significant difference between groups in either the PLR test or the bolus test. Thus, the PLR test immediately after surgery showed that 7 (47%), 10 (67%), and 9 (60%) patients in the GA+ESPB, GA+EA, and GA groups, respectively, were responders (*P*=0.53), while the bolus test showed 4 (27%), 7 (47%), and 7 (47%) responders (*P*=0.43). The lack of between-group differences in response to fluid therapy confirms previous findings [14]. In addition, no significant differences in mean PAP, PAWP, SVRI and PSR were observed between groups.

The agreement between PLR and bolus test in the GA+ESPB, GA+EA and GA groups was 0.53 (95% CI 0.12–0.94), 0.68 (95% CI 0.30–1.00) and 0.61 (CI 0.24–0.99), respectively. The same fluid responsiveness on both tests was found in 9–11 patients from each group, representing 80% of the total cohort. This suggests the limited consistency of PLR and bolus tests immediately after CABG and

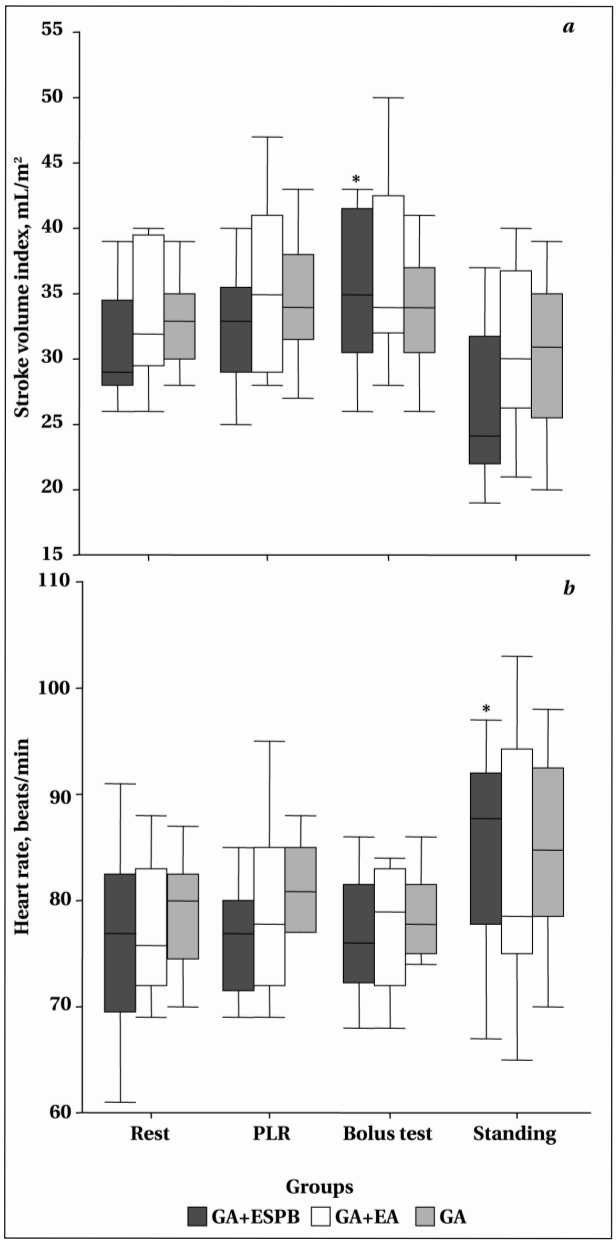

Fig. 3. Heart rate and stroke volume index at the end of first postoperative day.
Note. * — $P<0.05$ compared to the resting value within the group.

the need for cautious interpretation of their results at this stage. Based on different methods of CI assessment, several investigators have shown a moderate correlation between changes in CI during PLR and bolus testing [15]. In our study, the consistency of the tests increased at the end of the first postoperative day and was 0.70 (0.32–1.00), 0.84 (95% CI 0.55–1.00), and 0.82 (95% CI 0.47–1.00) in the GA+ESPB, GA+EA, and GA groups, respectively. The number of fluid responders in the groups was 6 (40%), 4 (27%), and 5 (33%) for the passive leg raise test (P=0.74) and 4 (27%), 3 (20%), and 4 (27%) for the bolus test (P=0.89). The same fluid responsiveness on both tests was found in 14 patients in the GA+ESPB group, 14 patients in the GA+EA group, and 14 patients in the GA group, representing 95% of patients in the total cohort.

The decrease in the number of responders on the first day after CABG may be a natural consequence of the positive postoperative balance. Thus, when analyzing within-group changes, the number of responders in the bolus test during the first postoperative day decreased from 18 to 3 with no between-group differences. On the other hand, 8 patients (18%) of the 27 non-responders immediately after surgery became responders to fluid therapy by the end of the first postoperative day despite a positive postoperative balance. This is probably due to the effect of myocardial revascularization and optimization of left ventricular inotropic and lusitropic function, transition to spontaneous breathing and improvement of right ventricular dysfunction [16], and tissue perfusion with fluid therapy [17]. However, the hemodynamic effects of fluid therapy administered to all patients in the postoperative period are often transient [18]. For example, even in responders, CI begins to decrease 60 minutes after bolus infusion [19], with complete loss of the volume effect of crystalloid solution in 120 min [20]. Meanwhile, the hemodynamic effects of fluid therapy can be prolonged when vasopressor support which reduces venous capacitance is used [21].

Although stable parameters, particularly CVP, have not been shown to be a reliable measure of preload [3], changes in CVP may reflect the severity of right ventricular dysfunction [7]. For example, Vlahakes et al. demonstrated that after pericardial closure and change in preload during cardiac surgery, the increase in left and right ventricular pressures no longer showed a linear relationship, reducing the potential for optimizing right ventricular preload to increase left ventricular performance [22]. While the changes in the passive leg-raise test were consistent in all groups, the subsequent bolus challenge resulted in an increase in CVP only in the EA and ESPB groups (Fig. 4). Several authors have suggested a decrease in right ventricular systolic function with the use of regional anesthetic techniques, particularly EA [23], but these results are controversial [24]. For example, Cooke et al. found that the maximal hemodynamic effect of fluid was in those patients who did not have an increase in CVP with increasing MAP and CI after bolus infusion [25]. However, the PLR test, which increases CVP, may have potential limitations. In addition, non-responders to dynamic testing may include patients with a decrease in cardiac output of more than 15% in response to bolus infusion [25], which is particularly undesirable in cardiac surgery.

The lack of between-group differences in CI values at the end of the first postoperative day during upright positioning of patients suggests a minor contribution of the studied regional anesthetic

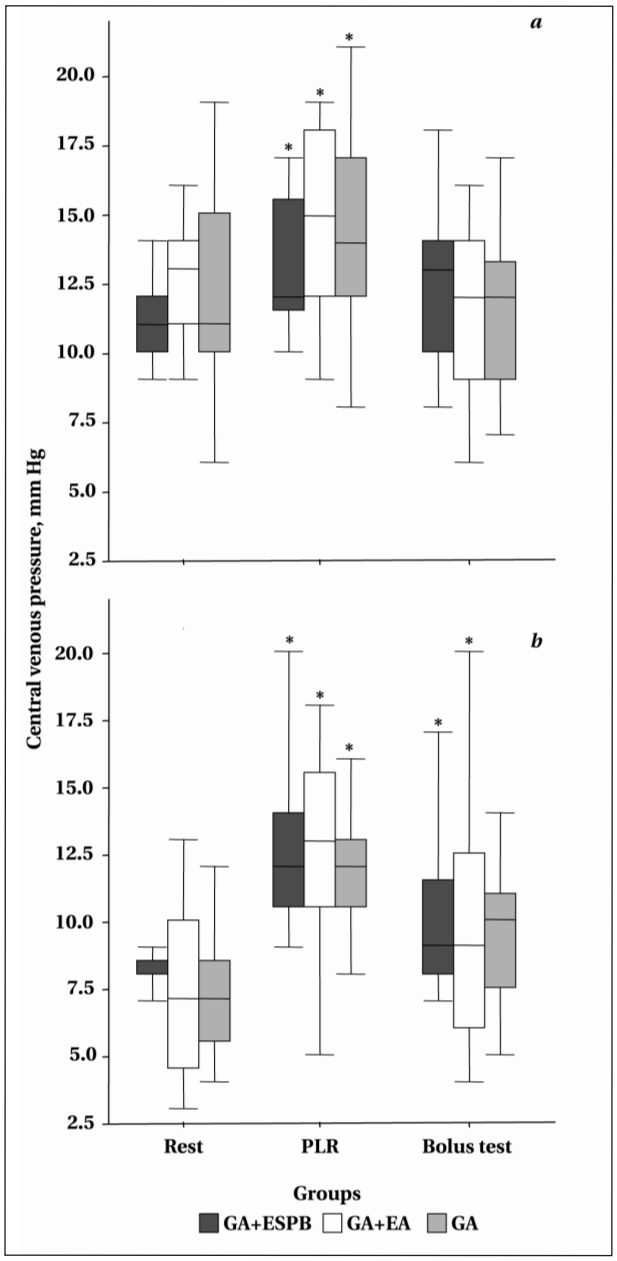

Fig. 4. Central venous pressure at the ICU admission (*a*) and at the end of first postoperative day (*b*).
Note. * — $P<0.05$ compared to the resting value within the group.

techniques at the thoracic level, in particular EA and ESPB, to the severity of autonomic nervous dysfunction [26]. Discomfort during upright positioning occurred in 15 patients, again without differences between groups, with two patients (one each from the GA and GA+ESPB groups) refusing the orthostatic test due to low tolerance. Although the effect of upright positioning is equivalent to a loss of 500–1000 mL of volume [24], we did not observe significant changes in CI and SVI, except for an increase in HR in the sitting (P=0.004) and standing (P=0.001) positions, which highlights the complexity of the adaptation mechanisms to changes in circulating blood volume, that are difficult to predict.

Limitations of the assessment of CI changes in PLR and upright positioning tests include the fact that the bolus test, which has an irreversible and independent effect on volume status, was performed between these two tests, and the combined hemodynamic effect of all three tests is poorly predictable.

No between-group differences were found when evaluating changes in blood gases and troponin T; troponin rise was 14.6 (10.3), 14.3 (11.5), and 11.2 (12.1) fold in the GA+EA, GA, and GA+ESPB groups, respectively (P=0.92). NT-proBNP levels at the end of the first postoperative days exceeded preoperative levels by 4.3 (3.6), 2.9 (2.3) and 2.9 (1.8) times, respectively (P=0.27), while postoperative fluid balance parameters did not differ between groups. Thus, the use of epidural anesthesia and ESPB does not cause excessive myocardial damage, and neurohumoral markers of systolic or diastolic dysfunction showed concordant changes attributed to perioperative surgical stress.

Another limitation of the study is its pilot nature without pre-specified statistical power. Further studies with a larger number of patients are warranted.

Conclusion

The use of EA and ESPB during off-pump CABG results in an increase in CVP when the bolus test is performed at the end of the first postoperative day. There were no differences in the severity of orthostatic response between groups. During upright positioning with ESPB, heart rate increased, whereas no changes in cardiac output and stroke volume were observed.

Thus, the use of regional anesthesia techniques does not significantly affect the responsiveness to fluid therapy after coronary artery bypass grafting and does not exacerbate perioperative myocardial injury or dysfunction. In the postoperative period after coronary artery bypass grafting, there is moderate concordance between the PLR test and the bolus challenge test, with a subsequent increase in concordance by the end of the first postoperative day, suggesting that responsiveness to fluid therapy on ICU admission could be assessed using the bolus test alone.

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Morphological and Functional Alterations of Respiratory Muscle Performance and Spirometry Parameters in Patients with Congestive Heart Failure

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Summary

The purpose of the study. To identify structural changes and functional modifications in respiratory muscle performance in patients with congestive heart failure.

Materials and methods. We conducted prospective observational study at the V. A. Almazov National Medical Research Center involving 118 subjects: 49 patients with congestive heart failure (CHF-group) and 69 healthy people (control group). NYHA functional classes of II to IV were taken as inclusion criteria in the CHF group, and respiratory diseases, abdominal pathology, morbid obesity, and anemia — as exclusion criteria.

Ultrasound imaging was used to assess the structural (thickness) and functional (thickening and excursion indices) diaphragmatic impairments during quiet (resting) and deep breathing. Facemask spirometry was used to assess pulmonary function.

Results. Patients with CHF were on average older than 59.0 years (53.0; 70.0) vs. 25.0 years (24.0; 26.0) in the control group, *P*=0.000001, had excessive body weight — 82.0 (73.0; 95.0) vs. 68.5 (55.0; 84.0) kg, *P*=0.000005 and higher body mass index — 28.4 (24.3; 31.3) vs 21.8 (19.9; 24.0) kg/m^2, *P*=0.000001, but did not differ in height 173.0 (166.0; 179.0) vs. 170.0 (165.0; 183.0) cm, 0.97.

Lower maximum inspiratory volume (MIV): 3000.0 (2300.0; 4000.0) vs. 3684.1 (3392.5; 4310.8) ml, *P*=0.0006, and negative inspiratory force (NIF) measured as max negative pressure generated by the respiratory muscles: 43.1 (–56.7; –33.0) vs. 53.5 (–58.8; –50.9) mBar, *P*=0.000082, respectively were found in patients with CHF. The diaphragm was significantly thicker (mm) in patients with CHF during quiet (eupnea) and deep breathing compared to healthy subjects. The thickness at the end of quiet inspiration was 3.0 (2.2; 3.6)/1.9 (1.5; 2.2) in the right hemi-diaphragm, *P*<0.001; and 3.0 (2.4; 3.5)/1.7 (1.4; 2.0) — in the left, *P*=0.000001; thickness at the end of quite expiration — 2.2 (1.8; 2.9)/1.5 (1.2; 1.7) in the right dome, *P*=0.000001; and 2.0 (1.7; 2.5)/1.4 (1.2; 1.5) — in the left, *P*=0.000001. Thickness at the end of deep inspiration was 5.1 (4.4; 6.1)/4.4 (3.6; 5.1) in the right dome, *P*=0.0005, and 4.9 (4.2; 6.2)/ 3.7 (3.1; 4.8) — in the left, *P*=0.000007.The diaphragm thickening index during deep breathing was lower in the CHF group than in the control group: 131.1 (82.5; 181.8) vs. 190.9 (150.0; 240.0) in the right dome, *P*=0.000004; and 148.8 (112.5; 190.3) vs. 175.2 (130.7; 227.7) — in the left, *P*=0.03, respectively.

Diaphragmatic excursions during quiet breathing were larger in patients with CHF than in healthy controls: 2.3 (1.6; 2.8)/1.7 (1.5; 1.9), *P*=0.0001 and 1.8 (1.5; 2.2)/1.5 (1.3; 1.9), *P*=0.03 of the right and left domes, respectively.

Conclusion. Congestive heart failure contributes to the development of structural and functional impairments of the diaphragm.

Keywords: congestive heart failure; ultrasound examination of the diaphragm; diaphragm; external respiration; diaphragm function; diaphragmatic dysfunction

Conflict of interest. The authors declare no conflict of interest.

Introduction

Chronic heart failure (CHF) can lead to a variety of symptoms, such as muscle dysfunction [1–3], including respiratory muscle dysfunction [2, 4, 5] that can be severe enough to cause ventilatory impairment [5]. Respiratory muscle dysfunction is considered a sign of multiorgan failure in advanced CHF [1] and is associated with increased dyspnea, reduced exercise tolerance and ultimately early death [1, 6–9].

Dysfunction of the diaphragm, as the most active respiratory muscle, is relevant to intensive care physicians and anesthesiologists because it may prolong ICU stay [10–12] and influence the choice of respiratory support [10, 13]. Meanwhile, current guidelines for preoperative assessment of respiratory function in patients with CHF include only lung auscultation [14].

The assessment of respiratory function in general and diaphragm performance in particular is especially relevant in CHF because it could help to determine the strategy of patient management in the ICU, including the choice of noninvasive and invasive ventilatory parameters [5, 10, 15].

While respiratory impairment in CHF is well known [2, 4, 5], diaphragmatic dysfunction, especially as assessed by ultrasound, has been poorly studied.

All the above provides a rationale for studying respiratory performance in critically ill patients with CHF, from both scientific and practical points of view.

Aim. To identify structural and functional changes in respiratory function in patients with chronic heart failure.

Materials and methods

A cross-sectional prospective descriptive study of structural and functional disorders of the respiratory system in patients with CHF was conducted at the Almazov National Medical Research Center from May 2022 to December 2022. The study followed the Helsinki Declaration of 2000 and was approved by the local ethics committee (protocol dated April 30, 2022).

Inclusion criterion for patients in the main (CHF) group was CHF II-IV NYHA functional class.

Exclusion criteria were respiratory diseases, morbid obesity, abdominal diseases, anemia (hemoglobin less than 120 g/l).

A total of 118 individuals were enrolled in the study (Figure 1), including 49 patients with CHF and 69 apparently healthy volunteers. The CHF group included 11 women and 38 men diagnosed with CHF at least one year prior to enrollment, with a mean age of 58.7±13.5 years, body weight of 84.7±18.3 kg, height of 172.8±8.6 cm, and BMI of 28.3±5.2 kg/m². Among all patients with CHF, 20 were diagnosed with NYHA class II, 15 with class III, and 14 with class IV. All patients were stable and receiving combination therapy including ACE inhibitors, beta-blockers, diuretics, aldosterone antagonists and statins. Patients with cardiac arrhythmias (atrial fibrillation) were on oral anticoagulants.

The control group consisted of 39 female and 30 male subjects with a mean age of 25.0±2.1 years, body weight of 68.56±15.62 kg, height of 173.4±10.7 cm, and BMI of 22.54±3.4 kg/m².

Ultrasound (US) examination of the diaphragm and measurement of respiratory function were performed in the supine position with the head end of the bed elevated 30 degrees, which is the basic position of patients in intensive care units. The structure (diaphragm thickness, DT) and function (diaphragm excursion, E; thickness fraction, DTF) of the diaphragm during quiet and deep inspiration/expiration were evaluated using a Philips CX50 ultrasound device (Philips Ultrasound, Inc., USA). Ultrasound parameters obtained only in patients with good visualization of the diaphragm were included in the final analysis.

Respiratory function was assessed using a Dräger Evita Infinity V500 ventilator (Germany) in noninvasive lung ventilation mode. A constant positive pressure of 0 mbar without pressure support and a FiO_2 of 0.21 were set on the device.

Tidal volumes during quiet (T_{vquiet}) and maximal deep (T_{vdeep}) breathing, inspiratory and expiratory times were measured. Neurorespiratory drive and respiratory muscle strength were estimated based on P0.1 (airway pressure at 100 ms of spontaneous attempt to breathe during occlusion of the breathing circuit) and NIF (negative inspiratory force, the minimum airway pressure during inspiration from a tightly closed circuit). Since NIF is conventionally used as a predictor of spontaneous breathing readiness with a weaning success threshold of less than –25 to –30 mBar [16], values above this threshold were considered indicative of weak respiratory muscles.

Hemoglobin oxygen saturation was assessed with a pulse oximeter.

The collected data were analyzed using STATISTICA 10.0 software and the Real Statistics Resource Pack Microsoft Excel add-on.

During the design phase, using the power analysis option of the STATISTICA-10 software, the sample size was estimated to be at least 100 subjects to achieve 80% power.

After the pilot study, calculations showed that the minimum number of subjects required in the main group was 28. In the end, 49 subjects were included in the study, giving a power of 0.9.

The pilot study was conducted according to the full study methodology to confirm the hypothesis of different diaphragm performance in patients with CHF, as well as to identify early the parameters with the greatest difference and to determine the minimum number of subjects required. First, the most different parameters, such as diaphragm thickness, were identified by taking the minimum number of observations necessary for statistical calculations according to the Mann–Whitney criterion, which was 3 healthy controls and 5 patients with CHF. Then, the group size was increased to 10 and the sample calculation for the whole study was performed. After obtaining

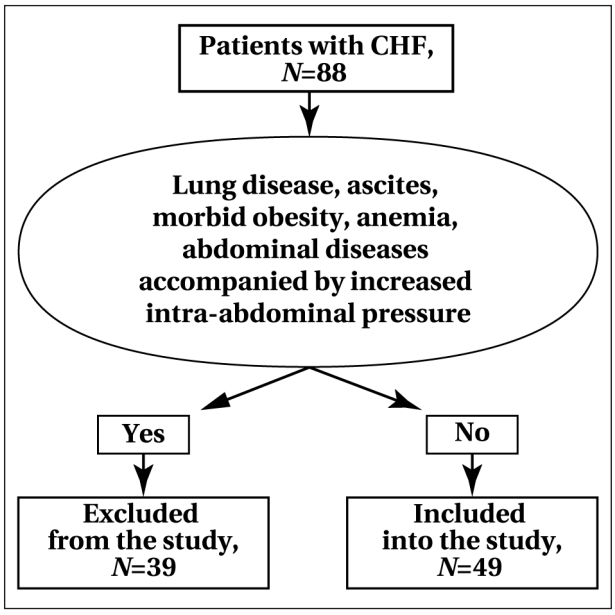

Fig. Patient inclusion flowchart.

the results and their statistical analysis in the initial stage, the study was continued in full length.

Normality of the distribution was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Independent groups were compared using the Mann–Whitney test. Data were presented as median *(Me)* and interquartile range *(Q1; Q3)*. Differences were considered significant at $P \leqslant 0.05$.

Results

The results obtained are shown in Tables 1–6.

As shown in Table 1, patients with CHF were older and had higher body weight and BMI.

As shown in Table 2, patients with CHF had a higher respiratory rate, shorter inspiratory and expiratory times, lower V_{tdeep} and inspiratory force. In addition, patients with CHF had lower hemoglobin oxygen saturation, although SpO_2 values remained in the reference range.

As shown in Table 3, the diaphragm thickness of patients with CHF was significantly higher during quiet and deep breathing, whereas the thickening fraction was smaller during deep breathing.

As shown in Table 4, a higher diaphragm excursion was observed during quiet breathing in patients with CHF, whereas no differences were found during deep breathing. Visualization of the diaphragm on the left side was not always possible, which explains the smaller number of subjects included in the statistical analysis.

As Table 5 demonstrates, the times of craniocaudal (contraction) and caudal-cranial (relaxation) diaphragm motion during quiet and deep breathing

Table 1. Anthropometric parameters of the study groups, median (*Q1; Q3*).

Parameter	Values in groups		*P*
	Controls, *N*=69	Patients with CHF, *N*=49	
Age, years	25.0 (24.0; 26.0)	59.0 (53.0; 70.0)	0.000001*
Body mass, kg	68.5 (55.0; 84.0)	82.0 (73.0; 95.0)	0.000005*
Height, cm	170.0 (165.0; 183.0)	173.0 (166.0; 179.0)	0.97
BMI, kg/m²	21.8 (19.9; 24.0)	28.4 (24.3; 31.3)	0.000001*

Note. BMI — body mass index; CHF — chronic heart failure; * — significant difference ($P \leqslant 0.05$, Mann–Whitney test).

Table 2. Respiratory function parameters, median (*Q1; Q3*).

Parameter	Values in groups		*P*
	Controls, *N*=69	Patients with CHF, *N*=49	
Respiratory rate	13 (11; 15)	15 (13; 17.5)	0.0009*
$V_{t\ quiet}$, ml	560.0 (493.5; 678.0)	548,0 (450.0; 666.0)	0.37
IT, s	1.5 (1.3; 1.6)	1,3 (1.1; 1.4)	0.0001*
ET, s	3.0 (2.5; 3.7)	2,8 (2.3; 3.1)	0.008*
$V_{t\ deep}$, ml	3684.1 (3392.5; 4310.8)	3000.0 (2300.0; 4000.0)	0.0006*
SpO_2, %	99 (99; 99)	97.4 (97.0; 98.0)	0.000001*
P 0.1, mbar	–2.1 (–3.0; –1.5)	–1.3 (–1.8; –0.9)	0.000003*
NIF, mbar	–53.5 (–58.8; –50.9)	–43.1 (–56.7; –33.0)	0.000082*

Note. $V_{t\ quiet}$ — tidal volume in quiet breathing; $V_{t\ deep}$ — tidal volume in deep breathing; IT — inspiratory time; ET — expiratory time; NIF — negative inspiratory force; P0.1 — airway occlusion pressure at 100 ms; * — significant difference ($p \leqslant 0.05$, Mann–Whitney test).

Table 3. Diaphragm thickness, median (*Q1; Q3*).

Parameter	Values in groups		*P*
	Controls, *N*=69	Patients with CHF, *N*=49	
$DT_{quiet-insp}$, right , mm	1.9 (1.5; 2.2)	3.0 (2.2; 3.6)	0.000001*
$DT_{quiet-exp}$, right, mm	1.5 (1.2; 1.7)	2.2 (1.8; 2.9)	0.000001*
$DT_{deep-insp}$, right, mm	4.4 (3.6; 5.1)	5.1 (4.4; 6.1)	0.0005*
$DT_{deep-exp}$, right, mm	1.1 (1.0; 1.4)	1.7 (1.3; 1.9)	0.000001*
$DT_{quiet-insp}$, left, mm	1.7 (1.4; 2.0)	3.0 (2.4; 3.5)	0.000001*
$DT_{quiet-exp}$, left, mm	1.4 (1.2; 1.5)	2.0 (1.7; 2.5)	0.000001*
$DT_{deep-insp}$, left, mm	3.7 (3.1; 4.8)	4.9 (4.2; 6.2)	0.000007*
$DT_{deep-exp}$, left, mm	1.1 (0.9; 1.2)	1.6 (1.3; 2.0)	0.000001*
$DTF_{quiet-insp}$, right, %	27.8 (20.0; 35.0)	30.4 (17.9; 44.8)	0.38
$DTF_{deep-insp}$, right, %	190.9 (150.0; 240.0)	131.1 (82.5; 181.8)	0.000004*
$DTF_{quiet-insp}$, left, %	23.6 (18.3; 33.0)	40.9 (28.5; 59.8)	0.000002*
$DTF_{deep-insp}$, left, %	175.2 (130.7; 227.7)	148.8 (112.5; 190.3)	0.03*

Note. DT — diaphragm thickness; DTF — diaphragm thickening fraction; insp — inspiration; exp — expiration; * — significant difference ($P \leqslant 0.05$, Mann–Whitney test).

were shorter in patients with CHF than in healthy controls.

Table 6 demonstrates that during quiet inspiration, the rate of cranial-caudal and caudal-cranial diaphragmatic movements was higher on both sides in the group of patients with CHF. During deep exhalation, only the right hemisphere of the diaphragm moved faster, while only a tendency for faster kinetics was observed on the left side.

Discussion

The diaphragm, along with the myocardium, is an almost continuously working muscle, which explains its significant oxygen consumption [17] and determines a significant susceptibility to over- or under-exertion as well as to oxygen delivery [18]. Chronic heart failure is associated with reduced oxygenation of the respiratory muscles, especially the diaphragm [5]. The data obtained indicate a

Table 4. Diaphragm excursion, median (*Q1; Q3*).

Parameter	Values in groups		*P*
	Controls	Patients with CHF	
Number of patients tested	69	49	—
E_{quiet}, right	1.7 (1.5;1.9)	2.3 (1.6;2.8)	0.0001*
E_{deep}, right	6.9 (6.0;8.0)	6.9 (5.8;9.1)	0.67
Number of patients tested	29	34	—
E_{quiet}, left	1.5 (1.3;1.9)	1.8 (1.5;2.2)	0.03*
Number of patients tested	29	26	—
E_{deep}, left	6.0 (5.3;6.9)	5.8 (4.5;7.3)	0.41

Note. E — excursion; quiet — quiet breathing; deep — deep breathing; * — significant difference ($P \leqslant 0.05$, Mann–Whitney test).

Table 5. Time parameters of diaphragm excursion, median (*Q1; Q3*).

Parameter	Values in groups		*P*
	Controls	Patients with CHF	
Number of patients tested	69	49	
$T_{contr/quiet}$, right	1.4 (1.1; 1.7)	1.2 (1.0; 1.4)	0.029*
$T_{relax/quiet}$, right	1.4 (1.2; 1.4)	1.1 (0.9; 1.4)	0.003*
$T_{contr/deep}$, right	2.2 (1.9; 2.8)	1.9 (1.5; 2.3)	0.001*
$T_{relax/deep}$, right	2.4 (2.0; 2.7)	1.8 (1.2; 2.6)	0.00002*
Number of patients tested	29	34	
$T_{contr/quiet}$, left	1.4 (1.1; 1.6)	1.2 (1.0; 1.4)	0.02*
$T_{relax/quiet}$, left	1.4 (1.1; 1.7)	1.0 (0.9; 1.4)	0.007*
Number of patients tested	29	26	
$T_{contr/deep}$, left	2.2 (1.8; 2.8)	1.9 (1.5; 2.3)	0.08
$T_{relax/deep}$, left	2.4 (2.0; 2.7)	1.8 (1.2; 2.6)	0.01*

Note. Contr — cranio-caudal excursion (contraction); relax — caudo-cranial excursion (relaxation); quiet — quiet breathing; deep — deep breathing; T — time; * — significant difference ($P \leqslant 0.05$, Mann–Whitney test).

Table 6. Velocity parameters of diaphragm excursion, median (*Q1; Q3*).

Parameter	Values in groups		*P*
	Controls	Patients with CHF	
Number of patients tested	69	49	—
$R_{contr/quiet}$, right	1.0 (0.8; 1.3)	1.8 (1.3; 2.3)	0.000001*
$R_{relax/quiet}$, right	1.1 (0.9; 1.4)	1.8 (1.3; 2.3)	0.000001*
$R_{contr/deep}$, right	3.3 (2.4; 4.5)	2.7 (1.2; 3,4)	0.7
$R_{relax/deep}$, right	2.9 (2.0; 3.5)	3.4 (2.7; 5.2)	0.001*
Number of patients tested	29	34	
$R_{contr/quiet}$, left	1.2 (0.9; 1.7)	1,7 (1.3; 2.3)	0.03*
$R_{relax/quiet}$, left	1.3 (0.9; 1.8)	1,7 (1.3; 2.3)	0.02*
Number of patients tested	29	26	
$R_{contr/deep}$, left	2.6 (2.2; 3.0)	2.7 (1.9; 4.0)	0.7
$R_{relax/deep}$, left	2.8 (2.1; 2.9)	3.3 (2.0; 4.1)	0.08

Note. R — rate; contr — contraction; relax — relaxation; quiet — quiet breathing; deep — deep breathing; * — significant difference ($P \leqslant 0.05$, Mann–Whitney test).

significant difference in almost all parameters, both spirometric evaluation of respiratory function and ultrasound structural and functional characteristics of the diaphragm in patients with CHF compared to healthy controls.

The lower hemoglobin oxygen saturation found in patients with CHF was anticipated [19]. Increased respiratory rate during quiet breathing in the group of patients with chronic heart failure allowed to compensate the decreased tidal volume [20]. However, during deep breathing, more severe structural and time-velocity disturbances were found, indicating reduced respiratory reserves [19, 20–22], probably due to impaired muscle function [21, 22] and the development of restrictive respiratory failure in CHF [19, 22, 23].

Respiratory muscle strength on inspiration was significantly lower in the group of patients with CHF. P0.1 is considered an indicator of respiratory drive, which is not quite equivalent to respiratory muscle strength [22, 24], so its interpretation requires caution. Researchers have not found changes in P0.1 in patients with CHF [22, 23], which is to some extent confirmed by our data. Despite the fact that P0.1 in patients with CHF was significantly lower (modulo) than in healthy individuals, in absolute terms the parameter did not exceed the reference range, which can be interpreted as the absence of significant disorders of neurorespiratory drive. The observed reduction of NIF modulo values in patients with CHF compared to healthy controls was expected and is consistent with the data of other researchers [1, 21, 22, 25].

Ultrasound structural (thickness) parameters of the diaphragm were significantly higher in patients with CHF on both sides during quiet and maximal deep inspiration and expiration. This does not agree with the results of Spiesshoefer J. et al [25], who found no differences in diaphragm thickness at the end of quiet expiration in healthy subjects and patients with CHF, whereas diaphragm thickness during deep breathing was greater in healthy subjects [25]. On the other hand, Miyagi M. et al [21] reported greater diaphragm thickness in patients with lower left ventricular ejection fraction, which is consistent with the results of our study.

Spiesshoefer J. et al. showed that the diaphragmatic thickening fraction during quiet breathing is lower in patients with CHF than in healthy subjects [25]. The data obtained are consistent with this finding, but only during maximal deep inspiration and with visualization of both right and left hemispheres. In general, it is rather difficult to compare the magnitude of diaphragmatic thickening because its calculation can be performed using different formulas [25, 26].

The assumption that diaphragm excursion decreases in the group of patients with CHF regardless of the depth of breathing has not been confirmed [27]. The amplitude of diaphragmatic motion during quiet breathing did not differ between healthy subjects and patients with CHF and preserved ejection fraction, whereas during deep breathing it was significantly lower in patients with reduced ejection fraction [25].

Our results differ from the above data: diaphragm excursion during quiet breathing was significantly greater in the group of patients with CHF than in the control group on both the right and left sides, whereas during deep breathing it did not differ significantly between the groups. The patients with CHF may already be doing relatively more work to maintain the effective tidal volume at rest, i.e., their metabolic and physiological «cost» of breathing increases.

The time-velocity parameters of diaphragmatic excursion are poorly studied. The authors of the previously mentioned study [25] did not find any changes in diaphragmatic kinetics during quiet inspiration in patients with CHF. According to our data, the time of diaphragmatic movements during inhalation and exhalation during quiet and deep breathing decreased on both sides and the velocity increased accordingly in the CHF group.

The observed increase in time-velocity characteristics during inspiration can be explained by the involvement of respiratory auxiliary muscles and the reduced ability to «hold the breath» due to dynapenia (decrease in muscle strength with preserved muscle mass) rather than by improved diaphragm performance. The increase in time-velocity characteristics during expiration can be explained by increased lung compliance.

All changes in respiratory function in CHF could be compensatory and explained by several pathophysiological mechanisms. Chronic heart failure associated with interstitial pulmonary edema causes increased elastic recoil (i. e., decreased compliance) of the lungs and chest wall stiffness [25, 28]. These changes are likely to increase the load on the diaphragm during inspiration as the reduced lung compliance must be overcome, ultimately leading to its hypertrophy.

However, these suggestions are not consistent with histologic data showing a decrease in muscle mass and its replacement by connective and adipose tissue [29]. Another hypothesis is that the diaphragm may be enlarged due to edema. In any case, the data in the literature [5, 6, 8, 25, 27] are controversial and limited by small samples, which calls for more research in this area.

Our study had several limitations. First, the patients with CHF were older and had higher body weight. Therefore, we cannot postulate that the results obtained were solely due to CHF. Second, all patients were relatively well compensated, whereas

the greatest changes in spirometric and ultrasound parameters would probably be expected in overt respiratory failure. Finally, functional class is a rather volatile indicator of severity in CHF patients, and changes in a patient's functional performance over a short period of time may influence the results obtained.

Conclusion

Anatomical and physiological evidence suggests that chronic heart failure is associated with impairment of the structure and function of the diaphragm, the main respiratory muscle. Its thickening, changes in the amplitude of cranio-caudal and caudal-cranial movements and time-velocity parameters indicate a decrease in the functional reserves of the respiratory system.

The strength of muscle contraction decreases, which leads to a decrease in tidal volume and, consequently, an increase in respiratory rate, i.e., the respiratory pattern changes to a more superficial and more frequent one.

Such a change in respiratory pattern, which is typical of patients with chronic heart failure, suggests that the structural and functional differences in diaphragm parameters found are also due to CHF rather than age. Further research into the impact of CHF on diaphragm performance is warranted.

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Risk Factors for the Development and Severe Course of Ventilator-Associated Tracheobronchitis in Patients with Prolonged Mechanical Ventilation

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Summary

Objective. Identification of risk factors for the development and severe course of ventilator-associated tracheobronchitis (VAT) in patients on prolonged mechanical ventilation (PMV).

Methods. VAT incidence rate in the intensive care unit of Academician V. Vakhidov Republican Scientific and Practical Medical Center for Surgery for the period 2018–2022 was evaluated retrospectively in 724 patients who were on PMV (more than 48 h). Patients' clinical and demographic characteristics were subjected to factor analysis. Mean age was 52.4±3.3 (18–81) years. VAT was diagnosed based on clinical signs (fever >38°C, leukocytosis >12 000 ctlls/ml, or leukopenia <4 000 cells/ml, purulent endotracheal secretions, or conversion to purulent), radiological (no progression of existing or emergence of new pulmonary infiltrates) and microbiological (polymorphonuclear lymphocytes with or without bacteria, moderate-to active growth of colonies of potentially pathogenic microorganisms) criteria. VAT prophylaxis was based on the use of bacterial filters and humidification of the respiratory gas; selective decontamination of the digestive tract; regulation of pressure in the tracheal cuff; sanitation of the oral cavity. Treatment of VAT included antimicrobial drugs administered i/v and/or inhalational, bronchodilators, expectorants and mucolytics.

Results. VAT incidence rate decreased over time from 24.7% to 10.1% (χ^2=9.52; *P*=0.003) with invariable practice of ventilator support. The incidence of the most severe VAT (hemorrhagic catarrhal purulent) also gradually decreased from 44.7% to 14.3% (χ^2=4.53; *P*=0.034).The duration of PMV and ICU stay in patients with VAT gradually decreased from 202.1±6.15 h to 125.3±7.81 h (*t*=7.73; *P*<0.0001), and from 9.7±0.25 days to 6.6±0.3 days (*t*=7.94; *P*<0.0001), respectively. In patients with VAT (*N*=122), in contrast to patients without VAT (*N*=602), the incidence of concomitant COPD was higher — 22.9% vs 10.6%, respectively (*P*<0.001). Gram-negative flora was the leading cause for development of severe tracheobronchitis, including *Acinetobacter* spp. — in 24% of cases, *Klebsiella pneumoniae* — in 11.6%, *Pseudomonas aeruginosa* — in 13.0%, *Esherichia coli* — 10.6%. Less frequently were isolated *Staphylococcus aureus* — in 5.3%, *Enterococcus* spp. — in 2.2% and *Candida fungi* — in 17.0%. The following predictors of severe VAT were identified: age over 60 years (OR=2.28; 95% CI 1.0–4.9), SAPS II > 40 scores (OR=5.9; 95% CI 2.6–13.8), duration of mechanical ventilation >144 h (OR=5.4; 95% CI 1.8–16.7) and the presence of malignant neoplasms (OR=2.83; 95% CI 1.2–6.9).

Conclusion. Decrease in VAT incidence rates, reduced duration of mechanical ventilation and ICU stay are indicative of adequate VAT prevention and treatment strategies within the analyzed period. Factors associated with VAT development and predictors of severe VAT can be used for identification of high risk patients.

Keywords: prolonged mechanical ventilation; ventilator-associated tracheobronchitis; risk factors

Conflict of interest. The authors declare no conflict of interest.

Introduction

Tracheobronchitis is one of the most common ventilator-associated complications. It is characterized by manifestations of respiratory infection without radiographic infiltrates in patients receiving prolonged mechanical ventilation for at least 48 h [1–4].

In the last decade, several epidemiological studies have shown that ventilator-associated tracheobronchitis (VAT) is a precursor of ventilator-associated pneumonia (VAP). VAT has an indirect effect on mortality, but once it develops, patient care costs increase in terms of ICU length of stay, antibiotic use, and duration of mechanical ventilation [5–8].

To date, many observational studies have shown an association between inadequate or no treatment of VAT and the subsequent development of VAP, but there are no randomized controlled trials demonstrating the benefits of VAT treatment [1, 7, 9, 10].

Meanwhile, the use of combined multi-zone decontamination of the upper airway, including the subglottic region, is known to reduce the risk of VAP development but does not affect the overall

incidence of various ventilator-associated infectious events [11].

The pathophysiological aspects and distinctive features of VAT as well as typical tracheobronchial morphogenetic patterns have been actively investigated worldwide [4, 5, 12–15].

The multicenter observational clinical study «Registry of Respiratory Therapy in Patients with Acute Cerebrovascular Accident (ACVA) (RETAS)», conducted under the auspices of the Russian Federation of Anesthesiologists and Reanimatologists, showed that in patients with acute cerebrovascular accident, the development of VAT and VAP is associated with increased duration of ventilatory support, delayed weaning time, prolonged stay in the intensive care unit, and poor outcomes. *Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* are the most common causes of VAP in ACVA [16].

Studies conducted during the COVID-19 pandemic showed that ventilated patients with severe and critical COVID-19 were more likely to develop nosocomial infections, which negatively affected the outcome of the disease. In more than half of the cases, the infection was caused by resistant strains of gram-negative bacilli [17].

A comprehensive analysis of clinical data may help to understand the pathophysiological aspects of VAT development and contribute to the management of this complication.

The aim of this study was to identify risk factors for the development and severity of VAT in patients receiving prolonged mechanical ventilation.

Materials and methods

The characteristics and frequency of VAT were retrospectively analyzed according to the clinical reports of the Department of Surgical Intensive Care of the V. Vakhidov Republican Scientific and Practical Medical Center of Surgery from 2018 to 2022. The study included cases of VAT in patients who were on a ventilator for more than 48 h and met the diagnostic criteria for VAT.

The diagnosis of VAT was based on the following clinical, radiological and microbiological criteria:

— body temperature >38°C, leukocyte count >12000/μL or leukopenia (leukocyte count <4000/μL) combined with purulent endotracheal discharge or change in sputum character;

— absence of new or progressive infiltrates;

— detection of polymorphonuclear lymphocytes with or without bacteria on Gram staining of endotracheal aspirate and moderate or intense growth of potentially pathogenic microorganisms according to the semiquantitative analysis of endotracheal aspirate culture.

Patients were excluded from the study if they had

— severe immunosuppression (leukocyte count <1000/μL or neutrophil count <50/μL);

— VAP without preliminary criteria for VAT.

The morphology of the tracheal and bronchial mucosa was examined using a stationary video bronchoscope with subsequent digital processing and archiving of the data obtained. Video-assisted tracheobronchoscopy allowed to minimize the frequency of individual decision making, expand the possibilities of team visualization, and assess the severity of disease and the impact of bronchoscopic treatment on the efficacy of VAT treatment.

Treatment of VAT included intravenous and/or inhaled antimicrobials, bronchodilators, and broncho- and mucolytics. The inhaled route of administration was reserved for aminoglycosides and polymyxin.

VAT prophylaxis included the use of bacterial filters and humidification of respiratory gas, selective decontamination of the digestive tract (administration of antibacterial drugs into the naso-intestinal tube), regulation of pressure in the tracheal cuff and oral hygiene.

The collection, correction, and arrangement of the raw data and the obtained results were performed in Microsoft Office Excel 2016 spreadsheets. Statistical analysis was done with the STATISTICA 13.3 (StatSoft.Inc) software. Means of normally distributed quantitative data were compared using Student's *t*-test. Differences were considered significant at a significance level of $P<0.05$. Non-numerical data were compared using Pearson's χ^2 test. The odds ratio (OR) was used as the measure of effect when comparing relative parameters. The limits of the 95% confidence interval (95% CI) were calculated to extrapolate the OR values to the general population.

Results

A total of 8170 patients underwent mechanical ventilation during the study period (2018–2022), of which 724 patients required prolonged ventilation (more than 48 h), with VAT diagnosed in 16.9% (122 of 724) of cases (Table 1).

The incidence of VAT (Fig. 1) decreased significantly over time from 24.7% (38 of 154) to 10.1%

Table 1. Number of patients who underwent ventilation and VAT frequency during the study period from 2018 to 2022.

Study period, year	Number of patients			VAT frequency, %
	Mechanically ventilated	Mechanically ventilated for >48 h	With VAT	
2018	1814	154	38	24.7
2019	1752	158	30	19.0
2020	1202	118	19	16.1
2021	1722	156	21	13.5
2022	1680	138	14	10.1
2018–2022	8170	724	122	16.9

(14 of 138) over 4 years, while the incidence of ventilator use in ICU patients remained unchanged (χ^2=9.52; *P*=0.003).

The frequency of the most severe VAT with hemorrhagic, catarrhal and purulent morphology was assessed. This parameter also gradually decreased significantly from 44.7% (17 cases out of 38 of all VAT in 2018) to 14.3% (2 cases out of 14 of all VAT in 2022) (χ^2=4.53; *P*=0.034) (Fig. 2).

Severe VAT cases were identified based on bronchoscopy, clinical, laboratory, and microbiologic data. Bronchoscopic findings in early tracheobronchitis included tracheobronchial mucosal erosions and moderate amounts of mucopurulent sputum. As the inflammation progressed, erosive and hemorrhagic phenomena such as confluent hemorrhagic erosions of the tracheal wall, thrombi in the bronchial mucosa and hemorrhagic sputum were observed.

The major causative agents of VAT are listed in Table 2.

Gram-negative bacteria predominated among the causative agents of severe tracheobronchitis: *Acinetobacter* spp. was isolated in 24% of cases, *Klebsiella pneumoniae* in 11.6%, *Pseudomonas aeruginosa* in 13.0%, *Escherichia coli* in 10.6%, while *Staphylococcus aureus* was identified in 5.3%, *Enterococcus* spp. in 2.2%, and *Candida* spp. in 17.0%.

All strains isolated from the trachea were highly resistant to almost all groups of antibiotics except polymyxin, vancomycin and linezolid, and in some cases imipenem, meropenem and amikacin. *Candida* spp. were highly resistant to antifungal agents in 90% of cases.

During all periods of the study, the duration of mechanical ventilation was defined as the total time spent with intubation and tracheostomy tube (if the latter was used).

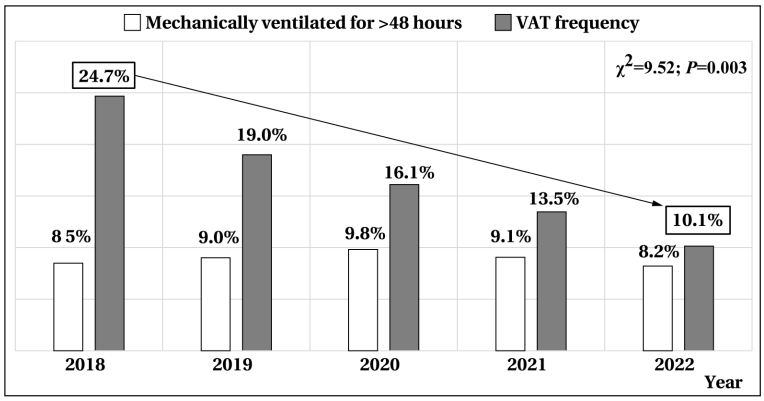

Fig. 1. Frequency of VAT during different periods of the study.

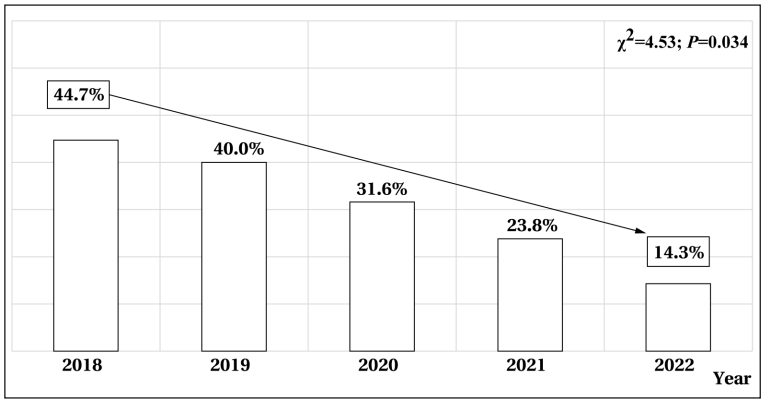

Fig. 2. Frequency of hemorrhagic catarrhal purulent VAT cases during the study period.

Table 2. Microorganisms isolated during 5 years.

Agent	Number of identifications per year				
	2018	2019	2020	2021	2022
Streptococcus spp.	1				
Enterococcus spp.		2		1	2
Staphylococcus aureus	1	2	1		1
Staphylococcus spp.	2	1		1	1
Acinetobacter spp.	10	6	11	4	8
Alcaligenes spp.		1			
Enterobacter cloacae			1		1
Esherichia coli	2	2	2	3	
Klebsiella pneumoniae		1	7		1
P. aeruginosa	1	2	2	2	3
Proteus mirabilis					
Proteus vulgaris					
Serratia marcesens	2			1	
Candida spp.					
Candida albicans	4	1	5	2	
Candida glabrata	2	1		2	
Candida kruseii	1			1	
Candida tropicalis					
Molds					
Total	24	23	29	17	17

The duration of mechanical ventilation in VAT patients gradually decreased from 202.1±6.15 h to 125.3±7.81 h between 2018 and 2022 (t=7.73; P<0.0001) (Fig. 3).

The minimum duration of mechanical ventilation during all 4 years of follow-up was 96 h and the maximum was 368 h.

As expected, the length of stay of patients with VAT in the ICU also decreased during this period from 9.7±0.25 days to 6.6±0.3 days (t=7.94; P<0.0001) (Fig. 4).

Clinical and demographic characteristics (Table 3) of patients receiving mechanical ventilation for more than 48 h (N=724) were analyzed. Patients with VAT (N=122) had a higher incidence of comorbid COPD compared to patients without VAT (N=602), 22.9% (28 of 122) vs. 10.6% (64 of 602) (P<0.001).

In addition, patients with VAT were found to have a higher mean SAPS II score (P<0.001), duration of ventilatory support (P<0.001), frequency of tracheostomy (P<0.001), and duration of ICU stay (P<0.001).

Several predictors of severe VAT were identified, including age greater than 60 years, male sex, severe comorbidities at baseline, and SAPS II score greater than 40 points (Fig. 5).

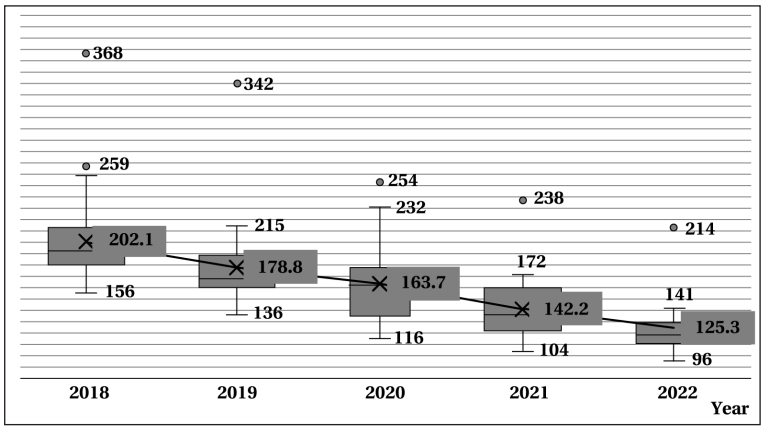

Fig. 3. Duration of mechanical ventilation in VAT, h.

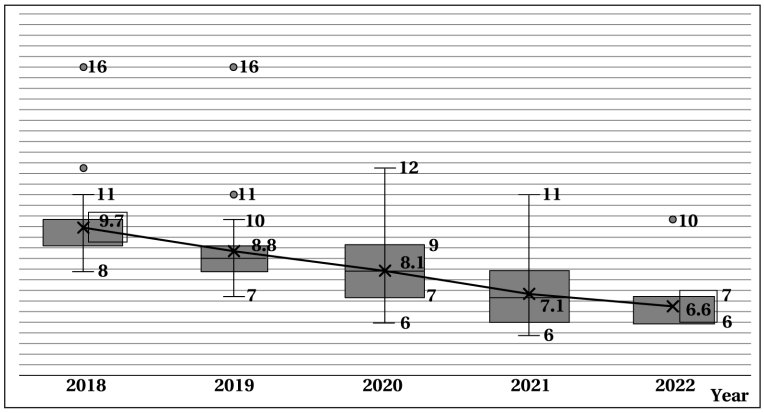

Fig. 4. Length of stay of patients with VAT in ICU, days.

Cardiac and vascular surgical procedures, which often require prolonged mechanical ventilation, and the presence of associated chronic lung disease did not significantly affect the development and progression of VAT.

The duration of mechanical ventilation over 144 h had a significant effect on the incidence of severe VAT.

The data obtained during the analysis of risk factors for severe VAT (Table 4) showed that such predictors as age over 60 years (OR=2.28; 95% CI 1.0–4.9), SAPS II over 40 points (OR=5.9; 95% CI 2.6–13.8), duration of ventilation more than 144 h (OR=5.4; 95% CI 1.8–16.7) and malignant surgical condition (OR=2.83; 95% CI 1.2–6.9) showed the most significant correlation between the factor and the outcome.

Discussion

In everyday clinical practice, hospital-acquired lower respiratory tract infections are commonly di-

Table 3. Clinical and demographic characteristics of patients with or without VAT who received ventilation for more than 48 h.

Parameters, units	Values		*P*
	Patients with VAT, *N*=122	Patients without VAT, *N*=602	
Mean age, *M*±*m* (range), years	52.4±3.3 (18–81)	54.8±3.6 (22–74)	0.623
Male sex, *n* (%)	76 (62.3)	398 (66.1)	0.482
Cardiovascular surgical conditions, *n* (%)	54 (44.3)	277 (46.0)	0.724
Pulmonary surgical conditions, *n* (%)	12 (9.8)	56 (9.3)	0.854
COPD, *n* (%)	28 (22.9)	64 (10.6)	<0.001
Malignancy, *n* (%)	26 (21.3)	107 (17.8)	0.429
Mean SAPS II score, *M*±*m* (range)	38.9±1.6 (11–81)	24.4±1.2 (11–56)	<0.001
Duration of ventilation, *M*±*m* (range), h	171.3±5.6 (96–368)	102.7±8.5 (56–172)	<0.001
Tracheostomy, *n* (%)	52 (42.6)	64 (10.6)	<0.001
Duration of ICU stay, *M*±*m* (range), day	8.4±0.4 (5–16)	4.7±0.3 (3–14)	<0.001

Table 4. Univariate factor analysis of the risk of severe VAT.

Parameter, *N* (%)	VAT severity		OR	95% CI
	Severe, *N*=42	Mild and moderate, *N*=80		
Age over 60 years	22 (52.4)	26 (32.5)	2.28	1.0–4.9
Malignancy	14 (33.3)	12 (15.0)	2.83	1.2–6.9
SAPS II more than 40 points	32 (76.2)	28 (35.0)	5.9	2.6–13.8
Ventilation time greater than 144 h	38 (90.5)	51 (63.8)	5.4	1.8–16.7

vided into intra-ICU and extra-ICU, often due to a conflict of interest in diagnosis. However, clinical studies in patients with such complications outside the ICU are limited due to bias in diagnostic approaches and limitations in microbiological identification [18, 19].

According to the literature, the incidence of VAT is estimated to be approximately 11.5%. The most common pathogens are *Pseudomonas aeruginosa, Acinetobacter* spp. and methicillin-resistant *Staphylococcus aureus*, although the infection may be polymicrobial [20].

Antimicrobial therapy in patients with VAT may not improve mortality, ICU length of stay, or duration of mechanical ventilation, but is usually associated with a reduction in the incidence of subsequent VAP [20].

Most clinicians believe that antibiotic therapy should be targeted and based on both combination and de-escalation approaches, as well as microbiological antibiotic susceptibility testing of the isolated agent. The results suggest that the prevalence of Gram-negative multidrug-resistant microflora among the pathogens and a high risk of fungal superinfection should be considered in the intensive care of patients with VAT.

The mean incidence of VAT over the 5-year study period was 16.9%, but decreased over time from 24.7% to 10.1%, despite an increase in high-tech major surgery, which often requires prolonged mechanical ventilation.

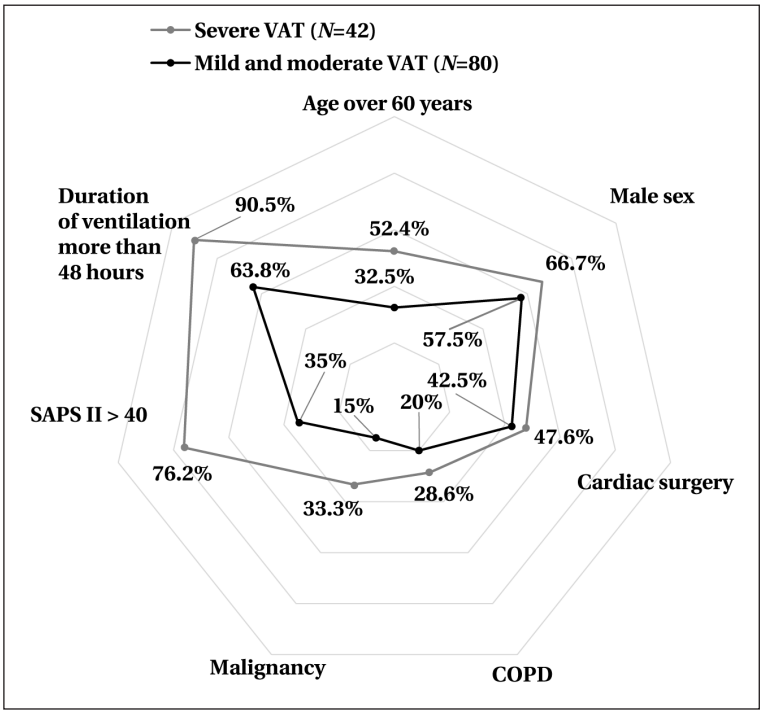

Fig. 5. Comparison of the frequency of identified risk factors for severe VAT.

Conclusion

Decrease in incidence of VAT, reduction in duration of mechanical ventilation and intensive care unit stay suggest adequate prevention and treatment of VAT during the study period. The identified factors associated with the development of VAT and predictors of severe VAT may provide a rationale for the identification of risk groups.

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Overtime Histological Changes in the Lungs after Intoxication with Baclofen Alone or in Combination with Ethanol (Experimental Study)

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Summary

The aim of the study was to evaluate the overtime histological changes in the lungs after intoxication with baclofen alone or in combination with ethanol.

Materials and methods. The experiment was carried out on 35 male Wistar rats weighing 290–350 g and aged 20 weeks. The animals were split into 7 equal groups (n=5); test drugs were administered via nasogastric tube: rats from Groups 1, 3 and 5 were treated with baclofen at 85 mg/kg; rats from Groups 2, 4 and 6 received similar dose of baclofen and 40% alcohol by volume at a dose of 7 ml/kg; control group rats were not administered with any drugs. Animals of all groups were removed from the experiment by cervical dislocation under anesthesia (chlorolase) after 3 h (Groups 1, 2), 4.5 h (Groups 3, 4) and after 24 h (Groups 5, 6, and the controls). Lung tissue samples were examined by light microscopy. The nonparametric Kraskel–Wallis test was used for multiple comparisons between the groups, and nonparametric Mann–Whitney test with Bonferroni correction was used for pairwise comparison.

Results. Light microscopy showed no pathological changes in the lungs of the Control group animals. Baclofen alone, or in combination with ethanol caused significant circulatory disorders (venular and capillary fullness, hemorrhages in the interalveolar septa (IAS) and alveoli, sludge phenomenon), emphysema, atelectasis and distelectasis, and pulmonary edema. IAS thickness in rats from all experimental groups was different from that in animals from the Control group, all differences confirmed by the Kruskel–Wallis test: H=748, P=0.00001.

In Group 1 animals IAS was 44.2% thinner (P=0.00052) vs the control Group, while in all remaining experimental groups it was, on the contrary, thicker: in Group 2 — 57.6% increase in thickness (P=0.000038), in Group 3 — 99 % (P=0.00001), in Group 4 — 2.2-fold increase (P=0.00001), in Group 5 — 2.1-fold (P=0.00001), in Group 6 — 2.5-fold increase (P=0.00001). Most significant increase in IAS thickness (6-fold, P=0.00001) occurred within the period from 3 to 4.5 h after administration of baclofen, while within the period from 4.5 to 24 h no statistically significant increase occurred (P=0.99). Co-administration of baclofen and ethanol caused 2.8-fold (P=0.00001) increase in IAS thickness after 3 h as compared to the effects of baclofen only. IAS thickness at 4.5 h after baclofen and ethanol co-administration increased by additional 41.8% as compared to thickness at 3 h (P=0.00001). IAS became 11.8% thicker at 24 h vs 4.5 h (P=0.87). At 24 h IAS was 21.7% (P=0.0011) thicker after baclofen and ethanol co-administration vs baclofen alone. The alveoli size increased by 69.4% (P=0.00001) in Group 1 animals vs the Control group, by 14.3% (P=0.43) — in Group 2, by 55% (P=0.00004) — in Group 3, by 26.3% (P=0.002) — in Group 4, by 45% (P=0.0003) — in Group 5 (baclofen, 24 h), by 43.3% (P=0.0004) — in Group 6 (baclofen and ethanol, 24 h). Co-administration of baclofen and ethanol initially caused a slight increase in alveoli size, bur 3 h later there was a visible shrinkage in the diameter of alveoli by 32.5% (P=0.003) vs baclofen mono, 4.5 h later — by 18.5% (P=0.062), and 24 h later — by 1.2% (P=0.99), that is, the differences were leveled.

Conclusion. The combined effects of baclofen and ethanol induce more severe alterations in pulmonary tissue compared to baclofen alone. The pathological changes in the lungs reached their maximum by 24 h, which confirmed by morphometric assessment. Morphological changes in pulmonary tissue alongside with established chemical properties of the two agents can be used to diagnose cases of intoxication either with baclofen alone or in combination with ethanol.

Keywords: baclofen; ethanol; lungs; histological changes; morphological changes; intoxication

Conflict of interest. The authors declare no conflict of interest.

Introduction

Poisoning is one of the leading causes of violent death nowadays [1–3]. The muscle relaxant baclofen is a common cause of poisoning [4–6]. Baclofen, unlike other substances in this class, is a β-p-chlorophenyl derivative of GABA (gamma-aminobutyric acid) [7–9]. Baclofen is a prescription medication available in both oral and intrathecal forms [7, 8].

Oral baclofen is indicated for the treatment of severe muscle spasticity, multiple sclerosis, tumors, trauma, spinal cord infections, acute cerebrovascular accidents, and meningitis. The efficacy of baclofen in patients with alcoholism [10–14] and drug addiction [14] has been studied, and several studies have shown its benefit in cerebral palsy [15, 16].

Baclofen has significant psychoactive effects [17–20]. For this reason, it is widely used by drug addicts, especially young people [21]. To achieve a narcotic effect, baclofen doses are increased many times over, up to 6–14 tablets. The drug is often combined with low-alcohol drinks. In this case, narcotic intoxication occurs in about half an hour. The main symptoms are nausea and vomiting, dizziness, impaired motor coordination, drowsiness, slurred speech [17–20].

Significant overdose with baclofen can lead to acute toxicity and death [18, 20, 22]. There is no specific antidote for poisoning with this drug [23].

In all suspected cases of baclofen poisoning, differential diagnosis with other poisonings is necessary for the most effective rehabilitation. A comprehensive understanding of the pathophysiology of the different stages of baclofen poisoning could help to provide timely help to this category of patients. In the case of fatal baclofen poisoning, a toxicologic investigation is necessary to determine the immediate cause of death [18, 22].

According to the literature, the lung is one of the target organs in baclofen poisoning [24]. The combined effect of baclofen and ethanol on the lung has been poorly studied.

The aim of the study was to evaluate the histologic changes in the lungs during baclofen or baclofen-ethanol poisoning.

Materials and methods

Thirty-five adult (20 weeks old) male Wistar rats weighing 290–350 g were included in the experiment. The animals were divided into 7 groups (5 rats in each group).

The experiments were conducted in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes [25].

Baclofen or its combination with ethanol was administered to animals under general anesthesia (chloralose) via a gastric tube.

Animals were divided into the following groups:

- Control (n=5) —animals receiving neither baclofen nor ethanol;
- Group 1 (n=5) — animals receiving baclofen 85 mg/kg, duration of experiment 3 h;
- Group 2 (n=5) — animals receiving a combination of baclofen 85 mg/kg and 40% ethanol 7 ml/kg, duration of experiment 3 h;
- Group 3 (n=5) — animals receiving baclofen 85 mg/kg, duration of experiment 4.5 h;
- Group 4 (n=5) — animals receiving a combination of baclofen 85 mg/kg and 40% ethanol 7 ml/kg, duration of experiment 4.5 h;
- Group 5 (n=5) — animals receiving baclofen 85 mg/kg, duration of experiment 24 h;
- Group 6 (n=5) — animals receiving a combination of baclofen 85 mg/kg and 40% ethanol 7 ml/kg, duration of experiment 24 h.

After drug administration, animals were awakened from anesthesia and left in the animal facility with free access to water but without food. After 3, 4, 5 and 24 h, animals were euthanized by cervical dislocation under anesthesia (chloralose). The thoracic cavity was opened and the lungs were removed and placed in 10% neutral formalin and embedded in paraffin. Lung sections, 5 μm thick, were mounted on slides and stained with hematoxylin and eosin using standard techniques. Histologic preparations were examined at ×400 magnification. A Nikon E-400 microscope with a video system based on a Watec 221S camera was used. Signs of impaired circulation (arterial, venous and capillary hemorrhage, sludge phenomenon, interalveolar septal hemorrhage, alveoli), atelectasis, dystelectasis and emphysema, fluid in bronchiolar lumen, epithelial desquamation in bronchiolar lumen, thickening of interalveolar septa due to edema were evaluated. Fisher's criterion was used to evaluate the statistical significance of histologic signs. A histologic feature was considered significant if it was observed in 4 or 5 cases in one group and in none in the other. Further morphometric examination of the specimens was performed using ImageScope 12.0. Alveolar diameter and interalveolar septa thickness were measured. We performed 30 measurements in each animal, so the sample contained 150 measurements in each group. The Shapiro–Wilk test showed a non-normal distribution of the obtained data, so the data were presented as median, lower and upper quartiles *[Me (QL;QH)]*. The nonparametric Kruskal–Wallis test was used for multiple comparisons between groups, and the nonparametric Mann–Whitney test with Bonferroni correction was used for pairwise comparisons. The number of pairs of comparisons was 13, and the critical significance level was 0.0038. Microsoft Excel and Statistica 12.0 software were used for statistical analysis of the data [26, 27].

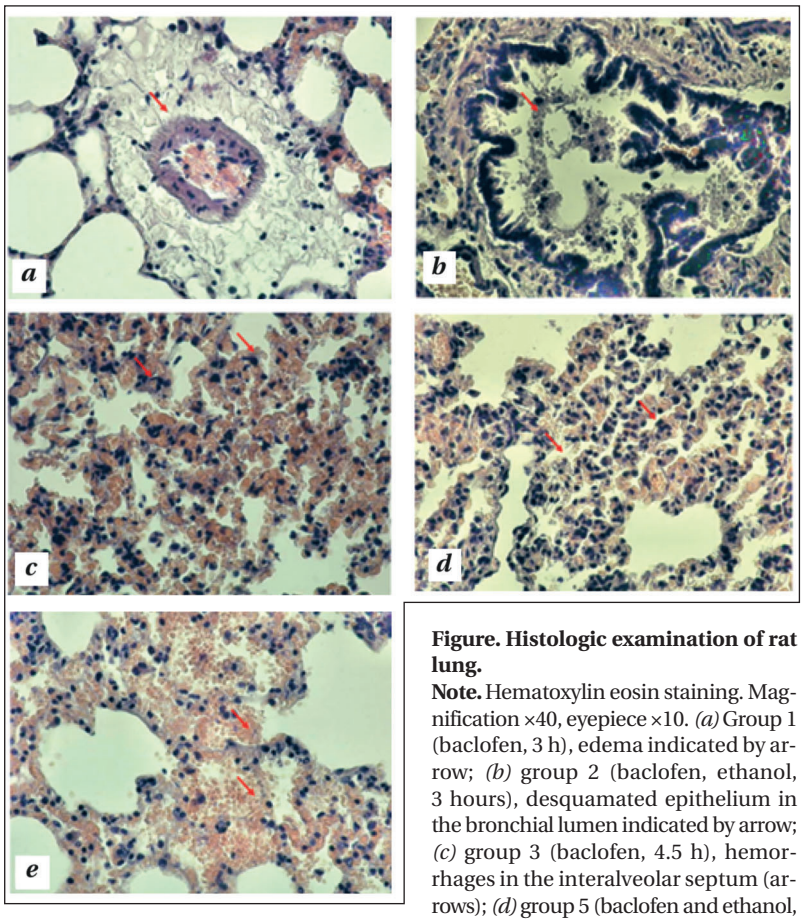

Figure. Histologic examination of rat lung.
Note. Hematoxylin eosin staining. Magnification ×40, eyepiece ×10. *(a)* Group 1 (baclofen, 3 h), edema indicated by arrow; *(b)* group 2 (baclofen, ethanol, 3 hours), desquamated epithelium in the bronchial lumen indicated by arrow; *(c)* group 3 (baclofen, 4.5 h), hemorrhages in the interalveolar septum (arrows); *(d)* group 5 (baclofen and ethanol, 4.5 hours), dystelectasis (arrows); *(e)* group 6 (baclofen, ethanol, 24 h), alveolar hemorrhages (arrows).

Results

No pathologic changes were observed in the lungs of rats of the control group.

The data obtained during the study of lungs of animals of group 1 confirm the results of our previous experiments [6]. Blood circulation disorders (venous and capillary hemorrhage), emphysema, atelectasis and dystelectasis, cellular response, thickening of interalveolar septa due to edema were noted in the lungs of animals of group 1 (Figure, *a*).

The data obtained when examining the lungs of group 2 animals also confirm the results of our previous experiments [6]. In addition to the histological changes described above for group 1, the presence of secretion and epithelial desquamation in the lumen of bronchioles was observed in the bronchi of animals in this group (Figure, *b*).

In group 3, characteristic features included arterial, venous and capillary congestion, sludge and hemorrhage in the IAS, which was not observed in the control group, neither in group 1 nor in group 2 (Figure, *c*). In addition, atelectasis, dystelectasis, IAS thickening due to edema, and emphysematous areas with thin IAS were observed in this group.

Blood circulation disturbances (venous, capillary, arterial hemorrhages, sludge, hemorrhages in IAS) were observed 4.5 h after administration of the combination of baclofen and ethanol. We also observed atelectasis and dystelectasis (Figure, *d*), emphysema (in the areas of thin IAS), fluid and desquamation of epithelium in bronchioles. All of these histologic changes were significant. Isolated hemorrhages appeared in the alveoli, which was not observed in group 1 or group 2.

In group 5 we observed venous and arterial congestion, sludge, hemorrhage in IAS. Atelectasis and dystelectasis, IAS thickening due to edema and emphysema were found. All of the above signs were significant. Isolated alveolar hemorrhage was also observed.

In group 6, the characteristic histological feature included venous, capillary and arterial hemorrhage, hemorrhage in IAS and in alveoli (Figure, *e*). Emphysema developed in the lungs of animals in this group. In addition, thickening of the IAS due to edema was observed. Fluid in the bronchioles and desquamation of the epithelium into the bronchial lumen were observed. Fluid in the bronchial lumen and epithelial desquamation in the bronchial lumen were observed only in the groups receiving both baclofen and ethanol (groups 2, 4, 6), but not in the groups receiving baclofen alone (groups 1, 3, 5).

The results of the morphometric study of the lungs after administration of baclofen and its combination with ethanol are shown in the Table.

Table. Interalveolar septum (IAS) thickness and alveolar diameter after administration of baclofen and its combination with ethanol, *Me* (*LQ; HQ*).

Group	Values	
	Thickness of IAS, μm	Alveolar diameter, μm
Control	7.7 (6.2; 9.3)	41.5 (35.2; 51.6)
1	4.3 (3.8; 5.1)[c]	70.2 (54.0; 86.3)[c]
2	12.2 (10.5; 13.9)[c,1]	47.4 (37.6; 56.3)[1]
3	15.4 (13.6; 17.9)[c,2]	64.3 (55.6; 75.0)[c,2]
4	17.3 (14.9; 19.9)[c,2,3]	52.4 (45.2; 60.2)[c]
5	15.9 (13.9; 18.4)[c,2,3]	60.1 (52.1; 70.6)[c]
6	19.4 (15.3; 22.8)[c,2,3,4]	59.4 (50.1; 69.3)[c]

Note. Differences are significant versus: [c] — control; [1] — group 1; [2] — group 2; [3] — group 3; [4] — group 4 at $P<0.0038$ (Mann–Whitney test).

The table shows that the IAS thickness in all experimental groups was different from the control. The Kruskal–Wallis test confirmed the existence of differences with H=748, P=0.00001. Meanwhile, IAS thickness in group 1 was 44.2% (P=0.00052) lower than in the control group, whereas in the other

groups it was higher: in group 2 by 57.6% (P=0.000038), in group 3 by 99% (P=0.00001), in group 4 by 2.2 times (P=0.00001), in group 5 by 2.1 times (P=0.00001), in group 6 by 2.5 times (P=0.00001). From 3 to 4.5 h after baclofen administration, a 3.6-fold increase in IAS thickness was observed (P=0.00001). No significant differences in IAS thickness were observed at 4.5 and 24 h (P=0.99). At 3 h after co-administration of baclofen and ethanol, a 2.8-fold (P=0.00001) increase in IAS thickness was observed compared to baclofen alone. IAS thickness 4.5 h after baclofen and ethanol administration was 41.8% higher than at 3 h (P=0.00001), and 24 h later it was 11.8% higher than at 4.5 h (P=0.87). Twenty-four h after administration of baclofen with ethanol, IAS thickness was 21.7% greater (P=0.0011) than after administration of baclofen alone.

Alveolar diameter was 69.4% greater (P=0.00001) in group 1, 14.3% greater (P=0.43) in group 2, 55% greater (P=0.00004) in group 3, 26.3% greater (P=0.002) in group 4, 45% greater (P=0.0003) in group 5 (baclofen, 24 h), and 43.3% greater (P=0.0004) in group 6 (baclofen and ethanol, 24 h) compared to the control group. A slight increase in alveolar diameter was observed after co-administration of baclofen and ethanol. At 3 h after co-administration of baclofen and ethanol, the alveolar diameter was 32.5% (P=0.003) smaller than after administration of baclofen alone, at 4.5 h by 18.5% (P=0.062), at 24 h by 1.2% (P=0.99), i.e., the differences disappeared.

Discussion

Baclofen administration decreases the tone of skeletal muscles, including the intercostal muscles. Excessive relaxation of these muscles leads to respiratory compromise and subsequent hypoxia [4, 7]. Baclofen is known to be a selective inhibitor of $GABA_B$ receptors, but at high enough doses it can cause stimulation of $GABA_A$ receptors, resulting in contraction of smooth muscle of bronchi and bronchioles with subsequent spasm and respiratory distress. In addition, stimulation of these receptors has been shown to increase vascular and tissue permeability [28]. In animal studies, we found that rats receiving baclofen alone (groups 3, 5) and a combination of baclofen and ethanol (groups 2, 4, 6) had significantly greater IAS thickness than the control group (except group 1), possibly due to stimulation of $GABA_A$ receptors by a subtoxic dose of baclofen and the development of hypoxia [29–30].

Thus, in all groups receiving a combination of baclofen and ethanol (2, 4, 6), IAS thickness was significantly greater than in the groups receiving baclofen alone. This confirms the hypothesis of a combined negative effect of baclofen and ethanol on the architecture of the air-blood barrier. As a result, the likelihood of impaired oxygen diffusion and more severe hypoxia increases.

Conclusion

After administration of the myorelaxant baclofen alone or in combination with ethanol, circulatory disturbances (venous and capillary hemorrhage, IAS and alveolar hemorrhage, sludge), emphysema, atelectasis and dystelectasis develop. With increasing hypoxia, vascular and tissue permeability increases and edema develops. The combined effect of baclofen and ethanol causes more severe changes in the lungs (epithelial desquamation and fluid in the bronchial lumen were observed only in the groups of animals receiving a combination of baclofen and ethanol). The pathological changes in the lungs showed progression, reaching the maximum severity at 24 h, which was confirmed by the results of the morphometric study. Data on morphological changes in the lungs can be extrapolated to the forensic material and later, together with the results of chemical tests, can be used to diagnose intoxication by baclofen and its combination with ethanol, as well as to determine the mode of drug administration (alone or in combination with ethanol).

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Destabilization of the Organized Structure of Ventricular Fibrillation During Reperfusion

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Summary

Aim: to study the effect of reperfusion on the organized frequency-amplitude structure of ventricular fibrillation (VF) in the dog heart.

Materials and methods. We conducted 4 experiments on 8 dogs. In each experiment, the isolated heart of one dog was perfused with the blood of the second (supporting) dog. In 4 experiments on an isolated artificially perfused heart, 6 episodes of 3 min ischemia and 10 min reperfusion of the heart were performed in VF (1–2 episodes of ischemia-reperfusion in one experiment). Each episode of 3 min ischemia in VF was preceded by a 10 min perfusion of the heart in VF. Ventricular electrogram was recorded during VF episodes. A frequency-amplitude (spectral) analysis of 1 sec segments of the electrogram was performed, and the proportion (in %) of 0.5–15 Hz frequency oscillations in 10 sec segments of the electrogram was determined in 6 episodes of perfusion, ischemia and reperfusion in VF ($M \pm m$, N=60). The VF frequency-amplitude structures during ischemia and reperfusion were compared with the stable VF frequency-amplitude structure during perfusion taken as the control. The nonparametric Welch criterion in the «The R Project for Statistical Computing» software environment was used to compare the VF parameters during perfusion, ischemia and reperfusion.

Results. 9–10 Hz frequency oscillations dominated in the VF frequency-amplitude structure during heart perfusion, taken as the control. In the first 30 sec of ischemia, the frequency and amplitude of the dominant oscillations did not significantly change vs VF control obtained during cardiac perfusion. A decrease of dominant oscillations frequency up to 6.5–7.5 Hz, and of the proportion of oscillations — up to 26% was documented at the 3rd min of ischemia. At the 1st min of reperfusion, the frequency of dominant oscillations increased to 13.5–14.5 Hz, but the proportion of oscillations remained reduced to 26%, as at the 3rd min of ischemia. At the 2nd min of reperfusion, the frequency of dominant oscillations decreased to 9.5–10.5 Hz, and the proportion of dominant oscillations increased to 33%. The frequency and amplitude of the dominant oscillations stabilized at 3–10 min of reperfusion: oscillations at 9–10 Hz frequency accounted for 32–33% of the spectral power.

Conclusion. Reperfusion in VF is characterized by transient destabilization of VF organized structure at the 1st min of the procedure. VF organized structure regains stabilization within 2–10 min of reperfusion. Cardiac perfusion in intentionally induced VF can be used instead of cardioplegia during major cardiac surgery to boost cardiac resistance to ischemia and prevent or reduce reperfusion complications.

Keywords: ventricular fibrillation; cardiac perfusion; cardiac ischemia; cardiac reperfusion; organized frequency-amplitude structure of fibrillation

Conflict of interest. The authors declare no conflict of interest.

Introduction

Ventricular fibrillation (VF) is a fatal cardiac arrhythmia and the most frequent cause of sudden cardiac death, which is the leading cause of mortality in many countries, including Russia [1, 2], and therefore remains an urgent problem. VF is considered to be a turbulent process [3, 4], in which, however, organized activity has been revealed both in mapping, reflecting local activity in VF [5, 6], and in electrocardiogram analysis, reflecting global VF activity [7].

We have shown that VF is characterized by stable organized activity during perfusion of the canine heart, which is confirmed by the dominance of 9–10 Hz oscillations, which are only 1/10 of the frequency range of 0.5–15 Hz, but contain more than 1/3 of the spectral power [8]. The frequency and amplitude parameters of the canine VF are applicable to humans because the frequency of the canine VF is close to that of the human VF [9].

In VF under cardiac ischemia, organized VF activity decreases along with the chances of successful defibrillation [5–7]. Therefore, restoration of myocardial perfusion is a prerequisite for restoration of cardiac rhythm during defibrillation. Organized VF activity, reduced by ischemia, should be restored during cardiac reperfusion, which would increase the chances of successful defibrillation.

However, myocardial reperfusion is associated with complications, including arrhythmias [10]. Therefore, VF may also be associated with reperfusion complications, including destabilization of the organized frequency and amplitude structure of VF.

Although VF and reperfusion are relevant issues, few studies have been devoted to the investigation of VF during reperfusion. In an isolated perfused heart from patients with cardiomyopathy who underwent donor heart transplantation, the dominant VF frequency decreased from 4.9 to 3.6 Hz during 200 sec of ischemia and increased to 4.7 Hz during 120 sec of reperfusion [11]. However, the functional activity of a nonviable (donor-replaced) heart cannot reflect the activity of a viable heart.

In VF induced during cardiac surgery, the dominant VF frequency increased from 5.3 to 6.4 Hz during 30 sec of perfusion, decreased to 4.7 Hz during 150 sec of ischemia, and increased to 7.1 Hz during 30 sec of reperfusion [12]. The reperfusion interval was reported to be shorter than the ischemia interval [11, 12], which we believe is insufficient to restore the organized frequency and amplitude structure of the VF during reperfusion. The dominant frequency of VF during perfusion used to compare changes in VF during ischemia and reperfusion was not specified[11], whereas in another study the dominant frequency of VF during the 30 sec perfusion before ischemia was variable during VF[12]. In addition, it is unclear how to characterize the frequency and amplitude structure of VF using a single, albeit dominant, VF frequency.

Thus, reperfusion complications of VF, including destabilization of the organized frequency and amplitude structure of VF during reperfusion, have not been clarified [11, 12]. Destabilization of the organized structure of VF during reperfusion may reduce the chances of successful defibrillation.

The aim of this study was to investigate the effect of reperfusion on the organized frequency and amplitude structure of VF in the canine heart.

Materials and methods

We performed 4 experiments on 8 mongrel dogs of both sexes weighing 20–30 kg according to the recommendations of the International Committee for Laboratory Animals, supported by the WHO, European Parliament Directive No. 2010/63/EU of 22.09.2010 «On the protection of animals used for scientific purposes».

Premedication was performed with subcutaneous atropine sulfate 0.1 mg/kg, followed 10 min later by intramuscular Zoletil® (Zolazepam hydrochloride 20–30 mg/kg (VIRBAC S.A., France). Five to 10 min after Zoletil® administration, the dog was placed on the operating table. Under intravenous thiopental anesthesia (10–15 mg/kg initial dose and 4–7 mg/kg hourly), mechanical ventilation was started and the heart was isolated from the thorax. The aorta was cannulated and the coronary arteries were perfused with the cardioplegic solution Custodiol® (Dr. F. Köhler Chemie GmbH, Germany). Supportive cardiac perfusion was then started with blood from another dog ventilated under thiopental anesthesia. The interval between cardioplegia and initiation of supportive perfusion did not exceed 10 min. Ischemia is known to be reversible without damage to cardiac structure and function when perfusion is initiated within 10 min of cardioplegia [13]. Arterial blood was supplied to the aorta of the isolated heart from the femoral artery of the supporting dog. The perfusion pressure in the aorta was 90–100 mmHg, resulting in aortic valve closure and retrograde perfusion of the coronary arteries with blood from the supporting dog. Venous blood from the atria of the isolated heart was returned to the femoral vein of the supporting dog. Heparin (500 IU/kg initially and 150 IU/kg hourly) was administered to prevent thrombosis. The heart was maintained in an enclosed chamber at 37°C.

In 4 experiments, 6 episodes of 3 min ischemia and 10 min reperfusion of the heart during VF were performed on an isolated perfused heart (1–2 episodes of ischemia-reperfusion per experiment). Each episode of 3 min of ischemia in VF was preceded by 10 min of cardiac perfusion in VF.

A clamp was placed on the aortic tube feeding the isolated heart to obtain ischemia during VF, and removal of the clamp resulted in reperfusion during VF.

Ventricular electrograms during perfusion, ischemia, and reperfusion of the heart during VF were recorded with bipolar electrodes in the right and left ventricles on a Cardiotechnica-EKG-8 cardiograph (Inkart, St. Petersburg) at a digitizing frequency of 1000 Hz.

Ventricular fibrillation was induced by frequent electrical stimulation of the heart through bipolar electrodes in the apex of the left ventricle. No pre-VF electrogram abnormalities were detected.

Frequency and amplitude (spectral) analysis of electrograms during perfusion, ischemia, and reperfusion of the heart in VF was performed using the Fast Fourier Transform method in the frequency range of 0.5–15 Hz, which is the best method for determining the spectral parameters of VF [14].

The spectral analysis of 1 sec electrogram segments was performed and the proportion (%) of 0.5–15 Hz oscillations in 10 sec electrogram segments was determined in 6 episodes of perfusion, ischemia, and reperfusion during VF ($M \pm m$, N=60). The stable frequency and amplitude structure of VF during perfusion served as a reliable control for comparing the spectral structure of VF during ischemia and reperfusion.

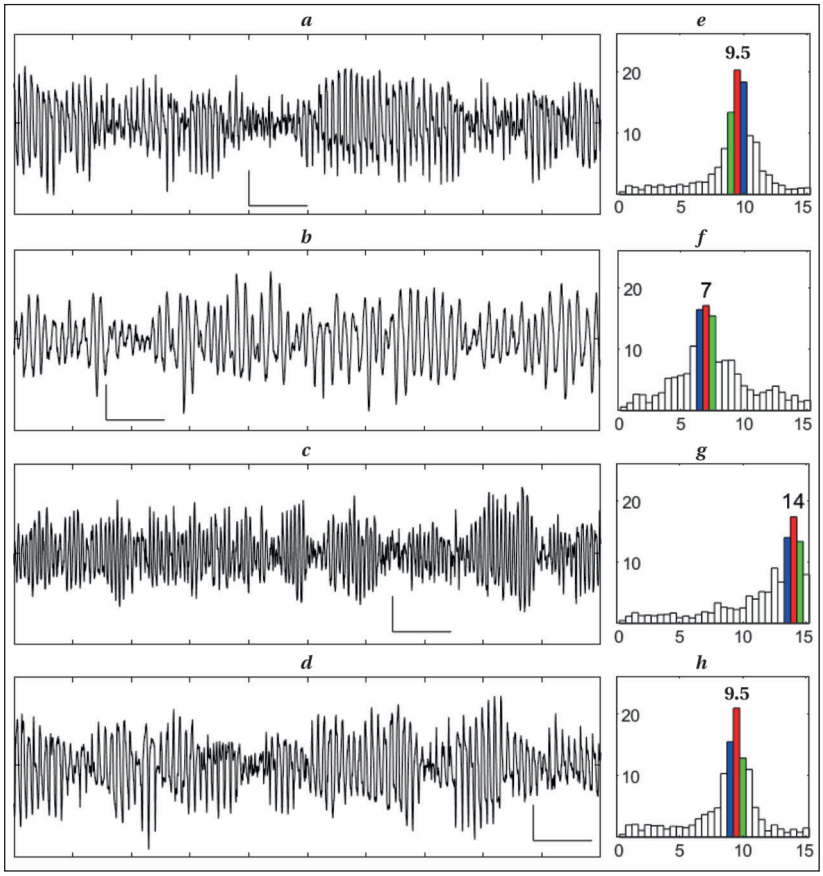

Fig. 1. Cardiac electrograms of the dog.
Note. *(a)* during perfusion; *(b)* at 171–180 sec of ischemia; *(c), (d)* at 51–60 and 581–590 sec of reperfusion of the heart in VF, respectively; *(e)–(h)* spectrograms of electrograms. Calibration of electrograms: 2 mV; 1 sec. Spectrograms: horizontal axis — frequency, Hz; vertical axis — amplitude, mV.

The ventricular fibrillation parameters during perfusion, ischemia, and reperfusion were compared by the nonparametric Welch criterion using R Project for Statistical Computing [15].

Differences at $P<0.05$ were considered significant.

Results

Oscillations of 9–10 Hz frequency dominated the electrogram and spectrogram during cardiac perfusion in VF (Fig. 1, *a, e*).

Oscillations of 6.5–7.5 Hz frequency were dominant at 3 min of ischemia during VF (Fig. 1, *b, f*). Oscillations of 13.5–14.5 Hz were dominant at the 1st min of reperfusion (Fig. 1, *c, g*), and oscillations of 9–10 Hz were dominant at the 10th min of reperfusion in VF (Fig. 1, *d, h*).

The dominant spectral structure on electrograms and spectrograms during ischemia and reperfusion of the heart in VF (Fig. 1) is shown in Fig. 2. During cardiac perfusion in VF, which served as a control, oscillations of 9–10 Hz frequency accounted for 32% of the spectral power and dominated the frequency and amplitude structure of VF (Fig. 2, *a*).

During the first 30 sec of ischemia, the frequency and amplitude of dominant VF oscillations did not change significantly (P=0.08) compared to the control during cardiac perfusion in VF (Fig. 1, *b*). At 3 min of ischemia, the frequency of dominant VF oscillations decreased to 6.5–7.5 Hz (P=0.001) and the proportion of dominant oscillations decreased to 26% (P=0.005) (Fig. 2, *c*), indicating a 20% decrease in proportion compared to the control during cardiac perfusion in VF.

At 1 min of reperfusion, the frequency of dominant VF oscillations increased to 13.5–14.5 Hz (P=0.001), while the proportion of dominant oscillations remained reduced to 26%, as at 3 min of ischemia (Fig. 2, *d*). At 2 min of reperfusion, the frequency of dominant oscillations decreased to 9.5–10.5 Hz (P=0.002), while the proportion of dominant oscillations increased to 33% (P=0.001) (Fig. 2, *e*).

At 3–10 min of reperfusion, the frequency amplitude of the dominant oscillations of the VF stabilized, i. e., oscillations of 9–10 Hz frequency accounted for 32–33% of the spectral power (Fig. 2, *f, g*).

Discussion

The dominant frequency and amplitude structure of VF during ischemia and reperfusion in VF was demonstrated, suggesting organized VF activity. However, the dynamics of VF during ischemia, when ischemia in VF was preceded by cardiac perfusion in VF, differed from those of cardiac VF in situ, when ischemia in VF was preceded by coordinated cardiac contractions. Ventricular fibrillation in situ is characterized by rapid changes during ischemia, as the dominant frequency and organized activity of VF begin to decrease as early as the first 10–15 sec of ischemia in VF [16, 17], and by the third min of ischemia, the dominant frequency of VF decreases by 5–6 Hz, while the organized activity of VF decreases 2-fold [5–7].

The frequency and amplitude structure of VF was stable in the first 30 sec of ischemia in VF, and at 3 min of ischemia, the frequency of dominant oscillations decreased by only 2.5 Hz (Fig. 2). Using the proportion of dominant VF oscillations as a quantitative parameter of organized VF activity, we can conclude that organized VF activity decreased by only 20% at 3 min of ischemia.

These data suggest that cardiac perfusion during VF increases the resistance of the organized spectral structure of VF to ischemia.

Reperfusion after 3 min of ischemia was characterized by a strong transient destabilization of the organized spectral structure of the VF, as confirmed by a 2-fold increase, from 7 to 14 Hz, in the dominant frequencies of the VF and a 20% decrease in organized activity at 1 min of reperfusion in the VF (Fig. 2). At least 2–3 min of VF reperfusion are required to restore and stabilize the frequency and amplitude structure of the VF. In our opinion, transient destabilization of organized activity during the 1st min of reperfusion should be considered a complication of reperfusion because such destabilization may provoke refibrillation during the first 30–60 sec of reperfusion after defibrillation. It can be hypothesized that chest compressions performed for 1 min during CPR destabilize VF activity, which is unfavorable for defibrillation, whereas chest compressions performed for longer periods of 2–3 min stabilize VF activity and improve defibrillation success.

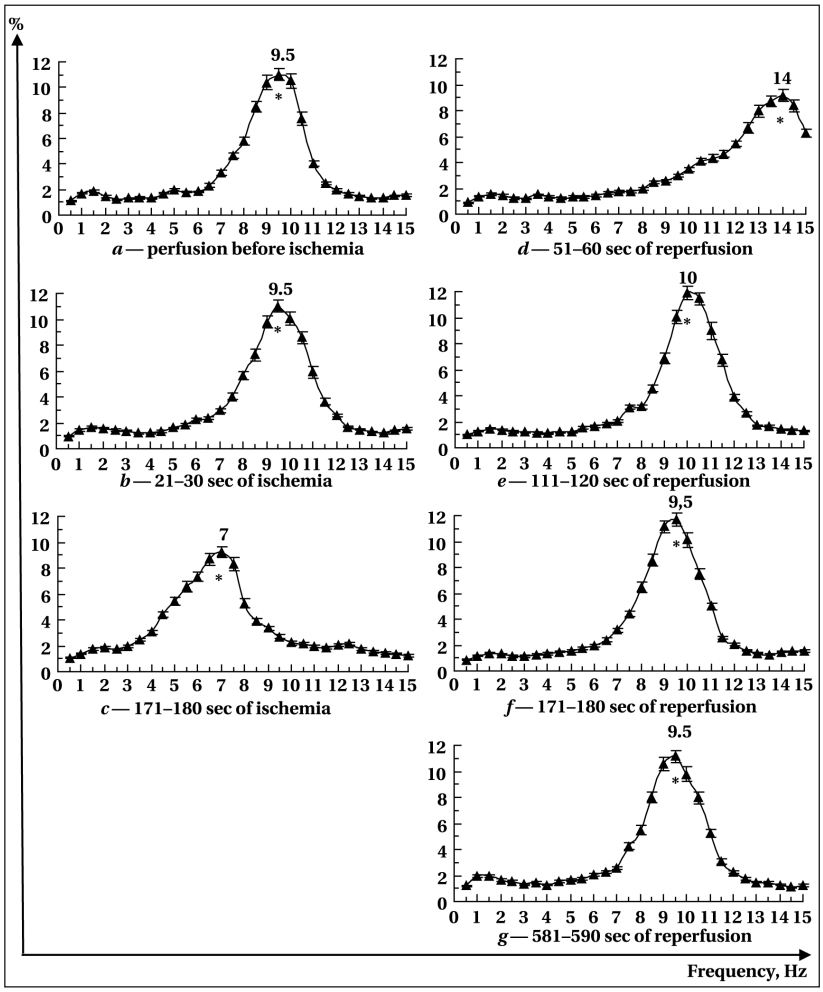

Fig. 2. Proportion of oscillations with frequency 0.5–15 Hz.
Note. *(a)* during perfusion; *(b)* at 1–3 min of ischemia; *(c)–(g)* at 1–10 min of reperfusion of the dog heart in ventricular fibrillation. Data are expressed as M±m, N=60. * $P<0.01$ when the three frequencies with the highest specific gravity were compared with the other frequencies.

Apparently, the organized structure of the VF becomes more resistant to ischemia after perfusion during VF because of the increase in ATP in the myocardium during perfusion during VF, as 10 times less energy is expended during VF than during coordinated contractions [13].

The increase in oscillation frequency at 1 min of reperfusion in VF indicates an increase in ATP synthesis, which suggests an acceleration of oxidative phosphorylation reactions and an increase in electron flow through the mitochondrial respiratory chain [18, 19]. However, the throughput of the respiratory chain is limited, and a pulse increase in the flow of high-energy electrons may lead to a slowing of the electron flow through the respiratory chain during reperfusion, which could cause thermal damage to the iron-sulfur centers and cytochromes of the respiratory chain. Damage to coenzymes of the respiratory chain can be considered the basis of in situ reperfusion injury of the heart, and increased permeability of the mitochondrial inner membrane and other mechanisms of myocardial reperfusion injury [20, 21] are the consequences of a primary alteration of the mitochondrial respiratory chain in the first 30–60 sec of reperfusion.

Based on our findings, we suggest that cardiac perfusion with induced VF can be used during prolonged cardiac surgery to increase cardiac resistance to ischemia and prevent reperfusion complications, which would improve the quality of surgery and postoperative cardiac recovery. During cardioplegia, which is used to «maintain» the operated heart, the myocardium still undergoes ischemia and reperfusion [22, 23], and several papers address myocardial protection from ischemia and reperfusion during cardioplegia [24, 25].

The issue of cardiac perfusion in induced VF during cardiac surgery needs further investigation. Our results were obtained in the isolated canine heart perfused with the blood of the supporting dog, whereas in the cardiac surgery clinic the human heart is perfused in situ with cardiopulmonary bypass and membrane oxygenator.

The limitation of the study was the lack of a control group with the same observation period but without ischemia-reperfusion. Cardiac electro-

grams obtained at a baseline (before ischemia-reperfusion) served as a control in the experiment. The baseline VF values represent generally accepted control for studying VF during ischemia and reperfusion [5, 6, 11, 12].

Hundreds of parasympathetic ganglia and tens of thousands of neurons affecting cardiac rhythm and conduction are localized around the sinus and atrial-ventricular node [26]. Nervous tissue is sensitive to ischemia and reperfusion [27], and ischemic and reperfusion injury to cardiac nervous tissue during cardioplegia can cause cardiac rhythm and conduction disturbances.

Perfusion of the operated heart instead of cardioplegia in induced VF may protect both myocardium and cardiac nervous tissue.

Conclusion

A transient destabilization of the organized structure of VF during the 1st min of reperfusion is typical of reperfusion in VF. The organized structure of VF becomes stable at 2–10 min of reperfusion. The use of cardiac perfusion instead of cardioplegia for induced VF during prolonged cardiac surgery could increase the resistance of the operated heart to ischemia and prevent reperfusion complications.

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Научно-исследовательский институт общей реаниматологии
имени В.А. Неговского ФНКЦ РР
г. Москва | **18 ноября 2023 года**

ОСНОВНЫЕ ТЕМЫ КОНФЕРЕНЦИИ

Экспериментальная хирургия как важная составляющая доклинических биомедицинских исследований
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Периоперационной мониторинг жизненно важных функций животного в доклинических исследованиях
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Гуманные методы эвтаназии лабораторных животных
Возможности и ограничения экстраполяции результатов фундаментальных и трансляционных доклинических исследований в клиническую медицину.

СПЕЦИАЛЬНЫЕ ВОПРОСЫ:

Экспериментальная физиология и патология кровообращения
Современные методы исследования сердечно-сосудистой системы в доклинических исследованиях
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