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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].

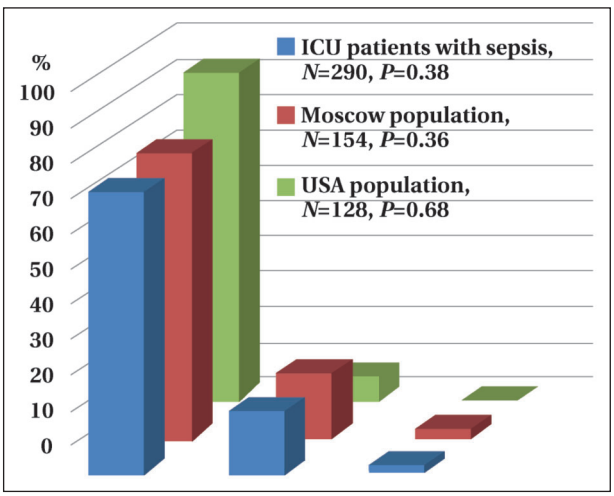

Fig. 1. *AQP*4 rs1058427 genotype frequencies among ICU patients, apparently healthy donors, and in USA population [23].

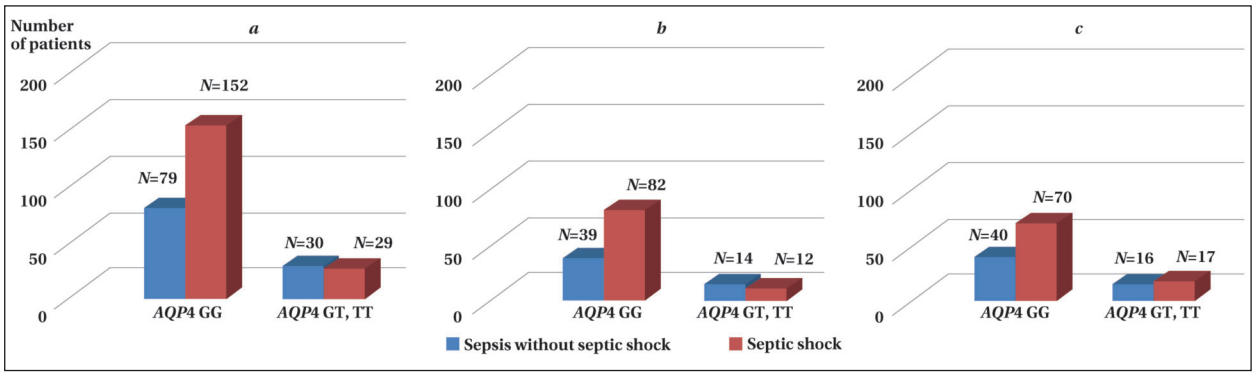

Fig.2. The incidence of septic shock in ICU patients with different *AQP*4 rs1058427 genotypes.
Note. *a* — all patients, *N*=290, *P*=0.009, Fisher`s exact test (FET), OR=1.99, 95% CI: 1.12–3.55. *b* — patients from ICU 2 and ICU 3, *N*=147, *P*=0.045, FET, OR=2.45, 95% CI: 1.04–5.79. *c* — patients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

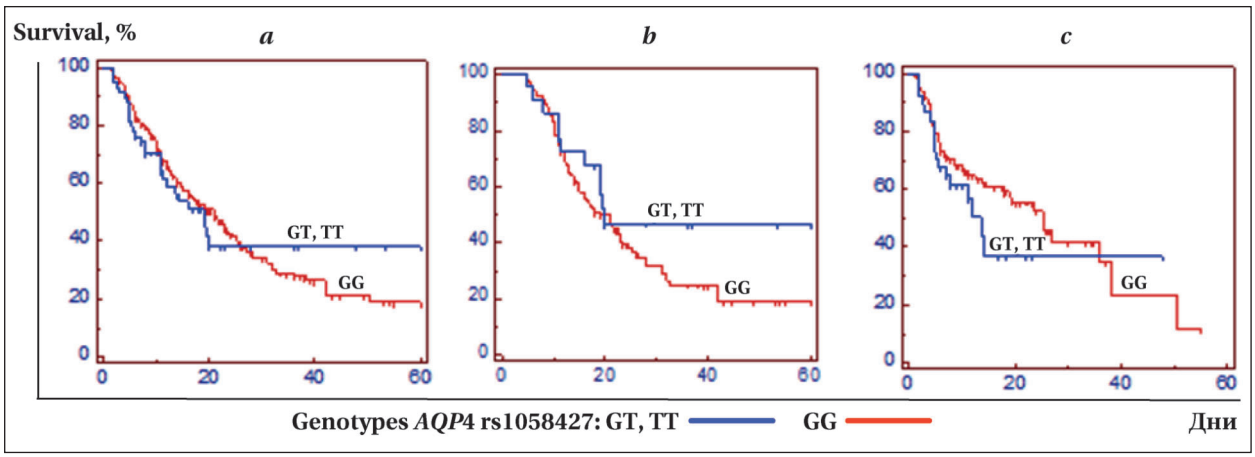

Fig. 3. Survival rate of sepsis patients with different *AQP*4 rs1058427 genotypes.
Note. *a* — all patients, *N*=290, *P*=0.995, log-rank test. *b* — patients who were on mechanical ventilation for 5 days or more, *N*=125, *P*=0.176, log-rank test. *c* — patients who were on mechanical ventilation less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

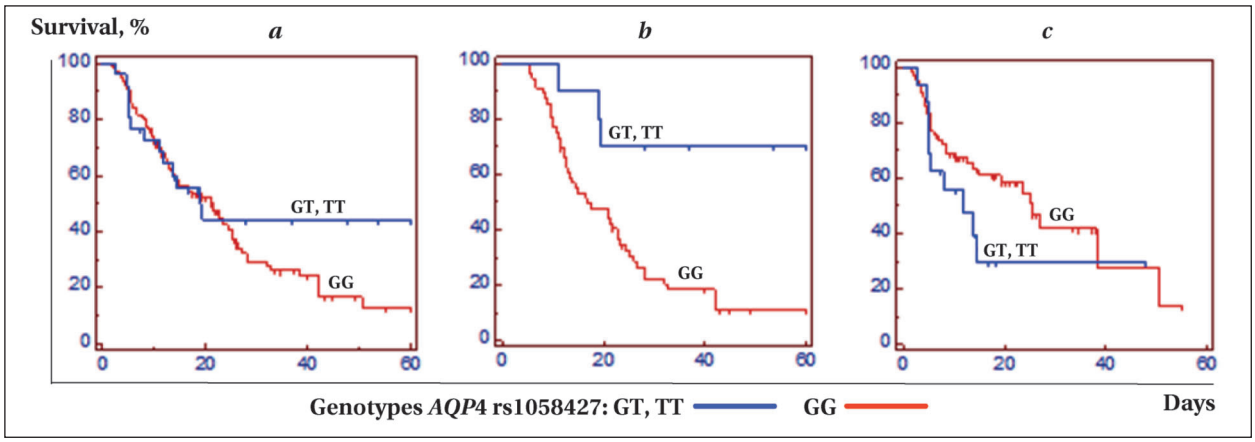

Fig. 4. Survival rate of ICU 2 and ICU 3 sepsis patients with multiple comorbidities with different *AQP*4 rs1058427 genotypes and duration of ventilation.
Note. *a* — all patients, *N*=147, *P*=0.32, log-rank test. *b* — patients who were on mechanical ventilation for 5 days or more, *N*=66, *P*=0.003,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

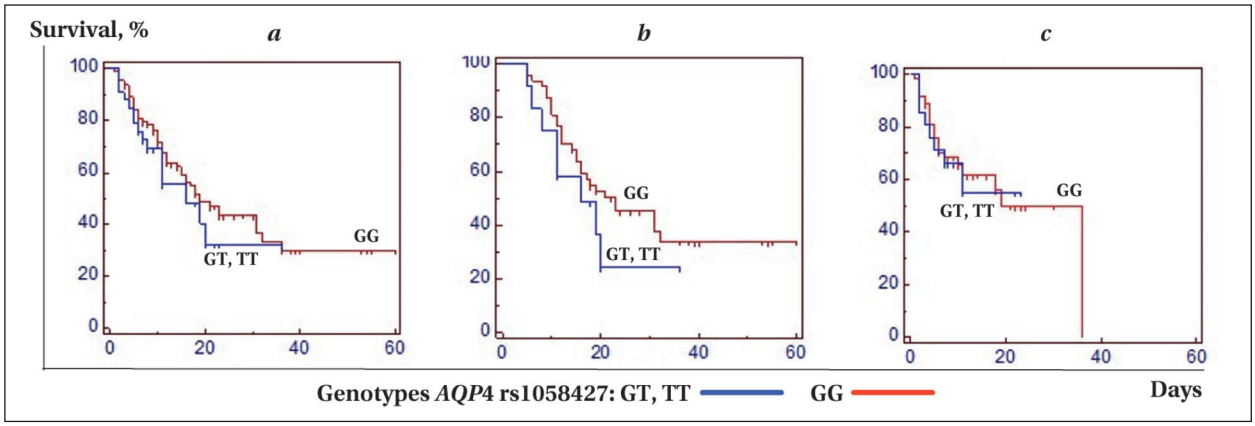

Fig. 5. Survival rate of ICU 1 sepsis patients without comorbidities with different *AQP*4 rs1058427 genotypes and duration of ventilation.
Note. *a* — all patients, *N*=143, *P*=0.330, log-rank test. *b* — patients who were on mechanical ventilation for 5 days or more, *N*=59, *P*=0.246, log-rank test.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