ORIGINAL ARTICLE

CONTENTS

The association of TLR4 gene polymorphisms with the severity of peritonitis in acute inflammatory diseases of the abdominal cavity organs

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ABSTRACT

Aim: To determine the role of TLR4 gene polymorphisms as risk factors for peritonitis severity in patients undergoing surgery for acute inflammatory diseases of the abdominal cavity.

Materials and Methods: The study included 139 patients who were operated on for acute abdominal diseases (acute appendicitis and cholecystitis, perforated gastric or duodenal ulcer, etc.). Depending on the number of points on the modified APACHE II scale, patients were divided into two groups: Group 1 - 1-3 points (63 patients, 45.3%) and Group 2 - 4 or more points (76 patients, 54.7%). Polymorphisms rs1927911, rs2149356 and rs4986790 were determined by polymerase chain reaction.

Results: The rs1927911 polymorphism of the TLR4 gene was protective for the development of peritonitis (according to the allelic model, OR 0.48; 95% CI 0.27-0.84; p=0.015). Regression analysis revealed a reduced (p=0.015) risk of severe peritonitis in rs1927911 A/A or G/A genotype carriers (OR 0.42; 95% CI 0.21-0.84) compared with G/G genotype carriers. There was no effect on the severity of peritonitis of TLR4 polymorphisms rs2149356 and rs4986790. There was a tendency to increase the frequency of the mutant G rs4986790 allele in patients with severe peritonitis ($\chi 2=2.17$; $p<0.001$). The analysis of the association of TLR4 gene polymorphisms with the phenotype of patients showed that carriers of mutant homozygotes and heterozygotes in the presence of severe peritonitis were older, had a tendency to coagulopathy, higher leukocytosis and leukocyte clotting rate.

Conclusions: Thus, the importance of TLR in the development of severe peritonitis was confirmed and the protective role of the rs1927911 promoter polymorphism was established.

KEY WORDS: regression analysis, peritonitis, rs4986790, rs1927911, rs2149356

Wiad Lek. 2024;77(1):35-41. doi: 10.36740/WLek202401105 DOI

INTRODUCTION

The high mortality rate in peritonitis, which ranges from 19% to 80%, makes it one of the most serious complications of abdominal diseases [1, 2].

Toll-like receptors (TLRs) are a family of so-called "pattern recognition receptors" that trigger cellular inflammatory and immune responses in response to exogenous and endogenous proinflammatory factors [3]. Activation of the proinflammatory signaling pathway mediated by Toll-like receptor 4 (TLR4) plays an important role in acute inflammation, sepsis, and chronic inflammatory diseases. Highly specific antibodies to TLR4 inhibited lipopolysaccharide-induced production of tumor necrosis factor-α, interferon-β, and interleukin-6 in mouse peritoneal macrophages by reducing phosphorylation of NF-κB and mitogen-activated protein kinase [4].

Changes in TLR function caused by genetic polymorphism can determine the incidence or severity of acute inflammatory diseases, as shown by acute pancreatitis [5] and inflammatory bowel disease in children [6].

Polymorphism of genes involved in the body's defense may be associated with the severity of local infection-inflammation in humans, in particular in acute appendicitis, which was shown for the IL-6 gene (-174G>C), but not for the TLR4 gene (896 A>G) [7].

On the other hand, a systematic review and meta-analysis that included the results of 1476 patients with urinary tract infection and 1449 healthy control patients showed that in Asian populations, the rs4986790 polymorphism of the TLR4 gene was associated with the risk of urinary tract infection [8].

A recent meta-analysis showed the role of TLR gene polymorphisms in inflammatory bowel disease in Caucasians [9]. Significant associations were found for the TLR1 rs5743611, TLR4 rs4986790, TLR4 rs4986791, and TLR6 rs5743810 polymorphisms. In addition, the TLR4 rs4986790 polymorphism was significantly associated

with the risk of inflammatory bowel disease in West Asians. The influence of TLR4 gene polymorphism on the risk of gastric cancer in northeastern China has also been shown [10]. Carriers of the mutant homozygote rs1927911 had a significantly reduced risk of the disease (OR 0.37; 95% CI 0.21-0.67; P=0.001).

AIM

The aim of the study was to determine the role of TLR4 gene polymorphisms as risk factors for peritonitis severity in patients undergoing surgery for acute inflammatory diseases of the abdominal cavity.

MATERIALS AND METHODS

A single-center, randomized, single-instance, open-label observational study was conducted on the basis of the Brovary Multidisciplinary Clinical Hospital in the period from 09.09.2021 to 24.10.2022. Patients were examined in accordance with the Declaration of Helsinki of the World Medical Association (Seoul, 2008), orders of the Ministry of Health of Ukraine (№ 281 of November 01, 2000, № 355 of September 25, 2002, № 356 of May 22, 2009 as amended by the order of the Ministry of Health of Ukraine № 574 of August 05, 2009, № 1118 of December 21, 2012).

All patients underwent clinical, laboratory, and genetic testing. All patients gave informed consent to participate in the study. The material for this article was based on the medical records of inpatients and the results of laboratory and genetic tests.

Inclusion criteria: patients with peritonitis over 18 years of age.

Criteria for non-inclusion in the study: patients with peritonitis of gynecological genesis, acute pancreatitis.

The study involved 139 patients who were operated on for acute abdominal diseases, including 71 patients with acute appendicitis, 51 with acute cholecystitis, 10 with perforated gastric or duodenal ulcer, 4 with tumor perforation, 1 with small intestine perforation, 1 with hernia, and 1 with cryptogenic peritonitis. Depending on the number of points on our modified APACHE II scale (Acute Physiology and Chronic Health Evaluation) [11], patients were divided into two groups: Group 1 - 1-3 points (63 patients, 45.3%) and Group 2 - 4 or more points (76 patients, 54.7%).

During the operation, the prevalence of the inflammatory process in the peritoneum (local, diffuse, spilled or general), the nature of the exudate (serous, serous-fibrinous, fibrinous-purulent, purulent, fecal or hemorrhagic), the stage of peritonitis (reactive, toxic or terminal) were determined. Prothrombin index (PTI, %), international normalized ratio (INR, units), prothrombin time (PT, min), fibrinogen content (g/l), as well as leukocyte count (G/l) and erythrocyte sedimentation rate (ESR, mm/h) were determined in the blood by biochemical laboratory methods.

TLR4 gene polymorphisms rs1927911 (Intron; Chr:9.117707776), rs2149356 (Intron; Chr:9. 117711921) and rs4986790 (896A/G; chr9:117713024; Asp299Gly) were determined by real-time polymerase chain reaction using Gene Amp® PCR System 7500 amplifier (Applied Biosystems, USA) and TaqMan Mutation Detection Assays Life-Technology (USA). Genomic DNA was extracted from venous blood using the PureLink® Genomic DNA Kit For Purification of Genomic DNA (INVITROGEN, USA).

Statistical processing of the results of the study was performed using EZR v.1.54 (graphical user interface for R statistical software version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) [12]. The Shapiro-Wilk test was used to check the distribution of quantitative indicators for normality; the distribution law differed from the normal one, so the median (Me) and the first and third quartiles (Q1-Q3) were calculated for presentation, and the Mann-Whitney, Wilcoxon, and Kruskal-Wallis ranked analysis of variance were used to compare samples. Differences in genotype and allele frequencies were compared by Fisher's exact method and Pearson's $\chi 2$ test. The statistical significance of differences in the distribution of genotype and allele frequencies in the case-control group was evaluated in the contingency tables (3×2 and 2×2, respectively). The degree of association of genotypes and alleles with the severity of peritonitis was determined by calculating the odds ratio (OR) and 95% confidence interval (95% CI). For regression analysis, the method of constructing logistic regression models was used [13], the initial sign was Y=0 (APACHE II 1-3 points - 63 patients), Y=1 (APACHE II 4 and more points - 76 patients). In all cases of statistical evaluation, the value of $p<0.05$ was considered significant.

RESULTS

When comparing the distribution of genotypes in patient groups, it was found (Table 1) that in patients of group 2, a decrease in the frequency of the G/A heterozygote, minor homozygote A/A and allele A was noted compared with patients of group 1, which was statistically significant at p=0.040 (for genotypes) and p=0.015 (for alleles).

Comparison of the dominant and recessive inheritance model showed statistical significance (p=0.022) of the dominant model (Table 2).

Table 1. Influence of the distribution of frequencies of genotypes and alleles of rs1927911 of the TLR4 gene on the severity of peritonitis and the degree of their association with the disease

Genotypes Alleles	Group, n (f)		χ^2	p	OR	95% CI
	2-nd	1-st				
G/G	53 (0,70)	31 (0,49)	6,42	0,040	Reference	
G/A	20 (0,26)	26 (0,41)			0,45	0,22 – 0,94
A/A	3 (0,04)	6 (0,10)			–	–
G	126 (0,83)	88 (0,70)	5,91	0,015	Reference	
A	26 (0,17)	38 (0,30)			0,48	0,27 – 0,84

Notes: n - number; f - frequency; χ2 - Pearson's correction for continuity; p - statistical significance of differences between groups; OR - odds ratio; 95% CI - 95% confidence interval for OR.

Table 2. Influence of the frequency distribution of TLR4 gene rs1927911 genotypes on the development of peritonitis (dominant and recessive models of inheritance)

	Genotypes 2-a	Group, n (f)		χ^2	p	OR	CI% BI
		1-a					
Dom.	G/G	53 (0,70)	31 (0,49)	5,24	0,022	Reference	
	G/A+A/A	23 (0,30)	32 (0,51)			0,42	0,21–0,84
Rec.	G/G+G/A	73 (0,96)	57 (0,90)	0,97	0,326	Reference	
	A/A	3 (0,04)	6 (0,10)			–	–

Notes: n - number; f - frequency; χ2 - Pearson's correction for continuity; p - statistical significance of differences between groups; OR - odds ratio; 95% CI - 95% confidence interval for OR.

Table 3. Influence of genotypes of rs1927911 polymorphism of TLR4 gene on the studied indicators

Indicator	Group	Genotypes		p
		G/G	G/A+A/A	
Age, years	1-st	44,2±16,7	41,4±14,8	0,489
	2-nd	47,5±17,1	51,8±13,7	0,282
p*		0,398	**0,011**	
PTI, %	1-st	105,0 (92,4–120,0)	98,6 (88,1–111,1)	0,202
	2-nd	87,9 (75,6–96)	88,4 (77,6–101,9)	0,931
p*		0,006	0,211	
INR	1-st	0,989 (0,940–1,05)	1,034 (0,970–1,080)	0,306
	2-nd	1,106 (1,055–1,163)	1,12 (1,020–1,160)	0,972
p*		**0,006**	0,134	
PT, min.	1-st	11,5 (10,3–11,9)	10,6 (10,3–11,0)	0,737
	2-nd	12,2 (11,5–13,0)	12,5 (10,7–13,3)	>0,999
p*		0,066	**0,020**	
Fibrinogen, g/l	1-st	8,41 (3,87–10,59)	4,58 (4,30–4,86)	0,845
	2-nd	5,13 (4,30–6,60)	4,22 (3,87–4,77)	0,702
p*		0,926	0,688	
Leukocytes, g/l	1-st	9,6 (6,4–12,6)	10,8 (6,8–12,6)	0,417
	2-nd	14,2 (11,5–17,5)	13,9 (11,1–17,5)	0,826
p*		<0,001	0,005	
ESR, mm/h.	1-st	13,9 (4,0–16,5)	21,3 (7,0–35,0)	0,184
	2-nd	25,5 (14,5–36,5)	42,0 (33,3–52,0)	0,400
p*		0,161	0,086	

Notes: p - statistical significance of differences in the group; p* - statistical significance of differences between groups. In the case of a normal distribution law, M±SD is presented, Student's test is used for comparison; in the case of a distribution law other than normal, Me (QI - QIII) and the Mann-Whitney test are used.

Table 4. Influence of rs2149356 genotype frequency distribution of TLR4 gene on the severity of peritonitis

Genotypes Alleles	Group, n (f)		χ^2	p	OR	95% CI
	2-nd	1-st				
G/G	33 (0,434)	28 (0,444)	0,02	0,992	Reference	
G/T	33 (0,434)	27 (0,429)			–	–
T/T	10 (0,132)	8 (0,127)				
G	99 (0,651)	83 (0,670)	0,00	>0,999	Reference	
T	53 (0,349)	43 (0,330)			–	–

Notes: n - number; f - frequency; χ2 - Pearson's correction for continuity; p - statistical significance of differences between groups.

Table 5. Influence of the frequency distribution of TLR4 gene rs4986790 genotypes on the severity of peritonitis

Genotypes Alleles	Group, n (f)		χ^2	p	OR	95% CI
	2-a	1-a				
A/A	57 (0,750)	52 (0,825)	2,97	0,227	Reference	
A/G	16 (0,211)	11 (0,175)			–	–
G/G	3 (0,039)	0 (0,000)				
A	130 (0,855)	115 (0,913)	1,66	0,198	Reference	
G	22 (0,145)	11 (0,087)			–	–

Notes: n - number; f - frequency; χ2 - Pearson's test with correction for continuity; p - statistical significance of differences between groups; OR - odds ratio; 95% CI - 95% confidence interval for OR

Table 6. Analysis of single-factor logistic regression models for predicting the risk of severe peritonitis by polymorphic genotypes of the TLR4 gene

Factor attribute		Model coefficient, b±m	Significance level of the difference between the OR and 1, p	The model's odds ratio, OR (95% CI)
rs1927911	*G/G*		Reference	
	A/A+G/A	-0,87 ± 0,35	0,015	0,42 (0,21 – 0,84)
rs2149356	*G/G*		Reference	
	G/T	0,04 ± 0,37	0,921	–
	T/T	0,06 ± 0,54	0,913	–
rs4986790	*A/A*		Reference	
	G/G+G/A	0,45 ± 0,42	0,284	–

Thus, the presence of the mutant allele A in the TLR4 gene rs1927911 genotype (for carriers of G/A and A/A genotypes) reduced the likelihood of severe peritonitis (OR 0.42; 95% CI 0.21-0.84), which allowed us to consider this polymorphism a prognostic factor.

The analysis of the association of rs1927911 with the phenotype is shown in Table 3. When stratifying patients by groups and genotypes of rs1927911, it was found that the oldest patients were carriers of the mutant allele A.

The PTI and INR were different in carriers of the ancestral G/G genotype, with a lower PTI (p=0.006) and higher INR (p=0.006) in patients with severe peritonitis than in those without. The PI was higher in patients carrying the A allele in the presence of severe peritonitis (p=0.020). The detected shifts indicated the presence of coagulopathy with a predisposition to hemorrhagic syndrome. Leukocytosis in the presence of severe peritonitis was higher in carriers of all genotypes (p≤0.005).

In the present study, there was no significant difference in the distribution of TLR4 rs2149356 and rs4986790 genotypes and their influence on the development of peritonitis (Table 4 and Table 5).

The analysis of the association of rs2149356 and rs4986790 with the phenotype of patients showed that mutant homozygotes and heterozygotes in the presence of severe peritonitis were older, had a lower PTI and higher INR, WBC, leukocytosis and ESR (p<0.05), which was similar to the distribution for rs1927911. Interestingly, in patients carrying the mutant homozygote G/G and heterozygote A/G rs4986790, who had mild peritonitis, ESR was the lowest among all groups stratified by diagnosis and genotype (Me 5.83 mm/h; QI-QIII 2 mm/h - 8 mm/h).

To confirm the role of the identified polymorphisms of the TLR4 gene, we used the method of building logistic regression models [13]. The analysis was performed to

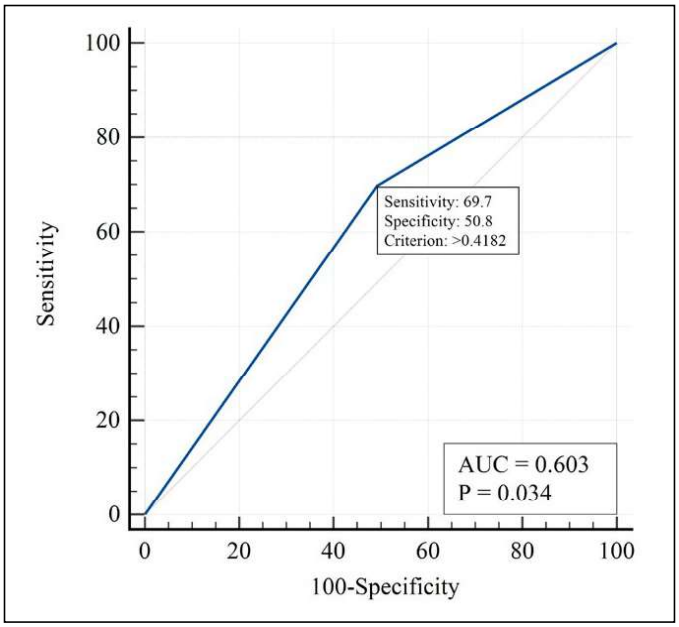

Fig. 1. ROC curve for predicting the risk of severe peritonitis by TLR4 rs1927911 genotypes.

identify the association of the risk of severe peritonitis with the factor characteristics for all patients. Table 6 shows the results of univariate analysis of the association of peritonitis risk with polymorphic genotypes of the TLR4 gene.

The analysis revealed a reduction (p=0.015) in the risk of severe peritonitis in carriers of the rs1927911 A/A or G/A genotypes (OR 0.42; 95% CI 0.21-0.84) compared with carriers of the rs1927911 G/G genotype.

Figure 1 shows the operating characteristics curve for this test.

The area under the ROC curve AUC=0.60 (95% CI 0.52-0.69), which indicated the presence of an association of severe peritonitis risk with rs1927911. When choosing the optimal threshold (rs1927911 G/G genotype), the test sensitivity was 69.7% (95% CI 58.1%-79.8%), specificity 50.8% (95% CI 37.9%-63.6%).

DISCUSSION

It is believed that the TLR4 rs4986790 gene polymorphism is a functional polymorphism that leads to a decrease in receptor sensitivity [10, 14]. In our studies, the frequency of the G rs4986790 mutant allele in patients of group 2 increased significantly ($\chi 2=2.17$; $p<0.001$), but the association with the disease for rs4986790 in this sample of patients was not confirmed (p=0.198; see Table 5). Thus, within the framework of the results obtained, it was possible to assert only the existence of a tendency for this polymorphism to be associated with severe peritonitis. This assumption is confirmed by the absence of an increase in ESR in patients carrying the mutant homozygote G/G and heterozygote A/G rs4986790 who had mild peritonitis.

A recent meta-analysis of 38 studies (10970 cases and 7061 controls) provided evidence that TLR4 rs4986790 is associated with susceptibility to Crohn's disease and ulcerative colitis in Caucasians but not in Asians [15].

The suppression of the immune response in the absence of the TLR4 gene was shown in another study [16]. In experiments with TLR4-deficient (TLR4-/-) mice, it was found that chronic inflammation and infiltration of the colon by macrophages, intestinal fibrosis and collagen deposition were reduced in a model of chronic colitis and colon fibrosis using 3% sodium dextran sulfate. In addition, the production of tumor necrosis factor-α, interleukin-12p40, and transforming growth factor-β was reduced in TLR4-deficient peritoneal macrophages.

Blockade of TLR4 by antibodies differentially suppressed the bacteria-induced production of profibrotic and inflammatory mediators by peritoneal leukocytes in peritoneal dialysis patients with uremia [17]. In addition, antibodies against TLR4 decreased the profibrotic responses of uremic leukocytes to endogenous components, whereas increased TLR-mediated inflammation increased fibrosis in vivo.

Analysis of the gene expression profile using the Gene Expression Omnibus database identified a panel of 446 key genes and relevant pathways involved in the formation of inflammation in postoperative peritoneal adhesion [18]. Functional analysis suggested that these genes were enriched in the TLR signaling pathway via myeloid differentiation primary response protein 88 (MyD88) and nuclear factor kappa B (NF-κB) - the TLR4/MyD88/NF-κB proinflammatory pathway/inflammatory cytokines/peritoneal adhesion. It was also found that the TLR4/MyD88/NF-κB signaling pathway was activated in the acute phase of appendicitis [19].

These results explain the general importance of TLR gene polymorphisms that cause a decrease in its functional activity. For example, TLR4 knockout enhances the clearance of infectious bacterial species that show excessive amounts in the abdominal cavity of mice after ligation and puncture of the cecum [20].

In our studies, we found an association of severe peritonitis with the promoter polymorphism rs1927911 of the TLR4 gene in a dominant model (OR 0.42; 95% CI 0.214-0.84; p=0.022). This polymorphism was protective for the development of severe peritonitis, which also confirmed the negative significance of wild-type TLR4 for the development of acute inflammation. The TLR4 gene polymorphism affects intracellular signal transduction and, as a result, changes the patterns of immune response not only in inflammatory but also in cancer, in particular cervical cancer [21].

Thus, our data confirmed the generally accepted view of the key role of TLR in the development of acute peritonitis and established the protective role of the promoter polymorphism rs1927911 and the tendency to risk value of the polymorphism rs4986790.

CONCLUSIONS

1. The rs1927911 polymorphism of the TLR4 gene was protective for the development of severe peritonitis (according to the allelic model, OR 0.48; 95% CI 0.27-0.84; p=0.015). Regression analysis revealed a decrease (p=0.015) in the risk of severe peritonitis in carriers of rs1927911 A/A or G/A genotypes (OR 0.42; 95% CI 0.21-0.84) compared with carriers of the G/G genotype.
2. There was no effect of rs2149356 and rs4986790 polymorphisms of the TLR4 gene on the development of peritonitis. There was a tendency to increase the frequency of the mutant G rs4986790 allele in patients with severe peritonitis ($\chi 2=2.17$; $p<0.001$).
3. The analysis of the association of TLR4 gene polymorphisms with the phenotype of patients showed that carriers of mutant homozygotes and heterozygotes in the presence of severe peritonitis were older, had a lower PTI and higher INR, PF, leukocytosis and ESR.

REFERENCES

1. Biliaieva OO, Karol IV. Peredoperatsiyna pidhotovka pry perytoniti [Preoperative preparation for peritonitis]. Ukrainskyi medychnyi chasopys. 2022. doi: 10.32471/umj.1680-3051.144.214004. (in Ukrainian). DOI
2. Spalding DR, Williamson RC. Peritonitis. Br J Hosp Med (Lond). 2008;69(1):M12-5. doi: 10.12968/hmed.2008.69.Sup1.28050. DOI
3. Lim KH, Staudt LM. Toll-like receptor signaling. Cold Spring Harb Perspect Biol. 2013;5(1):a011247. doi: 10.1101/cshperspect.a011247. DOI
4. Wang Y, Gong D, Yao C et al. Human monoclonal antiTLR4 antibody negatively regulates lipopolysaccharideinduced inflammatory responses in mouse macrophages. Mol Med Rep. 2020;22(5):4125-4134. doi: 10.3892/mmr.2020.11500. DOI
5. Matas-Cobos AM, Redondo-Cerezo E, Alegría-Motte C et al. The role of Toll-like receptor polymorphisms in acute pancreatitis occurrence and severity. Pancreas. 2015;44(3):429-33. doi: 10.1097/MPA.0000000000000272. DOI
6. Li X, Song P, Timofeeva M et al. Systematic meta-analyses and field synopsis of genetic and epigenetic studies in paediatric inflammatory bowel disease. Sci Rep. 2016;6:34076. doi: 10.1038/srep34076. DOI
7. Rivera-Chavez FA, Peters-Hybki DL, Barber RC et al. Innate immunity genes influence the severity of acute appendicitis. Ann Surg. 2004;240(2):269-77. doi: 10.1097/01.sla.0000133184.10676.26. DOI
8. Huang WL, Xu Y, Wan SP. Association of Toll-like 4 receptor gene polymorphism (rs4986790, rs4986791) with the risk of urinary tract infection: A systematic review and meta-analysis. Kaohsiung J Med Sci. 2020;36(3):206-211. doi: 10.1002/kjm2.12158. DOI
9. Wang H, Zhou S, Zhang J et al. Correlations between TLR polymorphisms and inflammatory bowel disease: a meta-analysis of 49 case-control studies. Immunol Res. 2019;67(1):142-150. doi: 10.1007/s12026-018-9061-0. DOI
10. Huang L, Yuan K, Liu J et al. Polymorphisms of the TLR4 gene and risk of gastric cancer. Gene. 2014;537(1):46-50. doi: 10.1016/j.gene.2013.12.030. DOI
11. Biliaieva OO, Karol IV. Modern Aspects Of Predicting The Course Of Acute Peritonitis. Kharkivska khirurhichna shkola. 2022; 1 (112): 65–70. doi: 10.37699/2308-7005.1.2022.12. DOI
12. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452-8. doi: 10.1038/bmt.2012.244. DOI
13. Guryanov VG, Lyakh YuE, Pariy VD et al. Posibnyk z biostatystyky. Analiz rezul'tativ medychnykh doslidzhen' u paketi EZR (R–statistics) [Handbook of Biostatistics. Analysis of the results of medical research in the package EZR (R-statistics)]. Kyiv: News. 2018, p.208. (in Ukrainian).
14. Latha M, Vaidya S, Movva S et al. Molecular pathogenesis of endometriosis; Toll-like receptor-4 A896G (D299G) polymorphism: a novel explanation. Genet Test Mol Biomarkers. 2011;15(3):181-4. doi: 10.1089/gtmb.2010.0178. DOI
15. Cheng Y, Zhu Y, Huang X et al. Association between TLR2 and TLR4 Gene Polymorphisms and the Susceptibility to Inflammatory Bowel Disease: A Meta-Analysis. PLoS One. 2015;10(5):e0126803. doi: 10.1371/journal.pone.0126803. DOI
16. Jun YK, Kwon SH, Yoon HT et al. Toll-like receptor 4 regulates intestinal fibrosis via cytokine expression and epithelial-mesenchymal transition. Sci Rep. 2020;10(1):19867. doi: 10.1038/s41598-020-76880-y. DOI
17. Raby AC, Colmont CS, Kift-Morgan A et al. Toll-Like Receptors 2 and 4 Are Potential Therapeutic Targets in Peritoneal Dialysis-Associated Fibrosis. J Am Soc Nephrol. 2017;28(2):461-478. doi: 10.1681/ASN.2015080923. DOI
18. Bian YY, Yang LL, Yan Y et al. Identification of candidate biomarkers correlated with pathogenesis of postoperative peritoneal adhesion by using microarray analysis. World J Gastrointest Oncol. 2020;12(1):54-65. doi: 10.4251/wjgo.v12.i1.54. DOI
19. Liu S, Pei F, Wang X et al. The immune impact of mimic endoscopic retrograde appendicitis therapy and appendectomy on rabbits of acute appendicitis. Oncotarget. 2017;8(39):66528-66539. doi: 10.18632/oncotarget.16236. DOI

20. Kuo PJ, Rau CS, Wu SC et al. Metagenomic analysis of the effects of toll-like receptors on bacterial infection in the peritoneal cavity following cecum ligation and puncture in mice. PLoS One. 2019;14(7):e0220398. doi: 10.1371/journal.pone.0220398. DOI
21. Moura EL, Santos IFD, Freitas PP et al. Polymorphisms in Toll-like receptors genes changes the host's immune response and is associated with cervical cancer. Immunobiology. 2022;227(2):152187. doi: 10.1016/j.imbio.2022.152187. DOI

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 23.07.2023
ACCEPTED: 08.11.2023