INVESTIGATING A CORRELATION BETWEEN THE LEVELS OF PERIPHERAL BLOOD CYTOKINES AND THE RISK FOR CARDIOVASCULAR COMPLICATIONS IN PATIENTS WITH STAGE II ESSENTIAL HYPERTENSION

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Essential hypertension (EH) is one of the most common modifiable risk factors for cardiovascular diseases and death. The aim of this study was to investigate a correlation between the levels of some cytokines (interleukins, adhesion molecules, tumor necrosis and growth factors, etc.) in the peripheral blood of patients with stage II EH and the rate of complications (myocardial infarction, acute cerebrovascular events, and transient ischemic attacks) occurring in a 5-year follow-up period. Twenty-eight cytokines were measured using ELISA, including IL1β, IL1α, IL1ra, IL18, IL18BP, IL37, IL6, sIL6r, LIF, sLIFr, IGF-1, IGFBP-1, TNFα, sTNF-RI, sVCAM-1, IL17, IL2, IL4, IL10, TGF-β1, IL8, CX3CL1, CXCL10, INFγ, M-CSF, IL34, VEGF-A, and erythropoietin, and a few vasoactive peptides, including NO, iNOS, eNOS, ADMA, SDMA, Nt-proCNP, and Nt-proBNP, in the peripheral blood samples of 200 patients with stage II EH who had been suffering from this condition for 10 to 14 years and were receiving comparable therapies to bring their blood pressure down. The patients were followed up for 5 years to keep track of complications. The retrospective analysis revealed that the group of patients who developed complications during the 5-year follow-up period exhibited a decline in the levels of IL1ra ($p < 0.001$) and IL10 ($p < 0.001$) and a rise in IL1β ($p < 0.001$), TNFα ($p < 0.001$) and M-CSF ($p < 0.001$) in comparison with the group of those who did not develop any complications. The multivariate Cox regression analysis was applied to the following parameters: IL1β > 18.8 pg/ml; IL1ra < 511 pg/ml; IL6 > 23.8 pg/ml; IL10 < 26.3 pg/ml; 389 pg/ml < M-CSF < 453 pg/ml; ADMA > 0.86 μmol/L; total cholesterol > 4.9 mmol/L; LDL> 3.0 mmol/L; HDL in men < 1.0 mmol/L; HDL in women < 1.2 mmol/L. The analysis revealed that M-CSF in the range from 389 to 453 pg/ml ($p < 0.001$) and LDL above 3.0 mmol/L ($p < 0.01$) correlated with an increase in the risk for end-organ damage in stage II EH. Changes in the cytokine levels can be regarded as a predictor of myocardial and cerebral damage in patients with stage II EH. Measurement of peripheral blood M-CSF can be included into the classic risk assessment schemes for the cardiovascular complications in the studied cohort of patients.

Keywords: cytokines, essential hypertension, myocardial infarction, stroke, M-CSF

Author contribution: Radaeva OA recruited patients, collected blood samples for the study took medical histories, interpreted the study results, and wrote the manuscript. Simbirtsev AS conceived and planned the study, analyzed the obtained data and revised the manuscript.

Compliance with ethical standards: the study was approved by the Ethics Committee of Ogariov Mordovian State University (Protocol 12 dated December 12, 2008). All patients gave their informed consent to participate. Blood samples were collected in full compliance with the Declaration of Helsinki (2000) and the Protocol of the Convention on Human Rights and Biomedicine of the Council of Europe (1999).

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АНАЛИЗ КОРРЕЛЯЦИИ СОДЕРЖАНИЯ ЦИТОКИНОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ С РИСКОМ РАЗВИТИЯ СЕРДЕЧНО-СОСУДИСТЫХ ОСЛОЖНЕНИЙ У БОЛЬНЫХ ЭССЕНЦИАЛЬНОЙ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ II СТАДИИ

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Эссенциальная артериальная гипертензия (ЭАГ) остается наиболее распространенным модифицируемым фактором риска сердечно-сосудистых заболеваний и смерти. Целью исследования было выявить корреляцию содержания цитокинов (интерлейкинов, молекул адгезии, факторов некроза опухоли, роста и др.) в сыворотке периферической крови и частоты осложнений (инфаркта миокарда, острого нарушения мозгового кровообращения) в последующие 5 лет у больных ЭАГ II стадии. У 200 пациентов с ЭАГ II стадии с длительностью заболевания 10–14 лет, получавших сопоставимую гипотензивную терапию и показавших целевые уровни АД, с помощью ИФА в сыворотке периферической крови определяли содержание 28 цитокинов (IL1β, IL1α, IL1ra, IL18, IL18BP, IL37, IL6, sIL6r, LIF, sLIFr, IGF-1, IGFBP-1, TNFα, sTNF-RI, sVCAM-1, IL17, IL2, IL4, IL10, TGF-β1, IL8, CX3CL1, CXCL10, INFγ, M-CSF, IL34, VEGF-A, эритропоэтина) и вазоактивных пептидов (NO, iNOS, eNOS, ADMA, SDMA, Nt-proCNP, Nt-proBNP). В течение последующих 5 лет фиксировали случаи развития осложнений. Ретроспективный анализ показал, что для группы с развитием осложнений в последующие 5 лет характерно предварительное снижение уровней IL1ra ($p < 0,001$) и IL10 ($p < 0,001$) на фоне повышения содержания IL1β ($p < 0,001$), TNFα ($p < 0,001$) и M-CSF ($p < 0,001$) в сыворотке крови при сравнении с группой без осложнений. Многофакторный анализ с включением в регрессионную модель Кокса ряда показателей: IL1β > 18,8 пг/мл; IL1ra < 511 пг/мл; IL6 > 23,8 пг/мл; IL10 < 26,3 пг/мл; 389 пг/мл < M-CSF < 453 пг/мл; ADMA > 0,86 мкмоль/л; общий холестерин > 4,9 ммоль/л; ЛПНП > 3,0 ммоль/л; ЛПВП у мужчин < 1,0 ммоль/л; у женщин < 1,2 ммоль/л выявил независимый характер «влияния» на повышение частоты повреждения органов-мишеней при ЭАГ II стадии следующих показателей: содержания M-CSF в диапазоне 389–453 пг/мл ($p < 0,001$), а также уровня ЛПНП более 3,0 ммоль/л ($p < 0,01$). Изменение уровня цитокинов является патогенетически обоснованным предиктором повреждения миокарда и головного мозга у больных ЭАГ II стадии, а определение уровня M-CSF в крови может дополнить классические схемы расчета риска развития сердечно-сосудистых осложнений у данной категории больных.

Ключевые слова: цитокины, эссенциальная артериальная гипертензия, инфаркт миокарда, острое нарушение мозгового кровообращения, колониестимулирующий фактор макрофагов M-CSF

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Соблюдение этических стандартов: исследование одобрено этическим комитетом МГУ им. Н. П. Огарева (протокол № 12 от 14 декабря 2008); все пациенты подписали добровольное информированное согласие; получение биологического материала для исследования (кровь) производили с учетом положений Хельсинской декларации ВМА (2000) и протокола Конвенции Совета Европы о правах человека и биомедицине (1999).

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Essential hypertension (EH) is one of the most common modifiable risk factors for cardiovascular diseases and death. According to some estimates, more than 1 billion adults suffer from EH. Their number is expected to have reached 1.5 billion in 2025 [1]. It was long thought that end-organ damage resulted from the increased hydrostatic pressure against blood vessel walls. However, recently a few research studies have been conducted [2–10] into the cytokine pathways in EH complications, including myocardial infarction (MI), acute cerebrovascular events (ACVE), and transient ischemic attacks (TIA), that pose a significant risk of death and morbidity to the working-age population. It is hardly disputable that end-organ damage is the ultimate sequela of chronic inflammation in the presence of elevated blood pressure and atherosclerosis. However, it is still unclear whether certain cytokines have a prognostic potential in the assessment of long-term risks for cardiovascular disorders in patients with stage II EH. The aim of this study was to investigate a correlation between peripheral blood concentrations of some cytokines in patients with stage II EH and the rate of cardiovascular complications (MI, ACVE, TIA) developed during the 5-year observation period.

METHODS

The study was carried out at the facilities of the Regional Vascular Center of Mordovian Republican Clinical Hospital No. 4, Mordovian Republican Clinical Hospital No. 3 and the Department of Immunology, Microbiology and Virology of Ogariov Mordovian State University in 2013 through 2018. The study recruited 490 participants with stage II EH. Of them, 200 patients were prescribed comparable antihypertensive therapies and then followed up for 5 years. Among them were 100 females and 100 males with the mean age of 57.5 ± 1.2 years at the beginning of the study.

The following inclusion criteria were applied: stage II EH lasting for 10–14 years; working age (females under 60, males under 65); comparable treatment regimens prescribed (monotherapy with ACE inhibitors or ACEIs+ diuretics); informed consent to participate. Patients with comorbidities, type I diabetes mellitus, metabolic syndrome, allergies, autoimmune conditions, secondary hypertension, infections or mental health problems 1 month before the study, alcoholism/drug abuse, and those who refused to participate were excluded from the study. The following cytokines were measured in the peripheral blood samples of 100 female and 100 male patients with stage II EH in order to assess their potential in predicting long-term cardiovascular complications (MI and ACVE): IL1β, IL1α, IL1ra, IL18, IL18BP, IL37, IL6, sIL6r, LIF, sLIFr, IGF-1, IGFBP-1, TNFα, sTNF-RI, sVCAM-1, IL17, IL2, IL4, IL10, TGF-β1, IL8, CX3CL1, CXCL10, INFγ, M-CSF, IL34, VEGF-A, erythropoietin, and a group of vasoactive peptides including NO, iNOS, eNOS, ADMA, SDMA, Nt-proCNP, and Nt-proBNP. These proteins were selected because they are either synthetized by vascular cells or the end organs have receptors recognizing the listed cytokines. Their concentrations were determined using immunoassays. In the 5-year follow-up period, the patients were surveyed on the phone using the original questionnaire; complications (MY, ACVE, TIA) were noted down and later correlated to the patients' medical records.

The obtained data were processed in *Statistica* 8.0 (*StatSoft*; ver 8.0). Normality of data distribution was tested using the one-sample Kolmogorov-Smirnoff test. In this article, the data are presented as an arithmetic mean (M), a standard deviation (SD) and 95% confidence interval for the mean (95% CI) for normal distribution. Student's t-test was used to compare the main groups. Absolute and relative risks of end-organ damage (MI, ACVE, TIA) were computed, as well as 95% CI for sensitivity (Se) and specificity (Sp). Uni- and multivariate Cox regressions were calculated.

RESULTS

We correlated the levels of IL1β, IL1α, IL1ra, IL37, IL18, IL18BP, IL6, sIL6r, LIF, sLIFr, TNFα, sTNF-RI, sVCAM, IL2, IL8, IL4, IL10, IFNγ, IGF-1, IGFBP-1, M-CSF, IL34, VEGF-A, CX3CL1, CXCL10, TGFβ1, IL17A, and erythropoietin in the peripheral blood of 200 patients in treatment for stage II EH who had been suffering from the condition for 10-14 years to the development of cardiovascular complications during the 5-year follow-up period. The retrospective analysis revealed a decline in IL1ra ($p < 0.001$) and IL10 ($p < 0.001$) concentrations and a rise in IL1β ($p < 0.001$), TNFα ($p < 0.001$), and M-CSF ($p < 0.001$) levels in the group of 47 patients who developed cardiovascular complications in the follow-up period, including MI (20 patients), ACVE (14 patients) and TIA (13 patients), as compared to the group of patients who did no develop any long-term complications during the study (Table 1). No significant differences were observed in the average values of other parameters between the groups of patients with and without complications ($p > 0.05$).

Further analysis of interquartile ranges for the patients with EH who developed complications after 6–10 years of observation demonstrated (Table 2) that at IL1ra < 513 pg/ml, the rate of complications over those 5 years increased 2.24 times ($p < 0.05$) reaching 45% (Sp was 83%, Se was 41.7%), $\chi^2 = 5.25$ ($p < 0.05$), C = 0,33 (a moderate correlation); at IL10 < 26.3 pg/ml the risk grew 1.94 times ($p < 0.05$), reaching 31% (Sp 54.9%, Se 66%), $\chi^2 = 6.26$ ($p < 0.05$), C = 0.17 (a weak correlation); at IL1β > 18.7 pg/ml the risk increased 2.37 times ($p < 0.05$) reaching 38% (Sp 59%, Se 80%), $\chi^2 = 7.59$ ($p < 0.05$), C = 0.22 (a moderate correlation); at IL6 > 23.8 the risk grew 1.76 times ($p < 0.05$) reaching 30% (Sp 54%, Se 63.8%), $\chi^2 = 6.45$ ($p < 0.05$), C = 0.17 (a weak correlation). At M-CSF concentrations in the blood serum ranging from 389 to 453 pg/ml the risk of cardiovascular complications in year 5 and in the next 5 years grew 3.87-fold ($p < 0.001$), as compared to M-CSF concentrations < 389 pg/ml and > 453 pg/ml, reaching 62% (Sp 81.6%, Se 66%), $\chi^2 = 32.5$ ($p < 0.001$), C = 0.6 (a relatively strong correlation). M-CSF was the most significant risk predictor in comparison with other cytokines. The analysis of interquartile ranges for the patients who developed cardiovascular complications in years 6–10 of the observation did not reveal any significant correlations between TNFα concentrations measured in year 5 and the analyzed risks.

The univariate Cox regression analysis confirmed a reliable correlation between the changes in the peripheral blood cytokine concentrations in patients with stage II EH (IL1β > 18.8 pg/ml; IL1ra < 511 pg/ml; IL6 > 23.8 pg/ml; IL10 < 26.3 pg/ml; 389 pg/ml < M-CSF < 453 pg/ml) and the risk of cardiovascular disorders during the observation period (Table 3).

The analysis of relationships between all studied parameters and vasoactive peptides (Tables 4, 5) demonstrated that cytokines that could be regarded as potential long-term predictors of cardiovascular complications in patients with stage II EH, namely IL1ra, IL10, IL1β, TNFα, and M-CSF, correlate with the serum levels of asymmetric dimethylarginine (ADMA), a vasoactive peptide. Therefore, when building the pathogenic model, we added ADMA to the list of variables subjected to the multivariate regression analysis to assess the independence of

the revealed risk factors. This has a pathogenic significance and affects the diagnostic and clinical value of the recorded changes. M-CSF concentrations in the blood serum of patients with stage II EH have the strongest correlation with ADMA. The model subjected to the multivariate analysis also included classic risk factors as recommended by the international and Russian guidelines for the risk assessment of complications in patients with hypertension. Based on the results yielded by the multivariate analysis, increased rates of end-organ damage in patients with stage II EH reported during the 5-year observation period correlated with M-CSF levels in the range between 389 and 453 pg/ml ($p < 0.001$) regardless of patients' sex, as well as with the classic risk factor: LDP > 3.0 mmol/L ($p < 0.01$) (Table 6).

DISCUSSION

Significant correlations have been reported between EH complications, including ACVE and MI, and the peripheral blood concentrations of IL17, IFNγ, TNFα, IL6 [2, 11], sTNF-RI [9], IL1 [12, 13], CXC chemokines [6], LIF [14], IL12 [15], and other cytokines. However, there have been few longitudinal studies of this problem, and only a small range of cytokines has been analyzed. Considering that the literature on the role of cytokines in the pathogenesis of EH is scarce, it is important to study the dynamics of these immunoregulatory factors in patients who develop EH complications and to identify factors that maintain their statistical and pathogenic significance when other cytokines or classic risk factors are introduced into the pathogenic model. We have found that changes in IL1ra, IL10, IL1β, IL6, and M-CSF levels correlate with the development of complications (MI, ACVE, TIA) during a 5-year period. In our study, the increase in the concentrations of anti-inflammatory IL1ra and IL10 correlated with a lower number of complications. Correlations between other analyzed cytokines and the risk for EH complications were not observed. Out findings are not fully consistent with the literature reporting correlations between LIF, IL1α, etc. and the risk for developing EH complications [10, 14]. This could be due to different inclusion criteria applied in different studies (the sex ratio, age, antihypertensive

Table 1. Cytokine levels in patients in treatment for stage II EH who developed cardiovascular complications during the 5-year follow-up period and those who did not have any complications. M (σ)

Cytokine levels (pg/ml)	Patients without complications $n = 153$	Patinates with EH and complications $n = 47$
IL1β	14.2 (4.42)	21.3 (3.32)*no complications
IL1α (f)	12.6 (3.21)	13.2 (2.91)
IL1ra	650 (112)	496 (93)*no complications
IL18	360 (64)	393 (87)
IL18BP	6790 (1170)	6440(1620)
IL6	21.7 (4.94)	24.9 (4.41)^no complications
sIL6r	1889 (323)	1733 (312)
TNFα	20. 2 (4.47)	26.6 (4.5)*no complications
sTNF-RI	2598 (680)	2873 (699)
sVCAM-1	577 (101)	591 (90)
IL2	10.6 (3.16)	10.9 (3.02)
IL8	28.7 (6.74)	30.6 (7.16)
IL4	19.8 (4.11)	20.8 (4.05)
IL10	29.3 (6.99)	23.8 (7.17)*no complications
IFNγ	18.4 (4.18)	18.1 (4.39)
IL37	93.2 (26.9)	90.1 (24.2)
IL17A	2.5 (0.56)	2.46 (0.49)
LIF (females)	7.28 (2.78)	7.76 (2.63)
sLIFr (females)	40500 (1120)	42100 (1600)
IGF-1	116000 (32300)	122000 (30800)
M-CSF	352 (88)	456 (69)*no complications
IL34	133 (40)	137 (36)
VEGF-A	339 (101)	344 (95)
CX3CL1	510 (105)	542 (120)
CXCL10	17.8 (4.33)	18.9 (3.92)
TGFβ1	21.8 (4.57)	22.1 (4.24)
Neopterin	8.81 (3.19)	8.23 (2.8)
Erythropoietin	11.4 (3.64)	16.6 (3.12)*no complications

Note: ^ — $p < 0.01$; * — $p < 0.001$ in comparison with patients without complications reported in the 5-year follow-up period.

treatment, normalized blood pressure, etc) and indicates the high significance of the observational group homogeneity, which affects the pathogenic significance of the obtained data. Importantly, our study shows that only cytokines correlating with ADMA levels can be regarded as potential predictors of end-organ damage in patients undergoing treatment against stage II EH and suffering from the pathology for 10–14 years. ADMA is a methylated analog of L-arginine (a substrate for NO synthesis) that competitively inhibits the functional activity of eNOS [16], curbing the NO synthesis and leading to its poor availability for vasorelaxation and vasoprotection [17]. This pathogenic pathway is important: the role of ADMA and SDMA in pathology is currently in the focus of scientific research. This study established that M-CSF was the only independent criterium (from the entire spectrum of the analyzed parameters) that had a high predictive value (even when compared to

Table 2. The relationship between changes in IL1ra, IL1β, IL6, TNFα, M-CSF, and IL10 concentrations measured at the beginning of the study AND the rate of complications (95% CI) during the 5-year follow-up period in patients in treatment for long-settled EH

	Quartile I	Quartile II	Quartile III	Quartile VI
	n = 50	*n* = 50	*n* = 50	*n* = 50
IL1ra	(330–511)	(512–575)	(574–633)	(634–820)
Complications (number of patients)	18	9	10	10
Absolute risk (%)	36 [22.7–49.3]	18 [7.35–28.6]	20 [8.91–31]	20 [8.91–31]
	36 [22.7–49.3]	19.3 [13–25.6]		
Risk ratio	Quartiles I / II + III + IV: 1.86 [1.13–3.05]*			
IL1β	(2.95–14.8)	(14.9–8.7)	(18.8–22.5)	(22.6–34.4)
Complications (number of patients)	1	8	18	20
Absolute risk (%)	2 [2.43–4.43]	16 [5.84–26.2]	36 [22.7–49.3]	40 [26.4–53.6]
			38 [28.5–47.5]	
Risk ratio	Quartiles II / III + IV: 2.37 [1.2 – 4.7]*			
Risk ratio	Quartiles I / II + III + IV: 1.86 [1.13–3.05]*			
IL6	(12.5–20.8)	(20.9–23.7)	(23.8–27.5)	(27.6–36.4)
Complications (number of patients)	8	9	14	16
Absolute risk (%)	16 [5.8–26]	18 [7.35–28.6]	28 [15.6–40]	32 [19–45]
	17 [8–22.1]		30 [21–39]	
Risk ratio	Quartiles I + II / III + IV: 1.76 [1.04 – 2.99]*			
TNFα	(10.3–18.3)	(18.6–23.7)	(21.4–24)	(24.1–32.4)
Complications (number of patients)	10	13	12	12
Absolute risk (%)	20 [8.9–31]	26 [13.8–38]	24 [12.2–35.8]	24 [12.2–35.8]
Risk ratio	Quartiles I / II: 1.3 [0.63–2.69]		Quartiles II / III + IV: 0.92 [1.04–1.65]	
M-CSF	(138–319)	(320–388)	(389–453)	(454–640)
Complications (number of patients)	0	7	31	9
Absolute risk (%)	0	14 [4.42–23.6]	62 [48.5–55.4]	18 [7.55–28.6]
Risk ratio	Quartiles II + IV / III: 3.87 [2.35–6.38]* Quartiles II/IV = 1.28 [0.52–3.18]			
IL10	(5.1–19.9)	(20–26.2)	(26.3–31.5)	(31.6–47.5)
Complications (number of patients)	15	16	8	8
Absolute risk (%)	30 [19–45]	32 [15.6–40]	16 [5.84–26.2]	16 [5.84–26.2]
	31 [22–40]		16 [5.84–26.2]	
Risk ratio	Quartiles I + II / III + IV: 1.94 [1.13–3.31]*			

Note: * — $p < 0.05$ for the comparison of absolute risks if the interval does not include 1.

Table 3. Correlations between IL1β, IL1ra, IL6, IL10, and M-CSF concentrations AND the rate of cardiovascular complications (95% CI) during the 5-year observation period in patients with stage II EH. The univariate Cox regression

Variables	Beta	Standard	*t*-value	Exponent Beta	*p*
IL1β (> 18.8 pg/ml)	1.99	0.35	5.68	2.37	0.006
IL1ra (< 511 pg/ml)	1.24	0.28	4.43	2.06	0.009
IL6 (> 23.8 pg/ml)	1.27	0.35	3.62	1.89	0.042
IL10 (< 26.3 pg/ml)	1.22	0.27	4.52	1.91	0.007
M-CSF (389–453) pg/ml	2.44	0.25	9.76	3.89	0.0004

Note: in our Cox regression models, Beta is a regression coefficient; Standard is a standard error of the regression coefficient; *t*-value is the *t*-statistic; Exponent Beta is the value of the relative risk indicating a connection with the range of changes in the analyzed factor; *p* shows statistical significance.

ADMA and classic risk factors) in the assessment of risks for developing ACVE, MI and TIA in patients undergoing treatment for stage II EH who had been suffering from this condition for 10 to 14 years. This confirms the priority of the cytokine in the M-CSF–ADMA correlational pathogenic model with a subsequent cascade of reactions causing progression of the pathology. Earlier, we published an article demonstrating a direct correlation between M-CSF concentrations > 453 pg/ml and the levels of VEGF-A in the peripheral blood, which was consistent with a significant increase in the myocardial collateral blood flow (coronary angiography) and might explain a low rate of MI in the studied cohort of patients [18], affecting the total risk for cardiovascular complications. M-CSF can activate MAP-kinases via the M-CSFR-1 receptor; the kinases play a key role in the production of VEGF-A by activating ERK, increasing the p38 and JNK promoter activity and stabilizing VEGF-A mRNA in a dose-dependent pattern [19].

CONCLUSIONS

The data yielded by this study prove that changes in the cytokine concentrations (IL1β > 18.8 pg/ml, IL1ra < 511 pg/ml, IL6 > 23.8 pg/ml, IL10 < 26.3 pg/ml) measured in the peripheral blood of patients suffering from stage II EH for 10 to 14 years and undergoing antihypertensive treatment correlate with a 5-year rate of cardiovascular complications (MI, ACVE, TIA). Only M-CSF at concentrations between 389 and 453 pg/ml can be regarded as a predictor of cardiac and cerebrovascular

Table 4. A correlation matrix for the cytokines in the peripheral blood of patients in treatment for stage II EH and the vasoactive peptides measured in the same patients

	IL37	LIF	sLIFr	IGF-1	IGFBP-1	M-CSF	IL34	VEGF-A	CX3CL1	CXCL10	TGFβ1	IL17A	Erythropoietin
AT II	-0.28 $p > 0.05$	0.51 $p < 0.05$	-0.36 $p > 0.05$	0.23 $p > 0.05$	0.23 $p > 0.05$	0.34 $p > 0.05$	0.24 $p > 0.05$	0.29 $p > 0.05$	0.12 $p > 0.05$	0.31 $p > 0.05$	0.23 $p > 0.05$	0.21 $p > 0.05$	0.23 $p > 0.05$
ET-1	-0.41 $p > 0.05$	0.31 $p > 0.05$	0.28 $p > 0.05$	0.21 $p > 0.05$	0.22 $p > 0.05$	0.43 $p > 0.05$	0.21 $p > 0.05$	0.42 $p > 0.05$	0.27 $p > 0.05$	0.35 $p > 0.05$	0.31 $p > 0.05$	0.23 $p > 0.05$	0.32 $p > 0.05$
NO	0.69 $p < 0.05$	0.42 $p > 0.05$	0.41 $p > 0.05$	0.23 $p > 0.05$	0.18 $p > 0.05$	0.32 $p > 0.05$	0.31 $p > 0.05$	0.34 $p > 0.05$	0.31 $p > 0.05$	0.39 $p > 0.05$	0.48 $p > 0.05$	0.21 $p > 0.05$	0.23 $p > 0.05$
ADMA	-0.32 $p > 0.05$	0.33 $p > 0.05$	0.49 $p > 0.05$	0.28 $p > 0.05$	0.45 $p > 0.05$	0.58 $p < 0.05$	0.24 $p > 0.05$	0.28 $p > 0.05$	0.22 $p > 0.05$	0.15 $p > 0.05$	0.34 $p > 0.05$	0.31 $p > 0.05$	0.32 $p > 0.05$
SDMA	-0.78 $p < 0.001$	0.28 $p > 0.05$	0.45 $p > 0.05$	0.38 $p > 0.05$	0.27 $p > 0.05$	0.52 $p < 0.05$	0.41 $p > 0.05$	0.31 $p > 0.05$	0.52 $p < 0.05$	0.5 $p < 0.05$	0.26 $p > 0.05$	0.31 $p > 0.05$	0.21 $p > 0.05$
eNOS	0.32 $p > 0.05$	0.38 $p > 0.05$	-0.39 $p > 0.05$	0.21 $p > 0.05$	0.34 $p > 0.05$	0.31 $p > 0.05$	0.24 $p > 0.05$	0.11 $p > 0.05$	-0.18 $p > 0.05$	-0.31 $p > 0.05$	0.23 $p > 0.05$	0.32 $p > 0.05$	0.34 $p > 0.05$
iNOS	-0.41 $p > 0.05$	0.28 $p > 0.05$	0.45 $p > 0.05$	0.34 $p > 0.05$	0.38 $p > 0.05$	0.17 $p > 0.05$	0.21 $p > 0.05$	0.21 $p > 0.05$	0.69 $p < 0.01$	0.71 $p < 0.01$	0.34 $p > 0.05$	0.21 $p > 0.05$	0.21 $p > 0.05$
NT-proCNP	-0.27 $p > 0.05$	0.41 $p > 0.05$	-0.28 $p > 0.05$	0.27 $p > 0.05$	0.37 $p > 0.05$	0.41 $p > 0.05$	0.25 $p > 0.05$	0.32 $p > 0.05$	-0.31 $p > 0.05$	-0.27 $p > 0.05$	0.52 $p < 0.05$	0.23 $p > 0.05$	0.24 $p > 0.05$
NT-proBNP	-0.78 $p < 0.01$	-0.65 $p < 0.05$	0.31 $p > 0.05$	0.31 $p > 0.05$	0.24 $p > 0.05$	0.45 $p > 0.05$	0.18 $p > 0.05$	0.34 $p > 0.05$	0.21 $p > 0.05$	0.2 $p > 0.05$	0.33 $p > 0.05$	0.27 $p > 0.05$	0.12 $p > 0.05$

Note: the data are presented as a coefficient of multiple correlation; the minus symbol indicates that the established correlation is negative; *p* shows the significance of differences.

Table 5. A correlation matrix for the cytokines in the peripheral blood of patients in treatment for stage II EH and the vasoactive peptides measured in the same patients

	IL1β	IL1α	IL1ra	IL18	IL18BP	IL6	sIL6r	TNFα	sTNF-RI	sVCAM-1	IL2	IL8	IL4	IL10	IFNγ
AT II	0.41 $p > 0.05$	0.34 $p > 0.05$	-0.19 $p > 0.05$	0.41 $p > 0.05$	-0.23 $p > 0.05$	0.43 $p > 0.05$	0.33 $p > 0.05$	0.41 $p > 0.05$	0.22 $p > 0.05$	0.21 $p > 0.05$	0.18 $p > 0.05$	0.37 $p > 0.05$	0.22 $p > 0.05$	-0.62 $p < 0.05$	0.16 $p > 0.05$
ET-1	0.68 $p < 0.05$	0.65 $p < 0.05$	-0.62 $p < 0.05$	0.34 $p > 0.05$	-0.36 $p > 0.05$	0.27 $p > 0.05$	0.41 $p > 0.05$	0.36 $p > 0.05$	0.24 $p > 0.05$	0.31 $p > 0.05$	0.25 $p > 0.05$	0.69 $p < 0.01$	0.24 $p > 0.05$	-0.36 $p > 0.05$	0.18 $p > 0.05$
NO	0.64 $p < 0.05$	0.46 $p > 0.05$	0.49 $p < 0.05$	-0.27 $p > 0.05$	0.64 $p < 0.05$	0.49 $p > 0.05$	0.33 $p > 0.05$	0.44 $p > 0.05$	-0.38 $p > 0.05$	0.25 $p > 0.05$	0.37 $p > 0.05$	-0.41 $p > 0.05$	-0.38 $p > 0.05$	0.33 $p > 0.05$	0.41 $p > 0.05$
ADMA	0.52 $p < 0.05$	0.4 $p > 0.05$	-0.58 $p < 0.05$	0.36 $p > 0.05$	-0.41 $p > 0.05$	0.57 $p < 0.05$	0.25 $p > 0.05$	0.38 $p > 0.05$	0.31 $p > 0.05$	0.38 $p > 0.05$	0.33 $p > 0.05$	0.27 $p > 0.05$	0.31 $p > 0.05$	-0.55 $p < 0.05$	0.24 $p > 0.05$
SDMA	0.34 $p > 0.05$	0.29 $p > 0.05$	-0.16 $p > 0.05$	0.48 $p > 0.05$	-0.71 $p < 0.01$	0.29 $p > 0.05$	0.23 $p > 0.05$	0.26 $p > 0.05$	0.25 $p > 0.05$	0.43 $p > 0.05$	0.72 $p < 0.01$	0.53 $p < 0.05$	0.25 $p > 0.05$	-0.23 $p > 0.05$	0.58 $p < 0.05$
eNOS	-0.62 $p < 0.05$	-0.67 $p < 0.05$	0.51 $p < 0.05$	0.22 $p > 0.05$	0.37 $p > 0.05$	-0.22 $p > 0.05$	-0.35 $p > 0.05$	0.4 $p > 0.05$	0.27 $p > 0.05$	0.31 $p > 0.05$	-0.13 $p > 0.05$	-0.31 $p > 0.05$	0.27 $p > 0.05$	0.46 $p > 0.05$	-0.19 $p > 0.05$
iNOS	0.78 $p < 0.01$	0.49 $p > 0.05$	-0.12 $p > 0.05$	0.36 $p > 0.05$	-0.39 $p > 0.05$	0.68 $p < 0.05$	0.56 $p < 0.05$	0.42 $p > 0.05$	0.41 $p > 0.05$	0.41 $p > 0.05$	0.62 $p < 0.05$	0.42 $p > 0.05$	0.41 $p > 0.05$	-0.4 $p > 0.05$	0.52 $p < 0.05$
NT-proCNP	-0.58 $p < 0.05$	0.68 $p < 0.05$	0.13 $p > 0.05$	0.31 $p > 0.05$	-0.37 $p > 0.05$	-0.61 $p < 0.05$	0.24 $p > 0.05$	0.52 $p < 0.05$	0.39 $p > 0.05$	0.24 $p > 0.05$	-0.74 $p < 0.01$	-0.37 $p > 0.05$	0.39 $p > 0.05$	0.29 $p > 0.05$	-0.64 $p < 0.05$
NT -proBNP	0.47 $p > 0.05$	0.29 $p > 0.05$	-0.31 $p > 0.05$	0.14 $p > 0.05$	-0.73 $p < 0.01$	0.17 $p > 0.05$	-0.12 $p > 0.05$	0.51 $p < 0.05$	0.24 $p > 0.05$	0.32 $p > 0.05$	0.32 $p > 0.05$	0.21 $p > 0.05$	0.24 $p > 0.05$	-0.22 $p > 0.05$	0.32 $p > 0.05$

Note: the data are presented as a coefficient of multiple correlation; the minus symbol indicates that the established correlation is negative; *p* shows the significance of differences.

Table 6. Correlations between IL1β, IL1ra, IL6, IL10, M-CSF, ADMA concentrations and classic risk factors AND the rate of cardiovascular complications (95% CI) in a 5-year observation period in patients with stage II EH; the table shows the Cox regression model; the multivariate analysis was applied

Variables	Beta	Standard	*t*-value	Exponent Beta	*p*
IL1β (> 18.8 pg/ml)	1.19	0.73	1.63	2.05	0.058
IL1ra (< 511 pg/ml)	1.04	0.62	1.67	1.34	0.067
IL6 (> 23.8 pg/ml)	1.07	0.63	1.69	2.17	0.062
IL10 (< 26.3 pg/ml)	1.06	0.66	1.61	1.32	0.072
M-CSF (389–453) pg/ml	2.17	0.34	6.38	2.53	0.0007
ADMA (> 0.86 μmol/l)	1.49	0.77	1.93	2.09	0.068
Total cholesterol > 4.9 mmol/l	1.18	0.73	1.62	1.63	0.062
LDL > 3.0 mmol/l	1.88	0.43	4.37	2.28	0.004
HDL in men < 1.0 mmol/l. in women < 1.2 mmol/l	1.12/1.19	0.71/0.68	1.58	1.38/1.32	0.071/0.069

Note: in our Cox regression models, Beta is a regression coefficient; Standard is a standard error of the regression coefficient; *t*-value is the *t*-statistic; Exponent Beta is the value of the relative risk indicating a connection with the range of changes in the analyzed factor; *p* shows statistical significance.

complications. Although the obtained data have a theoretical significance, M-CSF concentrations in the range from 389 to 453 pg/ml are a highly specific (81%) but lowly sensitive (66%) parameter in terms of predicting MI, ACVE and TIA in the studied cohort of patients. This means that an additional criterium should be added to the model to improve the diagnostic (prognostic) value of this cytokine. The role of patients' individual characteristics, such as genetic components including CSF1R TC/CA rs386693509: TC/CA variants in the established correlations should be further studied in patients with the progredient disease.

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