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The change in the circadian rhythm of macrophage colony-stimulating factor content in the blood of patients with essential hypertension

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ABSTRACT

The purpose of this research is to study the characteristics of the change in the circadian rhythm of macrophage colony-stimulating factor (M-CSF) content in the peripheral blood serum of patients with stage II essential hypertension (EH) based on 5 time points (8:00, 14:00, 20:00, 2:00, and 8:00) and analyze its connection with the frequency of cardiovascular events.

Materials and methods: Identified levels of M-CSF in the peripheral blood serum of 60 patients with stage II EH, before and after 1 year of antihypertensive therapy using enzyme-linked immunoassays (at 8:00, 14:00, 20:00, 2:00, and 8:00).

Results: The research demonstrated that stage II EH patients with a medical case history lasting 10–14 years have a greater content of M-CSF in their peripheral blood serum ($p > 0.001$). Before the start of antihypertensive therapy, they also have an increased variability in the circadian rhythm of M-CSF content in the bloodstream (when compared with healthy individuals) due to an increase at 20:00, decrease at 2:00 and recovery at 8:00. In 70% of those patients taking antihypertensive medication and have reached their target arterial blood pressure, the cytokine decrease stabilizes at 2:00 but the increase at 20:00 remains unchanged. Thirty percent of patients retained the rhythm characteristics of M-CSF content in the blood serum typical of patients before the start of therapy. This is a predictor of an increase in the five-year risk of developing cardiovascular complications, particularly myocardial infarction and acute cerebrovascular accident, in individuals with a comparable risk of cardiovascular complications or death on the Framingham risk score.

1. Introduction

Contradictory data about the role of macrophage colony-stimulating factor (M-CSF) in the development of cardiovascular diseases, in particular, the formation of myocardial infarction (MI) and acute disturbed cerebral circulation with underlying elevated blood pressure, has been published in literature. Schiopu A. et al. (2016) determined that high levels of M-CSF and MCP-1 in plasma correlate with a low risk of developing a MI [1]. At the same time, other studies [2,3] demonstrate a direct relation between the M-CSF content in peripheral blood serum and the frequency of myocardial infarction and acute disturbed cerebral circulation in people suffering from cardiovascular diseases. The researchers stress that the role of M-CSF in cardiovascular diseases is complex and possibly depends on additional factors, whose identification is topical. Recent studies have emphasized the importance of a 24-hour daily cycle, called the Circadian Cycle, in relation to the body's immune function and its influence on the development of cardiovascular disease [4]. A demonstrated relationship between the frequency of cardiovascular complications and the time of day suggests a hypothesis regarding the presence of circadian immune markers, in particular M-CSF, that determine the progression of EH. The purpose of this research is to study the change characteristics in the circadian rhythm of M-CSF content in the peripheral blood serum of patients with stage II EH based on 5 time points (8:00, 14:00, 20:00, 2:00, and 8:00) and analyze their connection with the frequency of cardiovascular events (MI, acute cerebrovascular accident (ACA)).

2. Materials and methods

The study on “Cytokines in the Pathogenesis of EH” was carried out

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Table 1
The content of M-CSF (pg/ml) in the peripheral blood serum of stage II EH patients at 8:00, 14:00, 20:00, 2:00 and 8:00 (the next day), depending on the use of antihypertensive therapy and the progression of cardiovascular complications over the subsequent 5 years of observation (Me (Q25%-Q75%)).

Groups		8:00 (day 1)	14:00 (day 1)	20:00 (day 1)	2:00 (day 1)	8:00 (day 2)	p
1		2	3	4	5	6	7
EH Patients (before therapy) n = 60 (individuals)	a	474 [406–549]	489 [410–559]	551 [487–683]*2,3	356 [314–422]*2,3,4	473 [407–556]*4,5	$p_{3-2} = 0.63$; $p_{4-2} = 0.002$; $_{p4-3=0.007;}$ $p_{5-2} < 0.001$; $p_{5-3} < 0.001$; $p_{5-4} < 0.001$; $p_{6-2} = 0.99$; $p_{6-3} = 0.32$; $p_{6-4} = 0.002$; $p_{6-5} < 0.001$
Comparison Group n = 29 (individuals)	b	209 [174–236]	209 [178–241]	206 [187–226]	203 [177–228]	202 [168–234]	$p_{3-2} = 0.45$; $p_{4-2} = 0.44$; $p_{4-3} = 0.45$; $p_{5-2} = 0.32$; $p_{5-3} = 0.27$; $p_{5-4} = 0.35$; $p_{6-2} = 0.46$; $p_{6-3} = 0.48$; $p_{6-4} = 0.48$; $p_{6-5} = 0.36$
		$*p_{b-a} < 0.001$	$*p_{b-a} < 0.001$	$*p_{b-a} < 0.001$	$*p_{b-a} < 0.001$	$*p_{b-a} < 0.001$	
EH Patients (after 1 year of therapy) n = 60 (individuals), of which:	c	398 [320–480]	396 [311–477]	450 [351–570]	375 [318–456]*4	398 [332–463]	$p_{3-2} = 0.44$; $p_{4-2} = 0.018$; $p_{4-3} = 0.014$; $p_{5-2} = 0.13$; $p_{5-3} = 0.17$; $p_{5-4} = 0.005$; $p_{6-2} = 0.36$; $p_{6-3} = 0.42$; $p_{6-4} = 0.023$; $p_{6-5} = 0.11$
		$*p_{c-a} = 0.002$	$*p_{c-a} < 0.001$	$*p_{c-a} < 0.001$	$p_{c-a} = 0.46$	$*p_{c-a} = 0.003$	
		$*p_{c-b} < 0.001$	$*p_{c-b} < 0.001$	$*p_{c-b} < 0.001$	$*p_{c-b} < 0.001$	$*p_{c-b} < 0.001$	
Subsequent 5 years of observation - MI, ACA n = 15 (individuals)	c1	524 [481–591]	533 [474–603]	641 [560–730]*2,3	420 [375–477]*2,3,4	530 [486–588]*4,5	$p_{3-2} = 0.49$; $p_{4-2} = 0.005$; $_{p4-3} = 0.004$; $p_{5-2} = 0.005$; $p_{5-3} < 0.001$; $p_{5-4} < 0.001$; $p_{6-2} = 0.5$; $p_{6-3=}0.49$; $p_{6-4=}0.005$; $p_{6-5} < 0.001$
Subsequent 5 years - no complications n = 45 (individuals)	c2	356 [305–413]	353 [298–414]	409 [321–489]	359 [293–422]	350 [310–397]	$p_{3-2} = 0.43$; $p_{4-2} = 0.041$; $_{p4-3} = 0.037$; $p_{5-2} = 0.29$; $p_{5-3} = 0.24$; $p_{5-4} = 0.008$; $p_{6-2} = 0.42$; $p_{6-3} = 0.36$; $p_{6-4} = 0.049$; $p_{6-5} = 0.36$
		$*p_{c2-c1} < 0.001$	$*p_{c2-c1} < 0.001$	$*p_{c2-c1} < 0.001$	$*p_{c2-c1} < 0.001$	$*p_{c2-c1} < 0.001$	

Note: conclusive when compared with the specified group (Bonferroni correction) - $p < 0.007$ - *.

Table 2
The deviation percentage of M-CSF (pg/ml) levels in the peripheral blood serum of stage II EH patients at 14:00, 20:00, 2:00 and 8:00 (the next day) from the 8:00 concentration on the first observation day, depending on the use of antihypertensive therapy and the progression of cardiovascular complications during the subsequent 5 years of observation (Me (Q25% -Q75%)).

Groups			8:00 (day 1)	14:00 (day 1)	20:00 (day 1)	**2:00 (day 1)**	8:00 (day 2)
1			2	3	4	5	6
Patients (before therapy) n = 60 (individuals)	a	–	1.89 [0.81–3.4]		17.2 [14.7–21.5]*[3]	–22 [− 26.7 − (− 18.6)]*[3,4]	0.38 [− 2.11 to 2.5]*[4,5]
Comparison Group – n = 29 (individuals)	b	–	1.31 [− 2.39 to 4.69]		1.44 [− 4.1 to 6.82]	−1.03 [− 3.12 to 4.06]	−1.17 [− 3.95 to 2.6]
			$p_{b\text{-}a} > 0.05$		*$p_{b\text{-}a} < 0.001$	*$p_{b\text{-}a} < 0.001$	$p_{b\text{-}a} > 0.05$
Patients (after 1 year of therapy) – n = 60 (individuals), of which:	c	–	1.03 [− 0.58 to 2.66]		16 [13.8–19.1]*[3]	−1.65 [− 3.46 to 2.48]*[4]	0.37 [− 1.6 to 2.04]*[4]
			$p_{c\text{-}a} > 0.05 - *p_{c\text{-}b} > 0.05$		$p_{c\text{-}a} > 0.05 - *p_{c\text{-}b} < 0.001$	*$p_{c\text{-}a} < 0.001 - p_{c\text{-}b} > 0.05$	$p_{c\text{-}a} > 0.05 - p_{c\text{-}b} > 0.05$
Subsequent 5 years of observation - MI, ACA – n = 15 (individuals)	c1	–	0.11 [− 2.24 to 2.19]		19.2 [17.9–20.6]*[3]	−21.2 [− 24 − (− 18.4)]*[3,4]	0.22 [− 81.35 to 2.33]*[4,5]
			$p_{c1\text{-}a} > 0.05 - *p_{c1\text{-}b} > 0.05$		$p_{c1\text{-}a} > 0.05 - *p_{c1\text{-}b} < 0.001$	$p_{c1\text{-}a} > 0.05 - *p_{c1\text{-}b} < 0.001$	$p_{c1\text{-}a} > 0.05 - p_{c1\text{-}b} > 0.05$
Subsequent 5 years – no complications n = 45 (individuals)	c2	–	−0.79 [− 2.08 to 2.53]		11.8 [9.81–15.9]*[3]	1.29 [− 0.65 to 3.71]*[4]	1.12 [− 1.03 to 2.43]*[4]
		–	$p_{c2\text{-}c1} > 0.05 - p_{c2\text{-}b} > 0.05 - p_{c2\text{-}a} > 0.05$		*$p_{c2\text{-}c1} < 0.01 - *p_{c2\text{-}b} < 0.001 - *p_{c2\text{-}a} < 0.001$	*$p_{c2\text{-}c1} < 0.001 - p_{c2\text{-}b} > 0.05 - *p_{c2\text{-}a} < 0.001$	$p_{c2\text{-}c1} > 0.05 - p_{c2\text{-}b} > 0.05 - p_{c2\text{-}a} > 0.05$

Note: conclusive when compared with the specified group (Bonferroni correction) - $p < 0.007$ - *.

during the period from 2008 to 2018 at the Immunology, Microbiology, and Virology Department in the Institute of Medicine at N.P. Ogariov Mordovia State University, Regional Vascular Center in the SBHI RM Republican Clinical Hospital No. 4. In 2013, an additional group of 60 stage II EH patients (30 men and 30 women) was formed to study the change in M-CSF content in peripheral blood serum over the course of 24 h.

Inclusion criteria: stage II EH, disease duration of 10–14 years, not on antihypertensive medication at the time of enrollment into the study, comparable antihypertensive therapy over the course of the following year (ACE inhibitor ± diuretic), born in 1955 or 1956, comparable levels of total cholesterol (< 5.0 mmol/L), LDL < 3.0 mmol/L, HDL > 1.0 mmol/L, triglycerides < 1.7 mmol/L, glucose < 5.5 mg/dL, BMI < 25 kg/m^2, patient's signed informed consent.

Exclusion criteria: associated clinical conditions (MI, ACA and more), total cholesterol (> 5.0 mmol/L), LDL > 3.0 mmol/L, HDL < 1.0 mmol/L, triglycerides > 1.7 mmol/L, glucose > 5.5 mg/dL, BMI > 25 kg/m2, diabetes mellitus types I and II, autoimmune and allergic diseases, secondary hypertension or patient's refusal to participate in a long-term study.

The comparison group is a conditionally healthy 30 people (15 men and 15 women) with an systolic blood pressure ranging from 100 to 120 mmHg and a diastolic blood pressure of 70–89 mmHg, comparable in age and key biochemical parameters with the observation group.

Biological material for the study (blood) was obtained in accordance with the provisions of the WMA Declaration of Helsinki (2000) and the protocol of the Council of Europe Convention on Human Rights and Biomedicine (1999). This study was approved by the ethical committee of Mordovia State University (protocol No. 12 on 14 December 2008). All patients signed a voluntary informed consent. Blood sampling was performed twice (before the beginning of antihypertensive therapy and after 1 year of therapy) at 8:00, 14:00, 20:00, 2:00, and 8:00 (an interval of no food intake for at least 6 h. M-CSF detection in the blood serum was performed at the laboratory of the Department of Immunology (Russian Federation perpetual license No. 13.01.04. 0001. L.000005.06.11, dated 23 June 2011) using R&D Systems (USA) enzyme-linked immunoassay test systems with a measurement range of 47.3–5000 pg/ml on the Personal Lab TM (Adaltis, Italy) immunoassay analyzer. We utilized the annual "telephone survey" method on the research participants to confirm the absence of complications (MI, ACA, transient disturbed cerebral circulation) during the observation, with a diagnosis confirmation based on medical history data: clinical and diagnostic, such as electrocardiogram, echocardiogram, troponins level test, computerized brain imaging.

Statistical data was processed using the application packages StatSoft Inc. 10.0 (USA). The distribution normality of the values was determined using the Kolmogorov-Smirnov one-sample test. The data were presented as a median (Me) and percentile ($Q_{0.25}$-$Q_{0.75}$). The normality determination of the data distribution justified the use of the paired student *t* test within the patient group with stage II EAH before therapy, the Wilcoxon test within the group "after 1 year of therapy" and the comparison group, and, between the groups, the Mann-Whitney test (in independent samples) and the Wilcoxon test (dependent samples) to compare the results at 8:00, 14:00, 20:00, 2:00, and 8:00. When dividing groups into subgroups, researchers used the Bonferroni correction for multiple comparisons, which confirmed the validity of the statistical analysis data specified in the article if $p < 0{,}007$. Absolute and relative risks of end-organ damage (MI, ACA) with 95% CI. Four-field tables were analyzed using Fisher's exact criteria (two-sided), φ, and the normalized value of the Pearson coefficient (C').

3. Results and discussion

Significant quantitative and qualitative variations in the circadian rhythm of M-CSF content were recorded in the peripheral blood serum

from stage II EH patients, with a disease history of 10–14 years and no antihypertensive medication, when compared with healthy individuals (the comparison group). M-CSF levels at 8:00, 14:00, 20:00 and 2:00 are 1.5–2.5 times higher ($p < 0.001$) in patients with stage II EH than in those without EH (Table 1). The growth of M-CSF by 17.2% ($p < 0.001$) at 20:00 and the 22% ($p < 0.001$) decrease at 2:00 are significant when compared with the results at 8:00 and 14:00 (Table 2). In the healthy group, the values at 8:00, 14:00, 20:00 and 2:00 did not vary ($p > 0.05$). Experimental literature indicates that one of the earliest responses to the effects of M-CSF is the stimulation of Na+/K+ metabolism, an increase in the activity of Na+ [5] and apoptosis of the smooth muscle cells of the vascular wall, with a synthesis imbalance of type III MMPs [6,7]. This affects the morphological stage of arteriogenesis of medium-sized arteries (progression of EH). According to our data [8], the change in the circadian rhythm of M-CSF content in blood serum is linked to the development of a pathologic blood pressure circadian rhythm («Non – dipper» и «Night-peaker»). In addition, M-CSF growth determines the increase of monocytes/macrophages in the periphery (macrophage recruitment is a critical step in the pathogenesis of hypertension [9]), with a potential accumulation of macrophages in the adipose tissue and an adipokine synthesis imbalance [10], which determines the progression of EH-related metabolic disorders.

Protective effects were recorded after 1 year of antihypertensive medication: a decrease in M-CSF at 8:00, 14:00, and 20:00 in the blood serum of patients with stage II EH when compared with a period without therapy ($p < 0.01$), but concentrations remain higher than in the healthy group ($p < 0.001$). There is no cytokine decrease at 2:00, characteristic of the “before therapy” stage, but maintains the growth at 20:00 (Tables 1 and 2). Eighteen patients who were taking antihypertensive medication and had reached their target blood pressure retained the circadian rhythms of M-CSF content in the blood characteristic of the “before antihypertensive therapy” stage (peak at 20:00, decrease at 2:00) (Table 2). In the subsequent 5 years of observation, in this group 13 out of 18 patients registered complications (MI – 8, ACA − 6). The absolute risk is equal to 72.2% [51.5–92.9]. In in the group under the effects of antihypertensive therapy and partial recovery of M-CSF circadian patterns (with the absence of a pronounced decrease at 2:00) 2 out of 42 patients developed ACA (the absolute risk of cardiovascular complications was 4.76% [−1.68 to 11.2]). The risk ratio of the two groups is 15.2 [3.8–60.4], the specificity of potential criterion is 0.89, the sensitivity is −0.87, φ is 0.0000 ($p < 0.05$), and C' is 0.82 (the link is very strong). M-CSF induced the production of VEGF-A in cardiomyocytes through the Akt signaling pathway and MAP kinase, which enhanced the promoting activity of p38 and JNK, stabilized the mRNA of VEGF-A, and implemented cardio- and angioprotective functions, but with a dose-dependent nature of the effects [6,7]. It may possibly be the changing dynamics of the M-CSF content in the blood during the 20:00 to 2:00 interval (with a marked decrease at 2:00) that persists in EH patients during antihypertensive therapy, impairs the dose-dependent protective effects of M-CSF and is one of the pathogenetic elements associated with the rise in the incidence of MI in this category of patients.

4. Conclusions

The results of the study have proven that stage II EH patients with a 10–14 year history of the disease are characterized by higher levels of M-CSF in the peripheral blood serum and by an increased variability in the circadian rhythm of the M-CSF content in the blood (when compared with healthy individuals), due to growth at 20:00, a decline of 2:00 and recovery at 8:00. Thirty percent of patients retained the rhythm characteristics of blood serum M-CSF content typical of patients before the start of therapy, which is a predictor of an increase in the five-year risk of developing cardiovascular complications, particularly MI and ACA. The data presented opens up the prospect for further examination of the role M-CSF plays in maximizing the personalization in the analytical findings of cytokine variation, which is the basis for future implementation in practical medicine.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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