ALTERED CIRCADIAN EXPRESSION OF CYTOKINES IN BLOOD OF PATIENTS WITH ESSENTIAL HYPERTENSION FOLLOWING COVID-19

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In spite of a vast number of trials devoted to quantitative characteristics of the cytokine profile in patients with various diseases, no exact reference intervals are presented. It is just as important that there is a limited number of trials analyzing circadian rhythms of cytokine synthesis in patients with arterial hypertension. The purpose of the work was to analyze the characteristics of circadian rhythms of cytokines (IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR) in patients with Grade II essential arterial hypertension, and to detect pathogenetically significant characteristics developed following Covid-19. Blood samples were taken at 7.00–8.00, 12.00–13.00, 19.00–20.00.00, 00.00–1.00 to determine the levels of IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR in 18 patients (56 (95% CI (54–69) years) with essential arterial hypertension (EAH) within three days prior to and following Covid-19. For this, the immunoenzyme method was used. The obtained data demonstrated altered circadian expression of cytokines in the peripheral blood of patients with essential hypertension depending on whether they have EAH or not, and their additional distortion following Covid-19, which is stable in the majority of cases. It is preserved for six months as low IL18 BP ($p < 0.001$), and twofold increase of sLIFr and MCSF ($p < 0,001$) at 18.00. A significant association is determined between the circadian rhythms of sLIFr and altered systolic BP resulting in the abnormal rhythm with BP rise at night (night peaker) in patients with EAH following COVID-19. The obtained fundamental data offer prospects for new research of immunopathogenesis following COVID-19 in patients with hypertension taking into account circadian rhythms of cytokines in the blood.

Keywords: essential hypertension, LIF, sLIFr, M-CSF, circadian rhythms

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Compliance with ethical standards: The trial was approved by the Ethics Committee of the National Research Mordovia State University (protocol № 12 as of December 14, 2008, protocol № 85 as of May 27, 2020). All patients signed a voluntary informed consent form. Biological sample (blood) was obtained in accordance with the provisions of Declaration of Helsinki of BMA (2013), protocol of Council of Europe Convention for the Protection of Human Rights and Biomedicine (1999) and additional protocol to Convention for the Protection of Human Rights and Biomedicine in the area of biomedical research (2005).

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ИЗМЕНЕНИЯ ЦИРКАДИАННЫХ РИТМОВ УРОВНЕЙ ЦИТОКИНОВ В КРОВИ ПАЦИЕНТОВ С ЭССЕНЦИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ В ПОСТКОВИДНОМ ПЕРИОДЕ

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Несмотря на большое число исследований, посвященных количественным характеристикам цитокинового профиля пациентов с различными заболеваниями, нет точных референсных интервалов, и что не менее важно, ограничено число исследований, анализирующих циркадианные ритмы синтеза цитокинов у лиц с артериальной гипертензией. Целью работы было проанализировать особенности циркадианных ритмов содержания цитокинов (IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR) в крови пациентов с эссенциальной артериальной гипертензией II стадии и выделить патогенетически значимые особенности, сформированные после перенесенной новой коронавирусной инфекции. Проводили забор крови в 7.00–8.00, 12.00–13.00, 19.00–20.00.00, 00.00–1.00 для определения иммуноферментным методом уровней IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR в течение трех суток у 18 пациентов (56 (95% ДИ (54–69) лет) с эссенциальной артериальной гипертензией (ЭАГ) до и после перенесенной новой коронавирусной инфекции. Полученные данные демонстрируют изменения циркадианных ритмов содержания цитокинов в периферической крови у пациентов в зависимости от наличия ЭАГ, а также их дополнительное искажение в постковидном периоде, что в значимом проценте случаев имеет стойкий характер с сохранением более шести месяцев в виде снижения содержания IL18 BP ($p < 0,001$), двукратного увеличения sLIFr и MCSF ($p < 0,001$) в 18.00. Определена достоверная связь между циркадианными ритмами содержания sLIFr в крови и изменения уровня систолического АД с формированием патологического ритма с ростом АД в ночное время (Найт-пикер) у лиц с ЭАГ в постковидном периоде. Полученные фундаментальные данные открывают перспективы для новых исследований иммунопатогенеза постковидного периода у лиц с гипертензией с учетом циркадианных ритмов содержания цитокинов в крови.

Ключевые слова: эссенциальная гипертензия, LIF, sLIFr, M-CSF, циркадианные ритмы

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The trials devoted to the role of cytokines from post-COVID perspective are pressing as a large amount of knowledge related to the altered link of immune regulation during COVID-19 is obtained. The restoration is a long-term process, including both early, and late periods of reconvalescence. Meanwhile, in the post-COVID era, virus-mediated changes of the cytokine profile can determine modification of non-infectious disease pathogenesis due to multiple organ action, including patients with essential arterial hypertension (EAH). It should be noted that in spite of a vast number of trials devoted to quantitative characteristics of the cytokine profile in patients with various diseases, no exact reference intervals are presented. It is just as important that there is a limited number of trials analyzing circadian rhythms of cytokine synthesis in patients with certain diseases. The things mentioned above are most significant for patients with EAH as daily curves of BP are essential and have been taken into account in practice for long. An increased rate of cardiovascular complications and development of the metabolic syndrome are detected in the post-COVID era. This can be associated with methods of therapy (wide use of corticosteroids) and role of coronavirus depending on pre-existing immunopathogenetic and clinical characteristics of patients. Cytokine synthesis concentrations are not only factors, which are subject to daily patterns. In case of clinical or subclinical imbalance, they can influence the main center of the circadian clock such as the suprachiasmatic nucleus in the brain. It can also promote the loss of synchronization between the main center and peripheral 'clocks' [1, 2]. The chronobiology is perspective. It has a vast scope of significant scientific data [3, 4] and needs to expand a number of trials in the post-covid era. It should be noted that the individual analysis of daily curves of cytokines in the peripheral blood allows to be declarative of more complex patterns as compared to two-phase day and night changes. According to some data, the amount of IL2 in the serum forms a single peak approximately at noon, whereas IL10, TNF and GM-CSF display a two-phase pattern [5]. In the subjects, the first peak and second peak of TNF occur at 07:30 and 13:30, respectively. So, it can't be considered as a cytokine of rest. The levels of IL10 are also displayed a biphasic pattern with one peak at 07:30 and the other one at 19:30 in 12 hours. According to these results, the temporal patterns are unique for every cytokine with daylight peaks and night minima [2]. Thus, fundamental data about altered circadian rhythms of cytokine synthesis following the previous novel coronavirus can be both of a scientific and practical interest in the future.

The purpose of the work was to analyze the characteristics of circadian rhythms of cytokines (IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR) in patients with Grade II EAH, and to detect pathogenetically significant characteristics developed following Covid-19.

METHODS

The trial was conducted in 2019-2023 at the departments of immunology, microbiology, virus ology of the National Research Mordovia State University (microbiological and immunological laboratory — license № 13.01.04. 0001. Л.000005.06.11, permanent), Katkov SV National Clinical Hospital, North Caucasus Sanatorium Resort of the Ministry of Defense of Russia.

27 patients (10 females and 17 males) with Grade 2 EAH (group 1) and 16 healthy people (7 females and 9 males) with normal BP (group 2) have undergone through a dynamic complex (clinical, laboratory ad instrumental) observation. In 2019 (prior to pandemic), they had their blood cytokine levels analyzed (7.00–8.00, 12.00–13.00, 19.00–20.00, 00.00–1.00) within three days. In 2020 (Wuhan-Hu-1 circulation), 18 patients

Table 1. Analysis of cytokines in the blood of patients with EAH prior to COVID-19

Time interval		7.00–8.00	12.00–13.00	19.00–20.00	0.00–1.00
		1	2	3	4
IL18, pg/ml	Day 1	356 [271–460]	402 [258–437]	523 [391–639]*1.2	610 [489–720]*1.2.3
	Day 2	317 [286–451]	398 [310–469]	551 [406–668]*1.2	689 [464–783]*1.2.3
	Day 3	368 [239–490]	417 [267–452]	584 [390–627]*1.2	621 [429–718]*1.2.3
IL18 BP, pg/ml	Day 1	6100 [3800–6900]	6300 [4100–7200]	5900 [3700–6800]	5500 [3500–6400]
	Day 2	5790 [3240–6450]	6100 [4320–7400]	6400 [4300–7100]	5800 [3200–6700]
	Day 3	5900 [3400–6600]	5800 [4200–6700]	6200 [4500–6900]	5700 [3500–6900]
LIF, pg/ml	Day 1	7.48 [4.21–1o1.3]	7.64 [4.37–11.5]	12.3 [8.25–14.1]*1.2	10.1 [9.88–11.9]^1.2'3
	Day 2	7.33 [4.18–12.1]	7.42 [3.91–11.9]	12.6 [8.12–14.3]*1.2	9.97 [10.2–12.2]^1.2.3
	Day 3	7.54 [4.12–11.8]	7.48 [4.11–12.5]	12.7 [7.96–14.4]*1.2	10.2 [9.97–12.5]]^1.2'3
sLIFr, pg/ml	Day 1	3850 [2600–5100]	4100 [3600–4900]	5400 [4500–7300]*1.2	2900 [2300–3800]*1.2.3
	Day 2	3910 [2770–5300]	3870 [3540–5200]	5640 [4800–7200]*1.2	3100 [2500–4200]*1.2.3
	Day 3	3960 [2650–5250]	3840 [3590–5300]	5700 [4900–7400]*1.2	3720 [2400–4100]*1.2.3
M-CSF, pg/ml	Day 1	371 [308–493]	410 [290–482]	419 [327–494]*1^2	395 [315–526]
	Day 2	350 [292–471]	421 [287–475]	415 [347–484]*1^2	405 [321–519]
	Day 3	363 [315–488]	442 [293–490]	426 [351–492]*1^2	398 [309–524]
IL34, pg/ml	Day 1	127 [96.7–152]	144 [102–168]	93.6 [81.2–105]*2	80.2 [68.1–85.5]*1.2.3
	Day 2	118 [90.8–141]	136 [96–153]	98.5 [85.6–117]*2	81.9 [66.2–87.7]*1.2.3
	Day 3	131 [101.1–159]	148 [108–172]	101.7 [90.1–120]*2	92.7 [69.3–90.5]*1.2.3
MCSFR, μg/ml	Day 1	22.8 [17.4–28.3]	18.7 [12.2–31.1]	20.6 [12.5–35.7]	19.9 [17.9–31.2]
	Day 2	24.4 [18.1–29.8]	19.3 [13.7–32.6]	23.2 [13.9–38.5]	26.2 [19.7–33.3]
	Day 3	23.6 [17.8–27.7]	17.5 [12.8–31.9]	22.5 [13.1–37.2]	21.7 [18.5–32.7]

Note: * — significant level $p < 0.001$, ^ — $p < 0.01$, ' — $p < 0.05$ (dependent and independent samples were compared with the Wilcoxon and Mann–Whitney tests respectively) indicating time intervals (1–7:00; 2–12:00; 3–19:00; 4–00:00.)

Table 2. Analysis of cytokines in the blood of healthy people prior to COVID-19

Time interval		7.00–8.00	12.00–13.00	19.00–20.00	0.00–1.00
		1	2	3	4
IL18, pg/ml	Day 1	185 [143–201]	164 [138–194]	214 [144–229]	301 [246–385]*1.2.3
	Day 2	178 [135–194]	157 [127–183]	196 [124–205]	289 [235–369]*1.2.3
	Day 3	190 [148–210]	171 [145–201]	220 [151–235]	314 [253–390]*1.2.3
IL18 BP, pg/ml	Day 1	4720 [4210–5110]	4830 [4160–5200]	4660 [4320–5170]	6100 [5720–6930]*1.2.3
	Day 2	4810 [4320–5230]	4920 [4210–5350]	4730 [4460–5250]	6450 [5840–7010]*1.2.3
	Day 3	4780 [4270–5160]	4870 [4190–5280]	4690 [4410–5190]	6320 [5800–7000]*1.2.3
LIF, pg/ml	Day 1	1.38 [1.15–1.68]	1.47 [1.31–1.73]	1.33 [1.26–1.75]	1.35 [1.24–1.63]
	Day 2	1.40 [1.17–1.71]	1.51 [1.35–1.77]	1.35 [1.29–1.78]	1.37 [1.27–1.66]
	Day 3	1.41 [1.19–1.75]	1.54 [1.40–1.81]	1.37 [1.31–1.82]	1.41 [1.30–1.69]
sLIFr, pg/ml	Day 1	3920 [3130–4870]	4260 [3450–4610]	4440 [3610–4830]	4160 [3780–4520]
	Day 2	3870 [3070–4790]	4990 [3390–4650]	4380 [3580–4770]	4130 [3690–4430]
	Day 3	4010 [3210–4980]	5180 [3520–4700]	4530 [3720–4950]	4240 [3870–4610]
M-CSF, pg/ml	Day 1	220 [143–283]	187 [137–240]	235 [138–281]	357 [315–394]*1.2.3
	Day 2	237 [160–299]	198 [149–243]	253 [155–310]	369 [328–404]*1.2.3
	Day 3	228 [151–295]	191 [142–239]	244 [147–293]	361 [321–398]*1.2.3
IL34, pg/ml	Day 1	162 [112–203]	170 [126–212]	169 [147–235]*2	158 [127–216]
	Day 2	158 [107–196]	167 [122–208]	165 [141–233]*2	155 [121–210]
	Day 3	165 [116–210]	174 [131–218]	172 [150–239]*2	163 [133–215]
MCSFR, μg/ml	Day 1	31.6 [25.7–39.2]	27.9 [19.3–37.1]	44.7 [32.1–49.8]*2.3	67.7 [42.1–93.6]*1.2.3
	Day 2	28.9 [23.5–38.7]	26.5 [18.2–35.3]	41.9 [29.9–48.3]*2.3	63.6 [38.8–89.7]*1.2.3
	Day 3	32.1 [26.3–40.1]	28.6 [20.4–39.2]	45.3 [33.7–50.6]*2.3	69.5 [44.6–96.3]*1.2.3

Note: * — significant level $p < 0.001$, ^ — $p < 0.01$, ' — $p < 0.05$ (dependent and independent samples were compared with the Wilcoxon and Mann–Whitney tests respectively) indicating time intervals (1–7:00; 2–12:00; 3–19:00; 4–00:00.).

from group 1 (8 females and 10 males) and 9 patients (4 females and 5 males) from group 2 were diagnosed with COVID-19 as per relevant temporary methodical recommendations related to prevention, diagnostics and treatment of moderately to severely novel coronavirus infection, pneumonia (grade I–II CT). Comparable treatment regimens were used (16 mg of dexamethasone per day, 500 mg of Sumamed TID, heparin (a group of researchers failed to influence the therapy).

Inclusion criteria in 2019: Grade II EAH for 10 years (first stage of the trial), comparable hypotensive therapy (ACE inhibitors ± diuretic), 55–60 years, total cholesterol less than 5.0 mmol/L, LDL less than 3.0 mmol/L, HDL more than 1.0 mmol/L, TG less than 1.7 mmol/L, IMT less than 0.9 mm, glucose less than 5.5 mg/dl, BMI less than 30 kg/m^2, comparable characteristics of day regimen (sleeping from 23.00 to 6.00, last food intake at 20.00, no disturbed sleep, intake of hypnotic agents and/or melatonin-containing preparations (as assessed by a neurologist), signing of an informed consent from by the patient.

Additional inclusion criteria in 2020: participation in the trial of circadian rhythm in cytokines administration in patients with EAH in 2019, a history of COVID-19 (day 10 following clinical and laboratory convalescence in comparable hospitalization duration of 14–16 days), whereas group 2 included people without a history of EAH + COVID-19 (day 10 following clinical and laboratory convalescence in comparable hospital stay of 14–16 days), COVID-19 was confirmed with the PCR method and results of a serologic examination, moderate to severe, with involvement of the lungs (grade I–II CT), comparable therapy of COVID-19, and a written consent form.

In 2019 and 2020, there were general exclusion criteria such as type 1 or 2 diabetes mellitus, allergic/autoimmune diseases, mental diseases, symptomatic AH, smoking, lack of readiness for a long-term examination, use of antiviral and immunomodulating medicinal agents during COVID-19 and six months prior to the trial. Patients who had to take antihypertensive medicinal agents apart from ACE inhibitors and/or thiazide/thiazide-like diuretics (only for patients with Grade II EAH) were excluded from the trial.

Blood sampling times (7.00–8.00, 12.00–13.00, 19.00–20.00.00, 00.00–1.00) and specter of cytokines (IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR) were determined based on scientific trials of circadian rhythms of human cytokines biology [6, 7] and previous own trials of cytokines in EAH pathogenesis [8]. Based on our 10-year trial, the levels of IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR are associated with the rate of cardiovascular complications in patients with EAH in a statistically independent mode. It explains the data about the specter of cells having the cytokines receptors [9] and their relation with metabolism of vasopressors and vasodilators.

Blood sampling was done at 7.00–8.00, 12.00–13.00, 18.00–19.00.00, 00.00–1.00 (three days) using BD Vacutainer (Becton Dickinson; USA (for blood serum release), blood was centrifuged (1,500-2,000 rounds per minutes, 15 min) to get serum, which will be subsequently stored in labeled test tubes at –30° C for no longer than 30 days. Time from blood sampling to freezing was 60 min. Cytokines (IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR) were determined by a certified professional with an enzyme-linked immunosorbent assay and Personal Lab TM immunoassay analyzer (Adaltis; Italy). Test systems produced by eBioscience (Bender MedSystems; Austria) and R&D Systems (USA).

Daily characteristics of patients were determined as per the following classification: dippers (BP decline from 10 to 20%), nondippers (BP declines less than 10%) and night-peakers (rise at night). Daily monitoring was done to all patients. It was

Table 3. Analysis of blood cytokines levels in patients with EAH 10 days following the novel coronavirus infection

Time interval		7.00–8.00	12.00–13.00	19.00–20.00	0.00–1.00
		1	2	3	4
IL18, pg/ml	Day 1	460 [396–510]	445 [368–474]	590 [453–670]	792 [373–821]*1.2.3
	Day 2	446 [370–482]	435 [352– 459]	582 [442–649]	759 [338–803]*1.2.3
	Day 3	454 [387–503]	440 [361–467]	588 [447–658]	781 [352–811]*1.2.3
IL18 BP, pg/ml	Day 1	6300 [4110–7500]	6110 [3730–7460]	5100 [3700–6400]*1.2	5310 [3230–6370]*3
	Day 2	6150 [4030–7350]	5980 [3610–7320]	4820 [3550–6240]	4830 [3050–6180]
	Day 3	6220 [4090–7460]	6050 [3690–7380]	4950 [3630–6330]	4910 [3170–6250]
LIF, pg/ml	Day 1	7.69 [4.35–10.8]	7.92 [4.51–10.5]	13.1 [8.74–15.6]*1.2	9.83 [9.12–11.7]*3
	Day 2	7.33 [4.25–9.9]	7.63 [4.00–10.2]	12.6 [8.11–14.9]	9.10 [8.92–10.8]*3
	Day 3	7.51 [4.31–10.4]	7.88 [4.32–10.4]	12.9 [8.55–15.4]	9.58 [9.05–11.3]*3
sLIFr, pg/ml	Day 1	4100 [2720–5000]	3820 [2910–4500]	7900 [6200–8400]*1.2	3870 [3100–4200]*3
	Day 2	3850 [2680–4930]	3530 [2850–4320]	7590 [6030–7990]	3480 [2860–4080]
	Day 3	3980 [2700–4980]	3660 [2890–4470]	7770 [6120–8250]	3750 [2970–4110]
IL34, pg/ml	Day 1	630 [570–810]	593 [492–685]	830 [540–973]*1.2	601 [518–710]*3
	Day 2	605 [561–792]	561 [477–661]	812 [521–953]	582 [501–695]
	Day 3	616 [569–802]	582 [485–672]	826 [530–964]	593 [512–693]
MCSFR, µg/ml	Day 1	9.18 [8.3–31.5]	10.23 [7.16–12.4]	10.5 [8.53–13.2]	9.51 [6.42–12.3]
	Day 2	9.05 [8.1–29.6]	9.81 [6.91–11.5]	9.93 [8.41–12.8]	9.11 [6.30–11.8]
	Day 3	9.10 [8.2–30.2]	10.12 [7.02–12.0]	10.4 [8.49–13.1]	9.26 [6.39–12.1]

Note: * — significant level $p < 0.001$, ^ — $p < 0.01$, ' — $p < 0.05$ (dependent and independent samples were compared with the Wilcoxon and Mann–Whitney tests respectively) indicating time intervals (1–7:00; 2–12:00; 3–19:00; 4–00:00.).

accompanied by blood sampling to determine the levels of cytokines.

The obtained results were statistically processed using StatTech v. 2.8.8 (StatTech, Russia) and Stat Soft Statistica 10.0 (USA). The results were presented as a median (Me) and percentiles ($Q_{0,25}$–$Q_{0,75}$). It was assessed whether qualitative characteristics corresponded to normal distribution with the Shapiro–Wilk test. Dependent and independent samples were compared with the Wilcoxon and Mann–Whitney tests respectively. Regression Cox model was used, and the regression coefficient was assessed. Differences at $p < 0.05$ were taken as statistically significant.

RESULTS

Analysis of altered concentration of investigated blood cytokines in patients with EAH allowed to determine the differences from regular patterns found among healthy persons (Table 1) within two days of the prepandemic period (Table 2).

Within the system of IL18–IL18BP, an increase of IL18 in the peripheral blood was observed at 19.00 and 0.00 when compared with the results obtained at 7.00 and 12.00 ($p < 0.001$) with no growth of IL18 during the observation period. No increase of IL18 was found among healthy people. However, an increase of IL18 BP ($p < 0.05$) was detected at 00.00. Meanwhile, qualitative levels of IL18 are significantly increased in patients with hypertension ($p < 0.001$). During the early reconvalescence following novel coronavirus, patients with EAH had an additional decrease of 18 BP at 19.00, which was preserved at 00.00 ($p < 0.001$) when compared with data obtained at 7.00, 13.00, 19.00 (Table 3). This is how the group was extinguished from healthy people with an increased level of IL18 at 00.00 but with a compensatory increase of IL18 BP at 20.00 (Table 4).

It was found out during analysis of LIF–sLIFR prior to SARS-CoV-2 that patients with EAH had experienced a growth of two components at 19.00, which was decreased at 00.00 (Table 1). These changes were not observed among healthy people (table 2). During reconvalescence following the previous novel coronavirus, patients with EAH had a more pronounced (200%) increase of sLIFr at 19.00 with preserved changes and qualitative characteristics of LIF.

Levels of M-CSF and its receptors in patients with EAH went through no changes during a day of the pre-COVID period (Table 1). Meanwhile, healthy people had seen a growth of M-CSF at 00.00 against the background of increased M-CSF at 19.00 and 00.00 with restoration at 7.00. Qualitative characteristics of M-CSF were significantly higher in patients with hypertension during all time points ($p < 0,001$) as compared with healthy people. During reconvalescence following COVID-19, a peak increase of M-CSF was seen at 19.00 with a decrease at 00.00 among patients with EAH (not previously detected) with no changes of M-CSFR. Healthy people had no changes of circadian rhythms as compared to data obtained prior to SARS-CoV-2.

It should be noted that during monitoring of similar cytokines performed six months following COVID-19 85% of patients with EAH had circadian patterns developed after COVID-19 as a decrease of IL18 BP at 19.00, two-fold increase of sLIFr and MCSF (Table 5) with restored patterns of post-COVID era among healthy people (Table 6).

Compared characteristics of circadian rhythms of analyzed cytokines during the post-COVID era and altered course of EAH allowed to find out (during the intraquartile analysis) that an increase of sLIFr by 200% and more (quartiles III and IV of altered sLIFr in 8 people) was accompanied with a rise in systolic BP above 150 mm Hg at 19.00 in spite of antihypertensive therapy, which provided a therapeutic effect prior to the previous novel coronavirus therapy and in the morning/in the afternoon following the infection. It was required to change therapy (time of intake of a medicinal product) and increase the dose of basic preparations. Moreover, 8 of 18 patients with EAH included into the trial during the post-COVID era had a daily rhythm of BP as per the results of Holter monitoring. It was night peaker

Table 4. Analysis of blood cytokines levels in patients with EAH 10 days following the novel coronavirus infection

Time interval		7.00–8.00	12.00–13.00	19.00–20.00	0.00–1.00
		1	2	3	4
IL18, pg/ml	Day 1	151 [144–203]	158 [129–214]	169 [136–223]	291 [247–383]*1.2.3
	Day 2	138 [133–196]	141 [120–203]	161 [128–215]	273 [233–375]*1.2.3
	Day 3	145 [139–201]	149 [125–211]	165 [131–220]	282 [242–379]*1.2.3
IL18 BP, pg/ml	Day 1	4670 [4170–5120]	4590 [4210–5310]	6400 [5370–7510]*1.2	6100 [5330–7820]*1.2.3
	Day 2	4320 [3890–5010]	4250 [4030–5100]	6050 [5150–7270]*1.2	5880 [5140–6510]*1.2.3
	Day 3	4590 [3960–5090]	4420 [4110–5230]	6230 [5230–7440]*1.2	6010 [5220–6680]*1.2.3
LIF, pg/ml	Day 1	1.39 [1.12–1.64]	1.47 [1.24–1.72]	1.36 [1.05–1.55]	1.36 [1.21–1.7]
	Day 2	1.25 [1.09–1.54]	1.38 [1.21–1.68]	1.33 [0.98–1.43]	1.34 [1.15–1.63]
	Day 3	1.32 [1.11–1.61]	1.41 [1.22–1.70]	1.28 [0.91–1.20]	1.31 [1.08–1.56]
sLIFr, pg/ml	Day 1	4120 [3620–4510]	3960 [3310–4580]	4210 [3640–4660]	4170 [3580–4790]
	Day 2	4030 [3430–4360]	3650 [3050–4270]	4090 [3400–4530]	3960 [3410–4530]
	Day 3	4090 [3580–4420]	3820 [3220–4390]	4130 [3550–4610]	4080 [3490–4670]
M-CSF, pg/ml	Day 1	190 [138–236]	171 [139–247]	182 [121–268]	333 [287–412]*1.2.3
	Day 2	175 [129–227]	160 [121–236]	171 [115–253]	292 [263–393]*1.2.3
	Day 3	183 [131–230]	169 [128–241]	179 [119–261]	314 [275–403]*1.2.3
IL34, pg/ml	Day 1	146 [121–198]	152 [127–231]	221 [169–262]*1.2	155 [127–193]*3
	Day 2	131 [115–179]	135 [113–219]	203 [153–248]*1.2	138 [115–174]*3
	Day 3	142 [119–183]	148 [121–227]	217 [161–254]*1.2	144 [123–188]*3
MCSFR, µg/ml	Day 1	31.6 [24.3–39.7]	29.6 [21.2–36.5]	51.9 [34.2–58.8]*2.3	66.2 [38.5–101.3]*1.2.3
	Day 2	27.6 [21.7–35.3]	26.8 [19.8–33.7]	48.3 [31.8–52.3]*2.3	59.1 [33.2–97.5]*1.2.3
	Day 3	29.4 [23.8–38.2]	28.3 [20.9–35.9]	50.2 [33.4–55.7]*2.3	63.5 [35.9–99.7]*1.2.3

Note: * — significant level $p < 0.001$, ^ — $p < 0.01$, ' — $p < 0.05$ (dependent and independent samples were compared with the Wilcoxon and Mann–Whitney tests respectively) indicating time intervals (1 — 7:00; 2 — 12:00; 3 — 19:00; 4 — 00:00.).

(increase of BP at night). Prior to SARS-CoV-2, all patients were classified as dippers. The patients had alternated circadian rhythms mentioned above such as a drop of IL18 BP for more than 58% and two-fold increase of sLIFr and MCSF. Meanwhile, a multiple correlation analysis of the relations between cytokine levels (IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR) during all analyzed time points and level of systolic arterial pressure (as per results of daily monitoring of BP) has seen a statistically independent relation between the level of sLIFr and systolic BP: regression coefficient of 2.48, standard error of 0.22, t-criterion of 9.83, relative risk of increased systolic BP after 18.00 equal to 4.2 and Wald criterion of 16.3.

DISCUSSION

The obtained data show that circadian rhythms of cytokines in the peripheral blood are alternated depending on the presence of EAH and their additional distortion during the post-COVID era. In the majority of cases, it is stable and can be preserved for over six months. Taking into account previously published data about the correlation between increased sLIFr over 5,000 pg/ml and increased NT-proBNP ($r = 0.87$; $p = 0.0007$), and the correlation between MCSF and SDMA ($r = 0.83$; $p = 0.0008$) [7] it is shown that there is a pathogenetic significance of discovered circadian changes as EAH progresses. Thus, in spite of ability for vasodilation, which produces a positive effect in case of EAH, the secondary growth of NT-proBNP will result in progression of chronic cardiac failure and an increased risk of cardiovasclar complications [10]. The positive relation between MCSF and IL18 and growth of SDMA [8] is more frequently of a renal origin [11]. It can determine the vector of hypertension progression by canceling the essential nature. It is known about its production by endothelial cells under the influence of proinflammatory cytokines [12], which can be induced by viruses in this case.

Fundamental trials devoted to circadian rhythms of synthesis of certain regulatory peptides require high comparability of included patients (this was achieved during the

Table 5. Analysis of blood cytokines levels in patients with EAH 6 months following the novel coronavirus infection

Time interval		7.00–8.00	12.00–13.00	19.00–20.00	0.00–1.00	7.00–8.00
		1	2	3	4	5
IL18, pg/ml	Day 1	457 [388–514]	449 [374–493]	589 [431–661]	791 [370–818]*1.2.3	461 [369–505]
IL18 BP, pg/ml	Day 1	6280 [4100–7490]	6070 [3670–7350]	5210 [3280–6500]*1.2	5380 [3510–6400]*3	6220 [4010–7090]
LIF, pg/ml	Day 1	7.26 [4.03–9.9]	7.88 [4.19–10.7]	12.9 [7.81–16.1]*1.2	10.1 [8.12–12.3]*3	7.31 [4.15–10.1]
sLIFr, pg/ml	Day 1	4020 [2510–5100]	3790 [2880–4430]	7870 [6150–8390]*1.2	3690 [3110–4180]*3	3990 [2490–5020]
M-CSF, pg/ml	Day 1	621 [554–816]	588 [461–625]	815 [593–954]*1.2	650 [521–733]*3	618 [560–831]
MCSFR, µg/ml	Day 1	9.04 [7.3–13.5]	11.12 [6.76–13.1]	11.3 [8.41–13.9]	9.42 [7.12–13.1]	8.95 [6.81–12.8]

Note: * — significant level $p < 0.001$, ^ — $p < 0.01$, ' — $p < 0.05$ (dependent and independent samples were compared with the Wilcoxon and Mann–Whitney tests respectively) indicating time intervals (1 — 7:00; 2 — 12:00; 3 — 19:00; 4 — 00:00.).

Table 6. Analysis of blood cytokines levels in patients with EAH 6 months following the novel coronavirus infection

Time interval		7.00–8.00	12.00–13.00	19.00–20.00	0.00–1.00	7.00–8.00
		1	2	3	4	5
IL18, pg/ml	Day 1	143 [138–193]	151 [126–210]	163 [128–264]	273 [232–357]*1.2.3	154 [129–201]
IL18 BP, pg/ml	Day 1	4530 [4030–4990]	4490[4010–5170]	6340 [4970–6960]*1.2	5990 [5330–7340]*1.2.3	4710 [3980–4920]
LIF, pg/ml	Day 1	1.31[1.05–1.44]	1.39 [1.13–1.55]	1.29 [0.98–1.45]	1.32 [1.19–1.68]	1.33 [1.01–1.38]
sLIFr, pg/ml	Day 1	4060 [3490–4330]	3830 [3110–4270]	4100 [3340–4220]	4160 [3390–4580]	4020 [3530–4290]
M-CSF, pg/ml	Day 1	179 [128–216]	168[123–221]	181 [118–246]	311 [269–403]*1.2.3	184 [131–226]
IL34, pg/ml	Day 1	139 [110–173]	154 [129–242]	218[159–258]*1.2	144 [107–173]*3	140 [114–185]
MCSFR, μg/ml	Day 1	28.7 [23.8–37.7]	29.1 [20.3–36.4]	52.2 [35.7–60.1]*2.3	64.9 [36.5–110.1]*1.2.3	30.1 [21.4–32.9]

Note: * — significant level $p < 0.001$, ^ — $p < 0.01$, ' — $p < 0.05$ (dependent and independent samples were compared with the Wilcoxon and Mann–Whitney tests respectively) indicating time intervals (1 — 7:00; 2 — 12:00; 3 — 19:00; 4 — 00:00.).

trial and allowed to reduce the sample scope) and stable daily rhythms of life, alternated day and night phases; they are rather of a theoretical significance. However, in the analyzed clinical situations, there exist essential connections that determine clinical characteristics of hypertension with altered treatment regimens due to loss of control over BP when the daily rhythm developed prior to infection with SARS-CoV-2 is altered.

The lack of a compensatory increase of cytokines with protected properties in relation to progressive endothelial dysfunction (IL18 BP, M-CSFR) was seen in patients with EAH during the post-COVID era. IL18-BP reduces the risk of renal damage on ischemia and reperfusion animal models due to anti-inflammatory and antioxidant activity [13]. It is essential in AH. A new prospect of IL18 BP as a therapeutic target in cardiovascular diseases was recorded in a number of publications [13, 14].

A detailed analysis confirms the necessity of assessing four time points; the largest prognostic significance of the analyzed cytokines was determined at 19.00. It is essential for further related trials, which examine the role of cytokines in the pathogenesis of cardiovascular diseases and post-COVID syndrome. However, they are oriented towards a morning blood sample. It is probably less significant than blood sample taken at 19.00.

CONCLUSIONS

Analysis of the role of infection with SARS-CoV-2 in the altered circadian rhythms of cytokines (IL18, IL18 BP, LIF, sLIFr, M-CSF, MCSFR) in the blood of patients with EAH opens up prospects for the analysis of the role and other infectious agents in the change of the cytokine component of progressing endothelial dysfunction. Altered cytokines levels were assessed within a day at several time points. During surveillance of a patient over several days it was verified whether the observed regular patterns were repeated. Chronobiology requires diversified examination to expand the scope of fundamental data, which can explain clinical features of socially significant diseases and can be the basis of new principles of therapy in the future.

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