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In vitro cell-based Hyperuricemia-hemotest bioassay for cytokine status evaluation in patients with gouty arthritis

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Abstract

Aim – to develop an *in vitro* method for assessing the activity of the inflammasome under conditions of hyperuricemic stimulation of inflammatory interleukins.

Material and methods. Whole blood cells of donors and patients with hyperuricemia and exacerbation of gouty arthritis diluted with RPMI were cultured *in vitro* in the presence of different concentrations of uric acid. The production of cytokines in the cell growth media of hematopoietic cells stimulated with uric acid was evaluated using an enzyme-linked immunosorbent assay (ELISA).

Results. By simulating the hyperuricemia *in vivo*, an *in vitro* cell-based bioassay was developed to stimulate blood cells of individual donors with uric acid. Using the developed *in vitro* Hyperuricemia-hemotest bioassay, quantitative differences were found in the production of inflammatory cytokines by the blood cells of potentially healthy donors and patients with hyperuricemia and gouty arthritis.

Conclusion. As a new approach in personalized diagnostics, a hyperuricemic (HU)-hemotest system was developed, which can serve as an *in vitro* cell model for studying the activation of inflammasome by inflammatory signaling molecules in gouty arthritis.

Keywords: cell biology, cytokines, hyperuricemia, gouty arthritis, personalized medicine.

Conflict of interest: nothing to disclose.

Citation
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In vitro клеточная гиперурикемическая гемотест-система для определения цитокинового статуса пациентов с подагрическим артритом

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Аннотация

Цель – разработать *in vitro* метод оценки активности инфламмасомы в условиях гиперурикемической стимуляции воспалительных интерлейкинов.

Материал и методы. Клетки цельной крови доноров и пациентов с гиперурикемией и обострением подагрического артрита культивировались *in vitro* в культуральной среде RPMI в присутствии разных концентраций мочевой кислоты. Продукция цитокинов, вырабатываемых в культуральную среду клетками крови, стимулированных мочевой кислотой, проводилась с помощью сравнительного иммуноферментного анализа.

Результаты. Имитируя условия гиперурикемии *in vivo*, была разработана *in vitro* клеточная тест-система стимуляции клеток крови индивидуальных доноров мочевой кислотой. С помощью разработанной гиперурике-

мической гемотест-системы *in vitro* обнаружены количественные различия в продукции воспалительных цитокинов, вырабатываемых клетками крови потенциально здоровых доноров и пациентов с гиперурикемией и подагрическим артритом.

Выводы. В рамках создания персонифицированной диагностики разработана гиперурикемическая гемотест-система, которая может служить клеточной моделью *in vitro* для изучения активации сигнальных молекул инфламмасомного воспаления при подагрическом артрите.

Ключевые слова: клеточная биология, цитокины, гиперурикемия, подагрический артрит, персонифицированная медицина.

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Список сокращений
МУН – моноурат натрия; ГУ – гиперурикемия; МК – мочевая кислота; IL-1β – интерлейкин 1β; IL-6 – интерлейкин 6; Il-18 – интерлейкин 18; TNF-α – фактор некроза опухоли-альфа; PBMC – мононуклеарные клетки периферической крови; NLRP3 – инфламмасома NOD-подобного рецепторного белка 3.

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INTRODUCTION

Gouty Arthritis is a systemic disease characterized with deposition of monosodium urate (MSU) crystals and formation of tophi [1]. Acute gouty arthritis usually starts with sudden acute pain. Very characteristic is the damage of the metatarsophalangeal joint of the hallux (hence the name 'podagra'). Tophi inside and outside joints may restrict the range of motions and cause deformations resulting in the development of gouty arthritis. This pathology often develops in persons with hyperuricemia (HU), or elevated level of uric acid (UA) in the blood serum. While HU with no gouty arthritis present is described as 'asymptomatic', the latest research indicate a string connection between the HU and risk of development of arterial hypertension, kidney failure [2] and cardiovascular diseases [3–5]. UA is the final product of purine metabolism in the human body that is synthesized predominantly in the liver, the intestinal tract, and in the vascular endothelium [6]. The level of UA in the serum above 420 umol is officially considered hyperuricemia. At the same time, women, prior to the menopause, demonstrate UA levels of ≤360 umol, and after the menopause they elevate to concentrations seen in men. For children and adolescents, the normal UA level is considered at ≤330 umol [7]. In water solutions, with concentrations of 420 umol the UA salts transform from soluble form into crystalline form, but the threshold of urate solubility in the plasma is much higher, and their concentrations may reach >600 umol without formation of crystals. An acute attack of gouty arthritis is always associated with salting out of UA salt crystals in the joint cavity. Epidemiological data points at a close correlation between the hyperuricemia and the risk of development of gouty arthritis [8]. The accumulated clinical evidence, however, show this correlation not to be linear [9, 10]. Moreover, deposit of UA crystals is found in the articular cartilage in patients having no episodes of gouty arthritis [11]. This may be explained by the individual sensitivity of the inflammasome responsible for the synthesis of inflammatory interleukins and activated under HU. The low sensitivity of the inflammasome may well account for a symptom-free accumulation of UA salts in the articular tissue. On the other hand, the attacks of gouty arthritis are always attributed to the synthesis of inflammatory cytokines. High levels of 1β (IL-1β), IL-6 and TNF-α cytokines are found in the synovial fluid of patients with gouty arthritis in the periods of aggravation [12]. The elevated levels of IL-6 and TNF-α cytokines were also found in the plasma of patients with gouty arthritis in the period of aggravation but not in patients with gouty arthritis during the gout interval [13]. Studies comparing the levels of cytokines IL-1β, IL-6, IL-8, IL-17A, IL-18, IL-22 and IL-23 in the blood serum and the clinical manifestations of the gouty arthritis demonstrated direct correlation only with respect to IL-18, whereas only a minor elevation of the IL-6 was found in the serum of patients with the tophaceous gout [14]. Elevated values of IL-18 and IL-17A were found in the serum of patients with gouty arthritis [15]. It is interesting to note that in patients with gout no high levels of IL-1β are found [13, 14]. Still, there is enough clinical evidence to the key role played by the IL-1β in the progress of the gout. Clinical studies of the IL-1β inhibitors showed efficiency in the suppression of the inflammatory process during the gout [16].

Currently, the gouty arthritis is regarded as a systemic inflammatory disease caused by the priming of the NLRP3 inflammasome (NOD-like receptor protein 3) [17, 18]. Inflammasomes are multi-molecular complexes created in the cellular cytoplasm as a response to cellular damage and infections [19]. The generally accepted two-signal system of activation of the NLRP3 innflammasome is shown in **Fig. 1**. Only the combination of two signals mediates and induces a series of cascade reactions of gouty inflammation with hematopoietic cells, i.e. macrophages, monocytes and neutrophils [14, 17, 20, 21]. The nature of those signals is not fully established yet. Signal 1 is considered to sensitizes the cells via PRR, pattern-recognition receptors (priming), and only after the priming of the cells, Signal 2 (specifically, UA) causes activation of the inflammasome NLRP3. It was

originally considered that UA causes inflammation via activation of NLPR3 inflammasome only due to the formation of the crystals [22]. It was demonstrated later that the soluble form of UA penetrates the cells through special transporters and is able to activate the NLRP3 inflammasome, thus causing the release of inflammation mediators [18]. Signal 1 transcripts the components of the inflammasome, pro-Caspase-1 and pro-IL-1β through the activation of the NF-kB transcription factor. It is to be mentioned that unlike the IL-1β, the IL-18 transcription is constructive therefore, the IL-18 mRNA is always available to synthesize the IL-18 protein forms [23, 24]. Only with the presence of the two signals, there occurs the formation of the Gasdermin-D – regulated membrane pores, and the release of mature IL-1β and IL-18 interleukins to the environment, which is vital for the initiation of the acute inflammatory reaction in the gouty distress.

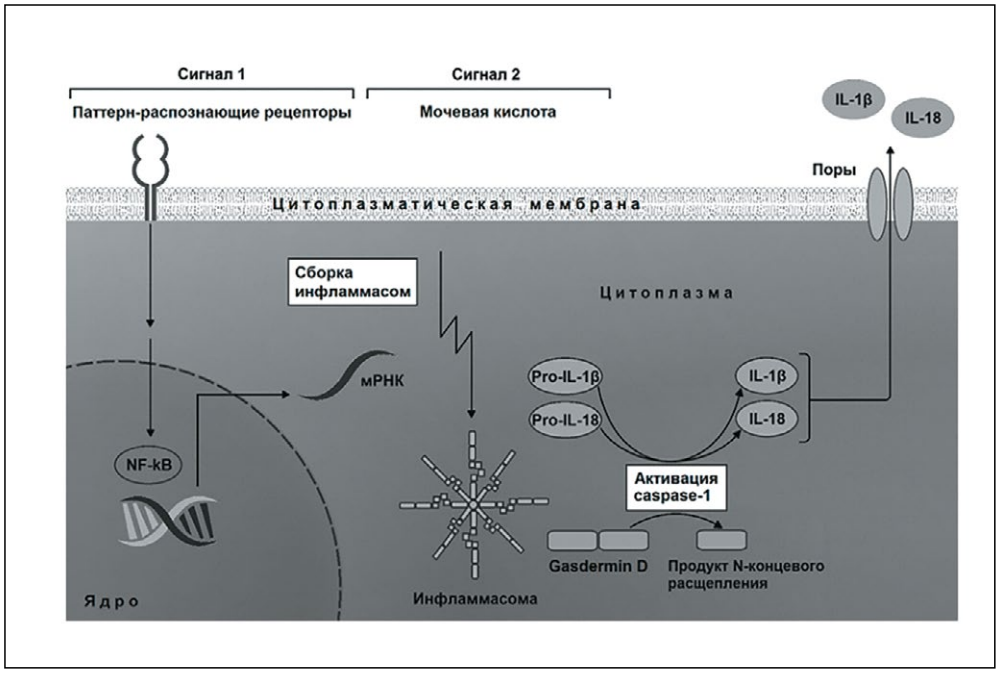

Figure 1. *Two-signal pathway activation of the NLRP3 inflammasome. Activated NLRP3 inflammasomes promote the release of Caspase-1, which converts the inactive forms of pro-IL-1β and pro-IL-18 to mature active IL-1β and IL-18, respectively. Gasdermin-D-mediated inflammatory signaling cascade provides the formation of membrane pores through which mature IL-1β and IL-18 are released into the environment.*

Рисунок 1. *Двухсигнальная активация NLRP3 инфламмасомы. Активированные инфламмасомы NLRP3 способствуют высвобождению Caspase-1, которые переводят неактивные формы про-IL-1β и про-IL-18 в зрелые активные IL-1β и IL-18 соответственно. Gasdermin-D-опосредованный каскад воспалительных реакций обеспечивает образование мембранных пор, через которые идет выброс зрелых IL-1β и IL-18 в окружающую среду.*

The NLRP3 inflammasome may be activated by viruses, bacteria, fungi, toxins, ATP, particles of crystals of alumen, silicic acid, asbestos, cholesterol, UA in soluble and crystalline form, chemical irritants, UVB light, amyloid-β and amylin of the pancreatic islets [25–27]. Considering the varied nature of the NLRP3 activators, a suggestion was made to the effect that not all activators link directly to the inflammasome [28]. Most of them likely act by selecting or modifying the original mediator that is the true ligand of NLRP3, whose nature is still unknown. In the current model of the NLRP3 inflammasome activation, the UA is seen to act as Signal 2, whereas the nature of the Signal 1, priming the cells, is not yet clear.

We suggested that the blood cells from patients with hyperuricemia are already primed *in vivo* with Signal 1 and may be used as individual cellular test systems in vitro with the addition of a controlled concentration of the Signal 2 (UA) in order to see the individual manifestation of production of inflammatory cytokines. Traditionally, the analysis of cytokine stimulation *in vitro* is performed on isolated peripheral blood mononuclear cells (PBMC). In the previous paper, we demonstrated a donor-specific production of cytokines by blood mononuclear cells in vitro under the influence of immunomodulatrs [29]. However, segregation of PBMC is a labor-intensive process that poses limitations on processing of a large number of specimens required for a scaled diagnostics. In the process of PBMC segregation, both signal molecules of inflammation and the cellular populations are lost. In the gout, that is characterized with systemic signs of inflammation, the cellular sources of inflammation mediators are not properly characterized. In the recent years, the diagnostics seems to show a trend to use whole blood for the *ex vivo* stimulation of cytokine production, which imitates in a closer way the physiological *in vivo* conditions vs. methods that require PBMC isolation [30]. Physiological concentrations of some cytokines in the whole blood may be sufficiently high, therefore, in order to minimize their effect on the cell stimulation *in vitro*, we deemed it reasonable to use blood of donors diluted with RPMI growth medium designed to cultivate cells in vitro. The use of diluted donor blood allows maximum preservation of the population set of hematopoietic cells that are already primed with Signal 1 *in vivo* and to decrease, at the same time, the level of concentration of Signal 2 molecules.

In this paper, we performed a screening of pro-inflammatory cytokines in the donor serum and checked the conditions of induction of these cytokines *in vitro* at high concentrations of UA using the cells of diluted blood of individual donors.

AIM

Perform a screening of pro-inflammatory cytokines in the individual donor serum, check *in vitro* conditions of stimulating the NLRP3 inflammasome activity using UA and ascertain the production of which pro-inflammatory cytokines may be induced *in vitro* by high concentrations of uric acid.

MATERIAL AND METHODS

Blood cells of potentially healthy persons from the Samara Region Clinical Blood Transfusion Center (SRCBTC) and those of patients with hyperuricemia and gouty arthritis from the Clinical Hospital of the Samara State Medical University (SSMU Clinics). The research involved formalization of permits of the SSMU Committee for Bioethics (Protocol No. 215 dated 20.01.2021). In 100% cases of SRCBTC patients, the patients were male. Patients from the SSMU Clinics were male and female. The characteristics of patients with gouty arthritis follow in Table 1.

Selection of research participants from the donor register of the SRCBTC was randomized. Each participant signed an informed consent form for the processing of personal data, and for the handover of biological material (venous blood) within the scope of research. The venous blood was sampled to EDTA vacutainer tubes (Kometaline, Russia). The sampling was performed following an overnight fast. The vacutainers with blood were stored at room temperature not more than 4 hours following sampling. To determine the cytokine status of donors, production of IL-1β, IL-6, IL-18 and TNF-α cytokines in the donors' serum was studied.

Preparation of soluble ureic acid (99+%, Thermo Fisher Scientific, USA) was performed as described in [18]. The initial UA solution (stock) was prepared as follows: 60 mg of UA was dissolved in 70 ml distilled water heated to 30°C. Then, using 0.5 M NaOH, the solution pH was brought to 7.3. The final concentration of UA in 100 ml of solution was 3.5 mM. The solution was filtered through a 0.22 micron filter, and the subsamples were stored in sterile tubes. On the day of the experiment, the stock solution of UA was heated to 37°C and diluted with heated nutritional medium RPMI ("BioloT", Russia) containing 50 U/ml of penicillin and 50 mcg/ml of streptomycin ("BioloT", Russia). In the polarized light of the microscope, the crystals were not found neither in the original 3.5 mM solution, not in the nutritional medium containing 1 mM UA (data not provided).

Research design. The whole donor blood was diluted with the nutritional medium RPMI, содержащей containing 50 U/ml of penicillin and 50 mcg/ml of streptomycin ("BioloT", Russia). 200 mcl subsamples of decimally diluted blood with added UA in the concentration of 0.5 and 1 mM were poured into the wells of a 96-well plate (TPP, Switzerland) in multiple replicas. The plates were incubated at 37°C and 5% CO2 for 16±4 hours. In the assessment of the cytokine status of patients with acute gouty arthritis, quintuple diluted blood with added UA in the concentration of 1 mM were used; the samples were poured 200 mcl per well of a 96-well plate (Biofil, China). The plates were incubated at 37°C and 5% CO2 for 22±4 hours. After the time specified, 100 mcl of conditioned medium were sampled into 1.5 ml microtubes that were frozen at -20°C for the enzyme-linked immunelectrodiffusion assay (ELISA).

Enzyme-linked immunoelectrodiffusion assay (ELISA). The samples of serum and conditioned medium of cells were analyzed as per instructions of the manufacturer of the ELISA test "Vector-Best" ("Vector" LLC, Russia). The sensitivity of the test was 1; 0.5; 2 and 1 pg/ml for IL-1β, IL-6, IL-18, and TNF-α, respectively.

Patient No.	P UA 372.12	P UA 375.8	P UA 450	P UA 519	P UA 620
Sex	F	F	F	M	M
Age	68	66	63	47	43
Diagnosis	Gouty arthritis, exacerbation				
Serum UA, umol/l	372,12	375,8	450	519	620
Number of deformed joints	0	0	3	2	2

Table 1. *Profile of patients with gouty arthritis from the Clinics of the Samara State Medical University*

Таблица 1. *Характеристика пациентов с подагрическим артритом из Клиник СамГМУ*

RESULTS AND DISCUSSION

Usually, in order to evaluate the cytokine status of patients the level of cytokines in serum or plasma is studied. First, we performed a screening of pro-inflammatory cytokines in the donor serum. The targets were some pro-inflammatory molecules IL-1β, IL-6, IL-18 and TNF-α described in the literature as participating in the pathogenesis of gouty arthritis. Following the published data, the average level of IL-1β, IL-6, IL-18 and TNF-α in the serum was 3.6±1.01; 4.7±0.84; 267.1±14.63; and 1.2±0.13, respectively [31]. The analysis of cytokines in the serum of healthy donors (SRCBTC) with the ELISE test ("Vector-Best") showed the lowest possible levels of IL-1β and TNF-α at the ELISE threshold of detection, and showed a donor-specific content of IL-6 and IL-18 cytokines (Fig. 2A). It is interesting to notice that the levels of inflammasome-regulated cytokines IL-1β and IL-18 in the donor serum were drastically different: IL-1β was present in minimum quantities (<10 pg/ml), whereas the IL-18 concentration was up to 200 pg/ml.

This is likely related to the constitutive expression of the IL-18 gene and the alternate pathways of regulation of the synthesis of mature forms of the IL-18 cytokine [24, 32]. Our goal was to test the priming of the blood cells with uric acid simulating the condition of the gouty arthritis. Considering that the formation of crystals *in vivo* is possible with the serum levels of UA at >420–600 μmol/l, we chose two concentrations of UA: 0.5 mM, which aligns with hyperuricemia, and 1 mM, which equals the ultimately high concentration of uric acid under which formation of crystals is still possible. However, the study of the RPMI medium with 1 mM of uric acid did not find any crystals (data not shown). To be sure that the concentrations of UA match the added quantities, a donor was selected with a boundary amount of UA in the serum at 392.1 μmol/l, and the concentrations of UA were identified in the conditioned medium of 10-fold diluted blood of the said donor with added UA in the final concentration of 0.5 and 1 mM (Table 2). The results of analysis of UA in the conditioned medium of 10-fold diluted donor blood align with the added quantities of uric acid.

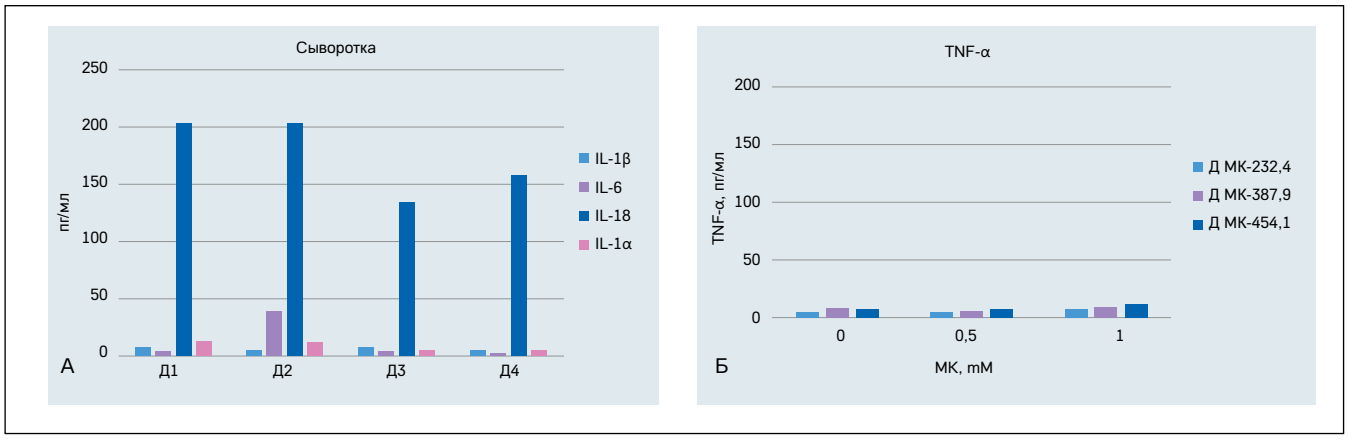

Figure 2. *(А) Analysis of interleukins in donor serum by ELISA. (Б) The amount of TNF-α in the conditioned medium of blood cells, obtained from donors with normouricemia (Д МК 232.4 and Д МК 387.9, with an indicator of uric acid in the blood of 232.4 and 387.9 μmol/l, respectively) and hyperuricemia (ГУ МК 454.1, with an indicator of uric acid in the blood 454.1 μmol/l) in the absence (0) and the presence of uric acid (UA) at 0.5- and 1-mM concentration.*

Рисунок 2. *(А) ИФА интерлейкинов в сыворотке доноров. (Б) Содержание TNF-α в культуральной среде клеток крови доноров с нормоурикемией (Д МК 232,4 и Д МК 387,9, с показателями мочевой кислоты в крови 232,4 и 387,9 мкмол/л соответственно) и гиперурикемией (ГУ МК 454,1, с показателем мочевой кислоты в крови 454,1 мкмол/л) при отсутствии (0) и присутствии мочевой кислоты (МК) в концентрации 0,5 и 1 мМ.*

Using the conditions tested in vitro with addition of 0.5 and 1 mM of uric acid to the conditioned medium of cells, some pilot tests were performed to evaluate the production of inflammatory cytokines by donors' blood cells whose UA cerum values varied from normal (232.4 μmol/l) to ones seen in hyperuricemia (454.1 μmol/l). The values of UA in the donor serum are shown in the donors' ID: 232.4, 387.9 and 454.1 μmol/l, respectively. The results of the ELISA test showed background indicators of TNF-α in the conditioned medium of donors' blood cells incubated in the presence of elevated concentrations of uric acid (0.5 and 1 mM). Th sensitivity of the ELISA test ("Vector-best") for TNF-α was 1 pg/ml. Even though the concentration of TNF-α in the conditioned medium was growing along the increase of uric acid concentration, the values of TNF-α varied in the range of 5–10 pg/ml, which is not sufficient for the diagnostically significant validity of results considering the donor-specific variations (**Fig. 2Б**). In the analysis of production of the inflammasome-regulated cytokine IL-1β, we faced the problem of significant qualitative variability of results, which calls for deeper research (data not shown).

The blood cells of the same donors produced significant quantities (hundreds of picograms per milliliter) of pro-inflammatory cytokines IL-6, IL-18 in response to the elevated concentrations of uric acid in the conditioned medium (**Fig. 3**).

At the same time, the uric acid in the concentration of 0.5 mM did not cause any significant changes in the production of IL-6, IL-18 interleukins (**Fig. 3**), and only in the presence of 1 mM of uric acid was there a marked stimulating effect on the production of the said cytokines by the donors' blood cells. The most significant, 30- to 40-fold increase of production was seen for the inflammasome-regulated cytokine IL-18 making it a potential marker for the analysis in patients with gouty arthritis. Interestingly, the evaluation of inflammatory cytokines IL-6 in the same samples of conditioned media showed a reverse effect of production of this cytokine as compared to the IL-18. In the samples of the patient's

	Donor serum	Decimally diluted donor blood		
Added UA(μmol/l)	0	0	535	1075
Results of UA analysis (μmol/l)	392,1	43,1	607,3	916,8

Table 2. *The concentration of uric acid (UA) in the serum and cell growth medium of donor cells. Donor blood with a serum UA of 392.1 μmol/L was diluted 10-fold with RPMI medium containing 0.535 and 1075 μmol/L UA and then the concentration of UA in the cell growth medium was determined using a BM/Hitachi 902 blood analyzer*

Таблица 2. *Концентрация мочевой кислоты (МК) в сыворотке и культуральной среде клеток донора. Кровь донора с показателем МК в сыворотке 392,1 мкмол/л была десятикратно разбавлена средой RPMI, содержащей 0,535 и 1075 мкмоль/л МК, и затем концентрация МК в культуральной среде была определена с помощью анализатора крови BM/ Hitachi 902*

blood cells with high concentration of uric acid (454.1), there was seen a minimal production of IL-6. The IL-6 is described as a cytokine whose values are elevated in the plasma of patients with gouty arthritis in the acute phase but not in the patients with remission of gouty arthritis [13]. We were interested in the effect of reverse correlation of IL-18 and IL-6 production in the course of *in vitro* stimulation of donor blood cells with uric acid. Patients with gouty arthritis in the acute phase were selected, and an *in vitro* analysis of production of IL-18 and IL-6 by blood cells of these patients was performed to be compared with that of potentially healthy donors (**Fig. 4**). In the clinical exacerbation of gouty arthritis, the production of IL-6 to the conditioned medium by the patient's blood cells (П МК) was lower, whereas the cells of potentially healthy donors continued to produce the IL-6 (**Fig. 4А**). The analysis of the same conditioned media showed the activity of cells of the patients with gouty arthritis in the acute phase to produce elevated concentrations of IL-18 in the presence of uric acid (**Fig. 4Б**), which matches the literature data on the elevated content of IL-18 in the serum of patients in the acute phase of gouty arthritis.

Despite the pilot research with a modest sampling of patients, it can be said that these results confirm our

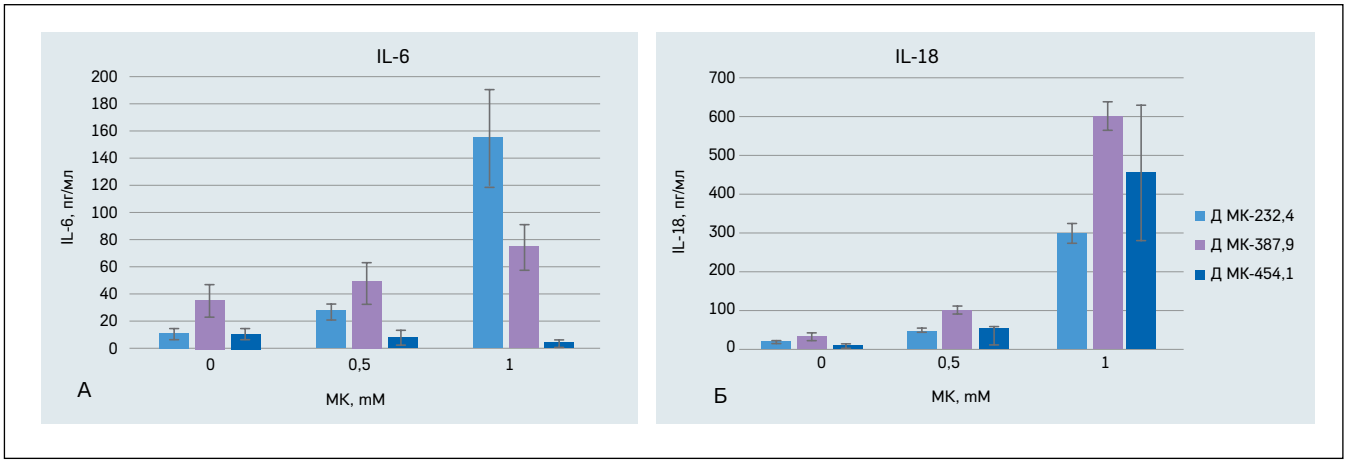

Figure 3. *ELISA analysis for IL-6 and IL-18 in a cell growth medium containing blood cells from donors (Д with UA in blood 232.4 and 387.9 μmol/L, respectively) and a patient with hyperuricemia (ГУ with UA in blood 454.1) in the absence of (0) and the presence of UA (0.5 and 1 mM).*

Рисунок 3. *ИФА IL-6 и IL-18 в культуральной среде, содержащей клетки крови доноров (Д с показателями МК в крови 232,4 и 387,9 мкмол/л соответственно) и пациента с гиперурикемией (ГУ с показателем МК в крови 454,1) в отсутствии (0) и присутствии МК (0,5 и 1 мМ).*

supposition that the blood cells of patients with gouty arthritis sensibilized *in vivo* with Signal react to the stimulation with uric acid in a different manner than the blood cells of healthy donors.

The developed *in vitro* hyperuricemic haemotest system for the analysis of hyperuricemia-stimulated activity of inflammasomes of patients is a more adequate model that the whole blood of serum since it uses the blood cells of patients already sensibilized *in vivo* with Signal 1. The nature of the latter is not yet fully studied. Specifically predefined concentrations of uric acid (Signal 2) may be added to the conditioned medium to optimize the experiment in vitro. The serum values of the majority of cytokines are at the borderline of detection, and their accumulation in the serum depends on multiple factors. The hyperuricemic haemotest system allows improvement of cytokine detection levels as compared with the analysis of the serum and, at the same time, a precise control of concentration of signal molecules characteristic for some disease or other.

The drawback of this approach is the cell sensitivity to fluctuations of temperature, composition of the nutritional medium, manufacturer of well plate and ELISA, which influences the quantitative values of cytokines. Specifically, we faced this challenge in the analysis of production of the inflammasome cytokine IL-1 (data not shown). The solution of the problem of quantitative variations may be in the finding of a regular correlation in the production of several cytokines in similar conditions. Following a scaled study of patients, it will be possible to introduce correlation indices characteristic of different pathologies.

The marked effect of reverse correlation between the production of the inflammasome interleukin IL-18 and the broad spectrum cytokine IL-6 by the blood cells of patients with hyperuricemia (including patients with

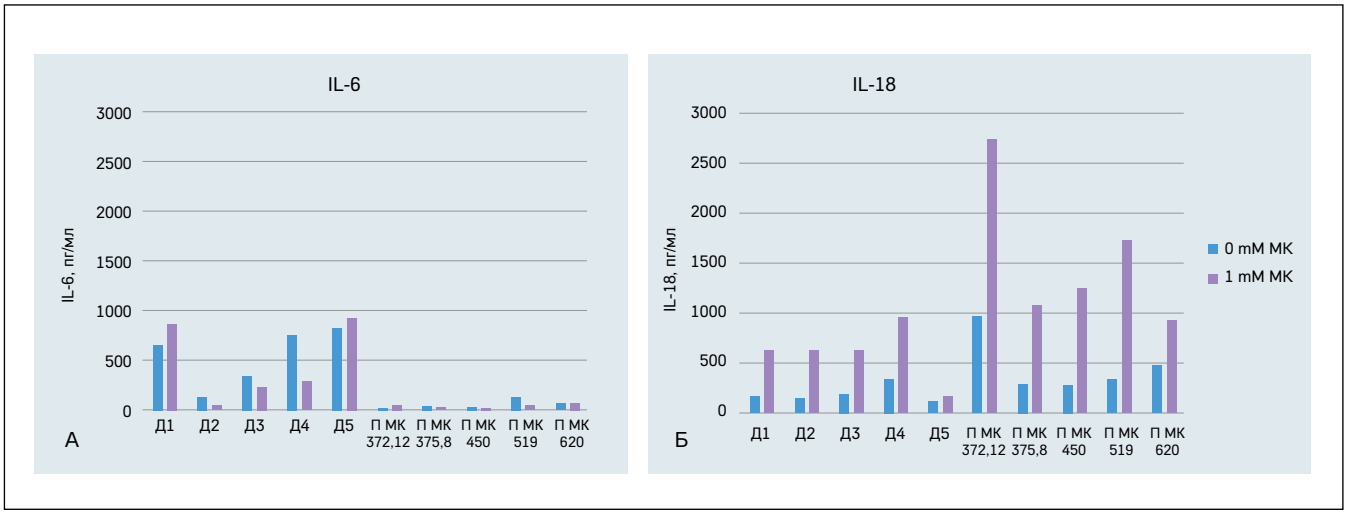

Figure 4. *ELISA analysis of IL-6 and IL-18 in a cell growth medium containing blood cells of donors (Д) and patients with acute gouty arthritis in the absence (0) and the presence of uric acid (1 mM UA).*

Рисунок 4. *ИФА IL-6 и IL-18 в культуральной среде, содержащей клетки крови доноров (Д) и пациентов с подагрическим артритом в период обострения в отсутствии (0) и присутствии мочевой кислоты (1 мМ МК).*

gouty arthritis in the acute phase) *in vitro* is of special scientific interest and requires further, broader research in the scope of creating the hyperuricemic haemotest system for personalized diagnostics.

CONCLUSIONS

As part of development of personalized cell test system, conditions were designed for the *in vitro* stimulation of blood cells from individual donors by uric acid. It was demonstrated that the developed hyperuremic haemotest system based on the use of blood cells from individual donors diluted with nutritional medium may serve as an adequate *in vitro* cell model to study the impact of signal molecules of the inflammation. The results of the analysis of the *in vitro* hyperuremic haemotest system using diluted blood of potentially healthy donors and patients, whose blood cells were sensibilized in vivo against presence of specific factors characteristic of gouty arthritis, showed variations in the production of IL-18 and IL-6 cytokines. This may be used in the personalized diagnostics to forecast development not only of gouty arthritis but of other pathologies as well.

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Conflict of Interest. The authors declare that there are no obvious or potential conflicts of interest associated with the content of this article.	***Конфликт интересов.*** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с содержанием настоящей статьи.
Contribution of individual authors. L.T. Volova – initiated and managed the study; was responsible for manuscript editing. E.I. Pugachev – conducted experimental studies; provided detailed analysis of study results. T.V. Starikova – conducted experimental studies. P.A. Lebedev – managed theoretical and clinical part of the study; provided interpretation of study results. I.A. Shafieva – coordinated the laboratory material collection. S.I. Kuznetsov – coordinated the laboratory material collection. O.A. Gusyakova – managed the laboratory tests. G.N. Svetlova – coordinated the study. N.K. Osina – was responsible for study design; provided interpretation of study results. All authors gave their final approval of the manuscript for submission, and agreed to be accountable for all aspects of the work, implying proper study and resolution of issues related to the accuracy or integrity of any part of the work.	***Участие авторов.*** Л.Т. Волова – инициирование и организация исследования; редактирование рукописи. Е.И. Пугачев – проведение экспериментальных исследований; анализ результатов исследования. Т.В. Старикова – проведение экспериментальных исследований. П.А. Лебедев – организация теоретической и клинической части исследований; интерпретация результатов исследования. И.А. Шафиева – координация сбора лабораторного материала. С.И. Кузнецов – координация сбора лабораторного материала. О.А. Гусякова – организация и проведение лабораторных исследований. Г.Н. Светлова – координация исследования. Н.К. Осина – оформление дизайна исследования; интерпретация результатов исследования. Все авторы одобрили финальную версию статьи перед публикацией, выразили согласие нести ответственность за все аспекты работы, подразумевающую надлежащее изучение и решение вопросов, связанных с точностью или добросовестностью любой части работы.

REFERENCES / ЛИТЕРАТУРА

1. Lebedev PA, Garanin AA, Novichkova NL. Pharmacotherapy of gout – modern approaches and prospects. *Sovremennaya revmatologiya*. 2021;15(4):107-112. (In Russ.). [Лебедев П.А., Гаранин А.А., Новичкова Н.Л. Фармакотерапия подагры – современные подходы и перспективы. *Современная ревматология*. 2021;15(4):107-112]. https://doi.org/10.14412/1996-7012-2021-4-107-112

2. Roumeliotis A, Dounousi E, Eleftheriadis T, et al. Dietary antioxidant supplements and uric acid in chronic kidney disease: a review. *Nutrients*. 2019;11(8):1911. https://doi.org/10.3390/nu11081911

3. Bos MJ, Koudstaal PJ, Hofman A, et al. Uric acid is a risk factor for myocardial infarction and stroke: The Rotterdam Study. *Stroke*. 2006;37(6):1503-1507. https://doi.org/10.1161/01.STR.0000221716.55088.d4

4. Kim K, Kang K, Sheol H, et al. The Association between Serum Uric Acid Levels and 10-Year Cardiovascular Disease Risk in Non-Alcoholic Fatty Liver Disease Patients. *Int J Environ Res Public Health*. 2022;19(3):1042. https://doi.org/10.3390/ijerph19031042

5. Duan X, Ling F. Is uric acid itself a player or a bystander in the pathophysiology of chronic heart failure? *Med Hypotheses*. 2008;70(3):578-581. https://doi.org/10.1016/j.mehy.2007.06.018

6. Yanai H, Adachi H, Hakoshima M, Katsuyama H. Molecular biological and clinical understanding of the pathophysiology and treatments of hyperuricemia and its association with metabolic syndrome, cardiovascular diseases and chronic kidney disease. *Int J Mol Sci*. 2021;22(17):9221. https://doi.org/10.3390/ijms22179221

7. Feig DI, Johnson RJ. Hyperuricemia in childhood primary hypertension. *Hypertension*. 2003;42(3):247-252. https://doi.org/10.1161/01.HYP.0000085858.66548.59

8. Stamp L, Dalbeth N. Screening for hyperuricaemia and gout: A perspective and research agenda. *Nature Reviews Rheumatology*. 2014;10(12):752-756. https://doi.org/10.1038/nrrheum.2014.139

9. Bhole V, De Vera M, Rahman MM, et al. Epidemiology of gout in women: Fifty-two-year followup of a prospective cohort. *Arthritis Rheum*. 2010;62(4):1069-1076. https://doi.org/10.1002/art.27338

10. Richette P, Doherty M, Pascual E, et al. 2018 updated European League against Rheumatism evidence-based recommendations for the diagnosis of gout. *Ann Rheum Dis*. 2020;79(1):31-38. https://doi.org/10.1136/annrheumdis-2019-215315

11. Shiozawa A, Szabo SM, Bolzani A, et al. Serum uric acid and the risk of incident and recurrent gout: A systematic review. *J Rheumatol*. 2017;44(3):388-396. https://doi.org/10.3899/jrheum.160452

12. Scanu A, Oliviero F, Ramonda R, et al. Cytokine levels in human synovial fluid during the different stages of acute gout: Role of transforming growth factor β1 in the resolution phase. *Ann Rheum Dis*. 2012;71(4):621-624. https://doi.org/10.1136/annrheumdis-2011-200711

13. Jiang X, Li M, Yang Q, et al. Oxidized Low Density Lipoprotein and Inflammation in Gout Patients. *Cell Biochem Biophys*. 2014;69(1):65-69. https://doi.org/10.1007/s12013-013-9767-5

14. Cavalcanti NG, Marques CDL, Lins TU, et al. Cytokine Profile in Gout: Inflammation Driven by IL-6 and IL-18? *Immunol Invest*. 2016;45(5):383-395. https://doi.org/10.3109/08820139.2016.1153651

15. Verma AK, Hossain MS, Ahmed SF, et al. In silico identification of ethoxy phthalimide pyrazole derivatives as IL-17A and IL-18 targeted gouty arthritis agents. *J Biomol Struct Dyn*. 2022;41(1):1-15. https://doi.org/10.1080/07391102.2022.2071338

16. Tran AP, Edelman J. Interleukin-1 inhibition by anakinra in refractory chronic tophaceous gout. *Int J Rheum Dis*. 2011;14(3):33-37. https://doi.org/10.1111/j.1756-185X.2011.01629.x

17. So AK, Martinon F. Inflammation in gout: Mechanisms and therapeutic targets. *Nat Rev Rheumatol*. 2017;13(11):639-647. https://doi.org/10.1038/nrrheum.2017.155

18. Braga TT, Forni MF, Correa-Costa M, et al. Soluble Uric Acid Activates the NLRP3 Inflammasome. *Sci Rep*. 2017;7:1-14. https://doi.org/10.1038/srep39884

19. Spel L, Martinon F. Inflammasomes contributing to inflammation in arthritis. *Immunol Rev*. 2020;294(1):48-62. https://doi.org/10.1111/imr.12839

20. Cavalcanti NG, Bodar E, Netea MG, et al. Crystals of monosodium urate monohydrate enhance lipopolysaccharide-induced release of interleukin 1-β by mononuclear cells through a caspase 1-mediated process. *Ann Rheum Dis*. 2016;68(2):273-278. https://doi.org/10.1136/ard.2007.082222

21. Malyshev IY, Pihlak AE, Budanova OP. Molecular and Cellular Mechanisms of Inflammation in Gout. *Pathogenesis*. 2019;17(4):4-13. (In Russ.). [Малышев И.Ю., Пихлак А.Э., Буданова О.П. Молекулярные и клеточные механизмы воспаления при подагре. *Патогенез*. 2019;17(4):4-13]. https://doi.org/10.25557/2310-0435.2019.04.4-13

22. Martinon F, Pétrilli V, Mayor A, et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 2006;440(7081):237-241. https://doi.org/10.1038/nature04516

23. Prencipe G, Bracaglia C, De Benedetti F. Interleukin-18 in pediatric rheumatic diseases. *Curr Opin Rheumatol*. 2019;31(5):421-427. https://doi.org/10.1097/BOR.0000000000000634

24. Kaplanski G. Interleukin-18: Biological properties and role in disease pathogenesis. *Immunol Rev*. 2018;281(1):138-153. https://doi.org/10.1111/imr.12616

25. Takahashi M. NLRP3 inflammasome as a novel player in myocardial infarction. *Int Heart J*. 2014;55(2):101-105. https://doi.org/10.1536/ihj.13-388

26. Duewell P, Kono H, Rayner KJ, et al. Activated By Cholesterol Crystals That Form Early in Disease. *Nature*. 2010;464(7293):1357-1361. https://doi.org/10.1038/nature08938.NLRP3

27. Liu W, Yin Y, Zhou Z, et al. OxLDL-induced IL-1beta secretion promoting foam cells formation was mainly via CD36 mediated ROS production leading to NLRP3 inflammasome activation. *Inflamm Res*. 2014;63(1):33-43. https://doi.org/10.1007/s00011-013-0667-3

28. Pirozhkov SV, Litvitskiy PF. Inflammasomal diseases. *Immunologiya*. 2018;39(2):158-165. (In Russ.). [Пирожков С.В., Литвицкий П.Ф. Инфламмасомные болезни. *Иммунология*. 2018;39(2):158-165]. https://doi.org/10.18821/0206-4952-2018-39-2-3-158-165

29. Volova LT, Osina NK, Kuznetsov SI, et al. Donor-specific production of cytokines by blood cells under the influence of immunomodulators: New aspects of a personalized approach in medicine. *Science and Innovations in Medicine*. 2022;7(4):250-257. (In Russ.). [Волова Л.Т., Осина Н.К., Кузнецов С.И., и др. Донор-специфичная продукция цитокинов клетками крови под влиянием иммуномодуляторов: новые аспекты персонифицированного подхода в медицине. *Наука и инновации в медицине*. 2022;7(4):250-257]. https://doi.org/10.35693/2500-1388-2022-7-4-250-257

30. Damsgaard CT, Lauritzen L, Calder PC, et al. Whole-blood culture is a valid low-cost method to measure monocytic cytokines – A comparison of cytokine production in cultures of human whole-blood, mononuclear cells and monocytes. *J Immunol Methods*. 2009;340(2):95-101. https://doi.org/10.1016/j.jim.2008.10.005

31. Zaitseva GA, Vershinina OA, Matrokhina OI, et al. Cytokine status of blood donors and its components. *Fundamental research*. 2011;3:61-65. (In Russ.). [Зайцева Г.А., Вершинина О.А., Матрохина О.И., и др. Цитокиновый статус доноров крови и ее компонентов. *Фундаментальные исследования*. 2011;3:61-65].

32. Miyazawa H, Wada T. Immune-mediated inflammatory diseases with chronic excess of serum interleukin-18. *Front Immunol*. 2022;13:1-14. https://doi.org/10.3389/fimmu.2022.930141