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Experience of the successful treatment with canakinumab of a patient with NLPC4-associated autoinflammatory syndrome with enterocolitis

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The article shows the observation of rare NLPC4-associated autoinflammatory syndrome with enterocolitis and familial cold urticaria. Diagnosis is confirmed molecularly-genetically: previously not described mutation c.928C>T in the heterozygous state in NLRC4 gene is discovered by a method of the new generation sequencing. The use of a monoclonal antibody to the interleukin 1 canakinumab provided complete relief of fever and skin and intestinal symptoms in just 1 week of treatment. Later the signs of inflammation have disappeared completely; the patient's quality of life improved and life-threatening complications were prevented. The above example demonstrates the high clinical efficacy of canakinumab in the patient with NLRC4-associated autoinflammatory syndrome and suggests promising therapeutic use of interleukin 1 blockers in such patients. There were no adverse events during canakinumab therapy..
Keywords*: children, autoinflammatory syndromes, NLRC4, autoinflammatory syndrome with enterocolitis, canakinumab, human monoclonal antibody, interleukin-1.*

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Introduction

Autoinflammatory syndromes are rare monogenic genetic diseases, which are inherited according to autosomal recessive or autosomal dominant modes. They are characterized by intermittent febrile states, which are accompanied by various systemic symptoms including joints, skin and intestine affections [1].

Autoinflammatory diseases, which are associated with the mutations in the NLRP3 gene (encodes the cryopyrin protein), are called cryopyrin-associated periodic syndromes (CAPS). The NLRP3 protein is a crucial component in the activation of natural (antigen-nonspecific) immunity through a cytoplasmic multi-protein complex called inflammasome. The latter plays an important role in the production of the main pro-inflammatory cytokine – interleukin (IL) 1β – and in the development of systemic inflammation [2].

It is known that the majority of these mutations (leading to CAPS) are located within or in close vicinity of the area which encodes the NOD domain of the NLRP3 inflammasome. This domain is responsible for the processes of inflammasome activation [3]. These mutations affect the conformation of NLRP3 inflammasome elements, which lead to its uncontrolled activation and to the launching of an inflammatory cascade with the release of IL 1 and 18. Most of these mutations are missense mutations and are inherited according to the autosomal dominant mode [3].

However, studies show that genetic analysis confirms only 30-40% of clinically proven cases [3]. Furthermore, it is determined that mutations, which affect the synthesis of other proteins – NLRC4 and NLRP1 – can lead to the development of severe autoinflammatory syndromes [4, 5]. For example, with mutations in the NLRC4 gene, two previously unknown autoinflammatory syndromes (characterized by an intermittent febrile state and other systemic presentations) are associated [4, 5].

The following clinical observation describes the experience of diagnosing and treating a rare autoinflammatory syndrome – NLRC4-associated enterocolitis and familial cold urticaria.

Clinical example

Patient J., 4 years old, has been observed in the rheumatology department of the Scientific Center of Children's Health (Moscow) for 1.5 years. From the anamnesis it is known that the girl was from the 1st pregnancy (which was physiological), born from term birth at the 36th week through Caesarean section. Birth weight: 2650 g, body length: 51 cm, Apgar score: 6/7. The girl started holding her head at the age of 5 months, sitting – at 11 months, walking – at 1 year and 2 months. Early physical development corresponded to the age. Preventive vaccination was carried out according to the National calendar. The child's hereditary history is aggravated: the mother and the grandmother on the mother's side are being observed with Systemic lupus erythematosus, the father has dermatitis.

The girl's mother has had lymphadenitis from the age of 6, facial edemas and proteinuria up to 0,33 g/l (the upper limit is 0,14); since the age of 14 years she has had arthralgia, high blood pressure (up to 170/110 mm Hg) and erythematous face rash. She has also developed optic discs atrophy. She was treated with hydroxychloroquine and glucocorticosteroids per os - without substantial positive effect. Concerning the grandmother on the mother's side, arthralgia and episodes of high blood pressure appeared in her at the age of 21 - after childbirth. Then alopecia and optic discs atrophy developed. Since the age of 26 myalgia, knee joints arthritis, mild proteinuria and edemas have been observed. Also, peptic ulcer disease was diagnosed. Hepatosplenomegaly and increased erythrocyte sedimentation rate (ESR) (up to 35 mm/h) were found during examination at the age of 34.

Our patient has congenital recidivous maculopapular rash on her cheeks and subfebrile conditions. A local pediatrician observed the girl - her state was considered atopic dermatitis. Increased body temperature (up to 37,4 °C) and infrequent urination were registered at the age of 1 month. Urinalysis indicated leukocyturia up to 15-18 cells per field of view (upper limit is 5), at one point – erythrocyturia up to 10 cells per field of view (upper limit is 3). Urinary tract infection was diagnosed and furasidin prescribed. A second episode of body temperature increase and leucocyturia occurred at the age of 3 months. At the age of 6 months during the examination at the local medical institution, pyelectasia on the right side was detected. According to cystography, there were no signs of reflux. The child was registered with a nephrologist, diagnosed with Recidivous urinary tract infection. No further changes in urinalysis were observed.

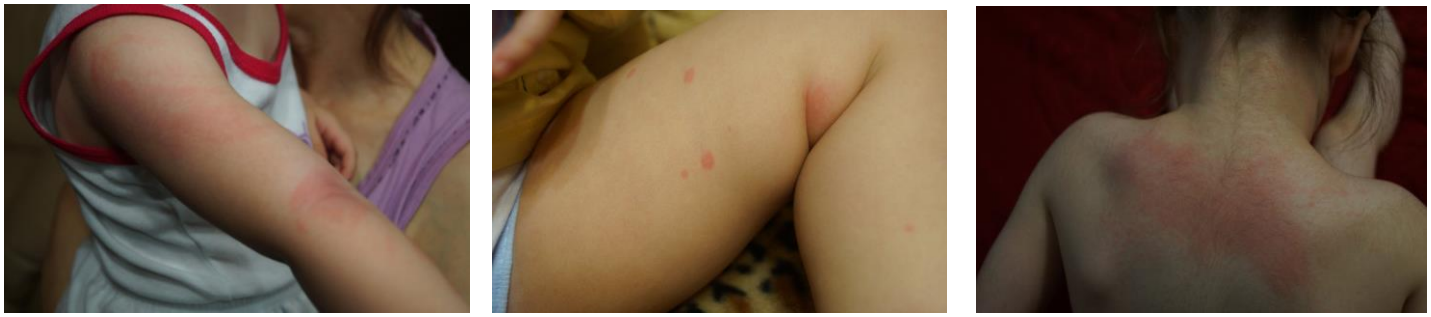
Starting from the age of 10 years, the girl started having episodes of non-motivated febrile temperature risings which lasted for 3-5 days, with a periodicity of approx. 1 month; recidivous stomach pains were noted. At the age of 1 year 11 months the body temperature rose to 39 °C, the patient developed first an erythematic and then - urticarial rashes on her shoulders, back and lower limbs. The child was examined by a dermatologist, who diagnosed erithema of unclear ethiology and prescribed antibacterial therapy with a penicillic preparation. The fever and rash stopped on the 4th day. However, the temperature rises started again after 1 week with antipyretic and antibacterial therapy being inefficient.

In order to clarify the diagnosis and to be treated, the child was directed for hospitalization to the rheumatological department of the Scientific Center of Children's Health. At the age of 2 years (april 2014) the patient was first hospitalized to the department. The child's condition at hospitalization was evaluated as of moderate severity. The severity was due to overall weakness and apathy. Examination revealed low-grade pyrexia, lymphadenopathy, hepatomegaly.

Menocelis was present on the face, body and limbs (fig. 1, a-c).

Fig. 1, a–c. *Overall looks of the patient before canakinumab treatment.*

A **B** **C**



Clinical blood analysis: leukocytosis up to $13,7\times10^9$/l (norm up to 9), trombocytosis up to 419×10^9/l (norm up to 370), ESR did not exceed 8 mm/h (norm up to 20).
All the indexes of biochemical and immunological analysis were within normal boundaries (table). Urinalysis indicated protein- (up to 0,33 g/l) and phosphaturia (12,5 mmol/l; normally phosphates are absent).

Table. *Dynamics of the clinical and laboratory indexes of disease activity in patient J. while treting with canakinumab.*

Canakinumab	**Before start of treatment**	**1 week after the start of treatment**	**12 weeks after the start of treatment**
Fever, ^{0}C	39,0	None	None
Rash	+	–	–
ESR, mm/h (N from 2 to 20)	8	4	6
Haemoglobin, g/l (N 120–145)	105	112	120
Thrombocytes, $\times10^9$/l (N 150–440)	419	354	272
Leucocytes, $\times10^9$/l (N 5,6–11,5)	13,7	9,4	7,2
C-reactive protein, mg/l (N to 5)	2	1	1
Proteinuria, g/l (N to 0,14)	0,033	Traces	None

According to ultrasound examination: the right liver lobe increased to 89 mm, diffused inhomogenity and an increased parenchima echogenicity; the size of kidneys and the calices-pelvis systems, did no exceed the norms, however, the examination demonstrated an echogenicity increase of the kidney cortex layer, bloodflow weakening on the periphery, enduration of the walls of the calices-pelvis system. There was no pathology revealed in other organs and systems.
The child underwent echocardiography, lung and abdominal computer tomography, magnetic-resonance imaging of the brain - no data for serositis, space-occupying neoplasms or inflammatory changes was received.
The girl was examined by an ophthalmologist and surdologist: no sense organ pathologies revealed; the nephrologist established phosphaturia.
The geneticist, having examined the child, recommended a molecular-genetic analysis in order to establish mutations that lead to the development of TRAPS-syndrome, familial Mediterranean fever and CAPS. It was also necessary to exclude mevalonic aciduria.
Urinalysis did not show an increase in the secretion of mevalonic acid, the level of which in the blood serum was not increased as well.

All coding exones and nearby intronic regions of the NLRP3, MEFV, TNFRSF1A genes were investigated using the direct automatic sequencing method. A homozygous c.605G>A polymorphism was found in the 2nd exon of the MEFV gene, which is characteristic for periodic disease patients [6]. Keeping in mind the clinical picture (fever, rash, aggravated hereditary anamnesis), familial Mediterranean fever was suspected in the patient.

According the modern international protocols for treating familial Mediterranean fever [7], the girl was prescribed with colchicine at a dose of 0.5 g/day.

Over the next 10 months 3 exacerbations were noted, each 5-7 days long and accompanied by hectic fever, polymorphous rash, pronounced weakness, sweatiness, arthralgia. At the age of 3 the child started getting blood in her stool.

The patient was hospitalized again to the rheumatological department of the Scientific center of children's health, where she underwent esophagogastroduodenoscopy, as a result of which multiple erosions were found in the antral section of the stomach, hyperemia and swelling of the mucosa of the stomach, duodenum and jejunum. Antral erosive-hemorrhagic gastritis was diagnosed, together with bulbitis, duodenitis and jejunitis. According to colonoscopy, the endoscopic picture of the reviewed parts of the digestive tract was in line with the norm.

Thus, the early debut of the disease, recidivous episodes of fever, rash, urinary and intestine syndromes and aggravated hereditary anamnesis witnessed in favour of an auto-inflammatory syndrome. However, colchicine therapy turned out to be ineffective, there were no mutations in the NLRP3 and TNFRSF1A genes. In order to establish the mutations that can lead to the development of auto-inflammatory syndromes, we conducted a molecular-genetic investigation using the new generation sequencing method[1].

In our study we used the Roche 454 platform together with the SewCap EZ target enrichment technology (NimbleGen, Germany). For investigation we used the SeqCap EZ hybridization probe library (IRN 150803_HG19), which contains specific oligonucleotide sequences in order to analyze all the coding, adjacent intronic and aldo 5' - and 3' -non-translatable areas of the NLRP3, NLRC4, IL1RN, TRNT1, TMEM173, PSMB8, TNFRSF1A, MVK, LACC1, MEFV, NOD2, LPIN2, NLRP12, CECR1 genes.

As a result of investigating exon 4 of NLRC4 gene, we found a heterozygous nucleotide replacement c.928C>T, which lead to the termination of the p.R310X translation. The nucleotide replacement has not been described previously, according the the computer analysis (Alamut Visual) it is pathogenic.

Diseases that are associated with NLRC4 mutations include an auto-inflammatory syndrome with enterocolitis and familial cold urticaria (AIFEC, OMIM 47810) [5].

Fig. 2, a, b. *Overall looks of the patient while treating with canakinumab.*

a)  **b)** 

Familial cold auto-inflammatory syndrome-4 is a rare autosomal-dominant disease which is characterised by repeating episodes of maculopapular rash, combined with artro- and myalgias, fever, chills, limb swellings,

[1] New generation sequencing (NGS) is a method of determining the nucleotide sequence of the DNA and RNA, which allows to study several areas of the genome at the same time, which is its main difference from other sequencing methods [8]

conjunctivitis - which are triggered by the impact of cold. Auto-inflammatory syndrome with enterocolitis is an autosomal-dominant disease which is distinguished by repeating inflammatory episodes arising in early babyhood and accompanied by febrile fever, digestive symptoms, splenmegaly, artro- and myalgias [4, 5].
Based on the anamnesis data, the clinical picture and the results of laboratory and instrumental examination, including the results of a molecular-genetic test, the patient was diagnosed with «NLRC4-assiociated auto-inflammatory syndrome with enterocolitis». Keeping in mind the pathogenetic mechanisms of the disease, the child's pronounced invalidization, and the poor prognosis, we decided to prescribe an IL 1 inhibitor.
The preparation of choice became canakinumab. The preparation was indicated in a dose of 4 mg/kg of body mass every 4 weeks. 2 injections were performed at the hospital department. After 1 day from the first injection a significant improve in the patient's state was noted. The rash and sweatiness stopped, the stool became normal, the girl became more active and mobile (fig. 2).

The child underwent a planned examination after 6 months and the number of leucocytes, trombocytes, ESR were within norm in the clinical analysis (table). There were no proteins and phosphates noticed in the urinalysis.
According to ultrasound examination - the liver size normalized, parenchima homogenity of the liver and kidneys, as well as the blood circulation in kidneys were restored.
The patient received canakinumab for 14 months, for subcutaneous injection at a dose of 4 mg/kg of body weight every 4 weeks. No adverse effects have been registered. Over the course of therapy only 1 episode of disease exacerbation: when the interval between injections was unwillingly increased to 7 weeks - rash and fever appeared but stopped in 1 day after the injection of the IL1 antagonist.

Discussion

The analysis of our patient's anamnesis - age of the disease onset, aggravated hereditary anamnesis, systemic inflammatory reaction, skin syndrome allowed us to suspect, while the molecular-genetical test - to confirm the diagnosis of a rare hereditary disease: NLRC4-associated auto-inflammatory syndrome. The c.928C>T mutation in the NLRC4 gene, found in the patient, which leads to the termination of the p.R310X translation, is located in a conservative area which encodes the NBD-domen of the NLRC4-inflammosome and, according to the computer analysis data (Alamut Visual), which uses SIFT, PolyPhen2 and Mutation Taster prediction modules, leads to the synthesizing of a defective protein and can lead to NLRC4-associated syndromes. The NLRC4 gene is located in the 2p33.3 chromosome area and codes one of the domains of the macromolecular complex of the NLRC4-inflammosome [9]. Mutations in the NLRC4 gene lead to the hyperproduction of IL 1 and 18 and the launch of an inflammatory cascade mediated by them [11]. The group of NLRC4-associated syndromes is inherited according to the autosomal-dominant type, while most mutations in the NLRC4 gene are de novo mutations [5]. Most mutations that have been described up to the present day, lie in the area coding the NBD-domain of the NLRC4-inflammasome, which is responsible for its activation. These mutations can affect the conformation structure of the NLRC4-inflammosome, which, in turn, leads to its uncontrolled self-activation and the launching of an inflammatory cascade with the release of free IL 1 and IL 18 [11]. This pathogenetic mechanism is similar to the inflammatory cascade which is launched in cryopyrin-associated periodic syndromes (CAPS) [3]. However, as in the case with CAPS, which are caused by mutations in the NLRP3 (CIAS1) gene coding one of the NLRP3-inflammosome subunits, a clear phenotype classification of the disease, which is caused by mutations in the NLRC4 gene, is absent [4, 5].
Enterocolitis that develops at early ages has been described in patients with NLRC4 gene mutations, which is not characteristic for cryopyrin-associated syndromes [4, 5].
According to N. Romberg et al., who described the first registered patient, the disease debuted during the first weeks of life with a body temperature increase and diarrhea without any signs of an infectious disease [4]. Inflammation markers were increased, ferritine concentration was 4840 hg/ml (normal range from 18 to 370), the number of NK-cells was low. Over the next 2 weeks the child's state was worsening catastrophically: macrophage activation syndrome developed (pancytopenia, hypertriglyceridemia, blood clotting discruptions). The patient died on the 23rd day from diffused alveolar bleeding. Autopsy showed active macrophages in the central nervous system and a total damage of intestinal tissue.

2 days after the child died, his father developed fever, subarachnoid bleeding and an acute respiratory distress-syndrome. Examination revealed high levels of ferritine (29 200 ng/ml), C-reactive protein, IL 18 and a low count of NK-cells. As in his son, no infectious cause was found. Therapy was prescribes for the father: dexamethasone, cyclosporine, normal human immunoglobuline for IV injection. Anamnesis revealed that he had often fever seizures throughout his life, as a child he was often hospitalized with vomiting, diarrhea and fever. The last episode of intestinal syndrome was recorded at the age of 42. As an adult the patient developed joint and skin syndrome and psoriatic arthritis was diagnosed. Further hereditary investigation showed that the child's step-brother had fever episodes, stomach pains and intestinal syndrome at the age of 5; intestinal biopsy revealed villi atrophy.

Because a hereditary genetic disease was suspected, both father and son received exom sequencing. 34 nucleotide replacements were identified, classified as possible pathogenic. One of them was a p.Val341Ala replacement in the NLRC4 gene, which was diagnosed in the child. Clinical and anamnesis data , an increase in NLRC4-inflammasome activation markers and the finding of a mutation in the NLRC4 gene all evidenced in favor of a genetically-caused hereditary auto-inflammatory syndrome.

S.W. Canna at al. reported a 7-year-old girl of European origin with an auto-inflammatory syndrome with a recidivous syndrome of macrophage activation [5]. The clinical picture included periodically happening episodes of fever, vomiting, rash and splenomegaly. Keeping in mind the disease onset at the age of 6 months, the absence of infectious and oncological reasons, an auto-inflammatory syndrome was suspected. The child was prescribed with colchicine and glucocorticoids. Examination revealed high levels of triglycerids and ferritine, anemia, thrombocytopenia, and an increase in the inflammatory markers, which is not characteristic for CAPS. Apparently, the laboratory results evidenced in favour of the macrophage activation syndrome, which is a complication of juvenile idiopathic arthritis and some auto-inflammatory syndromes.

However, unlike a secondary macrophage activation syndrome, developing secondary to an auto-inflammatory disease, the patient had a normal NK-cell function, while the molecular-genetical tests for periodic syndromes did not reveal any mutations.

In order to verify the diagnosis, exom sequencing was carried out, which identified a p.Thr337Ser replacement in the NLRC4 gene. Researchers supposed that the identified mutation could lead to an increase in the activity of the NLRC4-inflammosome and an NLRC4-mediated hemophagocytic syndrome. Supporting this hypothesis, a high level of IL 18 was identified in the girl's blood serum (from 8,316 to 17,355 pg/ml as compared to the norm of 56–105 in healthy controls and 102–1281 — in CAPS patients).

In two clinical cases described in literature concerning patients with NLRC4-associated syndromes, the macrophage activation syndrome was diagnosed [4 ,5]. However the exact mechanisms, which leads to such syndromes in patients with NLRC4 mutations, have not been established yet.

Unlike patients with CAPS syndromes, children with the NLRC4-periodic syndrome had intestinal syndrome and had no signs of central nervous system lesions [4]. In our patient the illness also debuted at a very early age with a fever and abdominal syndrome. We did not establish a macrophage activation syndrome (normal levels of ferritine, triglycerides in blood serum, no cytopenia).

The choice of treatment tactics was justified. Clinical studies have established the central role of IL 1 n the development of many auto-inflammatory syndromes [12, 13]. Today the usage of IL 1 inhibitors (anakinra, canakinumab, rilonacept) is the only effective way of treating. Actually, it is «salvage therapy» [14-16]. Canakinumab - a totally humanized anti-IL 1β-monoclonal antibody, which selectively binds with soluble IL 1β - is the only IL 1 blocker registered in Russia.

In cases of NLRP3-associated auto-inflammatory syndromes, the inflammasome spontaneously increases its activity [10], which leads to a high concentration of IL1 and 18 and to various symptoms, including fever, aseptic meningitis, arthralgies and different rashes. Mutations in the NLRC4 gene also lead to hyperactivation of the NLRC4-inflammosome and have common pathogenetic features with NLRP3-syndromes [11]. Keeping in mind the pathogenetic characteristics of the disease, one must suppose that the most effective therapeutic target in these patients is the blockage of IL1 and 18. S.W. Canna et al. discovered that prescribing therapy with an IL 1 antagonist decreases the frequency and expression of exacerbations, normalizes the levels of C-reactive protein in the blood serum and allows to abstain from prescribing dexamethazone [5].

The described clinical case demonstrates the high effectiveness of canakinumab treatment at stopping an acute inflammatory process in a patient with an NLRC4-associated auto-inflammatory syndrome. Therapeutic

effectiveness analysis demonstrated that in a girl that was ill with the disease for 3 years, the prescription of canakinumab allowed for a significant decrease of disease activity (common criteria not developed) even after 1 week of treatment. In 8 weeks the acute inflammatory changes stopped in the damaged organs and tissues. Using canakinumab stopped the fever, the intestinal syndrome, the rash, normalized the laboratory activity indicators and returned the child to full life.

Conclusion

The analysis of the offered clinical case demonstrates a successful usage of canakinumab for subcutaneous injection at a dosage of 4 mg/kg of body weight every 4 weeks for the treatment of a severe auto-inflammatory syndrome associated with NLRC4. Canakinumab therapy made it possible not only to stop the fever, rash, restore the functional activity of the patient, but also improved the quality of life by preventing the development of life-threatening conditions.
The results of treating our patient make it possible to conclude that the prescription of canakinumab for patients with an NLRC4-associated auto-inflammatory syndrome allows to reach remission and prevent increasing invalidisation and the development of life-threatening complications. Molecular diagnosis of auto-inflammatory syndromes is a especially important task, keeping in mind the multiplicity of their clinical manifestations. Using such highly effective methods as new generation sequencing allows to diagnose timely, and, having identified the origin of the disease, to commence treatment.

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Conflict of interests

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