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THE ROLE OF PROINFLAMMATORY CYTOKINES IN THE DEVELOPMENT OF CHRONIC DISCOGENIC PAIN SYNDROME

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The purpose of this review is to update the knowledge of neurologist doctors about the role of pro-inflammatory cytokines in the evelopment and chronicity of discogenic pain syndrome in patients with intervertebral disc degeneration.

Keywords: cytokines; cytokine status; disk degeneration; biomarker; chronic inflammation, discogenic pain syndrome.

Introduction: Intervertebral disc degeneration (IDD) is a multifactorial chronic recurrent pathological process that primarily affects the nucleus pulposus (NP) of the intervertebral disc and then spreads to the annulus fibrosus (AF) and other elements of the spinal motion segment (SMS). The pathogenetic mechanism manifests itself under certain conditions in various (reflex, compression, compression-reflex and reflex-compression) neurological syndromes [1]. The new International Classification of Diseases, 11th revision (ICD XI), has a separate block for coding discogenic pathology - block FA80 "Degeneration of the intervertebral disc with and without involvement of the nervous system." The new ICD-11 does not provide for the use of the terms "dorsopathy" and "dorsalgia". The term "Dorsopathy" can only be used in the case of category FB1Z - "conditions associated with the spine, unspecified." Non-discogenic back pain (not associated with damage to the intervertebral disc (IVD)) can be coded as ME84 - "back pain, the sources of which are muscles, nerves, bones, joints or other structures of the spine" or MG30.3 "chronic secondary musculoskeletal pain." Other categories that are directly related to IVD are highlighted separately. They are presented in table 1 [2].

One of the main mechanisms of IDD is the loss of proteoglycans [3], which results in a decrease in osmotic pressure in the disc matrix and loss of water molecules, which is manifested by a change in the mechanical properties of the IVD. Gradually, these processes lead to protrusion and a decrease in the height of the IVD. The loss of proteoglycans promotes the movement of serum proteins and cytokines into the extracellular matrix (ECM), which affect the PN cells and accelerate the process of IDD [3]. The activity of matrix metalloproteinases (MMPs) and cathepsins plays an important role in the denaturation and breakdown of collagen, fibronectin and proteoglycans. The consequence of this process is osteoarthritis degeneration, protrusion of ligaments into the spinal canal with compression of neural structures [39]. The degenerative cascade involves the roots of the spinal nerves, which causes chronic pain mainly due to their compression and partly due to neoangiogenesis (ingrowth of the smallest nerve endings into the degenerating IVD and their activation due to the constant release of inflammatory mediators, including proinflammatory cytokines) [36].

In recent years, cytokine imbalance is considered as one of the most important mechanisms for the formation of persistent vertebrogenic pain syndrome [41], which consists in a shift in the balance of cytokines towards an increase in the content of pro-inflammatory cytokines and the maintenance of chronic inflammation in the degenerating IVD and surrounding tissues [7]. The release of a large number of pro-inflammatory cytokines is mediated by NP and AF cells, as well as macrophages, neutrophils, T- and B-lymphocytes. Proinflammatory cytokines cause a chain of pathophysiological reactions leading to degeneration [12,25], oxidation [12,18], autophagy [19,34], aging [31,38] and apoptosis [34] of IVD cells (Table 2).

Accordingly, the delicate balance between pro-inflammatory and anti-inflammatory cytokines determines the overall effect of the inflammatory response in patients with IDD and the expected therapeutic response to prescribed drugs [26], as well as the possibility of using more high-tech treatment methods in case of severe IDD and persistent discogenic back pain [17].

Damage caused by proinflammatory cytokines in intervertebral disc degeneration. Cytokines (interleukins (IL), lymphokines, monokines, interferons and chemokines) are important components of the immune system (Fig. 1) [7]. They act in conjunction with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. Imbalances in cytokine production or cytokine receptor expression and/or dysregulation of cytokine balance contribute to the development and progression of IDD. Cytokines are classified into two large groups: pro-inflammatory and anti-inflammatory. Time-dependent imbalance of pro- and anti-inflammato-

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Table 1

Coding of intervertebral disc degeneration according to ICD-11 and ICD-10

ICD-11 block/code	Block/code name		ICD-10 block/code
FA80	"Intervertebral disc degeneration"	“Other dorsopathies associated with damage to the intervertebral disc”	M50-M53
8B93.6 8B93.8 8B42	"Radiculopathy due to damage to the IVD" "Radiculopathy due to spondylosis" "Myelopathy in spondylosis"		M50-M53 M47.1 M47.2
FA8Z	"Spondylosis"		M47
ME84	"Back pain"	"Dorsalgia"	M54
MG30	"Chronic pain"		-
FB1Z	"Spine-related conditions, unspecified"		-

ry cytokines determines the outcome of the inflammatory response as one of the main mechanisms for the development of IDD [7].

Pro-inflammatory cytokines play a central role in the development of IDD, are produced predominantly by activated macrophages and are involved in enhancing inflammatory reactions, serve to contain and eliminate inflammatory foci by activating local and systemic inflammatory reactions, and can directly modulate cell activity in various structures of the IVD, including the NP, AF and ECM [25]. The main proinflammatory cytokines responsible for early responses are interleukin 1 alpha (IL-1α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). Other proinflammatory mediators include members of the IL-20 family, IL-33, leukemia inhibitory factor (FIL), interferon gamma (IFN-γ), oncostatin M (OCM), ciliary neurotrophic factor (CNTF), transforming growth factor beta (TGFR-β), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-11, IL-12, IL-17, IL-18, as well as other chemokines that chemoattract inflammatory cells. IL-1, IL-6 and TNF-α manifest their action as endogenous pyrogens that enhance the synthesis of secondary mediators and proinflammatory cytokines by macrophages and mesenchymal cells, stimulating the production of acute phase proteins or attracting inflammatory cells. IL-1β, TNFα, IFN-γ, IL-12 and IL-18 are well characterized as proinflammatory cytokines [7].

Interleukin 1β – IL-1β is one of the key pro-inflammatory cytokines that is involved in the regulation of the innate immune response [28]. Apoptotic macrophage cells release only IL-1, but not IL-6 or TNF-α, suggesting that *in vivo* apoptosis of macrophages in the IVD is the source of cytokine release. IL-1β may promote the expression of matrix metalloproteinases in the IVD. IL-1β stimulates disintegrin and metalloprotease with thrombospondin-1-like domains (ADAMTS), which may regulate the production of ADAMTS-4 and ADAMTS-5 in the IVD, promoting ECM loss and development of IDD [24]. There is a connection between IL-1β and premature stress-induced aging of IVD cells. In IL-1β stimulated NP cells, the levels of β-galactosidase are significantly increased [38], which leads to an increase in the number of senescent IVD cells and a decrease in the ability of cell self-renewal. IL-1β promotes the production of pro-apoptotic proteins, including cleaved caspase 3 (apoptosis coordinator enzyme) and Bax (apoptosis promoter protein), and reduces the production of anti-apoptotic content in the IVD. IL-1β stimulation dramatically increased caspase-3 activity, cell apoptosis rate, and production of cleaved PARP (poly(ADP-ribose) polymerase), Bax, caspase-3, and cleaved caspase-3, but decreased the level of B-cell lymphoma 2 (Bcl- 2 - apoptosis inhibitor protein) in the IVD of rats. Stimulation of IL-1β leads to a sharp increase in the rate of apoptosis in rat AF due to increased caspase-3 activity, which is also suppressed by 17β-estradiol [44].

Also, IL-1β is associated with pyroptosis, a form of inflammatory programmed cell death associated with the secretion of IL-1β. The process of pyroptosis is pro-inflammatory and is triggered by the inflammatory domain NOD-like receptor 3 (NLRP-3) family pyrin (NLR family

Table 2

Theories about the role of cytokines in the development of intervertebral disc degeneration

Theory	Cytokines involved	Sources
Theory of structure degra-dation extracellular intervertebral disc matrix	IL-1β, IL-6, IL-8, IL-17, IL-18, IL-21, IL-23, TNF-α, IFN-γ	[8,12,25]
Oxidation theory	IL-1β, IL-6, IL-8, IL-17, IL-18, TNF-α, IFN-γ	[14,18]
Mechanical Load Theory	IL-1β, IL-6, IL-8, IL-17, TNF-α	[9,16]
Theory of programmed cell death (apoptosis)	IL-1β, IL-6, IL-17, IL-18, TNF-α	[19,30,33,35]
Theory of cell proliferation	IL-1β, IL-17, TNF-α	[11,22]
Theory of premature aging	IL-1β, IL-6, IL-18, TNF-α	[31,38]
Autophagy theory	IL-1β, IL-17, TNF-α, IFN-β1	[19,34]
Theory of angiogenesis and neoinnervation	IL-1β, IL-17, TNF-α	[10,14]
Theory of hypoxia	IL-1β, TNF-α	[12]
Cell cycle disruption theory	IL-1β, IL-17, TNF-α	[44]

Note: IL-1β – interleukin 1 β; IL-6 – interleukin 6; IL-8 – interleukin 8; IL-17 – interleukin 17; IL-18 – in-terleukin 18; IL-21 – interleukin 21; IL-23 – interleukin 23; TNF-α – tumor necrosis factor alpha; IFN-γ – interferon gamma; IFN-β – interferon beta.

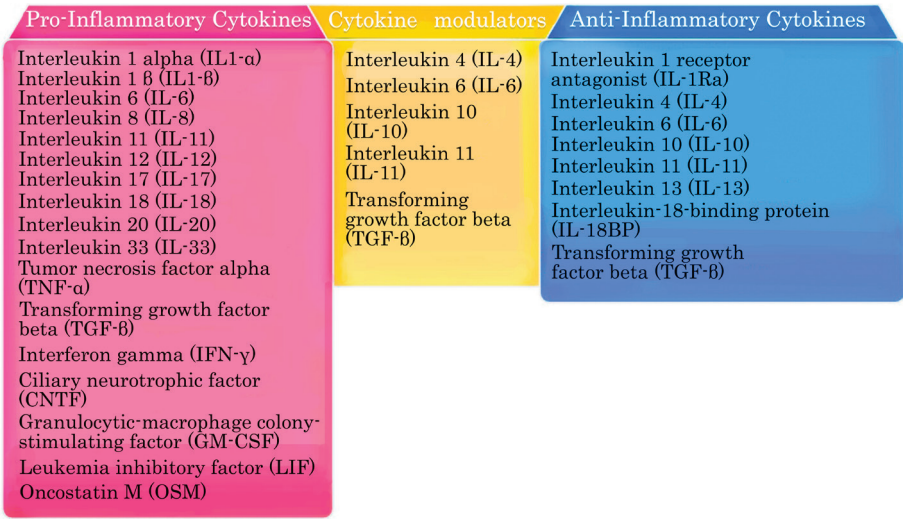

Fig. 1. Main groups of cytokines.

pyrin domain 3 (NLRP3)), which depends on the formation of oligomers of apoptosis-associated speck proteins known as pyroptosomes [35]. In addition, pyroptosis has been found to be associated with IDD mediated by the propiobacterium P. acnes. Increased levels of NLRP3, IL-1β, caspase-5, caspase-1 and gastermin D (a tumor suppressor) were found in NP cells after co-culture with P. acnes [40].

Many factors have been found that can modulate the proliferation of NP cells, such as thymosin beta-4, insulin-like granulocyte growth factor 1 (IGF-1) and leptin. IL-1β stimulation significantly suppresses IVD proliferation [11].

The processes of neoinnervation and neoangiogenesis likely increase the severity and rate of development of IDD. Overexpression of the most important proangiogenic factor, vascular endothelial growth factor (VEGF), leads to acceleration of IDD [14], as well as overexpression of neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BNF) [10].

Interleukin 2 – IL-2 is produced by mature T cells; this cytokine is involved in the maturation of T cells and B cells, functioning as a growth factor for them. Serum levels of IL-2 in patients with low back pain were significantly lower than in controls, similar to other factors including IL-6, IL-4, and MMP-1 [37]. It should be recognized that the role of IL-2 in the development of IDD is still being studied.

Interleukin 8 – IL-8 is produced by macrophages, T lymphocytes, neutrophils, and other cells in response to antigen; IL-8 is the most potent of the human chemokines [7]. IL-8 causes hyperalgesia by triggering local production of sympathetic amines, which increase the sensitivity of pain receptors [36]. Oxidative/nitrosative stress and mechanical

Table 3

The role of pro-inflammatory cytokines in the pathogenesis of intervertebral disc degeneration

Cytokine	Gene: OMIM	Role in the intervertebral disc	Clinical role	Sources
IL-1β	*IL1B*: 147720	Initiation of inflammatory, oxidative, degenerative, apop-totic cascades. Association with premature aging and cessation of cellular growth. Overexpression of VEGF, NGF, BNF.	+++	[24,35,38]
IL-8	*CXCL8:* 146930	Increased migration of neutrophils, T cells and monocytes. Indirect increase in oxidative stress, which can lead to IVD cell death. Participation in the pathogenesis of acute neuropathic pain.	+/-	[8,9,16,36]
IL-12A	*IL12A*: 161560	Stimulation of proliferation. Activation and increase in cytotoxicity of natural killer cells and T cells. Stimulation of Th-1 differentiation. Induction of secretion of IFN-γ and TNF-α, synergism with pro-inflammatory cytokines.	++	[7]
IL-17	*IL17A*: 603149	Initiation of the inflammatory and degenerative cascade. Association with cell growth arrest. Stimulation of angiogenesis.	+++	[19,43]
IL-18	*IL18:* 600953	Initiation of the inflammatory and degenerative cascade (activation of IFN-γ). Initiation of apoptotic and oxidative cascade. Association with premature aging of IVD cells.	+++	[42]
TNF-α	*TNF:* 191160	Initiation of inflammatory, apoptotic, oxidative and de-generative cascades of IVD cells. Association with premature aging and cessation of cell growth. Autophagy promoter.	+++	[7,24,35]
IFN-γ	*IFNG:* 147570	Initiation of the inflammatory and degenerative cascade in IVD cells. Participation in the pathogenesis of acute neuropathic pain in IDD.	++	[20]

Note: (+/-) – questionable prognostic role in the development of intervertebral disc degeneration (IVD); (+) – low prognostic role in the development of IDD; (++) – moderate prognostic role in the development of IDD; (+++) – significant prognostic role in the development of IDD; IFN-γ – interferon gamma; IL-12 – interleu-kin 12; IL-17 – interleukin 17; IL-18 – interleukin 18; IL-1β – interleukin 1β; IL-8 – interleukin 8; BNF - brain-derived neurotrophic factor; NK cells – natural killers; T cells – T lymphocytes; Th1 – T-helper type 1; TNF2 – tumor necrosis factor 2; TNF-α – tumor necrosis factor alpha; NGF – nerve growth factor; VEGF - vascular endothelial growth factor.

damage lead to increased levels of IL-8 in degenerating IVDs [9].

Unfavorable mechanical stress on human NP and AF cells and acute mechanical trauma ex vivo of the human IVD induces an increase in IL-8 expression [16]. An increase in the level of IL-8 after sterile inflammation of the IVD has been shown, including activation of toll-like receptors type 2 (TLRe2) in NP cells [13], TNFα treatment of NP cells, IL-1β treatment of NP cells with TPRe2 activation [5].

Interleukin 12 – IL-12 is secreted by macrophages and dendritic cells as a response to bacterial cell wall components. IL-12 causes stimulation of proliferation, activation and increased cytotoxicity of natural killer cells (NK cells) and T cells, participating in their differentiation into T-helper type 1 (Th-1) [7], which also plays a role in the development IDD. IL-12 induces the secretion of IFN-γ, TNF-α and has a synergistic effect with IL-18 [7]. IL-12 functions together with cytokines such as IFN-γ, and differences in the levels of these cytokines in IVD herniated fragments and in degenerating IVD cells have been noted [21]. Accordingly, both IL-12 and IFN-γ, as well as other cytokines (IL-4, IL-6) showed higher levels in IVD herniated fragments. Although, on the other hand, the expression levels of these cytokines obtained from the IVD at autopsy in patients with IDD did not have significant differences with their levels in the NP and AT in clinically healthy people [20].

Interleukin 17 – IL-17 is produced by Th-17, accelerates the development of IDD by stimulating the secretion of pro-inflammatory cytokines by macrophages [7], promotes ECM degradation, enhancing the inflammatory response, inducing neoangiogenesis and inhibiting autophagy and proliferation of NP cells.

High levels of IL-6, IL-17A and TNFα were noted in the blood of patients with lumbar spinal root lesions compared with patients with neuropathic pain syndrome, and the level of Th-17 was increased in the venous blood in patients with lesions of the lumbar spinal roots, compared with patients with neuropathic pain syndrome [43]. In an *in vitro* study, treatment of NP cells isolated from the IVD with IL-17A showed that IL-17A inhibits cell proliferation and ECM synthesis [22]. Treatment with IL-17A and anti-IL-17A neutralizing antibodies significantly reduces the response to IL-6, cyclooxygenase 2 (COX-2), MMP-3, and MMP-13. IL-17A can inhibit autophagy in human NP cells by activating the PI3K/Akt/Bcl-2 signaling pathway (the classical anti-apoptosis pathway in cells) (PI3K - phosphoinositide 3-kinase inhibitors; Akt - protein kinase B). Based on the protective role of autophagy in IDD, it can be concluded that IL-17A contributes to the development of IDD by inhibiting autophagy [19].

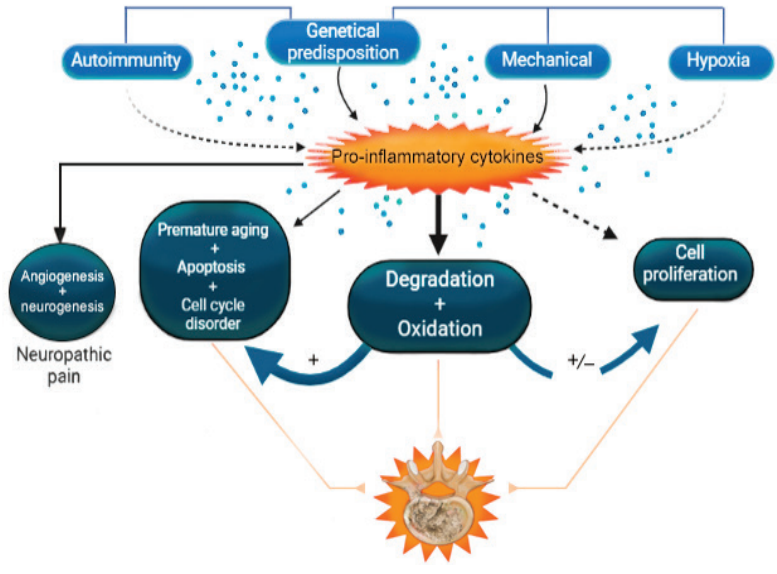

Fig. 2. Mechanisms of intervertebral disc degeneration [6]. Effects of proinflammatory cytokines in intervertebral disc degeneration (IDD). Note: (+) — interconnected mechanisms of development of IDD; (+/-) – possible connection between the mechanisms of development of IDD.

Interleukin 18 – IL-18, a member of the IL-1 superfamily with a structure similar to IL-1β, is a highly regulated proinflammatory cytokine that is cleaved by caspase-1 to produce a biologically active molecule. The level of IL-18 increases during inflammatory processes in the body [4]. The activation of T cells and NK cells mediated by this cytokine leads to the secretion of gamma interferon (IFN-γ), activation of macrophages secreting cytokines such as TNF-α and IL-1, which, in turn, cause increased degradation matrix both directly and through the activation of proteinases like MMPs [27].

IL-18 activates caspase-1 and the inflammasome system, increasing the production of reactive oxygen species in cells, which leads to further IL-18 production and neuronal apoptosis [30]. IL-18 can increase the level of anti-apoptotic proteins BCL-2 and BCL-XL (very large B-cell lymphoma) [33], and also cause inflammatory reactions in synoviocytes and chondrocytes, increasing the expression of pro-inflammatory factors such as TNF-α, prostaglan -din E2 (PGE-2) and COX-2, contributing to the acceleration of cartilage degeneration and the development of osteoarthritis [42].

Tumor necrosis factor alpha – TNF-α, as the most important pro-inflammatory cytokine, enhances the expression of COX-2 in IVD cells, and also increases the production of PGE-2 (which regulates the activity of various signaling pathways through the prostaglandin receptors of the G-protein family), which stimulates the work of the Wnt signaling pathway (intracellular signaling pathway regulating embryogenesis, cell differentiation and the development of malignant neoplasms) TNF-α-PGE-2 through the prostaglandin E3 receptor (EP3 receptor). TNF-α activates the p65, Janus kinase (JNK) and p38 sites of the mitogen-activated protein kinase (MAPK) signaling pathway in NP cells in the IVD. At the same time, stimulation of proliferative processes carried out through TNF-α depends on the interaction of NF-κB, JNK and p38 signaling pathways. Short-term exposure to TNF-α stimulates proliferative processes through the MAPK pathway without the involvement of extracellular signal-regulated kinases ½ (ERK1/2) [8]. In addition, TNF-α is involved in the activation of NLRP-3 inflammatory pathways [35].

Stimulation of TNF-α promotes the production of proinflammatory cytokines IL-8 and IL-6 in IVD AF in adults. The result of TNF-α stimulation is an increase in the level of substance P (SP), as well as induction of the expression of IL-1β, IL-6 and IL-8. TNF-α stimulates the synthesis of reactive oxygen species, which is subsequently associated with the severity of IDD. IL-17 and TNF-α can induce the secretion of inflammatory mediators in AF and NP cells in patients with surgery for IDD, including IL-6, NO and PGE2. IL-17 and TNF-α increase the level of intercellular adhesion molecule (ICAM (InterCellular Adhesion Molecule)-1) in IVD cells

[8]. TNF-α leads to stimulation of the expression of multiple MMPs and ADAMTS, inducing their expression through activation of the NF-κB/MAPK signaling pathway. TNF-α has a positive effect on the synthesis of MMP-1, MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 in the IVD *ex vivo*, leading to the degradation of aggrecan and collagen in the NP. Due to stimulation of TNF-α, the levels of MMP-3 and ADAMTS-5 significantly increase, and the production of type II collagen, on the contrary, decreases [24].

Interferon gamma – IFN-γ is a soluble cytokine that is predominantly released by Th-1, cytotoxic T lymphocytes and natural killer cells [7]. This is a pro-inflammatory cytokine activated in the IVD nucleus, which influences tissue-specific macrophages in NP cells in IDD. Any structural change in the gene encoding IFN-γ, which leads to an increase in the level of its expression, can theoretically participate in the pathogenesis of the disease, due to the activation of this cytokine during neuroinflammation, as well as its effect on the nociceptor system of the body [23].

Discussion. A summary of the role of proinflammatory cytokines in the development of DMD is presented in Table 3.

Effects of proinflammatory cytokines in intervertebral disc degeneration (IDD). Note: (+) — interconnected mechanisms of development of IDD; (+/-) – possible connection between the mechanisms of development of IDD.

Passive and active immune privileged barriers are both damaged during the development of IDD and various mechanisms are involved [29], disrupting the immune balance in the microenvironment of the degenerating IVD. This additionally involves various specific and non-specific immune cells in the IVD, which, together with cytokines secreted by the IVD cells, aggravate the pathological process, interfere with recovery and contribute to the development of acute and chronic discogenic pain syndromes. An increase in the expression of growth factors and pro-inflammatory cytokines in the area of the autoimmune reaction of the IVD contributes to the activation of neovascularization and neurogenesis [36]. Elevated levels of pro-inflammatory cytokines accelerate the development of IDD, enhance the degradation of aggrecan and collagen, contributing to changes in the phenotype of IVD cells and their microenvironment [15]. Moreover, proinflammatory cytokines can cause IVD cell death and degradation of ECM cells in degenerating IVDs, promoting further progression of IDD. Thus, although the inflammatory response may be involved in the onset of disease, it is also critical for maintaining tissue homeostasis. For example, with an optimal cytokine balance, it can promote restoration/regeneration of IVD tissue [32].

Conclusion: This narrative review provides new insight into the role of proinflammatory cytokines in the pathogenetic mechanisms of IDD, which, in turn, sets new targets for the future development of promising therapeutic strategies for patients with this pathology and with IDD-associated pain syndromes.

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POINT OF VIEW

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COMPARISON OF THE NANO-SIZED PARTICLES NUMBER IN BLOOD PLASMA AND ON THE ERYTHROCYTES SURFACE USING SCANNING ELECTRON MICROSCOPY IN A CERVICAL CANCER PATIENT

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To explain the more productive isolation of HPV DNA from the blood component compared to plasma in cervical cancer patients, using scanning electron microscopy images of venous blood were studied. It was revealed that there are more nanosized bioparticles on the erythrocytes surface than in plasma. It has been suggested that among them there may be tumor extracellular vesicles carrying HPV DNA. To confirm that the erythrocyte fraction of blood is a more productive biological sample for isolating HPV DNA, continued studies are needed.

Keywords: human papillomavirus, screening, extracellular vesicles.

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Despite widespread screening in Russia to prevent cervical cancer, mortality from it remains high and has not decreased significantly. Residents of the regions of the Arctic zone of the Russian Federation (AZRF) feel this to a greater extent [11]. In the territories of the Russian Arctic there are difficulties in solving government tasks to improve the standard of living of the population and provide them with quality goods and services. It is believed that the main reasons are the weather conditions, the size of the territory, insufficient or even absence of transport infrastructure, significant dispersion of settlements, low population density, nomadism, etc. [8].

These reasons also negatively affect the use and effectiveness of traditional methods of screening for cervical pathology in women living in the Arctic. Therefore, there is an urgent need to develop simple tests applicable in hard-to-reach settlements of the Russian Arctic, that