Immunoinflammatory predictors of the pelvic pain syndrome associated with adenomyosis

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ABSTRACT
The aim of the study was the analysis of immune inflammatory processes in the development of the pelvic pain syndrome associated with adenomyosis. For morphological examination were used 54 fragments of the myometrium obtained from patients after hysterectomy with pelvic pain on a background of diffuse adenomyosis of II–III degree, and 20 patients with painless form of adenomyosis. The identification of the macrophages distribution was held by means of an immune-histo-chemical analysis of MAT (monoclonal antibody) for CD68. (Clone PG-M1, ‘Diagnostic BioSystems’, USA). The results of the study showed a significantly higher expression of CD68 (49.3 ± 2.3 vs. 21.2 ± 1.7 units. p < .01) in patients with painful adenomyosis form in areas of the ectopic endometrium, in the perivascular regions of the myometrium, as compared to those areas in women with painless group. We assume that these factors increase neurogenic inflammation and sensitivity of nociceptors in myometrium, activation of peripheral nerve fibers and, can act as triggers of the pelvic pain syndrome associated with adenomyosis.

Introduction
Adenomyosis is a common benign gynecologic disease in which endometrial glands and stroma are present within myometrium, inducing myometrial inflammation and hypertrophy. Although some women with adenomyosis are asymptomatic, the condition often causes diffusely enlarged uterus, menorrhagia, dysmenorrhea, chronic pelvic pain and subfertility [1,2].

The pathogenesis of adenomyosis is poorly understood, but one hypothesis is that the basalis endometrium abnormally invades into the myometrium. The main mechanism of the pathogenesis of adenomyosis is disruption of the endometrial–myometrial borderline leading to the infiltration of endometrial cells, and survival of the endometrial cells within the myometrium [3,4].

We share an opinion that, being a special form of endometriosis, adenomyosis is defined by the intra-myometrial presence of glands and stroma derived from the basalis layer of the endometrium surrounded by the reactive hyperplastic or hypertrophic myometrium, causing chronic inflammation in the endometrium [5–7]. However, for now, pathogenetic mechanisms of the formation of pelvic pain in women with adenomyosis remain unclear. Theoretically, we can assume that inflammation of nerve terminals in myometrium is involved. With ongoing trauma, such sites of inflammation might accumulate and the increasingly produced estrogens interfere in a paracrine fashion with ovarian control over uterine peristaltic activity, resulting in permanent hyperperistalsis and a self-perpetuation of the disease process.

The proposed mechanism to explain the causality between the inflammation and tumor proliferation is the injury caused by the infection, which causes the growth of the extracellular matrix, cellular proliferation due to pro-inflammatory and growth factors, decreasing apoptosis and abnormal tissue repair [8].

The inflammatory process that occurs in adenomyosis, is accompanied by the release of various algogenic factors, contributes to functional and structural changes of nociceptors at the site of alteration and causes an increase in their excitability (peripheral sensitization) [9]. For example, interleukin-1 (IL-1) produced by activated macrophages, induces the synthesis of prostaglandins, the excessive concentration of which may be responsible for the occurrence of pain [10].

The aim of the study was the analysis of immune inflammatory processes in the myometrium in the development of adenomyosis and associated pelvic pain syndrome.

Methods
As the main method of this study, a morphological and immune-histochemical analysis of tissue materials was used. The samplings were obtained from two groups of patients: the main group and the control group.

The main group included macro-preparations of 54 uteruses tissue samplings, obtained after hysterectomy from patients with diffuse adenomyosis of the II–III degrees, accompanied by severe pelvic pain syndrome.

The control group consisted of 20 biopsies of the uterus of patients with a painless form of adenomyosis directed to hysterectomy for the reason of abnormal uterine bleeding. All the patients were operated in the proliferative phase of the cycle.

The diagnosis of adenomyosis was confirmed by ultrasonography (US) and magnetic resonance imaging (MRI). Ultrasound diagnostics was performed using the TOSHIBA APLIO MX...
scanner (Japan) with a function of the volumetric imaging and directional Doppler transabdominal and transvaginal convex sensors with a frequency of 4.0 to 7.0 MHz and 5.6 to 8.0 MHz. A record of echo graphic images in the form of digital images in two-dimensional mode, photos and videos, were produced on the hard disk of the device. The data were processed with the help of the ‘Astraia’ (Germany) computer program, archiving and processing of ultrasound data. MRI was performed on a Siemens 1.5 T MAGNETOM Avanto magnetic resonance tomograph (Germany).

After hysterectomy, parts of the uterus wall, including endometrium and myometrium, were fixed in neutral buffered 10% formalin (pH 7.4) for 24 h. After dehydration, the material was embedded in pure paraffin wax with polymer additives (Richard-Allan Scientific) at a temperature below 60°C. Sections of 5 μm thickness were obtained on a rotational Microm HM325 microtome with section. To identify estimates of the amount and spatial distribution of macrophages, a mouse MAT for CD68 was used. The diagnostic system of macrophages is Clone PG-M1, ‘Diagnostic BioSystems’, USA. For the purposes of immune phenotyping of lymphocytes, CD68 was used as the main marker of mature and activated macrophages.

For the purpose of objectification of morphological study, a comprehensive morphometric analysis was performed, using special Image Tool version 3.0. software and Adobe Photoshop CS4 Extended v.11.0.1. The images were made on the Olympus BX51 microscope with a DP70 (Olympus, Japan) digital camera.

Statistical processing of data was performed using the STATISTICA program for Windows 7.0. The differences veracity of the compared parameters were determined using the Wilcoxon – Mann–Whitney criterion. The differences were considered statistically significant when \( p < .05 \).

**Results and discussion**

A leukocytic infiltration was detected in the endometrium and myometrium samplings of patients of the both groups, however, the degree of the leukocytic infiltration was significantly higher in the main group \( p < .05 \). The infiltrates were located not only in the areas around the foci of adenomyosis, but freely in the stroma between bundles of smooth myocytes (Figure 1).

Moreover, if the infiltration of lymphocytes in the endometrium was mainly diffused (Figure 2), the infiltration of lymphocytes in myometrium was mainly observed in perivascular regions (Figure 3).

The number of CD68-positive cells in eutopic endometrium of patients with painful form of adenomyosis was significantly higher (49.3 ± 2.3 units) than in patients with a painless form (21.2 ± 1.7 units, \( p < .01 \)). Characteristically, while the macrophages were identified predominantly in the perivascular regions, some cells were found in close proximity to the uterine glands.

Numerous were CD68-positive cells and ectopic sites of endometrial growth. Here, macrophages were detected not only in the perivascular region, but in close proximity and in areas of invasion of the uterine glands and characterized by high-intensity staining (Figure 3). It is important to note that macrophages were detected not only in the foci of adenomyosis, but also in large enough quantities – in the myometrium, such as around major blood vessels and in the strata of the stroma between bundles of smooth myocytes and here naturally was perivascular localization of CD68-positive cells (Figure 4). In addition, a large number of macrophages were revealed in the areas of lymphocytic infiltration of the stroma, particularly around the foci of adenomyosis with signs of degeneration and desquamation of cells. Less numerous
were free macrophages, single located between the bundles of smooth myocytes. A comparative analysis of the number of macrophages in the myometrium in patients of the main group and the control group showed increased recruitment of these cells in adenomyosis complicated by the development of syndrome of pelvic pain. Moreover, CD68-positive cells were associated not only with areas of ectopic endometrial growth, but also the perivascular region and is the carrier of the nerves and center of the remodeling processes in the uterine wall in this pathology, as we have shown in previous studies [11,12].

Of course, we reaffirm the concept that nerve endings of fibers in the myometrium and the endometrial heterotopias can potentially be stimulated by various inflammatory substances, including histamine, serotonin, bradykinin, prostaglandins, leukotriene, interleukin, acetylcholine, growth factors (vascular endothelial (VEGF), epidermal growth factors, transforming growth factors-β, platelet and nerve growth factor (NGF). Many of the above substances secreted by macrophages themselves [13].

Increasing the density of macrophages and their secreted algogenic factors may be associated with the occurrence of pain in women with adenomyosis. Alternatively activated macrophages can promote the growth of nerve fibers due to the increased production of IL-1β and increased production of NGF [14]; of the increased expression of other growth factors derived from the brain-derived neurotrophic factor (brain-derived neurotropic factor – BDNF) is a key factor in the growth and differentiation of the peripheral nervous system [15]; the intensification of production of VEGF, which can act as a neurotrophic factor stimulating the growth of nerve fibers. There is strong evidence that macrophages are important in the regeneration process of nerve fibers in the peripheral nervous system. Peripheral nerve fibers injury alongside with macrophages reaction, produce neurotrophic factors influencing the excessive regeneration of nerve fibers and proliferation, including those in the Schwann cells. On the other hand, when the reaction of macrophages is disrupted, the ability of the sensory nerve fibers to regenerate is reduced. The viability and sustainability of the nerve fibers growth depends on many factors whose products are regulated by activated macrophages [13].

Conclusions

Adenomyosis is a chronic inflammatory disease, accompanied by dysfunction of the miometrium immune reactivity. An increase in number of activated macrophages in perivascular compartments and in areas of the myometrium remodeling, which are carriers of the nerves, leads to an increased neurogenic inflammation and hypersensitivity of nociceptors, activation of peripheral nerve fibers and serves as the main pathogenetic mechanism of the formation of pelvic pain in women with adenomyosis.

Disclosure statement

The authors report no conflict of interest.

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