DOI: 285-292. DOI: 10.20514/2226-6704-2022-12-4-285-292
EDN: KUOZBX
УДК 616.34-002.44-07

Е.В. Болотова[1], К.А. Юмукян[1,2], А.В. Дудникова*[1]

[1]— ФГБОУ ВО КубГМУ Минздрава России, Краснодар, Россия
[2]— Государственное бюджетное учреждение здравоохранения «Научно-исследовательский институт — Краевая клиническая больница № 1 им. С.В. Очаповского», Краснодар, Россия

НОВЫЕ ДИАГНОСТИЧЕСКИЕ ВОЗМОЖНОСТИ ОПРЕДЕЛЕНИЯ АКТИВНОСТИ ЯЗВЕННОГО КОЛИТА: РОЛЬ НЕЙТРОФИЛОВ

E.V. Bolotova[1], K.A. Yumukyan[1,2], A.V. Dudnikova*[1]

[1]— State budgetary educational institution of higher professional education «Kuban state medical university» Ministry of health of the Russian Federation, Krasnodar, Russia
[2]— State Public Health Budget Institution Scientific Research Institute — Ochapovsky Regional Clinic Hospital of Krasnodar Region Public Health Ministry, Krasnodar, Russia

New Diagnostic Possibilities for Determining the Activity of Ulcerative Colitis: The Role of Neutrophils

Резюме

Заболеваемость язвенным колитом в последние годы растет, и его развитие в молодом возрасте стало тенденцией, которая прогностически неблагоприятна. Клиническая картина язвенного колита часто расплывчата, что приводит к изначально ошибочному диагнозу. Оценка эффективности лечения и риска рецидива язвенного колита, требующая инвазивного вмешательства — одна из основных диагностических проблем. Целью исследования был анализ данных современной научной литературы о неинвазивных биомаркерах язвенного колита. Проанализированы данные зарубежных и отечественных статей по теме исследования, опубликованных в Pubmed и eLibrary за последние 5-10 лет. Биомаркеры нейтрофильного происхождения являются перспективным направлением в первичной диагностике и оценке активности язвенного колита.

Ключевые слова: *язвенный колит, воспалительные заболевания кишечника, нейтрофилы, неинвазивные биомаркеры*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

Статья получена 10.01.2022 г.
Принята к публикации 29.03.2022 г.

Для цитирования: Болотова Е.В., Юмукян К.А., Дудникова А.В. НОВЫЕ ДИАГНОСТИЧЕСКИЕ ВОЗМОЖНОСТИ ОПРЕДЕЛЕНИЯ АКТИВНОСТИ ЯЗВЕННОГО КОЛИТА: РОЛЬ НЕЙТРОФИЛОВ. Архивъ внутренней медицины. 2022; 12(4): 285-292. DOI: 10.20514/2226-6704-2022-12-4-285-292 EDN: KUOZBX

*Контакты: Анна Валерьевна Дудникова, e-mail: avdudnikova@yandex.ru
*Contacts: Anna V. Dudnikova, e-mail: avdudnikova@yandex.ru
ORCID ID: https://orcid.org/0000-0003-2601-7831

Abstract

The incidence of ulcerative colitis has been increasing in recent years, and its manifestation at a young age has become a trend that is prognostically unfavorable. The clinical picture of ulcerative colitis is often vague, which leads to an initially erroneous diagnosis. One of the main problems is to assess the effectiveness of treatment and the risk of recurrence of ulcerative colitis, which requires invasive intervention. The aim of the study was to analyze the data of modern scientific literature on noninvasive biomarkers of ulcerative colitis. The data of foreign and domestic articles on the research topic published in Pubmed and eLibrary over the past 5-10 years are analyzed. Biomarkers of neutrophil origin are a promising direction in the primary diagnosis and assessment of ulcerative colitis activity.

Key words: *ulcerative colitis, inflammatory bowel diseases, neutrophils, noninvasive biomarkers*

Conflict of interests

The authors declare no conflict of interests

Sources of funding

The authors declare no funding for this study

Article received on 10.01.2022

Accepted for publication on 29.03.2022

For citation: Bolotova E.V., Yumukyan K.A., Dudnikova A.V. New Diagnostic Possibilities for Determining the Activity of Ulcerative Colitis: The Role of Neutrophils. The Russian Archives of Internal Medicine. 2022; 12(4): 285-292. DOI: 10.20514/2226-6704-2022-12-4-285-292 EDN: KUOZBX

ANCA — anti-neutrophil cytoplasmic antibodies, CD — Crohn's disease, CRP — C-reactive protein, FC — faecal calprotectin, HNE — human neutrophil elastase, IBD — inflammatory bowel disease, IBS — irritable bowel syndrome, IL-6 — interleukin 6, LF — lactoferrin, MMP — matrix metalloproteinases, NGAL — neutrophil gelatinase-associated lipocalin, TIMPS 1–4 — tissue inhibitor of metalloproteinase 1–4, UC — ulcerative colitis

Introduction

Ulcerative colitis (UC) is one of the two primary subtypes of inflammatory bowel disease (IBD). The average prevalence of UC is 50–230 cases per 100,000 individuals, and its annual increase amounts to 5–20 per 100,000, with an upward tendency in all age groups [1]. The pathogenesis of UC is not completely studies; new studies on the development of new diagnostic techniques are performed which is especially important in view of the chronic and unpredictable course of UC. Spontaneous healing and persistent remission of UC with no drug treatment is rare; repeated ulceration and constant renewal of the epithelium increase the risk of colorectal neoplasia and cancer [2]. The healing of mucosa is a key therapeutic target in cases of inflammatory bowel disease (IBD), including UC, with endoscopy being the gold standard for diagnosis and treatment. [3]. However, this examination is invasive and stressful for patients, with monitoring of the mucous membrane condition required at different stages of the disease. In view of this fact, the search and implementation of new effective and minimally invasive UC activity markers remain relevant. Therefore, the objective of our review was to analyze the current literature data on potential biomarkers and their possible prognostic significance associated with UC.

Risk factors for the development and progression of UC

The etiology of this disease is currently not completely investigated. UC has historically been a disease of the population of European countries, however, in recent years there has been an increased incidence among non-European population groups, including African American and Asian, so, it was the reason for investigating the genetic determinants of IBD development [1, 4]. Results of studies have revealed about 200 susceptibility loci for IBD in the European population and at least 35 loci in the Asian population; some of the latter were identified as Asian-specific ones [4]. Many genetic tests resulted in the identification of IBD gene polymorphisms, including *NOD2/CARD15, IL-10, IL23R* [4]. Impaired intestinal homeostasis is currently considered as the main factor contributing to the pathogenesis and progression of intestinal inflammation associated with IBD [5]. Recent studies demonstrated that special genetic features contribute to the impairment of intestinal microbiome. So, prostaglandin EP4 receptor encoded by *PTGER4* gene is necessary to maintain the integrity of epithelial barrier, and its impaired structure is associated with the development of IBD [5].

Interaction of genetic and environmental risk factors is certainly important for the development of IBD. Thus, Min Zhao et al. in their review (2022) analyzed 255 studies and defined 25 risk factors of IBD development; seven of these factors were relevant to both eastern and western populations: family history of CD or UC, history of smoking, appendectomy, tonsillectomy, diet that includes meat and meat products, vitamin D deficiency [6]. Other factors, that is, living in an urban area, current smoking, use of antibiotics and oral contraceptives, caesarean section, use of isotretinoin, obesity, diet that includes fat, eggs, and non-alcoholic products, were associated with an increased risk of IBD in only one of these populations. Risk factors for IBD development in the eastern population were the following: diet that includes eggs, increased

consumption of fat and fatty acids (both monounsaturated and polyunsaturated) [6]. At the same time, the authors identified more than 20 protective factors in relation to IBD; eight of them became common for the eastern and western populations: contact with domestic and farm animals, many births, physical activity, history of breastfeeding, H. pylori infection, current smoking status, and coffee consumption [6]. It should be mentioned that the protective role of H. pylori was also previously demonstrated in a meta-analysis by Y. Zhong (2021): negative correlations were obtained between H. pylori and the prevalence of IBD, H. pylori had a protective effect against IBD, and according to the results of meta-analysis, eradication of H. pylori contributed IBD relapse [7].

A recent meta-analysis of 19 studies demonstrated the important role of nutrition in the development of IBD [8]. The objective of this paper was to summarize data on the daily diet of adults with IBD compared with healthy individuals of the same age and sex. It was discovered that adults with IBD do not get enough energy, fiber, fat-soluble vitamins, as well as important nutrients such as folic acid, vitamins B1, B2, B3, B6, potassium, magnesium and phosphorus. The adults with UC have been found to consume significantly more fat and copper, and CD patients consume significantly less protein, iron, and fiber compared to healthy controls. Another important result of this review was that the consumption of basic products that are considered to form the basis of a healthy diet, such as cereals, legumes, fruits, vegetables and dairy products, was found to be insufficient for people with IBD [8]. Based on the above, it is possible to define the groups of individuals with high risk of UC in order to provide earlier diagnosis of this disease, including non-invasive methods.

Instrumental examinations in UC

The preferred method for UC confirmation is endoscopic examination that allows to directly observe its macroscopic signs, as well as to obtain material for histological examination [9]. Endoscopic findings in UC include mucosal edema, loss/decrease of vascular pattern, pseudopolyps, loss of haustration, diffuse hyperemia, and mucosal granularity [2]. It should be mentioned that the aforementioned endoscopic signs can be observed in other colitis; therefore, differential diagnosis essentially depends on the type of endoscopic findings and the nature of their generalization in the intestine rather than on their range [3]. The histological findings typical for this disease include basal plasmacytosis and the altered structure of mucosa and/or crypts. The altered structure of mucosa and crypts includes several signs: crypt branching, changed size of crypts, atrophy and irregularity of mucous membrane. The abovementioned signs indicate the chronicity of the inflammatory process in colon mucosa; they appear when the underlying inflammation lasts more than 4 weeks and remain during remissions [3, 10]. The other signs of inflammation in the patients with UC exacerbation are: the groups of neutrophils are found in the lamina propria of the mucosa; neutrophils invade the superficial epithelium and the crypt epithelium with the development of “crypt abscesses”; erosions and granulation tissue are visualized. These signs indicate the active process; they are observed in the exacerbation phase with underlying signs of chronicity and resolve in inactive UC [11].

The ongoing clinical trials routinely include endoscopic evaluation of healing as an endpoint, and expert consensus recommends it as an important treatment goal in clinical practice. [11]. Despite progress in the drug treatment of UC, a significant part of patients have disease relapse [3, 11]. This is due to the fact that patients who have achieved mucosal healing according to endoscopy usually have active microscopic inflammation of colonic mucosa [12]. Many studies demonstrated persistent microscopic inflammation in most patients with the endoscopic diagnosis of remission what allows suggesting that the level of inflammation with underlying UC can not be fully characterized using just endoscopic evaluation [13, 14]. Thus, it is reasonable to assume that histological remission is associated with improved clinical outcome, and it is the parameter that may be the ultimate therapeutic goal in the management of UC.

The role of neutrophils in the pathogenesis of UC

Patients with UC have massive neutrophil infiltration of the intestinal wall followed by the production of reactive oxygen species and release of serine proteases, matrix metalloproteinases, and myeloperoxidase [15]. It has been established that neutrophils express more than 1,200 cellular proteins; 400 of these proteins are located in secretory vesicles, and almost 300 — in granules [16]. Disease activity corresponds to progressive neutrophil infiltration, crypt involvement, and neutrophil exudation, ranging from minimal inflammatory activity to severe ulceration [15, 16]. Thus, neutrophil infiltration is a special histopathologic feature of UC that indicates the central role of neutrophils as effector cells in mucosal damage [17]. Neutrophil infiltration into epithelium and lamina propria is the essential component in assessing the severity of UC, in particular, in its histological assessment using Riley and Geboes scores, as well as in the recently proposed Nancy histological index [17]. Neutrophil infiltration of mucosa correlates with the endoscopic severity of UC and such systemic indicators of inflammation as C-reactive protein (CRP) level in blood serum [16, 17]. Patients with UC also have altered neutrophil apoptosis that may be associated with the release of

anti-apoptotic cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) that prolongs the life span of granulocytes during mucosal inflammation [14]. Uncontrolled accumulation of neutrophils and their persistence in the intestinal mucosa in cases of active UC may delay timely improvement of intestinal inflammation [14–17]. Therefore, neutrophils are important in the pathogenesis of UC; they are also a valuable marker in defining disease activity/severity, as well as a potentially attractive drug target for therapeutic intervention.

Non-invasive biomarkers of neutrophilic origin in cases of UC

At present, the following faecal markers of neutrophilic origin are the most widely studied as potential non-invasive markers of UC activity: faecal calprotectin (FC) and lactoferrin (LF) [18].

Faecal calprotectin (FC) is a 36 kDa zinc- and calcium-binding protein. It is located mainly in neutrophils and, to a lesser extent, in monocytes and macrophages. Calprotectin makes up 60 % of the soluble cytosolic proteins of neutrophils and is used as a marker of neutrophil turnover. It can be found in different biological fluids, such as blood serum, saliva and urine, feces [18]. FC concentration in feces is proportional to the neutrophil migration into gastrointestinal tract; thus, calprotectin is the most widely used faecal marker [18, 19]. FC measurement is used in clinical practice for the differentiation between functional bowel disorders, mainly, irritable bowel syndrome and inflammatory bowel diseases [18]. It is used as a valuable non-invasive method for monitoring disease activity in patients with IBD [20].

Lactoferrin is an 80 kDa iron-binding protein that was first found in milk and is present in many other secretions of human body. Lactoferrin is released from secondary granules in neutrophils upon activation and has many functions. In addition to its antibacterial properties, it is involved in immune response, cell growth, and cell differentiation [14].

Several clinical trials were conducted concerning the usefulness of FC and LF in the differential diagnosis of IBD and irritable bowel syndrome (IBS), as well as for predicting relapse, and as a biomarker of disease activity in patients with UC [21, 22]. The recent ACERTIVE multicentre cross-sectional study that included 371 patients demonstrated that FC levels were statistically higher in patients with endoscopic and histological activity, and cut-off level of 150–250 μg/g was proposed [23]. The results of a large study conducted in 2013–2017 that involved 185 patients revealed that FC levels ≥170 μg/g were a predictive factors of endoscopic activity, and FC levels ≥135 μg/g predicted histological activity [24]. Therefore, lower threshold FC values may be chosen to optimize the identification of patients with persistent endoscopic and histological disease activity in clinical practice. A systematic review of FC and LF as surrogate markers for endoscopic monitoring in patients with UC performed by M.N. Mosli et al. (2017) demonstrated their high sensitivity and specificity (0.88 and 0.73 for FC and 0.82 and 0.79 for LF, respectively) [16]. In other publications, FC and LF sensitivity and specificity vary from 70 % to 90 % [25].

FC value in the patients with UC correlated with endoscopic disease activity with higher accuracy, reaching 89 %, in comparison to clinical activity index, increased CRP, and leukocytosis (overall accuracy: 73 %, 62 % и 60 %, respectively) [26]. Moreover, FC is used to differentiate the severity of colitis (sensitivity: 84 %, specificity: 88 %, AUC: 0.92) [27]. FC is a prognostic factor for assessing the management and the progress of disease (relapse and postsurgical relapse), remission (sensitivity: 92.3 %, specificity: 82.4 %, AUC=0.924) and exacerbation of UC (sensitivity 76 %, specificity 85 %) [28]. Decreased FC level in patients with UC treated with infliximab was a prognostic factor for disease remission [29, 30]. FC is used for the comprehensive evaluation of patients in clinical trials conducted to test new drugs [23–26].

LF was also used to predict UC relapse. LF cut-off value of 140 μg/g of feces predicted relapse with a sensitivity of 67 % and a specificity of 68 % [29, 30]. W.A. Faubion et al. (2018) performed a comparative assessment of biomarkers in patients with UC and CD compared with endoscopic parameters [31]. The markers that feature with the closet association with the endoscopic pattern included FC, LF, and lipocalin [31]. A systematic review conducted by Y. Wang et al. (2015) demonstrated that LF in feces is a sensitive and specific marker that can help to differentiate IBD from IBS, at least, at the cohort level [32]. The highest levels of LF were observed in patients with UC. At the same time, the informative value of LF as a biomarker of UC was questioned by D. Turner et al. (2010) due to the fact that LF has demonstrated limited value in predicting sensitivity to corticosteroids in severe pediatric UC [30].

Considering the strong association with IBD, FC is currently a common secondary endpoint in clinical interventional trials. M.T. Ostermann et al. (2014) found that increased doses of mesalazine resulted in a consistent decrease in FC levels what correlated with a lower relapse rate [33]. Several studies by R. Molander et al. (2013) demonstrated that normalization of FC levels after infliximab induction therapy predicts sustained clinical remission [34].

It is important to realize that both LF and FC are derived from activated neutrophils (as well as macrophages), and their levels correlate well with the amount of neutrophils in the intestine [35]. Both markers have antimicrobial properties including iron binding that is essential for bacterial replication and binding

of lipopolysaccharides [19, 21]. These proteins can be used as biomarkers is due to their resistance to proteolytic cleavage and stability in feces [23].

Other biomarkers of neutrophilic origin in the diagnosis of UC

Neutrophils are multifunctional cells that coordinate and initiate host immune response to an infectious agent or tissue damage. During the degranulation of activated neutrophils, leukocyte proteases are released on the cell surface and into extracellular space; they regulate the interaction of innate and adaptive immune systems by modulating the expression and activity of cell receptors produced by different cytokines [35]. Sensors for leukocyte and bacterial proteinases are proteolytically activated receptors expressed on the surface of platelets, blood leukocytes and macrophages, as well as of epithelial, endothelial, mast, dendritic and other cells involved in the development of inflammation and immune response [36]. Evaluation of the intensity of neutrophil degranulation can be important with regard to the pathogenesis of many diseases, as well as the assessment of the properties of immunostimulating agents.

The family of matrix metalloproteinases (MMPs) includes 24 zinc-dependent endopeptidases that are involved in the destruction of extracellular matrix in normal physiological processes [37]. Their activity is regulated by a tissue inhibitor of MMPs (TIMPS1-4) [38]. One of the most well-studied MMP enzymes is MMP-9 (matrix metalloproteinase-9, gelatinase B, or 92-kDa gelatinase) that is increased in serum and intestinal mucosa in patients with active UC [38]. In a study that involved 85 patients with UC, 64 patients with CD, and 27 control individuals, serum MMP-9 levels were positively correlated with disease activity and were significantly higher in patients with active IBD compared with inactive IBD, as well as in patients with active UC compared with those with active CD [39]. MMP-9 level demonstrated positive correlation with serum IL-6 level, platelet and WBC count in cases of UC. It was found that MMP-9 levels in feces significantly correlate with total Mayo score and serum levels of CRP and FCP [39]. Reported results of a phase I clinical trial concerning GS-574 (anti-MMP-9 antibody) demonstrated a clinical response rate of 43 % for patients with UC vs 13 % in placebo group [40].

In patients with active UC and CD, serum levels of neutrophil gelatinase-associated lipocalin (NGAL) are increased compared with controls what indicates its potential as a biomarker of UC activity [38]. M. de Bruin et al. in their two recent trials investigated MMP-9/NGAL complex as a surrogate marker of mucosal healing in both UC and CD [41]. They measured serum MMP9/NGAL levels in two independent infliximab-treated UC cohorts and observed that the decrease in MMP-9/NGAL levels found in the subjects could predict mucosal healing with specificity as high as 91 % [41].

Elafin (a peptidase-3 inhibitor, or antileukoprotease) is a neutrophil elastase inhibitor with broad spectrum antimicrobial activity. J. Wang et al. demonstrated that elafin levels in colon bioptates were increased in the presence of strictures in patients with IBD; this fact, according to the authors, demonstrated the altered balance of proteases and antiproteases [42]. However, W. Zhang et al. in a recently published paper demonstrated a statistically significant decrease in elafin mRNA in active UC and its increase during remission [43]. The relative expression of elafin mRNA in peripheral blood leukocytes in UC negatively correlated with erythrocyte sedimentation rate, C-reactive protein level, and modified Mayo score, and in patients with CD it negatively correlated with clinical activity index [43].

Human neutrophil elastase (HNE) of serine proteases family, stored in azurophilic granules of neutrophils has broad substrate specificity and can degrade structural proteins, including elastin, collagens, and proteoglycans [44]. Alongside with elafin, HNE extracellular activity is controlled by many other endogenous protease inhibitors, such as α1-antitrypsin (α1-AT), secretory leukoprotease inhibitor (SLPI), and α2-macroglobulin [45]. According to some authors, human neutrophil elastase level is increased in the mucosa of patients with UC, so it can be used as the disease activity biomarker [46].

The presence of autoantibodies against neutrophil cell proteins is a specific feature of many autoimmune diseases. A number of published papers include the description of different anti-neutrophil cytoplasmic antibodies (ANCA) that are biomarkers for the diagnosis and prognosis of UC [47, 48]. In particular, anti-proteinase-3 ANCA is significantly more common in UC than in CD patients [48]. This allows suggesting a possible role of anti-proteinase-3 ANCA as a serological biomarker not only for diagnosis but also for differentiating UC and CD.

Cat-G is another serine protease associated with UC. Cat-G expression was found to be higher in colon and stool samples of UC patients compared to healthy individuals in control group [48]. In these samples, PAR4 expression is not only higher but is also localized mainly in crypts. On the contrary, in samples of healthy volunteers, PAR4 expression is observed in the cytoplasm of non-epithelial cells [48].

Neutrophil surface markers CD16, CD177, CD64

The outer surface of neutrophils expresses molecules that can be biomarkers or drug targets. Thus, the potential significance of these molecules as biomarkers is extremely important [35, 36]. These molecules

are not just markers on the surface of neutrophils, they are also involved in the regulation of cellular functions. For example, CD16, or Fc gamma receptor IIIb that was found on the surface of neutrophils, as well as on the natural killer cells and monocytes/macrophages, is a Fc receptor with low affinity to IgG [36, 49]. *In vitro* studies demonstrated that CD16 was involved in the activation of neutrophils by immune complexes, however, takes no part in other neutrophil functions such as phagocytosis or bacterial killing. This makes CD16 a particularly attractive potential therapeutic target in inflammatory diseases, since its inhibition would not compromise host defense against infection [50]. Neutrophilic CD16 is also involved in therapeutic response in IBD [36, 49]. There is information in literature sources on infliximab-induced neutrophil-specific CD16-related autoantibodies [50].

CD177 is another surface marker that is selectively expressed by a distinct subset of neutrophils. It is interesting that CD177 expression on neutrophils was associated with clinical response to treatment with corticosteroids in severe UC [50]. CD177 transcript doubled in patients with UC with no response to systemic corticosteroid therapy; it became one of the top 10 indicators of steroid resistance in these patients during the test for prognostic value [50]. CD64 expression is relevant for the management of UC, as CD64 upregulation correlates with loss of infliximab efficacy, and CD64 mRNA expression in colon is increased in infliximab non-responders.

Conclusion

Neutrophil infiltration is central in the pathogenesis of UC. Currently available information on the role of biomarkers of neutrophilic origin in the diagnosis of UC is extremely vast and is of potential research and practical interest. The main challenges for their use at present are the variety of cut-off values, methods and timing of feces sampling, high cost of diagnostic tests. Further improvement in the understanding of pathophysiology and increased validation of biomarkers of neutrophilic origin are likely to help in the development of an optimal procedure that includes a number of clinical and laboratory markers and will help to reduce the need for invasive diagnostic procedures in routine practice.

Вклад авторов:

Все авторы внесли существенный вклад в подготовку работы, прочли и одобрили финальную версию статьи перед публикацией

Болотова Е.В. (ORCID ID: https://orcid.org/0000-0001-6257-354X): редактирование текста

Юмукян К.А.: (ORCID ID: https://orcid.org/0000-0001-9825-7610): сбор материала и анализ полученных данных, написание текста

Дудникова А.В.: (ORCID ID: https://orcid.org/0000-0003-2601-7831): анализ результатов, написание текста

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Bolotova E.V.: (ORCID ID: https://orcid.org/0000-0001-6896-877X): text editing

Yumukyan K.A. (ORCID ID: https://orcid.org/0000-0002-9377-5213): collection of material, analysis of the received data, writing text

Dudnikova A.V. (ORCID ID: https://orcid.org/0000-0003-2601-7831): analysis of the received data, writing text

Список литературы/ References

1. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2018; 390(10114): 2769–2778. doi: 10.1016/S0140-6736(17)32448-0
2. Маев И.В., Шелыгин Ю.А., Скалинская М.И., и др. Патоморфоз воспалительных заболеваний кишечника. Вестник Российской академии медицинских наук. 2020; 75(1): 27-35. doi:10.15690/vramn1219
   Maev I.V., Shelygin Y.A., Skalinskaya M.I., et al. The pathomorphosis of inflammatory bowel diseases. Annals of the Russian academy of medical sciences. 2020; 75(1): 27-35 doi:10.15690/vramn1219 [In Russian]
3. Тертычный А.С., Ахриева Х.М., Маев И.В., др. Проблемы диагностики гистологической ремиссии у больных с воспалительными заболеваниями кишечника. Архив патологии. 2017; 79(3): 3-9. doi:10.17116/patol20177933-9
   Tertychny A S, Akhrieva Kh M, Maev I V et al. Diagnostic problems of histological remission in patients with inflammatory bowel disease. Arkhiv Patologii. 2017; 79(3): 3-9 doi:10.17116/patol20177933-9 [In Russian]
4. Tang L, Xu M. Candidate polymorphisms and susceptibility to inflammatory bowel disease: A systematic review and meta-analysis. Gene. 2020; 30; 753: 144814. doi: 10.1016/j.gene.2020.144814
5. Wu PB, Qian R, Hong C, et al. Association between PTGER4 polymorphisms and inflammatory bowel disease risk in Caucasian: A meta-analysis. Medicine (Baltimore). 2020; 99(34): e19756. doi: 10.1097/MD.0000000000019756.
6. Zhao M, Feng R, Ben-Horin S, et al. Systematic review with meta-analysis: environmental and dietary differences of inflammatory bowel disease in Eastern and Western populations. Aliment Pharmacol Ther. 2022; 55(3): 266-276. doi: 10.1111/apt.16703
7. Zhong Y, Zhang Z, Lin Y, Wu L. The Relationship Between Helicobacter pylori and Inflammatory Bowel Disease. Arch Iran Med. 2021; 1; 24(4): 317-325. doi: 10.34172/aim.2021.44
8. Lambert K, Pappas D, Miglioretto C et al. Systematic review with meta-analysis: dietary intake in adults with inflammatory bowel disease. Aliment Pharmacol Ther. 2021; 54(6): 742-754. doi: 10.1111/apt.16549
9. Халиф И.Л., Шапина М.В., Головенко А.О., и др. Течение хронических воспалительных заболеваний кишечника и методы их лечения, применяемые в Российской Федерации (результаты многоцентрового популяционного одномоментного наблюдательного исследования). Российский журнал гастроэнтерологии, гепатологии, колопроктологии. 2018; 28(3): 54–62. doi:10.22416/1382-4376-2018-28-3-54-62

Khalif IL, Shapina MV, Golovenko AO, et al. Chronic inflammatory bowel diseases: the course and treatment methods in Russian Federation (Results of multicenter population-based onestage observational study). Russian Journal Gastroenterology, Hepatology, Coloproctology. 2018; 28(3): 54–62. doi:10.22416/1382-4376-2018-28-3-54-62 [In Russian]

10. Rath T, Atreya R, Neurath MF. Is histological healing a feasible endpoint in ulcerative colitis? Expert Rev Gastroenterol Hepatol. 2021; 15(6): 665-674. doi: 10.1080/17474124.2021.1880892
11. Solitano V, D'Amico F, Allocca M., et al. Rediscovering histology: what is new in endoscopy for inflammatory bowel disease? Therap Adv Gastroenterol. 2021; 14: 17562848211005692. doi: 10.1177/17562848211005692
12. Arkteg CB, Wergeland Sørbye S, Buhl Riis L, et al. Real-life evaluation of histologic scores for Ulcerative Colitis in remission. PLoS One. 2021; 16(3): e0248224. doi: 10.1371/journal.pone.0248224
13. Shah J, Dutta U, Das A, et al. Relationship between Mayo endoscopic score and histological scores in ulcerative colitis: A prospective study. JGH Open. 2019; 4(3): 382-386. doi: 10.1002/jgh3.12260
14. Muthas D, Reznichenko A, Balendran CA, et al. Neutrophils in ulcerative colitis: a review of selected biomarkers and their potential therapeutic implications. Scand J Gastroenterol. 2017; 52(2): 125-135. doi: 10.1080/00365521.2016.1235224.
15. Singh UP, Singh NP, Murphy EA, et al. Chemokine and cytokine levels in inflammatory bowel disease patients. Cytokine. 2016; 77: 44-49. doi:10.1016/j.cyto.2015.10.008
16. Arkteg CB, Wergeland Sørbye S, Buhl Riis L, et al.Real-life evaluation of histologic scores for Ulcerative Colitis in remission. PLoS One. 2021; 16(3): e0248224. doi:10.1371/journal.pone.0248224
17. Ayling RM, Kok K. Fecal Calprotectin. Adv Clin Chem. 2018; 87: 161-190. doi: 10.1016/bs.acc.2018.07.005
18. Drury B, Hardisty G, Gray RD, Ho GT. Neutrophil Extracellular Traps in Inflammatory Bowel Disease: Pathogenic Mechanisms and Clinical Translation. Cell Mol Gastroenterol Hepatol. 2021; 12(1): 321-333. doi: 10.1016/j.jcmgh.2021.03.002
19. Fu Y, Wang L, Xie C, et al. Comparison of non-invasive biomarkers faecal BAFF, calprotectin and FOBT in discriminating IBS from IBD and evaluation of intestinal inflammation. Sci Rep. 2017; 7(1): 2669. doi: 10.1038/s41598-017-02835-5
20. Nemakayala DR, Cash BD. Excluding inflammatory bowel disease in the irritable bowel syndrome patient: how far to go? Curr Opin Gastroenterol. 2019; 35(1): 58-62. doi: 10.1097/MOG.0000000000000493
21. Magro F, Lopes S, Coelho R et al. Portuguese IBD Study Group [GEDII]. Accuracy of Faecal Calprotectin and Neutrophil Gelatinase B-associated Lipocalin in Evaluating Subclinical Inflammation in UlceRaTIVE Colitis-the ACERTIVE study. J Crohns Colitis. 2017; 11(4): 435-444. doi: 10.1093/ecco-jcc/jjw170
22. Hart L, Chavannes M, Kherad O, et al. Faecal Calprotectin Predicts Endoscopic and Histological Activity in Clinically Quiescent Ulcerative Colitis. J Crohns Colitis. 2020; 14(1): 46-52. doi: 10.1093/ecco-jcc/jjz107
23. MH, Zou G, Garg SK, et al. C-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis. Am J Gastroenterol. 2015; 110(6): 802-19; quiz 820. doi: 10.1038/ajg.2015.120
24. Frin AC, Filippi J, Boschetti G, et al. Accuracies of fecal calprotectin, lactoferrin, M2-pyruvate kinase, neopterin and zonulin to predict the response to infliximab in ulcerative colitis. Dig Liver Dis. 2017; 49(1): 11-16. doi: 10.1016/j.dld.2016.09.001
25. Sakuraba A, Nemoto N, Hibi N, et al. Extent of disease affects the usefulness of fecal biomarkers in ulcerative colitis. BMC Gastroenterol. 2021; 21(1): 197. doi: 10.1186/s12876-021-01788-4
26. Grabherr F, Effenberger M, Pedrini A, et al. Increased Fecal Neopterin Parallels Gastrointestinal Symptoms in COVID-19. Clin Transl Gastroenterol. 2021; 12(1): e00293. doi: 10.14309/ctg.0000000000000293
27. Jangi S, Holmer AK, Dulai PS, et al. Risk of Relapse in Patients With Ulcerative Colitis With Persistent Endoscopic Healing: A Durable Treatment Endpoint.J Crohns Colitis. 2021; 15(4): 567-574. doi: 10.1093/ecco-jcc/jjaa184
28. Langhorst J, Boone J, Lauche R, et al. Faecal Lactoferrin, Calprotectin, PMN-elastase, CRP, and White Blood Cell Count as Indicators for Mucosal Healing and Clinical Course of Disease in Patients with Mild to Moderate Ulcerative Colitis: Post Hoc Analysis of a Prospective Clinical Trial, Journal of Crohn's and Colitis. 2016; 10(7): 786–794. doi: 10.1093/ecco-jcc/jjw044
29. Rubio MG, Amo-Mensah K, Gray JM, et al. Fecal lactoferrin accurately reflects mucosal inflammation in inflammatory bowel disease. World J Gastrointest Pathophysiol. 2019; 10(5): 54-63. doi: 10.4291/wjgp.v10.i5.54
30. Turner D, Leach ST, Mack D, et al. Faecal calprotectin, lactoferrin, M2-pyruvate kinase and S100A12 in severe ulcerative colitis: a prospective multicentre comparison of predicting outcomes and monitoring response. Gut. 2010; 59(9): 1207-12. doi: 10.1136/gut.2010.211755
31. Faubion WA Jr, Fletcher JG, O'Byrne S, et al. EMerging BiomARKers in Inflammatory Bowel Disease (EMBARK) study identifies fecal calprotectin, serum MMP9, and serum IL-22 as a novel combination of biomarkers for Crohn's disease activity: role of cross-sectional imaging. Am J Gastroenterol. 2018; 108(12): 1891-900. doi: 10.1038/ajg.2013.354
32. Wang Y, Pei F, Wang X, et al. Diagnostic accuracy of fecal lactoferrin for inflammatory bowel disease: a meta-analysis. Int J Clin Exp Pathol. 2015; 8(10): 12319-32
33. Osterman MT, Aberra FN, Cross R, et al. Mesalamine dose escalation reduces fecal calprotectin in patients with quiescent ulcerative colitis. Clinical Gastroenterology and Hepatology. 2014; 12(11): 1887,1893.e3.
34. Molander P, Sipponen T, Kemppainen H, et al. Achievement of deep remission during scheduled maintenance therapy with TNFa-blocking agents in IBD. J Crohn's Colitis. 2013; 7(9): 730-5. doi:10.1016/j.crohns.2012.10.018
35. Нестерова И.В, Колесникова Н.В., Чудилова Г.А., и др. Нейтрофильные гранулоциты: новый взгляд на «старых игроков» на иммунологическом поле. Иммунология. 2015; 4: 257-263
Nesterova I.V., Kolesnikova N.V., CHudilova G.A., et al. Neutrophil granulocytes: a new look at the "old players" in the immunological field. Immunology. 2015; 4: 257-263 [In Russian]
36. O'Sullivan S, Gilmer JF, Medina C. Matrix metalloproteinases in inflammatory bowel disease: an update. Mediators Inflamm. 2015; 2015: 964131. doi: 10.1155/2015/964131

37. Sandborn WJ, Bhandari BR, Fogel R, et al. Randomised clinical trial: a phase 1, dose-ranging study of the anti-matrix metalloproteinase-9 monoclonal antibody GS-5745 versus placebo for ulcerative colitis. Aliment Pharmacol Ther. 2016; 44(2): 157-69
38. Thorsvik S, Damås JK, Granlund AV, et al. Fecal neutrophil gelatinase-associated lipocalin as a biomarker for inflammatory bowel disease. J Gastroenterol Hepatol. 2017; 32(1): 128-135. doi: 10.1111/jgh.13598
39. de Bruyn M, Arijs I, De Hertogh G, et al. Serum Neutrophil Gelatinase B-associated Lipocalin and Matrix Metalloproteinase-9 Complex as a Surrogate Marker for Mucosal Healing in Patients with Crohn's Disease. J Crohns Colitis. 2015; 9(12): 1079-87. doi: 10.1093/ecco-jcc/jjv148
40. Wang J, Ortiz C, Fontenot L, et al. High circulating elafin levels are associated with Crohn's disease-associated intestinal strictures. PLoS One. 2020; 15(4): e0231796. doi: 10.1371/journal.pone.0231796
41. Zhang W, Teng G, Wu T, et al.Expression and Clinical Significance of Elafin in Inflammatory Bowel Disease. Inflamm Bowel Dis. 2017; 23(12): 2134-2141. doi: 10.1097/MIB.0000000000001252
42. Barry R, Ruano-Gallego D, Radhakrishnan ST, et al. Faecal neutrophil elastase-antiprotease balance reflects colitis severity. Mucosal Immunol. 2020;13(2):322-333. doi: 10.1038/s41385-019-0235-4
43. Jakimiuk K, Gesek J, Atanasov AG, et al. Flavonoids as inhibitors of human neutrophil elastase. J Enzyme Inhib Med Chem. 2021; 36(1): 1016-1028. doi: 10.1080/14756366.2021.1927006
44. Curciarello R, Sobande T, Jones S, et al. Neutrophil Elastase Proteolytic Activity in Ulcerative Colitis Favors the Loss of Function of Therapeutic Monoclonal Antibodies. J Inflamm Res. 2020; 13: 233-243. doi: 10.2147/JIR.S234710
45. Mizuochi T, Arai K, Kudo T, et al. Diagnostic accuracy of serum proteinase 3 antineutrophil cytoplasmic antibodies in children with ulcerative colitis. J Gastroenterol Hepatol. 2021; 36(6): 1538-1544. doi: 10.1111/jgh.15296.
46. Xu Y, Xu F, Li W, et al. The diagnostic role and clinical association of serum proteinase 3 anti-neutrophil cytoplasmic antibodies in Chinese patients with inflammatory bowel disease. Scand J Gastroenterol. 2020; 55(7): 806-813. doi: 10.1080/00365521.2020.178192
47. De Bruyn M, Ceuleers H, Hanning N, et al. Proteolytic Cleavage of Bioactive Peptides and Protease-Activated Receptors in Acute and Post-Colitis. Int J Mol Sci. 2021; 22(19): 10711. doi: 10.3390/ijms221910711
48. Malech HL, DeLeo FR, Quinn MT. The Role of Neutrophils in the Immune System: An Overview. Methods Mol Biol. 2020; 2087: 3-10. doi: 10.1007/978-1-0716-0154-9_1
49. Fujita M, Kawabata H, Oka T, et al. Rare Case of Adult Autoimmune Neutropenia Successfully Treated with Prednisolone. Intern Med. 2017; 56(11): 1415-1419. doi: 10.2169/internalmedicine.56.7619
50. Planell N, Masamunt MC, Leal RF, et al. Usefulness of Transcriptional Blood Biomarkers as a Non-invasive Surrogate Marker of Mucosal Healing and Endoscopic Response in Ulcerative Colitis. J Crohns Colitis. 2017; 11(11): 1335-1346. doi: 10.1093/ecco-jcc/jjx09