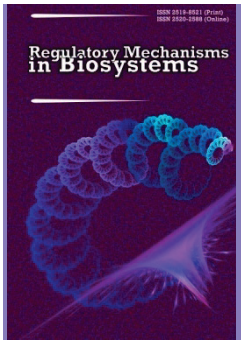

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Diagnosis of inflammatory bowel disease according to human IgG4 and possibilities of evaluating efficacy of the therapy

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Taking into account the progress of understanding diagnosis, course prognosis, evaluation of effectiveness of therapy of inflammatory bowel disease and also differentiation diagnosis between its main forms – ulcerative colitis and Crohn's disease, the search for efficient non-invasive markers for solving those issues is extremely relevant. The patients were divided into groups depending on nosology and severity of the course of the disease. All the patients had undergone endoscopic study for diagnosis verification and biopsy samples were taken for further detection of tissue IgG4 using the immunohistochemical method. Also, we determined concentration of serum IgG4. Increase in IgG4content in blood serum was determined in 54.0% of the cases of inflammatory bowel disease. Concentration of IgG4 in patients suffering ulcerative colitis was higher (by 2.31 and 2.46 times) compared with its level in the control group and patients with Crohn's disease, respectively. We found relationships between the concentration of serum IgG4 and the activity of the disease. In patients with ulcerative colitis, increased tissue IgG4 was found more often than in patients with Crohn's disease (by 2.77 times, $P < 0.05$). We determined the relationship between tissue IgG4 and histological activity. Simultaneous increase in serum IgG4 and presence of tissue IgG4 during ulcerative colitis were more frequent than during Crohn's disease (by 2.66 times). In all examined groups of patients, we determined decrease in serum IgG4 content (by 1.66 times) after treatment. Concentration of serum IgG4 and positive tissue IgG4 in ulcerative colitis patients exceeded such in Crohn's disease patients, which may be used for differentiation diagnosis between those disease types. We determined dependence of IgG4 concentration on severity and duration of the disease, which could be used as a prognostic marker. Decrease in IgG4 content in blood serum against the background of the therapy shows that this indicator could be used as a marker of treatment efficacy. Perspectives of further studies are as follows: parameters of concentration of serum IgG4 and presence of tissue IgG4 could be used as diagnostic and prognostic biomarkers and be introduced to practice for differentiation diagnosis between ulcerative colitis and Crohn's disease, and could be used as prognostic marker of severity of the disease and therapy efficacy.

Keywords: ulcerative colitis; Crohn's disease; biomarkers; humoral immunity; plasmatic cells.

Introduction

Inflammatory bowel disease remains one of the hardest problems for gastroenterology and therapy in general, because extraintestinal manifestations occur in various systems and the organs. Inflammatory bowel disease is a chronic disease of the gastrointestinal tract occurring in two main forms: ulcerative colitis and Crohn's disease. Though the etiology and pathogenesis of ulcerative colitis and Crohn's disease are still unknown, an important role in both cases is played by immunologic mechanisms (Uo et al., 2013; Şimşek et al., 2016; Chen et al., 2023). Oftentimes, it is hard to perform differentiation diagnosis between ulcerative colitis and Crohn's disease in patients with inflammatory bowel disease, manifestations of which are limited by the colon and are not characterized by typical endoscopic or histological data. Moreover, it is assumed that up to 15% of patients with inflammatory bowel disease have so called "indeterminate colitis", the term used for cases that could be classified neither to Crohn's disease or ulcerative colitis (Nyuyki et al., 2015; Şimşek et al., 2016). Standard diagnosis of inflammatory bowel disease is carried out by endoscopic examination of the colon (colonoscopy or sigmoidoscopy) and histological assay of bioptates (Uo et al., 2013; Cui et al., 2021). However, significant disadvantages of the assay are invasiveness and risk of trauma: in severe cases, during notable exacerbations, application of this method could be limited. Currently, non-invasive inflammation markers are used, such as fecal calprotein, myeloperoxidase, C-reactive protein, but the development of diagnostic criteria, study and introduction of new additional markers would only facilitate diagnosis and allow developing prediction criteria and tactics of treatment. A promising direction is the study of IgG4 as a diagnostic and prognostic marker (Şimşek et al., 2016; Stepanov et al., 2021; Fleming et al., 2022).

IgG4 accounts for the least share of all isotypes of IgG. Its role in various autoimmune disorders and even in cases of cancer has become an object of studies along with diagnosis and the widespread character of IgG4-related disease (Crescioli et al., 2016; Wang et al., 2018; Cui et al., 2021; Maslinska et al., 2022). The main peculiarity of IgG4 is absence of the effect of complement activation, allowing IgG4 to be a pathogen antibody, as well as have protective activity, and also be a fortuitous marker of aberrant inflammatory response (Trampert et al., 2018; Peerani et al., 2022; Martínez-Botas et al., 2023). The studies conducted by Watanabe et al. (2017) revealed that presence of a large amount of IgG4 in the conditions of chronic inflammation of the mucous membrane is an attempt to decrease the inflammatory process, conditioned by high activity of plasmatic cells that cause scars and traumatize the tissues.

Hamano et al. (2001) proposed distinguishing a separate nosological unit – IgG4-related disease in patients suffering autoimmune pancreatitis, who were observed to have increase in the level of serum IgG4 (Topal et al., 2014; Kotani et al., 2020). The study of autoimmune pancreatitis, specifically morphological changes in other organs in cases of this disease, was a starting point for distinguishing IgG4-associated disease in gastroen-

terology (Ishikawa et al., 2020; Tang et al., 2020). In 2012, there were determined diagnostic criteria of IgG4-related disease, including specific inflammatory process with infiltration by IgG4-positive cells, increase in concentration of serum IgG4 and progressing fibrosis, which has a storiform structure and obliterative phlebitis (Tanaka et al., 2014; Satou et al., 2020).

Despite the fact that IgG4 is a recognized marker for diagnosis of IgG4-related disease, recent studies have considered a possibility that IgG4 may perform a certain role in pathogenesis of inflammatory bowel disease (Chen et al., 2018; Tanaka et al., 2018). Despite the presence of heightened level of IgG4-positive plasma cells in the mucous membrane of the intestine during inflammatory bowel disease, this disease is not a condition of IgG4-related disease. The conditions of IBDs have no storiform fibrosis and phlebitis in their histological and clinical features, and therefore do not correspond to the profile of IgG4-related disease (Tanaka et al., 2018; Satou et al., 2020; Martín-Nares et al., 2022).

Only some cases of inflammatory bowel disease were observed to have typical cellular features and diagnostic criteria of IgG4-related disease and the data on diagnostic criteria are being given incompletely. In the global medical literature, the data on increase in tissue IgG4 in the mucous membrane of the intestine occurs rarely or is determined rarely. This is one of the largest unsolved issues of the modern gastroenterology. Scientists have revealed that presence of tissue IgG4 and increase in serum IgG4 are characteristic of patients suffering inflammatory bowel disease, and immunohistochemical staining can predict severity of ulcerative colitis. Determining IgG4 level could be a useful marker for differentiation diagnosis between ulcerative colitis and Crohn's disease (Harkness et al., 2020). However, almost all of the studies have been retrospective, without comparing levels of tissue and serum IgG4, and the effects of immunosuppressive therapy are yet to be studied (Tanaka et al., 2018; Cui et al., 2021; Koutroumpakis et al., 2021).

Therefore, in our prospective study, we compared the activities of inflammatory bowel disease according to Mayo score and W. R. Best's index, or Crohn's Disease Activity Index (CDAI), and level of infiltration of the intestinal mucosa by positive IgG4 plasmatic cells and concentration of IgG4 in blood.

The objective of the study was considering the possibility of using IgG4 as a marker of diagnosis and effective therapy of ulcerative colitis and Crohn's disease.

Materials and methods

The assays were carried out according to the bioethical norms and positions of the WMA, World Medical Association Declaration of Helsinki (2013) – Ethical principles for medical research involving human subjects, current legislation of Ukraine, approved by the Bioethic Committee of the State Institution the Institute of Gastroentorology of the National Academy of Medical Sciences of Ukraine. Prior to having blood and biomaterials drawn, all the patients gave informed consent for the involvement in the study, and their personal data is kept private.

We examined 100 patients suffering inflammatory bowel disease, including 75 patients with ulcerative colitis and 25 with Crohn's disease, who were treated at the ward for intestinal diseases of the SI the Institute of Gastroentorology of the National Academy of Medical Sciences of Ukraine, including 57 women and 43 men, their average age equaling 39.2 ± 1.8 and 36.5 ± 2.4 years, respectively. The patients were divided into two groups depending on the nosology: group I comprised 75 patients with ulcerative colitis and group II included 25 patients with Crohn's disease. Furthermore, the patients were divided into groups depending on severity of the disease, according to the Mayo Scoring System for ulcerative colitis patients and W.R. Best's index (CDAI) for Crohn's disease patients. The groups were as follows: mild ulcerative colitis – 7 patients, moderate ulcerative colitis – 54 patients, severe ulcerative colitis – 14 patients, moderate Crohn's disease – 17 patients, severe Crohn's disease – 8 patients. Endoscopic examinations of the large intestine were carried out for all the patients according to the generally accepted methods using video-colonoscope Pentax Olympus EVIS EXERA III (Olympus, Japan 2016). The goal of the endoscopic examination of the large intestine was diagnosing, in-detail evaluating the condition of the mucous membrane of the large intestine and obtaining biopsy specimens for histological, morphometric and immune-histological assays. To make paraffin histological preparations, the material had been fixed in 10.0% solution of neutral formalin, dehydrated in alcohols of ascending concentrations and was then embedded in paraffin. To study the general histological structure of the mucous membrane, 5–7-μm-thick sections of colon bioptates, cut using PM60-EKA rotary microtome, were stained using the standard hematoxylin and eosin method.

For the morphometric assays, the sections were photographed using XSZ-21 light microscope (MicroMed, Ukraine) and measured using ImageJ 1.45S (National Institutes of Health, USA) software. Morphometric assays of histological samples included: measuring depth of crypts (μm), height of the superficial epithelium (μm), height of crypt epithelium (μm), number of goblet cells of crypt epithelium per 100 cells, density of cellular infiltrate and number of eosinophile and neutrophilic leukocytes (per 1 mm^2 of the stroma).

Presence of IgG4-positive plasmatic cells (IgG4+) was determined using the immunohistochemical method. For the assay, we used monoclonal rabbit anti-human IgG4 (Abcam, USA). We determined the number of infiltrated plasma IgG4+ cells under a high-resolution microscope. Presence of ≥10 IgG4 cells in microscope high-power field (HPF; field ×40) was considered significant.

Concentration of IgG4 in blood serum was determined using the immunoenzymatic method. The assays were performed according to the instructions for each test kit. In the assays, we used tool kits manufactured by Hema, Ukraine. The material for the assays of immunological indicators was venous blood, drawn at the same time in the morning from veins of patients on an empty stomach in the amount of 10 mL. For this purpose, we used test tubes with no anticoagulants, and blood samples from healthy donors were collected in the same way.

The results were statistically analyzed using Statistica 6.1 software (StatSoft Inc., USA). Correspondence to types of distribution of features to the law of normal distribution was verified using the Shapiro-Wilk test. To evaluate the quantitative parameters, we calculated mean value (x) and standard error (SE). Mean values were compared using the Tukey test with Bonferroni correction, the difference considered significant at $P < 0.05$. For the analysis of interrelation between the parameters, we used correlation analysis taking into account the Spearman correlation coefficient (r) and their significances (P).

Results

As a result of the study, we determined that IgG4 concentration in blood serum was increased in 54.0% (54 patients) of the cases of inflammatory bowel disease. This parameter accounted for 60.0% (45 patients) in patients with ulcerative colitis, and 36.0% (9 patients) in patients with Crohn's disease. Concentration of IgG4 in the examined patients ranged within 0.1–6.3 g/L, the norm equaling 0.1–1.2 g/L. The median of the parameters was 1.50 (0.55; 2.25) g/L in ulcerative colitis patients and 0.60 (0.45; 0.80) g/L in Crohn's disease patients (Table 1). Concentration of IgG4 in the group of ulcerative colitis patients was higher by 2.31 times ($P < 0.05$) and by 2.46 times ($P < 0.05$), compared with its levels in the control group and Crohn's disease patients, respectively.

Table 1
Concentration of IgG4 (g/L) in blood serum of patients with inflammatory bowel disease (x ± SE)

Parameter	Inflammatory bowel disease (n = 100)	Ulcerative colitis (n = 75)	Crohn's disease (n = 25)	Control group (n = 15)
IgG4, g/L	1.24 ± 0.12^{a}	1.57 ± 0.14^{a}	0.69 ± 0.18^{b}	0.65 ± 0.13^{b}

Note: different letters indicate values that significantly differ one from another within one line of the table according to the Tukey test comparison taking into account Bonferroni correction.

When analyzing the parameters depending on severity of ulcerative colitis, we determined that IgG4 concentration in blood serum exceeded the norm by 42.8% in patients with mild ulcerative colitis, in 62.9% of patients with moderate ulcerative colitis and 57.1% of patients with severe ulcerative colitis. Increased IgG4 during moderate ulcerative colitis was 1.21 times higher than during severe colitis and even higher, by 1.15 ti-

mes, than during mild ulcerative colitis. Heightened concentration of IgG4 in blood serum was seen in 41.2% of the patients with moderate and 25.1% of the patients with severe Crohn's disease. In patients suffering from moderate Crohn's disease, we determined increase in IgG4 concentration, compared with the control and severe disease patients, equaling 1.92 and 1.64 times, respectively. Concentration of IgG4 in blood from the group of ulcerative colitis patients was 2.46 times higher ($P < 0.05$), compared with such in patients with Crohn's disease. We found relationships between IgG4 concentration in blood serum and the activity of the disease according to CDAI ($r = –0.455$; $P < 0.01$), duration of Crohn's disease ($r = –0.379$; $P < 0.05$), and tissue IgG4 ($r = 0.370$; $P < 0.01$).

During microscopic assays of colonobioplates of patients with ulcerative colitis and Crohn's disease (Fig. 1a, 1b), we detected such morphometric changes in the mucous membrane of the large intestine as inflammatory cellular infiltration in both lamina propria and crypt lumen, indicating development of cryptitis and formation of crypts abscesses. According to the data of the morphometric assays, cellular density of the infiltrate in the cases of Crohn's disease of colonobioptates was 1.35 times higher, compared with the parameter of cellular density of inflammatory infiltrate in the cases of ulcerative colitis.

Presence of cryptites and crypt abscesses is a characteristic microscopic trait of activity of ulcerative colitis. In the patients suffering from inflammatory bowel disease, the inflammatory infiltration was represented by neutrophilic and eosinophile leukocytes, macrophages, basophiles, fibroblasts and lymphocytes (Table 2). All the patients were subjected to immunohistochemical staining for detection of tissue IgG4 (Fig. 1c, 1d).

Presence of more than 10 IgG4-positive plasmatic cells in the microscope field of view was seen in 28 patients, accounting for 28.0%; in 23 patients (23.0%), the number of IgG4 accounted for 2–9 cells in the field of view; and no IgG4-positive cells were observed in 49 patients, accounting for 49.0%. Biopsy from the patients with ulcerative colitis revealed a significantly higher amount of IgG4-positive plasmatic cells than in patients with Crohn's disease (average number of IgG4 in the microscope high-power field (hpf) 9.8 against 2.8, $P < 0.001$).

In the ulcerative colitis patients, 33.3% (25 patients) had positive result of immunohistological assay for tissue IgG4. In the Crohn's disease patients, this value equaled 12.0% (3 patients), taking into account that increased tissue IgG4 was seen 2.77 times more often during ulcerative colitis than during Crohn's disease.

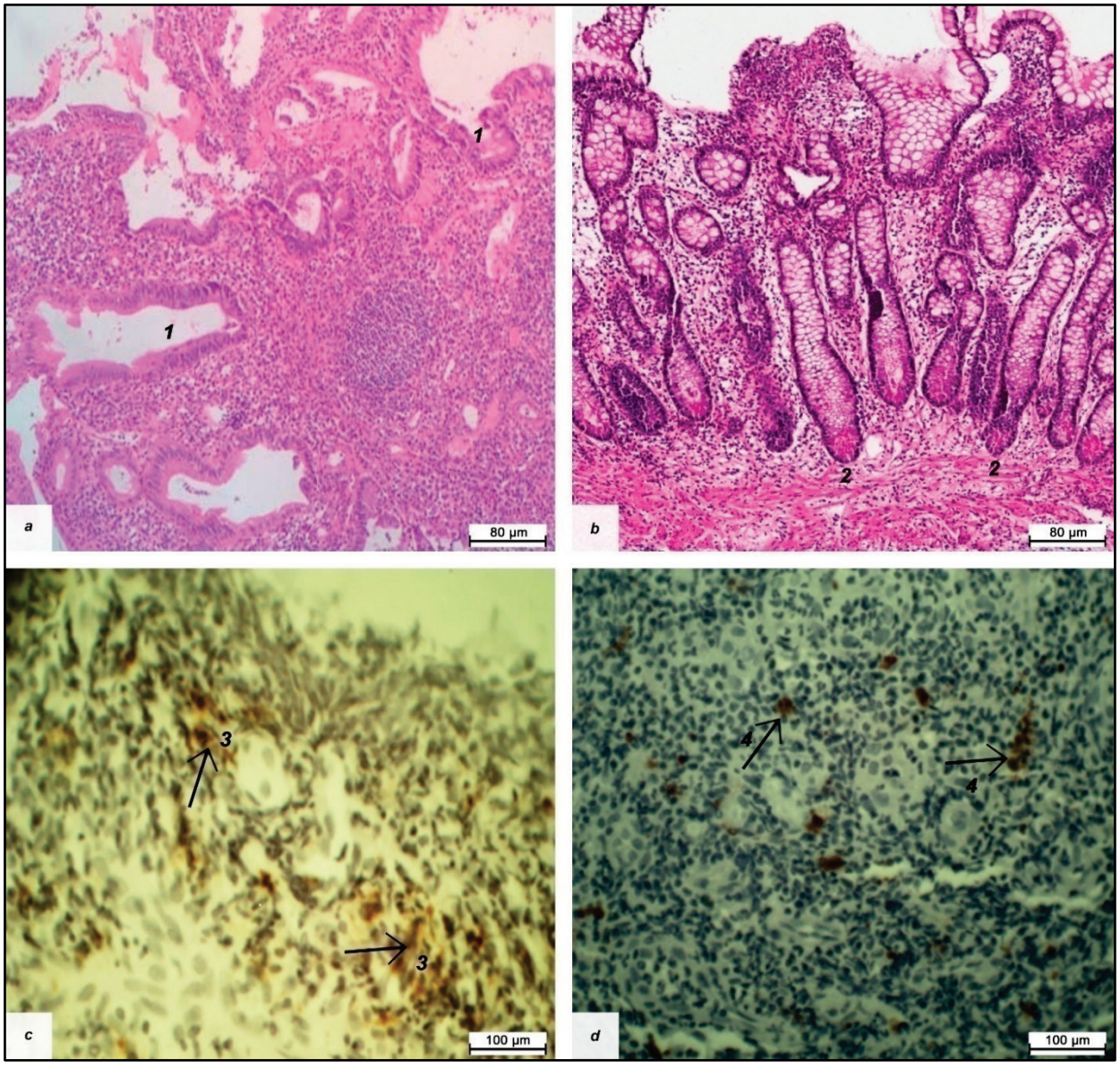

Fig. 1. Peculiarities of the structure of the mucous membrane of the large intestine during inflammatory bowel disease: *a* – ulcerative colitis, change in crypt architectonics (*1*); hematoxylin and eosin; *b* – Crohn's disease, inflammatory villus distortion, elongated, and deformed crypts (*2*); hematoxylin and eosin; *c* – ulcerative colitis, accumulation of IgG4-positive cells (*3*); indirect immune-histological reaction; *d* – Crohn's disease, accumulation of IgG4-positive cells in the stroma of the mucous membrane of the large intestine (*4*); indirect immunohistological reaction

Table 2
Morphometric changes in the mucous membrane of the large intestine in the cases of inflammatory bowel disease

Parameter	Ulcerative colitis (n = 75)	Crohn's disease (n = 25)	Control group (n=15)
Crypt depth, μm	427.8 ± 10.6[a]	423.0 ± 25.1[a]	40.7 ± 25.0[b]
Crypt width, μm	28.7 ± 0.7[a]	27.7 ± 1.9[a]	20.4 ± 2.3[b]
Epithelium height, μm	41.6 ± 1.2[a]	40.9 ± 1.1[a]	26.1 ± 1.4[b]
Superficial epithelium height, μm	41.1 ± 1.4[a]	40.9 ± 1.8[a]	34.4 ± 0.8[b]
Thickness of the mucous membrane, μm	418.2 ± 11.8[a]	414.0 ± 6.4[a]	427.0 ± 275.1[b]
Cellular density of infiltrate, per 1 mm^2	14431 ± 483[a]	19484 ± 848[a]	6780 ± 234[b]
Lymphocytes, per 1 mm^2 of the stroma	2923 ± 77[a]	2781 ± 22[a]	2465 ± 227[b]
Neutrophils, per 1 mm^2 of the stroma	212.2 ± 20.9[a]	385.2 ± 2.6[a]	23.1 ± 1.5[b]
Eosinophils, per 1 mm^2 of the stroma	332.5 ± 17.2[a]	575.9 ± 34.4[a]	23.2 ± 19.3[b]
Fibroblasts, per 1 mm^2 of the stroma	2300 ± 59[a]	2460 ± 33[a]	1058 ± 69[b]
Macrophages, per 1 mm^2 of the stroma	370 ± 16[a]	389 ± 10[a]	318 ± 32[b]
Basophils, per 1 mm^2 of the stroma	306.1 ± 10.6[a]	279.9 ± 4.3[a]	54.3 ± 2.1[b]
Goblet cells, per 1 mm^2 of the stroma	28.1 ± 0.9[a]	25.8 ± 2.4[a]	0.9 ± 0.1[b]

Note: different letters indicate values that significantly differ one from another within one line of the table according to results of the Tukey test comparison taking into account Bonferroni correction.

Correlation analysis revealed relationships between tissue IgG4 in patients with ulcerative colitis and presence of such morphological features as crypt depth, thickness of the mucous membrane, density of infiltration per 1 mm of the stroma, neutropile infiltration, macrophage infiltration, presence of fibroblasts, indicating possible role of IgG4 in the development and progression of the pathological process (Table 3). In the patients with Crohn's disease, content of tissue IgG4 was associated with erosion, granular appearance, thickness of the mucous membrane, height of the superficial epithelium, presence of fibroblasts and lymphocytes in bioptates, presence of ulcers and dysplasia (Table 4).

Table 3
Empirical correlation coefficient between tissue IgG4 and morphometric parameters in the cases of ulcerative colitis (n = 75)

Morphometric parameters	Tissue IgG4
Crypt depth	0.421 ± 0.105*
Mucous membrane thickness	0.468 ± 0.103**
Density of infiltration per 1 mm of the stroma	–0.552 ± 0.097*
Neutrophilic infiltration	–0.404 ± 0.106*
Macrophage infiltration	–0.350 ± 0.109***
Presence of fibroblasts	–0.438 ± 0.105**

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001.

Table 4
Empirical correlation coefficient between tissue IgG4 and morphometric parameters in the cases of Crohn's disease (n = 25)

Morphometric parameters	Tissue IgG4
Erosion	0.605 ± 0.163***
Granular appearance of the mucous membrane	0.376 ± 0.189*
Mucous membrane thickness	0.447 ± 0.183*
Superficial epithelium height	–0.343 ± 0.192*
Presence of lymphocytes	–0.371 ± 0.190*
Presence of ulcers	0.396 ± 0.187**
Presence of dysplasia	0.396 ± 0.187**
Presence of fibroblasts	–0.420 ± 0.185*

Note: see Table 3.

In all the examined patients with inflammatory bowel disease, simultaneous detection of increased serum IgG4 and positive tissue IgG4 occurred in 18.0% (18 patients), increased level of serum IgG4 with no positive tissue IgG4 was determined for 36.0% (36 patients), and detection of positive tissue IgG4 without increase in IgG4 level in blood accounted for 11.0% (11 patients). Presence of serum IgG4 and tissue IgG4 depending on nosology, in cases of ulcerative colitis, simultaneous increase in IgG4 in blood and positive plasmatic IgG4 was found in 21.3% of the cases (16 patients of 75), against 8.0% (2 patients of 25) of the cases of Crohn's disease. Based on this, simultaneous increase in serum IgG4 and presence of tissue IgG4 in the cases of ulcerative colitis occurred 2.66 times more often (P < 0.05) than in the cases of Crohn's disease. According to the degree of severity, simultaneous increase in blood IgG4 and presence of positive immunohistochemical staining was found in 27.7% of the moderate cases (15 patients of 54), 7.1% (1 patient of 14) of the severe cases. At the same time, there was no simultaneous detection of increased level of serum and tissue IgG4 in the mild cases. Therefore, in the cases of moderate ulcerative colitis, simultaneous increase in blood IgG4 and positive tissue IgG4 occurred 3.9 times (P < 0.05) more often than in the severe ulcerative colitis cases.

When performing the study, we analyzed the effect of the treatment on IgG4 level in blood serum of the patients with inflammatory bowel disease (Table 5). In all the examined groups, we determined decrease in IgG4 concentration in blood serum after treatment: 1.66-fold (P < 0.05) in the general group, 1.85-fold (P < 0.05) in the ulcerative colitis patients and 1.22-fold (P < 0.05) in the Crohn's disease group.

Table 5
Effects of the treatment on IgG4 concentration (g/L) in blood serum of the patients with inflammatory bowel disease (x ± SE)

Group	Prior to treatment	After treatment
Inflammatory bowel disease (n = 44)	2.03 ± 0.18[a]	1.28 ± 0.13[a b]
Ulcerative colitis (n = 36)	1.95 ± 0.16[a]	1.05 ± 0.17[a]
Crohn's disease (n = 8)	2.01 ± 0.21[a]	1.74 ± 0.23[b]

Note: concentration of IgG4 in the control group accounted for 0.65 ± 0.13 g/L; [a] – P < 0.05 significance of difference compared with the control; [b] – P < 0.05 significance of changes compared with prior and after the treatment.

Our study included data analysis in order to determine the effects of glucocorticoids (GCSs) on IgG4 level. For this purpose, the patients were divided into two groups: 23 patients having inflammatory bowel disease who had been taking glucocorticoids and 97 patients with inflammatory bowel disease who underwent no glucocorticoid treatment. The analysis of the obtained data revealed that patients who had undergone no glucocorticoid treatment were characterized by 1.86-fold (P < 0.05) increase in IgG4 concentration in blood serum and 1.38-fold (P < 0.05), compared with the control group and patients who had been taking glucocorticoids, respectively (Table 6).

Table 6
Concentration of IgG4 (g/L) in blood serum of the patients with inflammatory bowel diseases depending on glucocorticoid therapy (x ± SE)

Parameters, measurement U	Inflammatory bowel disease, after glucocorticoid treatment (n = 23)	Inflammatory bowel diseases, no glucocorticoid treatment (n = 97)	Control group (n = 15)
IgG, g/L	8.62 ± 0.34[a]	8.93 ± 0.18[a]	12.75 ± 0.49
IgG4, g/L	0.96 ± 0.29[a]	1.25 ± 0.21[a]	0.65 ± 0.13

Note: different letters indicate values that significantly differ one from another within one line of table according to results of Tukey test comparison taking into account the Bonferroni correction.

To evaluate the effects of the treatment, the patients were divided into two groups: 30 patients with inflammatory bowel disease (24 patients with ulcerative colitis and 6 patients with Crohn's disease) who had been taking glucocorticoids and 14 patients with inflammatory bowel disease (13 with ulcerative colitis and 1 with Crohn's disease) who underwent no glucocorticoid therapy. Therefore, greatest decrease in IgG4 concentration in blood serum was found in 87.5% (in 21 of 24 patients) of the cases with ulcerative colitis after glucocorticoid therapy and after the treatment, the

median of the level was significantly decreased by 2.27 times ($P < 0.05$), compared with the concentration prior to the treatment. At the same time, in 66.7% (4 of 6 patients) of the Crohn's disease patients after glucocorticoid therapy, we determined a tendency towards decrease. In the Crohn's disease patients who had not been taking glucocorticoids, we saw increase in IgG4 concentration in blood serum (Table 7). Concentration of IgG4 in blood serum decreased on average by 57.9% in the patients with ulcerative colitis who had undergone glucocorticoid treatment and by 27.2% in the patients with ulcerative colitis who underwent no glucocorticoid therapy (Fig. 2). In the Crohn's disease patients who had not been taking glucocorticoids, this decreases equaled 16.7% (Fig. 3).

Table 7

Effects of the treatment on IgG4 concentration (g/L) in blood serum of the patients with inflammatory bowel disease depending on glucocorticoid treatment (x ± SE)

Group	Prior to the treatment	After the treatment
Inflammatory bowel disease, after glucocorticoid treatment (n = 30)	2.13 ± 0.11^{a}	0.90 ± 0.08^{b}
Inflammatory bowel disease, no glucocorticoid treatment (n = 14)	1.95 ± 0.19^{a}	1.48 ± 0.16^{a}
Ulcerative colitis, after glucocorticoid treatment (n = 24)	2.14 ± 0.12^{a}	0.90 ± 0.10^{b}
Ulcerative colitis, no glucocorticoid treatment (n = 13)	2.06 ± 0.17^{a}	1.62 ± 0.18^{a}
Crohn's disease, after glucocorticoid treatment (n = 13)	1.80 ± 0.24^{a}	1.50 ± 0.21^{a}

Notes: concentration of IgG4 in the control group accounted for 0.65 ± 0.13 g/L; [a] – $P < 0.05$ significance of changes compared with the control; [b] – $P < 0.05$ significance of changes compared with prior and after the treatment.

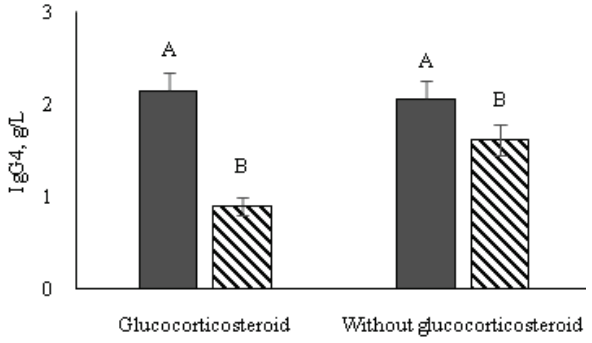

Fig. 2. Concentration of IgG4 (g/L) in blood serum of the patients with ulcerative colitis depending on glucocorticoid treatment (x ± SE): *A* – prior to the treatment, *B* – after the treatment

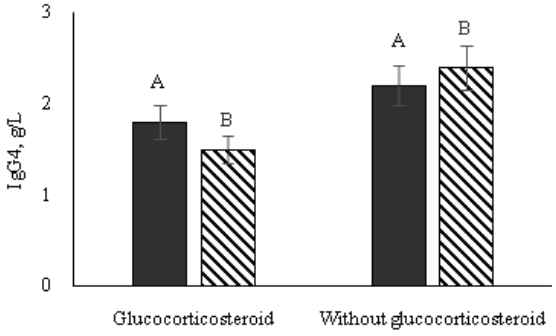

Fig. 3. Concentration of IgG4 (g/L) in blood serum of the patients with Crohn's disease depending on glucocorticoid treatment (x ± SE): *A* – prior to the treatment, *B* – after the treatment

Discussion

Inflammatory bowel disease includes chronic systemic inflammatory conditions impairing the gastrointernal tract – ulcerative colitis and Crohn's disease. Knowledge of epiopathogenesis, morphology and clinical manifestations of inflammatory bowel disease, which is expanding every year, has led to detailed phenotypical subclassification, becoming a complex interdisciplinary process (Fabián et al., 2022; D'Alessio et al., 2022). Difficulties in detection of the pathology and correct diagnosis are due to similarities in clinical pictures of the conditions of inflammatory bowel disease, absence of specific laboratory criteria for differentiation between ulcerative colitis and Crohn's disease (Şimşek et al., 2016; Dmochowska et al., 2018). The leading method for the diagnosis of inflammatory bowel disease is comparing results of endoscopic and morphological examinations. Morphological examination of colonobioptates in cases of inflammatory bowel disease is a gold standard of diagnosis which allows one to determine signs of the disease activity (Cui et al., 2021). From a clinical-morphologic perspective, to objectively evaluate the histological diagnosis, the most informative are morphometric parameters that most accurately characterize the course of inflammatory condition and reparative processes, recovery of morpho-functional condition of the mucous membrane of the intestine (D'Alessio et al., 2022). Morphometric assay of the crypt structure (their length and width, height of crypt epithelium) indicates that those parameters changed neither in the cases of ulcerative colitis nor Crohn's disease. Also, no significant difference was in the parameters of thickness of the mucous membrane of the large intestine, height of the superficial epithelium (Wang et al., 2019; D'Alessio et al., 2022; Fabián et al., 2022).

However, application of the standard assays is significantly limited due to risk of trauma and invasiveness, especially in case of severe course and aggravation stages (Şimşek et al., 2016; Stepanov et al., 2021). Currently, there are non-invasive markers that are already being used to improve the diagnosis of inflammatory bowel disease, but they do not always answer the questions of prognosis, evaluation of treatment efficacy, and therefore assays and use of new non-invasive biomarkers would improve both diagnostic and prognostic possibilities (Fleming et al., 2022; Chen et al., 2023). Such a biomarker could be IgG4. The peculiarity of this immunoglobulin is that not only can it act as pathogenic antibody, but also has a protective ability and can be a marker of inflammatory response (Peerani et al., 2022; Martínez-Botas et al., 2023). Over the recent years, numerous studies were conducted, dealing with the condition of IgG4-positive cells, as well as concentration of serum IgG4 in patients with inflammatory bowel diseases (Şimşek et al., 2016; Chen et al., 2018; Wang et al., 2018; Koutroumpakis et al., 2021). Those studies have not involved attempts to evaluate differences between patients that had and had not undergone immune-suppressing therapy. Those studies confirmed the relationship between IgG4 concentration and pathology of the intestines, and also determined the advantage of serum IgG4 in case of ulcerative colitis (Chen et al., 2018; Faria et al., 2022). The only shortcoming of those studies is their retrospective design, while there is a small number of promising studies that would evaluate the possibility of using IgG4 as a diagnostic and prognostic marker in patients suffering inflammatory bowel disease.

Our prospective study draws attention to the use of both tissue biomarkers and serum IgG4 for differentiation diagnosis between ulcerative colitis and Crohn's disease. We demonstrated that in cases of ulcerative colitis, number of IgG4-positive plasmatic cells is significantly higher than in cases of Crohn's disease, and increased value of serum IgG4 is much more frequent. The correlation analysis revealed relationships between tissue IgG4 in patients with ulcerative colitis and presence of such morphological features as crypt depth, thickness of the mucous membrane, density of infiltration per 1 mm of the stroma, neutrophilic infiltration, macrophage infiltration, presence of fibroblasts, indicating possible role of IgG4 in the development and progression of pathological process.

Morphometry provides quantitative evaluation of the parameters of cellular and tissue structures on histological and cytological preparations (or their photos). Using morphometry, one can determine the number of study objects per unit area, and also their sizes and shape. Changes of any morphological feature in number or extent allows for the use of adequate mathematical analysis in modeling process. Use of systemic analysis for those purposes expands the possibilities of morphological study of qualitative and quantitative changes, gives a possibility of deeper revealing general and individual regularities and describing them in more details (Wang et al., 2019). Relationship between morphometric data and presence of

positive plasmatic IgG4 cells allows for the use of this marker as an additional when determining histological activity during inflammatory bowel disease. The determined relationships between IgG4 concentration in blood serum and activity of diseases according to CDAI, with duration of Crohn's diseases, with presence of tissue IgG4, may suggest dependence of IgG4 concentration on severity and duration of the disease.

For the first time, there were obtained data that positive tissue IgG4 was more frequent in cases of moderate inflammatory bowel disease than in severe cases, and this may indicate protective role of IgG4 and be used as a marker of prognosis of the disease course. In the cases of ulcerative colitis, IgG4-positive cells occurred 2.77 times more often than in cases of Crohn's disease, and therefore this indicator may be used for differentiation diagnosis between those disease conditions. It is the first time there were obtained data on dependence of IgG4-positive cells on duration of the disease and localization of damage to the mucous membrane of the large intestine. As a matter of fact, those data could not be included in the routine use of tissue IgG4 for differentiation between ulcerative colitis and Crohn's diseases, but presence of > 10 IgG4 positive plasmatic cells in the field of view could additionally corroborate the diagnosis of ulcerative colitis. This is the first time when the concentration of serum IgG4 was studied in the dynamics of the treatment. The obtained data suggests that those indicators could be used as markers for choosing treatment tactics and evaluating treatment efficacy. Also, for the first time, the content of serum IgG4 in blood was studied depending on glucocorticoid therapy, both in anamnesis and treatment dynamics, the obtained data suggesting that those indicators could be used as markers to choose treatment tactics and evaluate treatment efficacy.

Conclusion

Concentration of IgG4 in blood of the group of ulcerative colitis patients was 2.46 ($P < 0.05$) times higher than its level in patients with Crohn's disease. In the cases of ulcerative colitis, positive tissue IgG4 occurred 2.77 ($P < 0.05$) times more often than in the cases of Crohn's disease. This could give an opportunity of using those parameters for differentiation diagnosis between those disease types. Increased serum IgG4 during moderate ulcerative colitis was 1.21 times higher than in severe cases and 1.15 higher than in the cases of mild ulcerative colitis, which could be used for prognostic purposes.

Simultaneous increase in serum IgG4 and presence of positive tissue IgG4 in the cases of ulcerative colitis occurred 2.66 ($P < 0.05$) times more frequently than in cases of Crohn's disease and 3.91 ($P < 0.05$) times more often in the cases of moderate ulcerative colitis, which could be used as a prognostic marker.

We determined dependence of IgG4 concentration on severity and duration of the disease and relationship between presence of tissue IgG4 and histological activity in the cases of inflammatory bowel disease.

In all the examined groups of patients, we determined a decrease in IgG4 concentration in blood serum after the treatment, making it possible to use IgG4 as a treatment efficacy marker.

Ulcerative colitis patients with heightened level of IgG4 better reacted to the therapy, the greatest drop in IgG4 concentration in blood serum was determined in the ulcerative colitis patients who had been taken glucocorticoids, allowing for the use of IgG4 as a marker in treatment efficacy and marker of choosing treatment tactics.

Perspectives of studying IgG4 as a non-invasive biomarker would make it possible to solve the issue of differentiation diagnosis between the types of inflammatory bowel disease, to predict the course of the disease and evaluation of therapy efficacy and are relevant in medical practice.

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