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Serum level of lactadherin (MFGE8) in inflamatory bowel disease

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Abstract: Objective: Evaluation of serum lactadherin level, its correlation with disease activity and certain biochemical parameters in IBD patients.
Methods: The study involved adult IBD patients, comprising 50 with ulcerative colitis (UC), 68 with Crohn's disease (CD), and 29 healthy controls.
Results: The MFGE8 median concentration was significantly higher in UC versus controls (1914.54 vs. 1392.21; p = 0.017), but not in CD. The median MFGE8 levels in UC and CD patient groups didn't significantly differ. There was a significant inverse correlation between MFGE8 and CRP (r = –0.283; p = 0.044) and fibrinogen (r = –0.362, p = 0.017) in UC. In active UC, MFGE8 median concentration was higher versus controls (1974.36 vs. 1392.21; p = 0.04) and negatively correlated with CRP (r = –0.482; p = 0.005), WBC (r = –0.391; p = 0.027), and fibrinogen (r = –0.473; p = 0.015). Inactive UC showed negative correlation only with fibrinogen (r = –0.567; p = 0.018). No correlations were found with disease activity measured using appropriate scales, age, BMI, or gender.
Conclusions: Active UC patients show higher MFGE8 levels. These increase inversely with inflammatory markers (CRP, WBC, fibrinogen) in active UC, but not in CD.

Keywords: MFGE8, lactadherin, inflammatory bowel disease, IBD, Crohn's disease, ulcerative colitis, apoptosis.

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Introduction

Inflammatory bowel disease (IBD) is a term denoting conditions which are mainly characterized by the presence of inflammatory lesions in the small and/or large intestine; the etiology of such lesions — being the effect of genetic, immune and environmental factors — has not been fully explained. The group of conditions includes

ulcerative colitis (UC) and Crohn's disease (CD). Despite numerous similarities, the two diseases also show significant differences involving their clinical and molecular aspects. To use some examples, inflammatory lesions in UC are situated solely in the colon, spread by continuity, are more rarely associated with complications, and their serological picture shows the predominance of antineutrophil cytoplasmic antibodies (pANCA) [1]. By contrast, CD may involve any segment of the gastrointestinal tract, inflammatory lesions develop segmentally and are often accompanied by abscesses, fistulas and strictures. The characteristic finding in the serological picture is the predominance of the anti-*Saccharomyces cerevisiae* antibodies (ASCA) [1].

In spite of a considerable progress in therapeutic management of IBD observed over the last decade, some patients, especially these diagnosed with CD, cannot avoid surgical interventions. The conditions known as IBD pose a significant clinical problem, especially in view of their increasing incidence that is also seen in geographical areas where the diseases have been rare until recently [2, 3]. Thus, understanding the etiology of IBD and searching for better and better therapeutic methods are of a key importance. The cause of IBD is complex and multifactorial, numerous factors regulating immune reactions cause inflammation and among such factors is lactadherin (MFGE8).

MFGE8 (BA46) is a secretory glycoprotein which is present in the membrane that encloses fat bubbles that are secreted into the milk of mammals. It is expressed in the majority of tissues and organs. The glycoprotein fulfils an important function within the intestinal epithelium through its participation in removing apoptotic cells via bridging the connections between phosphatidylserine and αVβ5 integrins on the surface of macrophages, as well as through limiting the production of some proinflammatory factors, such as interleukin 1β (IL-1β), tumor necrosis factor (TNFα) [4] and through its ability to inhibit the secretory form of phospholipase A2 [5]. Deficiency of the protein leads to accumulation of apoptotic cells and by the same token to pathological sustenance of the inflammatory reaction and tissue damage.

Breaching the intestinal barrier thus resulting in the increased exposure of the immune system to antigens present in the chyme additionally promote the development of inflammation [6]. To date it has been demonstrated in studies performed in mice that MFGE8 deficiency is associated with a more severe course of intestinal inflammatory diseases [4]. MFGE8 may also participate in the development of other immunological diseases, such as for example systemic lupus erythematosus [7, 8].

MFGE8 participates not only in the process of apoptosis, but also accelerates repairing of the intestinal epithelium in the process of the so-called restitution which involves the migration of normal, healthy epithelial cells to the sites damaged by the inflammatory process [9].

The majority of investigations of MFGE8 in IBD performed to date have focused on the level of its expression analyzed in biopsy materials originating both from

humans diagnosed with UC, animal models of the disease or directly from cell cultures. Some of the authors also concentrated on assessing the effect of MFGE8 on the course of UC.

The objective of the present paper is the assessment of the levels of plasma-dissolved lactadherin in patients diagnosed with IBD — both UC and CD — as well as a search for possible differences between the two conditions.

Material and Methods

The authors of the present report analyzed the population of 118 patients, including 68 individuals diagnosed with CD (46 subjects in the active stage of the disease and 22 in remission) as well as 50 individuals diagnosed with UC (31 patients in the active stage of the disease and 19 in remission). All the studied patients were >18 years of age and the diagnoses were established based on the results of histopathology, endoscopy and radiology. The subjects were included into the study regardless of the type of their treatment. The exclusion criteria were pregnancy as well as being diagnosed with other autoimmunological diseases, kidney failure, liver damage, uncorrected diseases of the circulatory system, diabetes and neoplastic diseases. The controls included 29 healthy volunteers.

A detailed medical history was taken from every subject, thus collecting information on significant elements related to the course of the disease, such as its duration, complications, therapeutic management, past surgical procedures and tobacco smoking.

IBD complications were defined as the presence of an abscess, fistula or extra-intestinal IBD complications. The analysis included only such surgical procedures that were a consequence of the IBD course. The patients were also subjected to a detailed physical examination.

The Montreal classification was employed to determine the location of inflammatory lesions in the intestines [10]. To assess the clinical activity of the diseases the authors used the CDAI scale [11] for CD and the DAI scale [12] for UC.

Additional analyses were performed following the division of the UC and CD groups into the "active" and "nonactive" subgroups. Active CD patients were defined as these who received >150 points as measured by the CDAI scale; in the case of UC patients, the criterion was >5 points on the DAI scale.

A fasting blood sample was collected from each patient in the morning; the sample was used in the following tests: complete blood count (white blood cells, erythrocytes, hemoglobin, hematocrit, blood platelets); the analysis also included the levels of CRP, fibrinogen, albumins, ALT and creatinine.

The samples collected for MFGE8 determinations were centrifuged and the resultant plasma was frozen at -80^{o}C until an appropriate number of samples was obtained.

All the patients granted their informed consent to participate in the investigation. The study was performed in keeping with the recommendations of the Helsinki Declaration.

Laboratory tests

Basic laboratory tests were performed in all the patients from the study group in the course of their hospitalization. Fasting blood samples were collected from the basilic vein in the morning hours. The following parameters were analyzed on the same day: blood count, albumins, fibrinogen and CRP.

CRP and albumins were tested using a Modular P clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Blood count was established employing a Sysmex XE-2100 hematology automated analyzer (Sysmex, Kobe, Germany). The levels of fibrinogen were determined using a Behring Coagulation System (BCS, Dade Behring, Marburg, Germany).

Human MFGE8 was determined in the serum using the immunoenzymatic test (Abcam, Great Britain). Absorbance was measured at the wave length of 450 nm using a microplate reader (Synergy HTX, Biokom, US).

Statistical analysis

The statistical analyses were performed using the IBM SPSS Statistics statistical package. The package served to analyze basic descriptive statistics with the Kolmogorov–Smirnov test, one-way intergroup analyses of variance, the Kruskal–Wallis tests, the ρ Spearman's rank correlation tests, the Mann–Whitney U test, the χ^2 tests and the Fisher's exact test. The significance level equaled the classic value $\alpha = 0.05$, yet the results of test statistical probability of $0.05 < p < 0.1$ were interpreted as significant at the statistical trend level.

Results

Table 1 presents the characterization of the groups.

The patients diagnosed with IBD did not significantly differ from each other and from the controls with respect to their gender, age and BMI; in the case of tobacco smoking they showed no significant differences from each other.

The median WBC count (9.86 vs. 6.17; $p = 0.001$) was significantly higher only in the UC group as compared to the controls, while CRP (UC: 17.0 vs. 0.84; $p \leq 0.001$; CD: 14.76 vs. 0.84; $p \leq 0.001$) and fibrinogen levels (UC: 3.88 vs. 2.83; $p \leq 0.001$; CD: 4.03 vs. 2.83; $p \leq 0.001$) were significantly higher both in the UC and CD group as compared to the control group when no disease activity was taken into consideration.

Table 1. Characteristics of patients with UC, CD, and the control group.

Parameter	CU n = 50	CD n = 68	control group (cg) n = 29	p
Age, year	37.6	35.6	39.0	0.518
Sex — male, n, (%)	26 (50.98%)	33 (48.5%)	15 (51.7%)	0.945
BMI kg/m^2, mean, (SD)	23.40 (4.6)	22.3 (3.8)	23.03 (3.1)	0.12
disease duration, year	7.6	6.1	—	0.62
complications, n, (%)	8 (15.7%)	36 (52.9 %)	—	<0.001
surgery, n, (%)	1 (1.96%)	29 (42.6%)	—	<0.001
smoking, n, (%)	12 (23.5%)	17 (25%)	—	0.85
disease activity, n, (%)	31 (62%)	46 (67.6%)	—	0.52
Laboratory tests				
CRP, mg/l	17 a	14.7 a	0.84 b	<0.001
WBC, ×10^3/μL	9.86 a	7.74 ab	6.17 b	0.001
RBC, ×10^6/μL	13.86	4.58	4.85	0.079
HB, g/dL	12.58 a	12.45 a	14.61 b	<0.001
PLT, ×10^3/μL	347.80 a	322.28 a	229.03 b	<0.001
fibrinogen, g/L	3.88 a	4.03 a	2.8 b	<0.001
albumin, g/l	40.6 a	39.5 a	45.83 b	<0.001
creatinine, umol/l	68.87	68.55	70.83	0.178
MFGE8, pg/ml	1914.54 a	1763.07 ab	1392.21 b	0.017
Treatment				
5-ASA, n, (%)	21 (41%)	19 (27.9%)	—	0.246
glucocorticost., n, (%)	11 (21%)	10 (14.7%)	—	
immunosupresion, n, (%)	9 (17.6%)	21 (30.8%)	—	
immunosupresion + glucocorticost., n, (%)	7 (13.7%)	10 (14.7%)	—	
none, n, (%)	3 (5.8%)	8 (11.7%)	—	
Localization				
E1/L1, n, (%)	2 (3.8%)	15 (19.2%)		
E2/L2, n, (%)	20 (38.4%)	9 (11.5%)		
E3/L3, n, (%)	30 (57.6%)	53 (67.9%)		
– /L4, n, (%)	—	0		

Abbreviations: BMI — body mass index; WBC — white blood cells; RBC-red blood cells; HB — hemoglobin; PLT — platelets; CRP — C-reactive protein; MFGE8 — lactadherin; 5-ASA — 5-aminosalicylic acid; E1 — proctitis; E2 — left-sided UC; E3 — pancolitis; L1 — CD with involvement of the small intestine; L2 — CD with involvement of the large intestine; L3 — CD with involvement of both the small and large intestine; L4 — CD with involvement of the upper gastrointestinal tract.

At the same time, the albumins level (UC: 40.6 vs. 45.83 p ≤0.001; CD: 39.50 vs. 45.83; p ≤0.001) was significantly lower in the two groups of patients when they were compared to healthy volunteers.

While assessing the concentration levels of MFGE8, the authors showed a statistically significant difference between the UC group and the controls, but no such difference was demonstrated when comparing the CD group and the controls. The difference of the median MFGE8 concentration values for the two groups (UC/CD) was statistically non-significant.

The UC group demonstrated a statistically significant inverse correlation between MFGE8 and CRP (r = –0.283, p = 0.044) and fibrinogen (r = –0.362, p = 0.017).

In view of the complex effect exerted by inflammation on the levels of MFGE8 in various diseases that has been described in the literature on the subject, in order to render the results more specific, the authors additionally divided the UC and CD patients into the active and nonactive disease subgroups in keeping with the formerly adopted criteria. The thus obtained subgroups continued to show no significant differences with respect to age, gender and BMI. Following the above-mentioned division, the median value of MFGE8 was significantly different solely in the UC patients with active disease — it was higher as compared to the controls (1974.36 vs. 1392.21; p = 0.04); in all the remaining cases the differences were statistically non-significant. The differences between the median concentration values of the acute phase proteins (CRP, fibrinogen) and leukocytes, erythrocytes, blood platelets and albumins seen following the division of the subjects into the active and nonactive subgroups are presented in Table 2.

Table 2. Comparison of median values of selected laboratory parameters within patient groups divided into active and inactive forms (together with the control group — 5 groups). Letters denote groups which medians showed a difference at the level of statistical significance in post-hoc tests.

Parameter, unit	CD active	CD non-active	CU active	CU non-active	Control group	p
CRP, mg/l	20.84 c	2.06 ab	25.19 c	3.2 b	0.84 a	$H(4) = 78.77$ $p < 0.001$
WBC, ×10^3/μL	8.37 bc	6.44 ab	12.03 c	6.20 ab	6.17 a	$H(4) = 35.29$ $p < 0.001$
RBC, ×10^6/μL	4.42 b	4.90a	19.22 b	4.85 ab	4.85 a	$H(4) = 22.27$ $p < 0.001$
HB, g/dL	11.57 b	14.26 a	11.73 b	13.99a	14.61a	$F(4.\ 143) = 22.44$ $p < 0.001$
PLT, ×10^3/μL	349.24 b	265.91 a	398.56 b	262.32 a	229.03 a	$H(4) = 45.5$ $p < 0.001$

Table 2. cont.

Parameter, unit	CD active	CD non-active	CU active	CU non-active	Control group	p
fibrinogen, g/L	4.52 b	3.14 a	4.44 b	3.02 a	2.83 a	$H(4) = 43.98$ $p < 0.001$
albumin, g/L	36.16 a	46.51 c	38.64 ab	43.92 b	45.83 c	$H(4) = 56.21$ $p < 0.001$
creatinine, umol/l	65.23 ab	75.50 b	66.13 a	73.72 b	70.83 ab	$H(4) = 17.26$ $p = 0.002$
MFGE8, pg/ml	1879.70 ab	1519.21 ab	1974.36 b	1813.79 ab	1392.21 a	0.04

Abbreviations: WBC — white blood cells; RBC — red blood cells; HB — hemoglobin; PLT — platelets; CRP — C-reactive protein; MFGE8 — lactadherin.

In none of the analyzed groups did the level of MFGE8 correlate with age, gender or BMI. In the controls, the authors detected no statistically significant correlation between CRP, WBC, fibrinogen and MFGE8. The correlations seen in the groups of patients with active and nonactive UC are presented in Table 3.

Table 3. Correlation of MFGE8 with CRP, WBC, and fibrinogen in the active and inactive form of UC.

Parameter	CU active (r)	p	CU non-active (r)	p
CRP	**–0.482**	**0.005**	–0.441	0.059
fibrinogen	**–0.473**	**0.015**	**–0.567**	**0.018**
WBC	**–0.391**	**0.027**	–0.309	0.198
RBC	–0.118	0.521	–0.153	0.533
HB	0.039	0.830	–0.270	0.264
PLT	–0.342	0.055	–0.100	0.684
albumin	0.321	0.102	–0.076	0.781
creatinine	0.314	0.080	0.175	0.488
disease duration	0.319	0.080	–0.104	0.672

Abbreviations: WBC — white blood cells; RBC — red blood cells; HB — hemoglobin; PLT — platelets; CRP — C-reactive protein.

In the case of the patients with diagnosed CD, a negative correlation between MFGE8 and fibrinogen was demonstrated in the active disease subgroup (r = –0.369, p = 0.025). The nonactive CD subgroup showed a negative correlation of MFGE8 with the level of albumins and a positive correlation with the activity of the disease measured using the CDAI scale (r = 0.443, p = 0.039). In the case of the active CD

subgroup as well as both the UC groups, the correlations with the disease activity scales did not even approximate the level of statistical trend. No statistically significant correlation was detected between MFGE8 and CRP, WBC and Hb in the two CD subgroups.

No statistically significant correlation was demonstrated either between MFGE8 and duration of the disease in all the analyzed groups.

Discussion

To the best knowledge of the authors, the present study is the first investigation where the plasma MFGE8 levels in patients with UC and CD are described and compared.

A search of the available literature failed to reveal any papers addressing the plasma MFGE8 levels in patients diagnosed with IBD. The majority of publications — whether referring to humans or animals — concentrated solely on the level of MFGE8 expression in UC as measured in samples collected during colon biopsies.

The available reports do not describe any studies on MFGE8 in patients with CD.

The publications on the plasma levels of MFGE8 addressed solely other diseases, although some of them also had an autoimmune background, as for example systemic lupus erythematosus (SLE) and rheumatoid arthritis; the results of such investigations showed discrepancies depending on the condition studied [13–20].

Lower values of the mean MFGE8 level as compared to the controls were reported in the case of patients with rheumatoid arthritis, coronary atherosclerosis and chronic obturative lung disease (COPD) [13–15].

Higher values were encountered in patients with SLE, chronic pancreatitis, type 2 diabetes, gestational diabetes and atherosclerosis of the internal carotid artery [16–20]. In the present study, the level of MFGE8 was significantly lower in the UC group as compared to the controls, while in the CD patients, the difference was at the statistical trend level. No statistically significant difference between the two diseases was observed.

In the case of COPD, an important factor affecting the level of MFGE8 was tobacco smoking which is among the main causes of the disease and that affected almost all the studied subjects. As it was shown by the present authors, individuals who smoked and patients who recently stopped smoking demonstrated significantly lover MFGE8 levels as compared to individuals who never smoked [15]. In the present study, smokers constituted slightly more than 20% of subjects in both groups (UC — 23.8%, CD — 21.79%), while the MFGE8 concentration values in smokers and non-smokers did not differ even at the statistical trend level; the observation was valid also following the division of the patient groups into the active and nonactive subgroups.

Dai *et al.* [14] described a lower mean MFGE8 concentration value in the group of patients with coronary disease. Regardless of the fact that male subjects predominated

in the study group, a decreased MFGE8 level persisted also when gender was taken into consideration. However, when the patients described by Dai *et al.* were compared to the present population of subjects, the former individuals were significantly older (mean age 61.41 years vs. 37.9 years — UC, 34.4 years — CD). Nevertheless, both in the afore-mentioned study and in the present investigation, CRP showed a negative correlation with the MFGE8 level.

While studying atherosclerosis, Zhao *et al.* observed a different result; their investigations demonstrated increased MFGE8 levels parallel with intensification of atheromatosis of the internal carotid artery, while in the controls, age was a factor that positively correlated with MFGE8 [20]. In the present study none of the investigated subgroups showed a correlation between MFGE8 and age.

In the case of rheumatoid arthritis, the population of patients described by Zhao *et al.* was to a higher degree similar to the presently described subjects in view of the therapeutic management which included glucocorticosteroids, immunomodulatory agents and biologic therapy. However, in the above-mentioned investigation, patients with medium or high disease activity were in a substantial minority (22.5%) [13], whereas in the present study the individuals defined as suffering from "active" disease accounted for 62% of the total number of the UC patients and 67% of the entire CD group.

Obesity has proven to be yet another important factor that affects the levels of MFGE8. In the study on type 2 diabetes, obese patients were characterized by significantly lower levels of the investigated substance as compared to slender individuals; also the mean MFGE8 level in patients with diabetes was higher as compared to the controls [18]. No effect of BMI on the MFGE8 levels was observed by the present authors.

As it follows from the report on SLE describing 40 newly diagnosed patients, the investigators demonstrated a higher level of MFGE8 as compared to the controls; the authors also suggested two different clinical types of SLE depending on the MFGE8 concentration level [16]. Taking into consideration the MFGE8 level, the patients were divided into "positive" (MFGE8 $\geq$7.5 ng/ml) and "negative" (MFGE8 <7.5 ng/ml); the MFGE8 value that differentiated the two groups (7.5 ng/ml) was selected in a manner allowing the control group to include <5% of MFGE8 "positive" patients or in other words individuals with MFGE8 of $\geq$7.5 ng/ml. The authors of the aforementioned publication suggested a more potent immune response expressed as a higher antibody titter in the group defined as MFGE8 "positive", while the MFGE8 "negative" group demonstrated a more intense inflammatory response, manifested mainly by higher CRP values [16].

Apart from the higher mean MFGE8 values in the UC group, the present authors also observed a negative correlation between MFGE8 and WBC, CRP and fibrinogen in active UC and with fibrinogen in nonactive UC and active CD.

In the aforementioned publication on SLE, the negative correlation between MFGE8 and CRP did not reach statistical significance, but the analysis involved only 16 selected MFGE8-"positive" patients [16].

The authors of two other reports on SLE also obtained significantly higher mean MFGE8 values in their patients as compared to the controls; they also noted a weak correlation of MFGE8 with disease activity [21]. One of the above investigators described the higher MFGE8 values to be associated with a complication manifested as changes in the brain detected by MRI [22].

The mean concentration value of MFGE8 in pregnant women was significantly higher as compared to the controls; the value increased even more in case of gestational diabetes. The latter group additionally demonstrated a negative correlation with CRP [23]. Hormonal changes in pregnancy — such as for example the increased prolactin concentration levels — might have had a significant effect on the plasma MFGE8 concentration levels [19].

While analyzing the results obtained by the UC and CD groups without division into active and nonactive subgroups, the present authors observed statistically higher MFGE8 concentration values as compared to the controls solely in the UC group; in the case of CD, the difference between the study group and the controls was not statistically significant.

The investigators who assessed the expression of MFGE8 in samples collected from the bowel of UC subjects, both in humans and in animal models, pointed to a decreased expression of the glycoprotein within the intestinal epithelium accompanying exacerbation of inflammation [24].

When it comes to investigations performed in humans, one publication described the expression of MFGE8 in patients diagnosed with UC to be negatively correlated with histological and clinical exacerbation of inflammation assessed using the MAYO scale, but no correlation with endoscopic findings was seen [4, 24, 25].

Similar results were obtained in animal models, although the findings did not fully coincide. The authors of the publication describing an animal model of UC induced by the administration of dextran sodium sulphate (DSS) demonstrated a decrease in the MFGE8 expression in mice during all days of DSS phase which was subsequently followed by increased expression in the healing phase [4]. The authors of another paper reported an intensified expression of MFGE8 in the colon of mice in the early phase of DSS and a decrease in the said expression in the late phase of DSS and the healing phase. Contrary to the situation in the remaining segments of the colon, in the same investigation the authors noted in the rectum alone an increasing expression of MFGE8 throughout the entire DSS phase and a decrease of the expression in the healing phase [25].

The intestinal epithelium is not the only source of MFGE8, both within the gastrointestinal tract and in the entire organism. A considerable expression of MFGE8

is noted in the lungs, spleen and kidneys [4]. Within the gastrointestinal tract, an additional important source of MFGE8 is provided by macrophages situated in the intestinal mucous lamina propria [26] and dendritic cells of lymphatic follicles [27] that are most likely also the principal source of MFGE8 in the spleen [27]. The dendritic cells of lymphatic follicles additionally participate in the elimination of B lymphocytes which show auto-aggressive properties and — precisely owing to the MFGE8 secretion — regulate the pace of removal of apoptotic cells [28], what may provide an explanation for the increased concentration values of this factor in such diseases as for example SLE.

And thus, the plasma MFGE8 level is determined by its general production in the organism which likely increases with increasing apoptosis [29], what may be yet another cause of discrepancies in results obtained in patients with various diseases since various conditions are characterized by different levels of apoptosis participation in the pathogenesis and sustenance of inflammation [30–40].

Yamaguchi *et al.* pointed out that both the deficiency and excessive levels of MFGE8 compromised the phagocytosis of apoptotic cells; by the same token, the authors suggested that the excess of the factor might also be associated with the pathogenesis of — to use an example — SLE [8]. The observation is compliant with theory, since in the process of efferocytosis, apart from binding with phosphatidylserine through MFGE8, GAS6, CCN1, ProS1 [41, 42], the macrophages also require a direct contact with phosphatidylserine. Given such a situation, the excess of MFGE8 that causes opsonization of apoptic cells might block the contact with the remaining receptors situated on the surface of the macrophages thus contributing to impairment of phagocytosis and in consequence to maintaining a high MFGE8 concentration level. On the other hand, a considerably low level of MFGE8 might be explained by a fast rate of consumption of the factor by the phagocytic cells in the course of inflammation.

The statement suggests that under special conditions, patients with severe inflammation might present both low and high values of MFGE8, the more so that — as it has been stated before — the very participation of apoptosis in the pathogenesis of various diseases is diverse. To use an example, intensified apoptosis is observed in SLE [30, 31], IBD [32–34] and type 2 diabetes [35], while decreased apoptosis is seen in rheumatoid arthritis [36–38], or COPD [39, 40].

It should be additionally mentioned here that the course of apoptosis and efferocytosis — the phenomena being directly related to MFGE8 concentration values — is also affected by the employed therapeutic management. To use an example, a study in vitro showed glucocorticosteroids to intensify the expression of stabilin 1 (Stab1) on the surface of the macrophages, what in turn may be an efferocytosis-triggering factor [43]; in turn infliximab was demonstrated to intensify apoptosis of T lymphocytes in the intestinal mucous lamina propria in patients with CD [44].

The presence of discrepancies in the reported results may be also triggered by genetic factors, such as polymorphism of a nucleotide [45] or mutation of the gene for MFGE8 [46].

In the latter case, patients with SLE were described as having a mutation which caused abnormalities of post-transcriptional processing and in consequence the formation of a shorter form of MFGE8 which continued to be capable of linking with phosphatidylserine and intensify phagocytosis of apoptotic cells, but was present in the circulation for a longer time [46].

It seems that the plasma MFGE8 level in IBD is a resultant of numerous processes occurring in the organism; its association with the two basic phenomena necessary to maintain homeostasis, i.e., apoptosis and efferocytosis, makes it an important factor in the pathogenesis of IBD.

The present paper has some limitations. Firstly, various therapeutic modalities were employed in the patients from the study groups; as it has been already reported, this phenomenon might have affected the results. Another limitation was the method of MFGE8 determination, which has an upper limit of 8000 pg/ml, leading the authors to discard results exceeding this threshold. The strongest effect was seen in the CD group, where such results accounted for 11.5% of the entire result pool. To use an example, in the UC group the percentage was 1.9% and only 3.3% in the controls. The present paper did not offer any evaluation of the MFGE8 expression in the intestinal wall.

Conclusions

The present authors demonstrated higher concentration values of MFGE8 in the patients with UC as compared to the controls and a negative correlation between MFGE8 and CRP and fibrinogen in this disease. In the case of CD, the difference between the median concentration value in the group as compared to the controls was only at the statistical trend level; this suggests that statistical significance might be achievable if the number of subjects were larger or if a different methodology for MFGE8 measurement were applied. Regardless of such considerations, in the patients with active CD the authors managed to obtain a correlation between MFGE8 and one of the inflammatory indicators, i.e., fibrinogen. As it may be concluded based on the present results, MFGE8 plays a greater role in active form of UC as compared to active CD. The discrepancies between the values of MFGE8 in various diseases remain still unsolved and this is why further studies are necessary.

Conflict of interest

None declared.

Abbreviations

ASCA — Saccharomyces cerevisiae antibodies
CD — Crohn's disease
IBD — Inflammatory bowel disease
MFGE8 — lactadherin
pANCA — antineutrophil cytoplasmic antibodies
UC — ulcerative colitis

References

1. *Lecis P., Germanà B., Papa N., et al.*: p-ANCA and ASCA Antibodies in the Differential Diagnosis Between Ulcerative Rectocolitis and Crohn's Disease. Recenti Prog Med. 2002 May; 93 (5): 308–313.
2. *Molodecky N.A., Soon I.S., Rabi D.M., et al.*: Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012; 142: 46–54.
3. *Cui G., Yuan A.*: Systematic Review of Epidemiology and Risk Factors Associated With Chinese Inflammatory Bowel Disease. Front Med (Lausanne). 2018 Jun 19; 5: 183.
4. *Monowar A., Ishihara S., Mishima Y., et al.*: MFG-E8 attenuates intestinal inflammation in murine experimental colitis by modulating osteopontin-dependent alphavbeta3 integrin signaling. J Immunol. 2009 Jun 1; 182 (11): 7222–7232.
5. *Nyegaard S., Novakovic V.A., Rasmussen J.T., Gilbert G.E.*: Lactadherin Inhibits Secretory Phospholipase A2 Activity on Pre-Apoptotic Leukemia Cells PLoS One. 2013 Oct 23; 8 (10): e77143.
6. *Kusunoki R., Ishihara S., Tada Y., et al.*: Role of milk fat globule-epidermal growth factor 8 in colonic inflammation and carcinogenesis. J Gastroenterol. 2015; 50: 862–875.
7. *Hanayama R., Tanaka M., Miyasaka K., et al.*: Autoimmune disease and impaired uptake of apoptotic cells in MFGE8-deficient mice. Science. 2004; 304: 1147–1150.
8. *Yamaguchi H., Takagi J., Miyamae T., et al.*: Milk fat globule EGF factor 8 in the serum of human patients of systemic lupus erythematosus. J Leukoc Biol. 2008; 83: 1300–1307.
9. *Bu H.-F., Zuo X.-L., Wang X., et al.*: Milk fat globule-EGF factor 8/lactadherin plays a crucial role in maintenanceand repair of murine intestinal epithelium. J Clin Invest. 2007; 117: 3673–3683.
10. *Satsangi J., Silverberg M.S., Colombel J.-F.*: The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006; 55: 749–753.
11. *Yoshida E.M.*: The Crohn's Disease Activity Index, its derivatives and the Inflammatory Bowel Disease Questionnaire: a review of instruments to assess Crohn's disease. Can J Gastroenterol. 1999 Jan–Feb; 13 (1): 65–73.
12. *Lewis J.D., Chuai S., Ellenberg J.E.*: Use of the Non-invasive Components of the Mayo Score to Assess Clinical Response in Ulcerative Colitis. Inflamm Bowel Dis. 2008 Dec; 14 (12): 1660–1666.
13. *Albus E., Sinningen K., Winzer M., et al.*: Milk Fat Globule-Epidermal Growth Factor 8 (MFG-E8) Is a Novel Anti-inflammatory Factor in Rheumatoid Arthritis in Mice and Humans. J Bone Miner Res. 2016 Mar; 31 (3): 596–605.
14. *Dai W., Li Y., Lv Y.N., Wei C.D., Zheng H.Y.*: The roles of a novel anti-inflammatory factor, milk fat globule-epidermal growth factor 8, in patients with coronary atherosclerotic heart disease. Atherosclerosis. 2014 Apr; 233 (2): 661–665.
15. *Zhang S., Xie J.G., Su B.T., et al.*: MFG-E8, a clearance glycoprotein of apoptotic cells, as a new marker of disease severity in chronic obstructive pulmonary disease. Braz J Med Biol Res. 2015 Nov; 48 (11): 1032–1038.
16. *Yamamoto N., Yamaguchi H., Ohmura K., et al.*: Serum milk fat globule epidermal growth factor 8 elevation may subdivide systemic lupus erythematosus into two pathophysiologically distinct subsets. Lupus. 2014 Apr; 23 (4): 386–394.

17. *Haese J.G.D., Demir I.E., Kehl T., et al.*: The impact of MFG-E8 in chronic pancreatitis: potential for future immunotherapy? BMC Gastroenterol. 2013; 13: 14.
18. *Li Y., Ran W., Zhang J., et al.*: Elevated Serum Milk Fat Globule-Epidermal Growth Factor 8 Levels in type 2 Diabetic Patients Are Suppressed by Overweight or Obese Status. IUBMB Life. 2017 Feb; 69 (2): 63–71.
19. *Aziz M.M., Ishihara S., Rumi M.A., et al.*: Prolactin induces MFG-E8 production in macrophages via transcription factor C/EBPbetadependent pathway. Apoptosis. 2008; 13: 609–620.
20. *Zhao H., Zhang H., Qin X.*: Age-related differences in serum MFG-E8, TGF-b1 and correlation to the severity of atherosclerosis determined by ultrasound. Mol Med Rep. 2017; 16: 9741–9748.
21. *Madkour L., Elgengehy F., Niazy M., Ghoneim S.*: Milk fat globule E-8 and interleukin 17 in systemic lupus erythematosus: partners in crime? Reumatologia. 2015; 53: 309–314.
22. *Kishi C., Motegi S.I., Ishikawa O.*: Elevated serum MFG-E8 level is possibly associated with the presence of high-intensity cerebral lesions on magnetic resonance imaging in patients with systemic lupus erythematosus. J Dermatol. 2017 Jul; 44 (7): 783–788.
23. *Li Y., Ran W., Zhang J., et al.*: Circulating milk fat globule-epidermal growth factor 8 levels are increased in pregnancy and gestational diabetes mellitus. J Diabetes Investig. 2017 Jul; 8 (4): 571–581.
24. *Zhao Q., Yu Y., Zuo X., Dong Y., Li Y.*: Milk Fat Globule–Epidermal Growth Factor 8 Is Decreased in Intestinal Epithelium of Ulcerative Colitis Patients and Thereby Causes Increased Apoptosis and Impaired Wound Healing. Mol Med. 2012 May 9; 18: 497–506.
25. *Chogle A., Bu H., Wang X., et al.*: Milk Fat Globule–EGF Factor 8 Is a Critical Protein for Healing of Dextran Sodium Sulfate–Induced Acute Colitis in Mice. Mol Med. 2011 May–Jun; 17 (5–6): 502–507.
26. *Laplant P., Brillant-Marquis F., Brissette M.J., et al.*: MFG-E8 Reprogramming of Macrophages Promotes Wound Healing by Increased bFGF Production and Fibroblast Functions. J Invest Dermatol. 2017 Sep; 137 (9): 2005–2013.
27. *Kranich J., Krautler N.J., Heinen E., et al.*: Follicular dendritic cells control engulfment of apoptotic bodies by secreting Mfge8. J Exp Med. 2008 Jun 9; 205 (6): 1293–1302.
28. *Yau I.W., Cato M.H., Jellusova J., et al.*: Censoring of self-reactive B cells by follicular dendritic cell-displayed self-antigen. J Immunol. 2013 Aug 1; 191 (3): 1082–1090.
29. *Brissette M.J., Lepage S., Lamonde A.S., et al.*: MFG-E8 released by apoptotic endothelial cells triggers anti-inflammatory macrophage reprogramming. PLoS One. 2012; 7 (4): e36368.
30. *Kuhn A., Herrmann M., Kleber S., et al.*: Accumulation of apoptotic cells in the epidermis of patients with cutaneous lupus erythematosus after ultraviolet irradiation. Arthritis Rheum. 2006 Mar; 54 (3): 939–950.
31. *Emlen W., Niebur J., Kadera R.*: Accelerated in vitro apoptosis of lymphocytes from patients with systemic lupus erythematosus. J Immunol. 1994 Apr 1; 152 (7): 3685–3692.
32. *Kadijani A.A., Javadinia F., Mirzaei A., et al.*: Apoptosis markers of circulating leukocytes are associated with the clinical course of inflammatory bowel disease. Gastroenterol Hepatol Bed Bench. 2018 Winter; 11 (Suppl 1): S53–S58.
33. *Hagiwara C., Tanaka M., Kudo H.*: Increase in colorectal epithelial apoptotic cells in patients with ulcerative colitis ultimately requiring surgery. J Gastroenterol Hepatol. 2002 Jul; 17 (7): 758–764.
34. *Di Sabatino A., Ciccocioppo R., Luinetti O., et al.*: Increased enterocyte apoptosis in inflamed areas of Crohn's disease. Dis Colon Rectum. 2003 Nov; 46 (11): 1498–1507.
35. *Butler A.E., Janson J., Bonner-Weir S., et al.*: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003 Jan; 52 (1): 102–110.
36. *Firestein G.S., Yeo M., Zvaifler N.J.*: Apoptosis in rheumatoid arthritis synovium. J Clin Invest. 1995 Sep; 96 (3): 1631–1638.
37. *Salmon M., Scheel-Toellner D., Huissoon A.P., et al.*: Inhibition of T cell apoptosis in the rheumatoid synovium. J Clin Invest. 1997 Feb 1; 99 (3): 439–446.
38. *Zhang J., Bárdos T., Mikecz K., Finnegan A., Glant T.T.*: Impaired Fas signaling pathway is involved in defective T cell apoptosis in autoimmune murine arthritis. J Immunol. 2001 Apr 15; 166 (8): 4981–4986.

39. *Pletz M.W., Ioanas M., de Roux A., Burkhardt O., Lode H.*: Reduced spontaneous apoptosis in peripheral blood neutrophils during exacerbation of COPD. Eur Respir J. 2004 Apr; 23 (4): 532–537.
40. *Schmidt-Ioanasa M., Pletzb M.W.R., de Roux A., Lode H.*: Apoptosis of peripheral blood neutrophils in COPD exacerbation does not correlate with serum cytokines. Respir Med. 2006 Apr; 100 (4): 639–647.
41. *Green G.R., Oguin T.H., Martinez J.*: The clearance of dying cells: table for two. Cell Death Differ. 2016 Jun; 23 (6): 915–926.
42. *Penberthy K.K., Ravichandran K.S.*: Apoptotic cell recognition receptors and scavenger receptors. Immunol Rev. 2015; 269: 44–59.
43. *Kzhyshkowska J.*: Multifunctional receptor stabilin-1 in homeostasis and disease. Scientific World Journal. 2010; 10: 2039–2053.
44. *Hove T., van Montfrans C., Peppelenbosch M.P., van Deventer S.J.H.*: Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. Gut. 2002 Feb; 50 (2): 206–211.
45. *Hu C.Y., Wu C.S., Tsai H.F., et al.*: Genetic polymorphism in milk fat globule-EGF factor 8 (MFG-E8) is associated with systemic lupus erythematosus in human. Lupus. 2009; 18: 676–681.
46. *Yamaguchi H., Fujimoto T., Nakamura S.*: Aberrant splicing of the milk fat globule-EGF factor 8 (MFG-E8) gene in human systemic lupus erythematosus. Eur J Immunol. 2010; 40: 1778–1785.