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Metalloproteinase-3 and -9 as Novel Markers in the Evaluation of Ulcerative Colitis Activity in Children

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Objectives. The enhanced activity of matrix endopeptidases (MMPs) involved in the degradation of connective tissue has been noted in tissue samples from the digestive tract in inflammatory bowel disease (IBD), however sera concentrations of MMPs, a potential tool for diagnostic tests in IBD patients, have not been established so far. The goal of the studies was to evaluate the concentrations of MMP-3 and MMP-9, in the sera of children suffering from ulcerative colitis (UC) in relation to disease activity.
Material and Methods. The study was comprised of 31 children with UC (aged 3–18 years) and 37 children in the control group (aged 1–18 years). Disease activity was estimated using the Truelove-Witts scale. MMP-3 and -9 concentrations were determined using ELISA tests.
Results. Median MMP-3 concentrations were 18.4 ng/mL (95% CI: 12.5–24.3) for moderate and severe, 4.35 ng/mL (95% CI: 2.5–7.8) for the mild form of UC and 1.8 ng/mL (95% CI: 1.2–2.5) for the control group. Median MMP-9 concentrations were 18.0 ng/mL (95% CI: 2.83–36.6) for moderate and severe, 1.55 ng/mL (95% CI: 0.4–3.0) for the mild form of UC and 1.3 ng/mL (95% CI: 0.7–1.96) for the control group. Serum MMP-3 and MMP-9 concentrations in the moderate group were higher than those in the mild and control groups ($p < 0.001$). MMP-3 concentrations in the mild group also differed from those in the control group ($p < 0.001$). Among the parameters studied (MMP-3, MMP-9, CRP and ESR), MMP-3 had the highest discriminative value (AUC = 0.9, $p < 0.001$, sensitivity = 71%, specificity = 92%) in distinguishing patients with UC from healthy individuals.
Conclusions. Elevation of MMP-3 and MMP-9 concentrations along with the disease activity suggests the possibility of their application in the evaluation of the clinical activity of UC (**Adv Clin Exp Med 2014, 23, 1, 103–110**).

Key words: metalloproteinases, ulcerative colitis, children.

Acute damage of tissues caused by the deregulation of the immune system in genetically prone individuals and in the presence of modulating environmental factors is nowadays a generally accepted hypothesis concerning the etiopathogenesis of inflammatory bowel diseases (IBD). Numerous cytokines have been demonstrated to participate in the maintenance of the disease process – the inhibition of tumor necrosis factor alpha (TNF-α) by specific antibodies has been successfully applied in practice and today it is one of the basic strategies in the treatment of IBD. Numerous studies have also been conducted on tissue effectors of the systemic inflammatory process, which cause ulcerations of the intestinal tract typical for IBD. It has been demonstrated that metalloproteinases of the extracellular

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matrix (MMPs) are involved in this process [1, 2]. Metalloproteinases are a family of zinc-dependent endopeptidases which, due to their ability to metabolize the elements of the extracellular matrix, are engaged in the remodeling of connective tissue [3]. Precise regulation of expression and the activation of MMPs, together with the specific tissue inhibitors of metalloproteinases (TIMPs), guard the state of functional balance of these enzymes. It has been observed that the hyperactivation of some of the MMPs leads to enhanced tissue remodeling in regions affected by the disease process [3, 4]. Numerous studies have demonstrated increased concentrations of MMPs in biopsy specimens collected from inflammatory altered sections of alimentary tract in the course of IBD [1, 2]. Therefore, MMPs have become a subject of studies on the possibility of their application in the inhibition of the disease process as well as on the evaluation of their potential as biomarkers of disease activity and exacerbation. At this moment there are no specific, sensitive and at the same time noninvasive markers that would permit the evaluation of the degree of disease activity and its exacerbation. Finding such biomarkers could, in the course of long-term care of the IBD patient, prevent the development of problems related to the severe stages of the disease as well as life-threatening complications.

The goals of our studies were: the evaluation of the concentrations of MMP-3 and -9 in the sera of patients suffering from ulcerative colitis, the determination of correlations between MMP-3 and -9 concentrations and the activity of the inflammatory state as well as indices of inflammation such as concentration of C-reactive protein (CRP), seromucoid and erythrocyte sedimentation rate (ESR). We also compared the diagnostic utility of the measurements of the studied MMPs in the estimation of disease severity with those of ESR and CRP.

Material and Methods

Study Group

Inclusion criteria: the studied group was comprised of 31 patients with UC (aged from 3 to 18 years, average 14.7) treated in the Department and Clinic of Pediatrics, Gastroenterology and Nutrition of Wroclaw Medical University. Ulcerative colitis was diagnosed on the basis of subjective and objective examination as well as laboratory and image findings using the algorithm proposed in Porto [5]. The clinical activity of ulcerative colitis was estimated using a modified Truelove-Witts scale, supplemented by the Cole index, which was a method applied in the determination of UC activity in the medical system at the time the study was conducted [6]. On this basis, the patients were divided into 3 groups: 16 patients, mean age 12.94 years, with a mild form of the disease (< 5 points), 14 patients, mean age 16.64, with a moderate form (≥ 5 to < 9 points) and only 1 patient, during the period of study, with severe disease activity (≥ 9 points). Patients with moderate and severe disease activity were gathered in one group. Mean disease duration in the group of patients with the mild form of the disease was 8.1 months and in the group with the moderate form of the disease 11.5 months. The differences between the groups were not statistically significant.

The patients enrolled in the study were treated, depending on the clinical disease activity, using sulfasalazine, steroids and azathioprine. The control group consisted of 37 children (mean age 10.1) who were hospitalized in the clinic because of abdominal pain and were diagnosed with functional disorders of the digestive tract, mostly functional dyspepsia and irritable bowel syndrome. The above control group was also used in the evaluation of serum concentrations of MMPs in Crohn's disease.

Exclusion criteria: inflammation associated with diseases other than UC, patients with extraintestinal manifestations of IBD were not included in the study. Treatment with monoclonal antibodies against TNF-alpha was also an exclusion criterion.

Preparation of the Material

Blood samples were drawn by venous puncture in a fasting state. Sera were obtained from clotted (30 min, room temperature) and centrifuged (15 min, 1500 × g) blood. Serum samples were stored at –80°C until analysis.

MMP-3 and MMP-9 concentrations were estimated by enzyme double-antibody indirect immunoassays with the Quantikine Human Total MMP-3 Immunoassay and DuoSet Human MMP-9/TIMP-1 Complex provided by R&D Systems (Minneapolis, MN, USA) in accordance with manufacturer protocol.

Data on C-reactive protein (CRP), seromucoid and erythrocyte sedimentation rate (ESR) was retrieved from the patients' medical records.

Statistical Methods

Due to the skewed distribution of the analyzed data, they were logarithmically transformed before analysis. The results are presented as median values accompanied by 95% confidence intervals

(95% CI). The significance of differences between groups was examined with a Mann-Whitney *U* test.

Correlation analysis between MMPs concentration and the degree of disease activity, as well as CRP, seromucoid and ESR, was conducted with a Pearson's test. All tests were two-sided and *p* values ≤ 0.05 were considered significant.

The diagnostic utilities of MMP-3, MMP-9, ESR and CRP were evaluated using a receiver operating characteristic (ROC) curves analysis. The overall performance was expressed as the area under the ROC curve (AUC) with 95% CI and p-statistics for the difference between the calculated AUC and AUC = 0.5 (marker without discriminative power). Cut-off values corresponding to the highest accuracy were determined and the related sensitivities and specificities together with likelihood ratios for positive and negative results (+LR and -LR) were calculated. The Youden index (sensitivity + specificity-1), a summary measure, was calculated as well. Statistical analysis was conducted using Statistica 9 and MedCalc programs.

Ethical Considerations

The study protocol was approved by the Medical Ethics Committee of Wroclaw Medical University, Wroclaw, Poland and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Results

MMP-3, MMP-9, CRP and ESR in the Sera of Patients with Ulcerative Colitis

Concentrations of MMP-3 in patients with UC were elevated in comparison to the control group (Fig. 1). The concentrations of this metalloproteinase also differed between subgroups of patients with different degrees of disease activity (Table 1). The highest concentrations were observed in a group of children with moderate activity of UC and they differed significantly from the concentrations in the group with mild activity as well as from the control group ($p < 0.001$).

Also, the concentrations of MMP-9 were higher in patients with UC than in the control group (Fig. 2). When the concentrations of MMP-3 were compared in the subgroups differing in disease activity, the elevated levels were observed in the groups with active disease (Table 1). In the group with mild disease activity, these concentrations were similar to those in the control group. Statistically significant differences were observed between the groups with moderate and mild disease activity ($p < 0.001$) as well as between the group with moderate disease activity and the control group ($p < 0.001$).

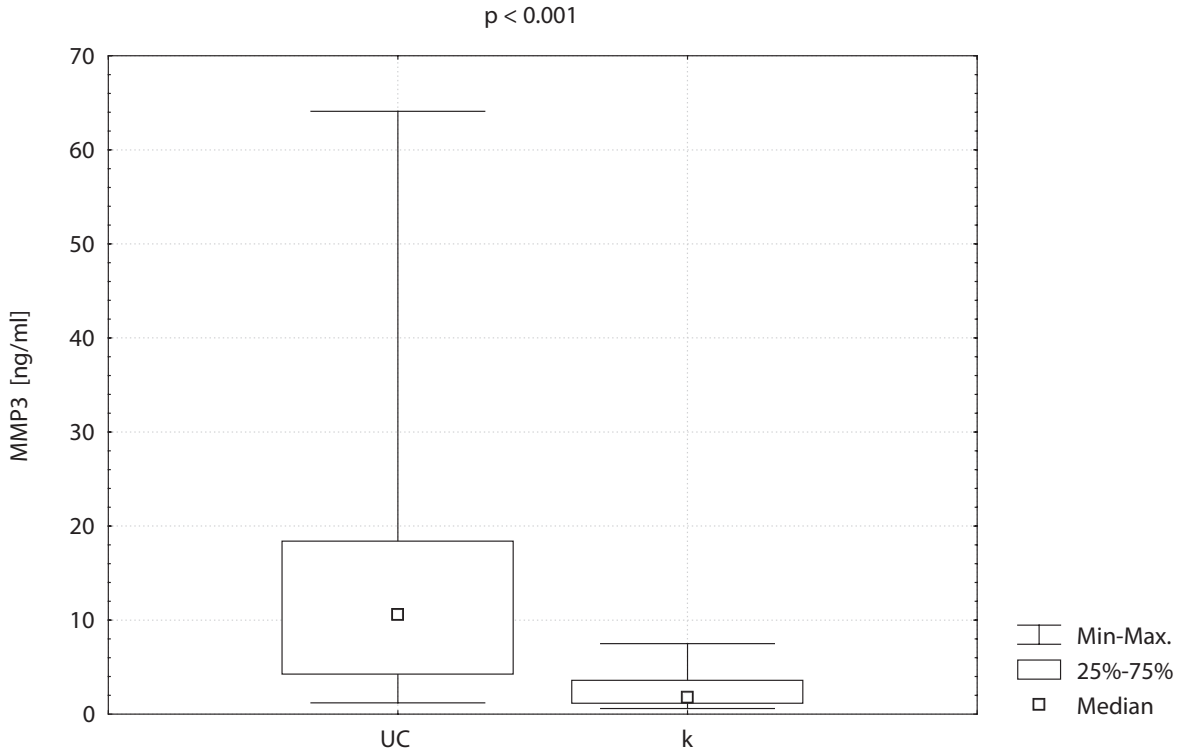

Fig.1. Concentrations of MMP-3 in the serum of patients with ulcerative colitis (UC) and the control group (C)

Table 1. MMP-3 and MMP-9 concentrations in the sera of patients with ulcerative colitis (UC) and in the control group

Diagnosis	Disease activity	n	MMP-3 [ng/mL]		MMP-9 [ng/mL]	
			MEDIAN	95% CI	MEDIAN	95% CI
UC	severe + moderate	1 + 14	18.4	12.5–24.3	18.0	2.83–36.6
	mild	16	4.35	2.5–7.8	1.55	0.4–3.0
	UC – total	31	10.6	6.1–14.9	3	1.4–7.3
Control group		37	1.8	1.2–2.5	1.3	0.7–1.96

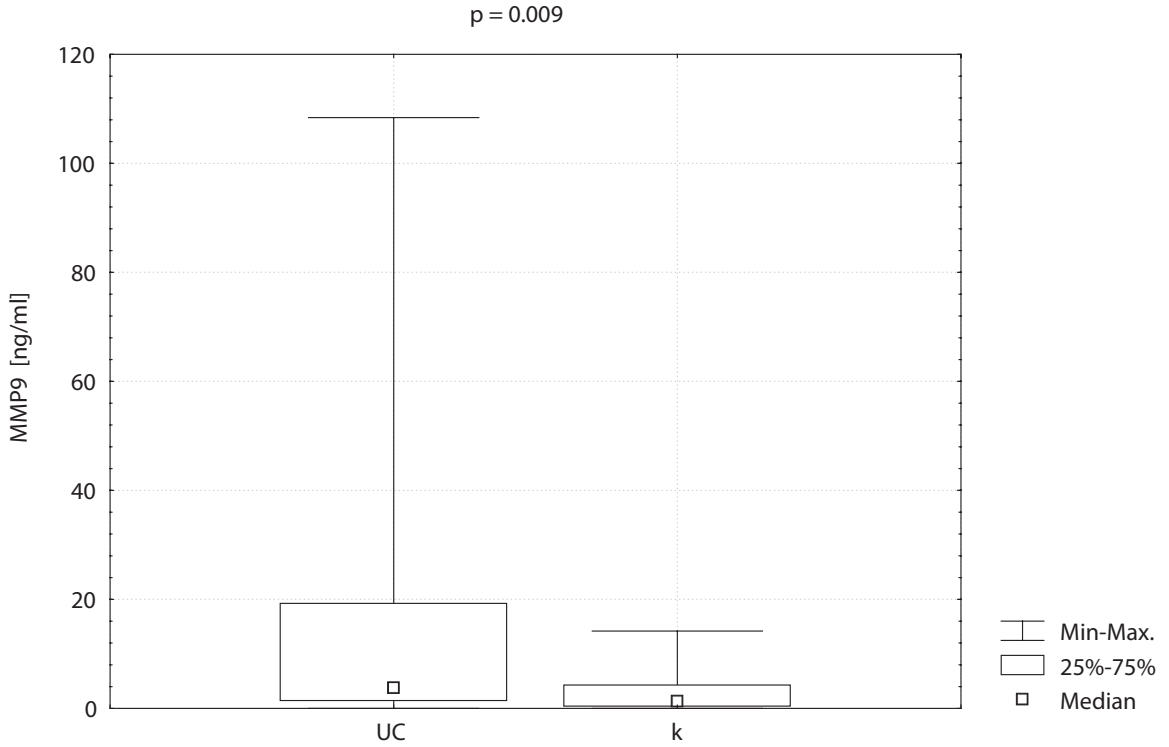

Fig.2. Concentrations of MMP-9 in the serum of patients with ulcerative colitis (UC) and the control group (C)

In the studied cohort of patients, we did not observe statistically significant differences in the concentrations of CRP and the levels of ESR between patients with UC and the control group (ESR/control group – p = 0.07, CRP/control group – p = 0.52).

Correlations Between Serum Concentrations of MMP-3 and MMP-9, Disease Activity and Selected Laboratory Indices

The concentrations of MMP-3 in the sera of UC patients positively correlated with the number of points evaluating disease activity in the Truelove-Witts scale (r = 0.58, p = 0.001) (Table 2). We did not observe statistically significant correlations between MMP-3 concentrations and ESR, CRP or seromucoid (Table 2).

Also, the concentrations of MMP-9 positively correlated with the degree of disease activity estimated by the Truelove-Witts scale (Table 2). Concentrations of MMP-9 positively correlated with CRP and seromucoid concentrations as well (Table 2). The concentrations of both metalloproteinases positively correlated with each other.

Diagnostic Utility of the Studied Parameters in the Evaluation of Ulcerative Colitis Activity

ROC analysis of the studied parameters in the patients with UC indicated that MMP-3 has a higher diagnostic utility than MMP-9, ESR and CRP as markers of disease activity. Diagnostic accuracies expressed as the areas under the ROC curves (AUC) for the studied parameters were as follows:

Table 2. Relations between MMP-3, MMP-9 and selected inflammatory parameters in children with ulcerative colitis

Parameter	n	MMP-3 (log)		MMP9 (log)	
		p	r	p	r
Disease activity (Truelove-Witts scale)	31	P = 0.001	0.58	P < 0.0001	0.69
MMP-9 (log)	31	P < 0.0001	0.69	–	–
MMP-3 (log)	31	–	–	P < 0.0001	0.69
ESR (log)	31	P = 0.08	0.32	P = 0.05	0.35
CRP (log)	31	P > 0.05	0.29	P = 0.001	0.57
Seromucoid	31	P > 0.05	0.19	P = 0.01	0.46

ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; r – Pearson's correlation coefficient.

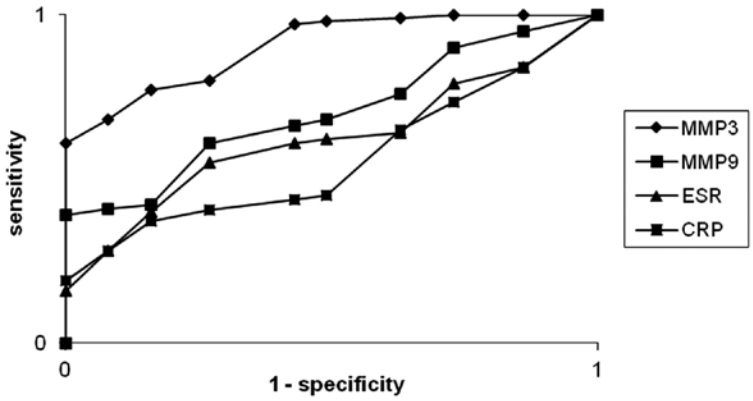

Fig. 3. Comparison of ROC curves for MMP-3, MMP-9, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)

Table 3. Diagnostic performance of the measured parameters

Parameter	Accuracy	p	Cut-off value	Sensitivity	Specificity	Youden index
MMP-3	0.898	< 0.001	> 5.4 ng/mL	0.71	0.919	0.629
MMP-9	0.686	0.005	> 2.46 ng/mL	0.613	0.730	0.343
ESR	0.629	0.058	> 13 mm/h	0.387	0.919	0.306
CRP	0.547	> 0.05	> 4.3 mg/L	0.258	0.943	0.201

ESR – erythrocyte sedimentation rate; CRP – C-reactive protein.

MMP-3 – 0.90 (95% CI – 0.80 – 0.96, p < 0.001) at the cut-off value of 5.4 ng/mL, MMP-9 – 0.69 (95% CI – 0.56– 0.79, p = 0.005) at the cut-off value of 2.46 ng/mL, ESR – 0.63 (95% CI – 0.50–0.74, p = 0.058) at the cut-off value of 13 and CRP – 0.55 (95% CI – 0.42–0.67, p > 0.05) at the cut-off value of 4.3 mg/L (Fig. 3). This indicates that there is 90% probability of correctly distinguishing a healthy person from a UC patient based on MMP-3 concentration. The highest Youden index was observed for MMP-3 and was equal 0.63, with the sensitivity of the test equal to 0.71 and specificity 0.92. This means that in 71% of patients with UC, MMP-3 concentrations were higher than the highest value in the control group. Both the sensitivity and specificity of MMP-9 were lower than that of MMP-3. In the studied cohort of individuals, ESR only tended to discriminate patients with UC from healthy ones (with the same specificity but much lower sensitivity than MMP-3) and CRP had no discriminative power towards diagnosis of UC. Sensitivities for both ESR and CRP were found to be lower in comparison to the studied metalloproteinases (Table 3).

Discussion

Revealing the mechanisms responsible for the development and exacerbation of symptoms in the course of inflammatory bowel diseases is an important goal from a scientific and diagnostic as well as therapeutic standpoint. Enhanced tissue remodeling leading to the formation of characteristic changes in the large bowel of patients with ulcerative colitis has become a stimulus for the search for the biological factors responsible for the initiation and maintenance of these pathological processes. Among such factors are metalloproteinases, elevated concentrations of which have been observed in biopsy specimens collected from the disease altered fragments of the alimentary tract [1, 2].

Matrix metalloproteinases play a key role in the remodeling of the extracellular elements of connective tissue. Influencing the pathways of immune cell migration, they are modulating the course of inflammatory process [7–10]. The imbalance in the synthesis and degradation of MMPs leads to the improper tissue remodeling, which can result in the formation of ulceration, including the ulceration of the alimentary tract. The description of MMPs function in IBD seems to be important in the elucidation of the pathomechanism of these still perplexing diseases. The studies published so far have confirmed the overexpression of MMPs, including MMP-3 and -9, both in ulcerative colitis and in Crohn's disease (CD). Alterations of MMPs activity depending on the degree of disease exacerbation have been observed in animal models with induced bowel inflammation [11, 12]. Elevated concentrations of MMPs have also been noted in biopsy specimens collected from inflamed fragments of the alimentary tract [1, 2, 13, 14]. Despite these reports, there are only a few accounts concerning the possibility of the measurement of MMPs in samples other than tissue. Increased concentrations of MMPs have been demonstrated in the urine of patients with IBD in comparison to healthy individuals [15]. Significant elevation of MMP-1 and TIMP-1 levels in plasma corresponding to mucosal expression have been shown by Wang YD et al. [16].

In our earlier studies, we already indicated that MMP-3 and -9 concentration measurement can be useful in the differentiation of patients with various degree of CD exacerbation estimated by the pediatric Crohn's disease activity index [17]. In our present studies we have demonstrated that also in the case of pediatric patients with ulcerative colitis there are significant differences in MMP-3 and MMP-9 concentrations in sera, which reflect the various stages of disease activity. Significantly higher concentrations of both MMP-3 and MMP-9 in the sera of patients with the active form of the disease (above 5 points on the Truelove-Witts scale) in comparison to the patients with the mild form of the disease as well as the control group indicate that application of the measurement of the studied metalloproteinases might be useful in the clinical evaluation of patients. Our studies indicate that the imbalance in MMPs activity can be observed both in ulcerative colitis and in Crohn's disease and, therefore, these markers are not specific to these diseases. In the majority of studies conducted so far, no distinction between particular forms of IBD has been taken into account, assuming that the studied metalloproteinases are the effectors of the systemic inflammatory reaction of the body [1, 2, 13]. This can be confirmed by the clinical application of antibodies against TNF-α, which, as a consequence, results in the inhibition of MMPs activity both in CD and UC.

Taking into account the possible impact of treatment on the concentration of MMPs, we did not enroll patients under biological therapy in the study. The possible effects of other drugs, including supplementation with iron, calcium preparations and other macro and micronutrients, which according to recent reports [18, 19] may influence the activity of MMPs, were not studied in this work. Patients with moderate disease activity receiving therapeutic doses of iron did not require the use of other supplements because of the normal function of the small intestine in UC.

Because the only studies evaluating the effects of macro- and micronutrients on MMPs have been conducted *in vitro*, the influence of these drugs requires further studies to confirm the results in *in vivo* experiments.

In spite of these limitations, to the best of our knowledge, this is the first report indicating the high diagnostic value of MMPs determination in the evaluation of UC activity. The concentrations of the studied metalloproteinases increased linearly along with the increases in the points on the Truelove-Witts scale. This high correlation with the Truelove-Witts scale indicates that these MMPs should be considered as possible indicators of the clinical course of UC. This is important from a routine clinical practice point of view, because currently there is no single marker characterized by specificity and sensitivity high enough to be used in a reliable evaluation of the severity of IBD or UC alone. Fecal calprotectin determination, with its 93% specificity and 95% sensitivity, appears to be an important marker of colorectal inflammation, however the elevation of calprotectin concentration in inflammations other than UC points to the need for further efforts in the search for non-invasive markers of this disease [20, 21].

Many studies have also discussed the diagnostic and prognostic value of commonly used indicators of inflammation. Elevation of CRP is reported in 70%–100% of patients with active inflammation in the course of CD and 50%–60% of patients with UC [22, 23]. Our results, indicating that in the cohort of patients which were included in our studies, CRP has no discriminative value in distinguishing UC patients from healthy individuals, corroborates these findings. Although we observed positive, statistically significant correlation between MMP-9 and CRP in the group of patients with UC, when the group of UC patients was combined with the control group, the correlation became questionable ($r = 0.32$, $p = 0.008$). Despite its limitations, CRP measurement is still commonly used in clinical practice. Moreover, every accompanying infection, for which children treated with drugs lowering immunity during the therapy for IBD are prone, decreases the specificity of a single marker. Simultaneous determination of several parameters increases the probability of proper evaluation, because it takes into account the differences in origins and activity of the estimated indicators. Nevertheless, it is necessary to look for new indicators which would be specific to a disease process.

In our cohort of patients, we also observed positive correlations between MMP-9 and seromucoid. We did not, however, find significant correlations between the studied metalloproteinases and ESR. Similar to the case of CD [17], MMP-3 and -9 concentrations positively correlated with the disease activity scale. Hence, the demonstration of the statistically significant, positive correlations between the number of points on the Truelove-Witts scale and the studied metalloproteinases supports the possibility of their application in the evaluation of the disease severity. Additionally, the lack of significant correlations between MMP-3 and the indices of inflammation (CRP, ESR, seromucoid), as well as between MMP-9 and ESR, might be evidence of the superiority of MMPs over standard markers. The estimation of MMPs might, therefore, be considered as an alternative or supplemental to CRP, seromucoid and ESR measurement in routine clinical practice. The high diagnostic utilities, as well as the sensitivities and specificities, especially those of MMP-3, seem to support such an idea. However, it has to be taken into account that metalloproteinases are not specific to IBD. The elevation of their concentration has been described in cancers, cardiovascular diseases and autoimmune diseases [24–26]. The presence of symptoms from outside of the alimentary tract or overlapping syndromes in patients may influence the result of MMPs determination in IBD without an indication of the dominance of symptoms from the alimentary tract. However, a similar limitation is also true in the case of ESR or CRP.

Our studies, demonstrating the high diagnostic potential of metalloproteinases in UC severity evaluation, also point to the need for further research in this direction, especially with respect to changes of MMPs on the tissue level and their connections to MMPs concentration and activity on a systemic level.

References

[1] **Meijer MJ, Mieremet-Ooms MA, van der Zon AM:** Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn's disease phenotype. Dig Liver Dis 2007, 39, 733–739.

[2] **von Lampe B, Barthel B, Coupland SE:** Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. Gut 2000, 47, 63–73.

[3] **Medina C, Radomski MW:** Role of matrix metalloproteinases in intestinal inflammation. J Pharmacol Exp Ther 2006, 318, 933–938.

[4] **Sternlicht MD, Werb Z:** How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001, 17, 464–516.

[5] IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis-the Porto criteria. J Pediatr Gastroenterol Nutr 2005, 41, 1–7.

[6] **Truelove SC, Witts LJ:** Cortisone in ulcerative colitis; final report on a therapeutic trial. Br Med J 1955, 2, 1041–1048.

[7] **Faveeuw C, Preece G, Ager A:** Transendothelial migration of lymphocytes across high endothelial venules into lymph nodes is affected by metalloproteinases. Blood 2001, 98, 688–695.

[8] **Delclaux C, Delacourt C, D'Ortho MP:** Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. Am J Resp Mol Cell Biol 1996, 14, 288–229.

[9] **Di Sebastiano P, di Mola FF, Artese L:** Beneficial effects of Batimastat (BB-94), a matrix metalloproteinase inhibitor, in rat experimental colitis. Digestion 2001, 63, 234–239.

[10] **Naito Y, Takagi T, Kuroda M:** An orally active matrix metalloproteinase inhibitor, ONO-4817, reduces dextran sulfate sodium-induced colitis in mice. Inflam Res 2004, 53, 462–468.

[11] **Santana A, Medina C, Paz-Cabrera MC:** Attenuation of dextran sodium sulphate induced colitis in matrix metalloproteinase-9 deficient mice. World J Gastroenterol. 2006, 12, 6464–6472.

[12] **Sykes AP, Bhogal R, Brampton C:** The effect of an inhibitor of matrix metalloproteinases on colonic inflammation in a trinitrobenzensulphonic acid rat model of inflammatory bowel disease. Aliment Pharmacol Ther 1999, 13, 1535–1542.
[13] **Louis E, Ribbens C, Godon A:** Increased production of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1 by inflamed mucosa in inflammatory bowel disease. Clin Exp Immunol 2000, 120, 241–246.
[14] **Gao Q, Meijer MJ, Kubben FJ:** Expression of matrix metalloproteinases-2 and -9 in intestinal tissue of patients with inflammatory bowel diseases. Dig Liver Dis 2005, 37, 584–592.
[15] **Manfredi MA, Zurakowski D, Rufo PA:** Increased incidence of urinary matrix metalloproteinases as predictors of disease in pediatric patients with inflammatory bowel disease. Inflamm Bowel Dis 2008, 14, 1091–1096.
[16] **Wang YD, Tan XY, Zhang K:** Correlation of plasma MMP-1 and TIMP-1 levels and the colonic mucosa expressions in patients with ulcerative colitis. Mediators Inflamm 2009, 275072. Epub 2009, 9.
[17] **Kofla-Dlubacz A, Matusiewicz M, Krzystek-Korpacka M, Iwanczak B:** Correlation of MMP-3 and MMP-9 with Crohn's Disease Activity in Children. Dig Dis Sci 2012, 57, 706–712.
[18] **Tezvergil-Mutluay A, Agee KA, Hoshika T:** The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. Dent Mater 2010, 26, 1059–1067.
[19] **Kato MT, Leite AL, Hannas AR:** Effect of iron on matrix metalloproteinase inhibition and on the prevention of dentine erosion. Caries Res 2010, 44, 309–316.
[20] **Fagerberg UL, Lööf L, Myrdal U:** Colorectal inflammation is well predicted by fecal calprotectin in children with gastrointestinal symptoms. J Pediatr Gastroenterol Nutr 2005, 40, 450–455.
[21] **Konikoff MR, Denson LA:** Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. Inflamm Bowel Dis 2006, 12, 524–534.
[22] **Vermeire S, Van Assche G, Rutgeerts P:** Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut 2006, 55, 426–431.
[23] **Vermeire S, Van Assche G, Rutgeerts P:** The role of C-reactive protein as an inflammatory marker in gastrointestinal diseases. Nat Clin Pract Gastroenterol Hepatol 2005, 2, 580–586.
[24] **Dollery CM, McEwan JR, Hanney AM:** Matrix metalloproteinases and cardiovascular disease. Circ Res 1995, 77, 863–868.
[25] **Tchetverikov I, Lard LR, DeGroot J:** Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. Ann Rheum Dis 2003, 62, 1094–1099.
[26] **Egeblad M, Werb Z:** New functions for the matrix metalloproteinases in cancer progression, Nature Rev Cancer 2002, 2, 161–174.

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