

• CLINICAL RESEARCH •

Elevated serum values of procollagen III peptide (PIIIP) in patients with ulcerative colitis who will develop pseudopolyps

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Abstract

AIM: To assess the impact of procollagen III peptide as a marker of collagenesis in the development of pseudopolyps in patients with ulcerative colitis.

METHODS: Development of pseudopolyps was monitored in 25 patients with ulcerative colitis classified according to Powell-Tuck index as mild ($n=12$) or moderate ($n=13$) form of disease. Patients with a mild form of disease were treated with oral mesalazine medication (2-4 g/day) and local mesalazine preparation (suppository). Patients with a moderate form of disease received oral mesalazine medication (2-4 g/day), local mesalazine preparation (suppository) and local methylprednisolone at an initial dose of 60 mg/day, followed by dose tapering. How many significant variables (previously determined by analysis of variance) were elevated in the groups with and without pseudopolyp development was observed. ROC analysis for calculation of new index was made.

RESULTS: Serum values of procollagen III peptide (PIIIP), C-reactive protein (CRP) and C4 complement component (C4) were statistically significantly lower in the group of patients free from pseudopolyp development than those who developed one or more pseudopolyps (0.45 ± 0.12 vs 1.42 ± 0.70 , $P < 0.0027$; 7.6 ± 4.7 vs 17.8 ± 9.17 , $P < 0.035$; and 0.46 ± 0.11 vs 0.34 ± 0.16 , $P < 0.068$, respectively) at endoscopic controls with pathohistologically samples during 13 months. There were no statistically significant differences in the values of C3, ceruloplasmin and IgM between the two groups ($P > 0.05$). Discrimination function analysis yielded highest standardized cannon coefficients for PIIIP (0.876), CRP (0.104), C3 (-0.534) and C4 (0.184) ($P < 0.036$). The elevation in two of three laboratory variables (PIIIP, CRP and C4) reached sensitivity of 93 % and specificity of 90 % in the development of pseudopolyps.

CONCLUSION: It is proposed that an increase in two of the three laboratory parameters (PIIIP, CRP and C4) could improve the accuracy of prediction of the development of pseudopolyps. When using PIIIP, CRP and C4 on decision

making, the positive predictive value and accuracy were 90 % and 92 %, respectively.

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INTRODUCTION

The role of procollagen and of its metabolites and enzymes involved in the synthesis and degradation of procollagen during the development of ulcerative colitis has already been investigated in a number of studies^[1-6]. Higher levels of procollagen transcripts have been reported in patients with ulcerative colitis as compared with healthy subjects^[4], pointing to an enhanced *de novo* synthesis of all types of collagen in patients with ulcerative colitis^[1,3,4]. Also, the expression of collagenase has been demonstrated to be higher in patients with ulcerative colitis than in normal subjects^[4]. These patients showed hyperexpression of procollagen III RNA transcripts. The elevated level of procollagen messenger RNA correlated with the rate of inflammatory infiltrations^[1,3,4], represented by inflammatory polyps (pseudopolyps). In the process of healing inflammatory desctructed mucosa is changed with the reparatory process^[1-9].

The development of pseudopolyps sometimes is seen in the stage of disease remission^[7,8]. The presence of procollagen and other materials is necessary for polyp formation^[1,9]. The measurement of procollagen may be helpful in the determination of the patient who will develop pseudopolyp formation. Insight to literature of the last 20 years, there were no studies into the predictive value of procollagen III peptide (PIIIP) for polyp development in patients with ulcerative colitis. The aim of the study was to assess the role of PIIIP as a marker of collagen synthesis in the development of pseudopolyps in patients with ulcerative colitis.

MATERIALS AND METHODS

Patients

Twenty-five patients with ulcerative colitis^[7], 11 men with median age of 34 years (aged 30-45) and 14 women with median age 35 years (aged 29-47), were included in the study. Only newly detected patients were enrolled in the study, thus to exclude the effect of previous therapy on collagen formation^[1-7]. Thus the patients were classified according to Powell-Tuck index^[7,8] for disease severity into the groups with mild ($n=12$) and moderate ($n=13$) form of disease. Mild form of disease had no system symptoms, had less then 4 stools over 24 hours. This form of disease was without significant rectal bleeding, had no signs of anemia, had normal body temperature, normal puls rate and had sedimentation rate under 30 mm per hour. Moderate form of disease had 4-6 diarrhoic stools per day, crampy abdominal pain, elevated body

temperature, increased pulse rate, tachycardia, anemia, elevated sedimentation over 30 mm per hour and extraintestinal symptoms (arthritis). Severe form of disease with more than 6 diarrhoic stools per day, more rectal bleeding and severe intestinal and extraintestinal complications, *etc.* were not included in the study, while the therapy for this form of disease can influence the collagen formation^[1-12].

The course of disease was monitored clinically, endoscopically and histologically. The development of pseudopolyps was observed by using endoscopy^[1,7,9-12]. The formation of intraluminal mucosal enlargement with one or more polyps in former or newly inflamed mucosa was observed. Histological criteria for inflammatory polyps (pseudopolyps) were: only the finding of a diffuse colitis with nonspecific inflammation, no granulomas, and involved rectum would be consistent with ulcerative colitis; however, even in cases that the patient might still have some other form of diffuse colitis and the diagnosis of ulcerative colitis is only established by exclusion of all other causes^[13]. The criteria for the diagnosis of epithelial dysplasia and its distinction from the inflammatory and reparative^[14,15] lesions and neoplasms^[16] that regularly occur in these patients have been established.

Clinical and endoscopic controls were done once monthly during 12 months (12 times), and then once after six months again, what meant totally 13 controls^[7].

Laboratory measurements and new index calculation

PIIIP was measured by using RIA-gnost PIIIP method (Berhingwerke). CRP, C3, C4, IgM and ceruloplasmin were measured by using Turbox Immunonephelometry method (Orion diagnostics).

The significant laboratory variables were determined by using analysis of variance. The contribution of each variable was determined by using the discriminant canonical function on Statistica 5.0 software.

The indexes from three most significant variables were calculated by using ROC analysis. How many significant variables were elevated above laboratory reference values for each patient in two groups (with and without pseudopolyps) was observed. ROC analysis was used to determine the sensitivity, specificity, accuracy and positive predictive value of our new index.

Therapy

The patients with a mild form of disease were treated with oral mesalazine medication (2-4 g/day) and local mesalazine preparation (suppository)^[7]. The patients with a moderate form of disease received oral mesalazine medication (2-4 g/day), local mesalazine preparation (suppository) and oral methylprednisolone at an initial dose of 60 mg/day followed by methylprednisolone dose tapering^[7]. Severe form of disease was excluded with Powell-Tuck index, while therapy for severe form of disease can influence on inflammatory polyps formation^[1-7].

RESULTS

In the group of patients without pseudopolyp development ($n=15$), the levels of PIIIP, C-reactive protein (CRP) and C4 complement component (C4) were statistically significantly lower than those in the group of patients developing pseudopolyps (0.45 ± 0.12 vs 1.42 ± 0.70 , $P<0.0027$; 7.6 ± 4.7 vs 17.8 ± 9.17 , $P<0.035$; and 0.46 ± 0.11 vs 0.34 ± 0.16 , $P<0.068$, respectively). Other parameters, i.e. C3 complement component (C3), ceruloplasmin and IgM, showed no statistically significant differences between the groups of patients with and without pseudopolyp development. Analysis of the discriminative canonical function yielded highest

standardized canonical coefficients for PIIIP (0.876), CRP (0.104), C3 (-0.534) and C4 (0.184) ($P<0.036$), which were then used for subsequent data analysis.

The use of PIIIP, CRP and C4 levels showed that an increase in two of these three laboratory parameters improved the accuracy of prediction of pseudopolyp development. When using PIIIP, CRP and C4 (ROC analysis) on decision making sensitivity was 93 % and specificity 90 %, the positive predictive value and accuracy were 90% and 92%, respectively.

DISCUSSION

In ulcerative colitis patients, inflammatory mucosal destruction is changed by regenerative process (inflammatory polyps (pseudopolyps))^[1-7,13-16]. Collagen is a constituent of connective tissue, thus also of polyps^[1]. In inflammatory bowel diseases, elevated levels of collagen I, III and V are found^[1-3]. Serum level of PIIIP was found to be higher in patients with ulcerative colitis and liver damage, then in patients without liver damage^[9]. Collagenase is regulated by the processes involved in the collagen synthesis (N-terminal propeptide of type III procollagen and C-terminal propeptide of type I procollagen) and degradation (C-terminal telopeptide of type I collagen)^[1]. Degradation of collagens is highly regulated by a cascade of matrix metalloproteases and their tissue inhibitors taken by endoscopic biopsies in patients with inflammatory bowel disease^[8]. The histological severity degree of acute inflammation was correlated well with the expression of metalloproteases gene^[8].

Collagenase can be influenced by glucocorticoid therapy, therefore only newly detected patients were enrolled in the study^[1]. The effect of glucocorticoids on collagenase and collagen degradation has not yet been fully clarified, however, a number of speculative theories have been proposed^[1,3,5,6,9,17]. So, that was the reason why we did not include severe forms of disease in the study, while there was a lot of factors that could have influence on the synthesis of collagen and therefore lead to misinterpretation of results (we tried to succeed "a homogenous sample" according to collagenesis). We found no data about any study predicting pseudopolyps development in patients with inflammatory bowel disease. The development of pseudopolyps is sometimes seen in disease relaps, but not strongly^[1].

Many studies have tackled the issue of predicting disease relapse, however, little has been reported on predicting remission of the disease and none using PIIIP^[1,8,18-22].

The levels of interleukin-1, a potent CRP stimulus, were also monitored^[23]. Serum levels of interleukin-1 receptor antagonist (IL-1ra) may also be an index of ulcerative colitis activity, being low in disease remission^[24]. This parameter may be useful in the differential diagnosis against other IBDs. A group of authors from Aachen prefer the level of interleukin-6 to CRP^[25]. In the present study, CRP levels were among those that, used in combination with other parameters, improved the accuracy of predicting pseudopolyp development, probably pointing not only to inflammation expression but also to the cellular reparatory potential.

The levels of total sialic acid remained increased after the therapy, especially in patients with poor response to therapy with 5-aminosalicylic acid and corticosteroids, while the levels of CRP were normalized after three weeks in most of the patients, irrespective of their therapeutic response^[26].

Endoscopic monitoring of IBD activity should be supplemented by the noninvasive measurement of the levels of α_1 -antitrypsin in stool and serum albumin^[27], the more so, Moran *et al* recommend them as routine markers of the disease endoscopic activity^[28].

The levels of immunoglobulin proved helpful in the

assessment of intestinal resorption, however, in spite of previous belief, had no practical clinical relevance in the determination of disease activity^[29,30]. The results of our study were consistent with these concepts. So, only the values of C4 complement component could be used for subsequent evaluation, and these only in combination with other parameters. Neither were the values of ceruloplasmin as an early inflammation reactant useful for further analysis.

According to Schmoud *et al.*, age is an unfavorable prognostic factor for disease relapse in patients with inflammatory bowel disease (IBD)^[31]. Therefore, the patients included in our study were matched by both sex and age, thus to minimize the impact of these factors on study results.

In the present study, we used the ever more popular method including a combination of factors, providing more accurate information on the real state than each of the factors alone. The role of procollagen should be investigated in a larger sample. Studies with tissue collagen determined before and after therapy may also be expected to yield interesting results. In addition, studies in more severe forms of the disease would be highly interesting, although it might be difficult to differentiate between the collagenase involved in the connective tissue formation in the intestinal wall and the collagenase formed by systemic stimulation of other tissues due to the disease severity^[1,2,6,8,9].

In conclusion, based on the study results, it is proposed that elevation in two of the three laboratory parameters (PIIP, CRP and C4) can improve the prediction of the development of pseudopolyps in patients with ulcerative colitis. When PIIP, CRP and C4 are used in the assessment of pseudopolyp development, the positive predictive value and accuracy were as high as 90 % and 93 %, respectively.

REFERENCES

- Kjeldsen J**, Schaffalitzky OBM, Junker P. Seromarkers of collagen I and III metabolism in active Crohn's disease. Relation to disease activity and response to therapy. *Gut* 1995; **37**: 805-810
- Graham MF**, Diegelmann RF, Elson CO, Lindblad WJ, Gitschalk N, Gay S, Adam J. Collagen content and types in the intestinal strictures of Crohn's disease. *Gastroenterology* 1988; **94**: 257-265
- Matthes H**, Herbst H, Schuppan D, Stallmach A, Milani S, Stein H, Riecken EO. Cellular localisation of procollagen gene transcripts in inflammatory bowel disease. *Gastroenterology* 1992; **102**: 431-442
- Matthes H**, Stallmach A, Matthes B, Herbst H, Schuppan D, Riecken ED. Indications for different collagen metabolism in Crohn's disease and ulcerative colitis. *Med Clin* 1993; **88**: 185-192
- Berg S**, Brodin B, Hesselvik F, Laurent TC, Maller R. Elevated levels of plasma hyaluronan in septicaemia. *Scand J Clin Lab Invest* 1998; **48**: 727-732
- Kaushner I**. The phenomenon of the acute phase response. *Ann NY Acad Sci* 1982; **389**: 39-48
- Rutgeerts P**. Medical therapy of inflammatory bowel disease. *Digestion* 1998; **59**: 453-469
- von Lampe B**, Barthel B, Coupland SE, Riecken EO, Rosewicz S. Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. *Gut* 2000; **47**: 12-14
- Lidenius MH**, Risteli LT, Risteli JP, Taskinen EI, Kellokumpu IH, Hockerstedt KA. Serum aminoterminal propeptide of type III procollagen (S-PIIP) and hepatobiliary dysfunction in patients with ulcerative colitis. *Scand J Clin Lab Invest* 1997; **57**: 297-305
- Walsley RS**, Ayres RCS, Pounder RE, Allan RN. A simple clinical activity index. *Gut* 1998; **43**: 29-32
- Stallmach A**, Schuppan D, Riese HH, Matthes H, Riecken ED. Increased collagen type III synthesis by fibroblasts isolated from strictures of patients from Crohn's disease. *Gastroenterology* 1992; **102**: 1920-1929
- Modigliani R**. Definition of patient groups: remission. In: Campieri M, Bianchi-Poro G, Fiocchi G, Scholmerich J. eds. Clinical challenges in inflammatory bowel disease- diagnosis, prognosis and treatment. Dordrecht, Boston, London: *Kluwer Academic Publishers* 1998: 85-94
- Goldman H**. Interpretation of large intestinal mucosal biopsy specimens. *Hum Pathol* 1994; **25**: 1150-1159
- Riddell RH**, Goldman H, Ransohoff DF. Dysplasia in inflammatory bowel disease: Standardized classification with provisional clinical applications. *Hum Pathol* 1983; **14**: 931-968
- Pascal RR**. Consistency in the terminology of colorectal dysplasia. *Hum Pathol* 1988; **19**: 1249-1250
- Pascal RR**. Dysplasia and early carcinoma in inflammatory bowel disease and colorectal adenomas. *Hum Pathol* 1994; **25**: 1160-1171
- Dionne S**, Ruemmele M, Seidman EG. Immunopathogenesis of inflammatory bowel disease: role of cytokines and immune cell-enterocyte interactions. In: Bistran BR, Walker-Smith JA. editors. *2nd Nestle Nutrition Workshop Series Clinical & Performance Programme* 1999: 41-62
- Mahmud N**, Stinson J, O'Connell MA, Mantle TJ, Keeling PW, Feely J, Weir DG, Kellehr D. Microalbuminuria in inflammatory bowel disease. *Gut* 1994; **11**: 1599-1604
- Nielsen OH**, Langholz E, Hendel J, Brynskov J. Circulating soluble intercellular adhesion molecule-1 (sICAM-1) in active inflammatory bowel disease. *Dig Dis Sci* 1994; **39**: 1918-1923
- Rowe FA**, Camilleri M, Forstrom LA, Batts KP, Mullan BP, Thomforde GMDunn W, Zinsmeister AR. A pilot study of splenic and whole body retention of autologous radiolabeled leukocytes in the assessment of severity in inflammatory colitis. *Am J Gastroenterol* 1995; **90**: 1771-1775
- Weldon MJ**, Masoomi AM, Britten AJ, Gene J, Finlayson CJ, Joseph AE, Maxwell MD. Quantification of inflammatory bowel disease activity using technetium-99m HMPAO labelled leukocyte single photon emission computerised tomography (SPECT). *Gut* 1995; **36**: 243-250
- Patel RT**, Bain I, Youngs D, Keighely MR. Cytokine production in pouchitis is similar to that in ulcerative colitis. *Dis Colon Rectum* 1995; **38**: 831-837
- Mazlam MZ**, Hodgson HJ. Interrelations between interleukin-6, interleukin-1 beta, plasma C-reactive protein values, and *in vitro* C-reactive protein generation in patients with inflammatory bowel disease. *Gut* 1994; **35**: 77-83
- Propst A**, Propst T, Herold M, Vogel W, Judmaier G. Interleukin-1 receptor antagonist in differential diagnosis of inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 1995; **11**: 1031-1036
- Hotkamp W**, Stollberg T, Reis HE. Serum interleukin-6 related to disease activity but not disease specificity in inflammatory bowel disease. *J Clin Gastroenterol* 1995; **20**: 123-126
- Ricci G**, D' Ambrosi A, Resca D, Masotti M, Alvisi V. Comparison of serum total sialic acid, C-reactive protein, alpha 1-acid glycoprotein and beta 2-microglobulin in patients with non-malignant bowel disease. *Biomed Pharmacother* 1995; **49**: 259-262
- Lindgren S**, Floren CH, Lindgren T, Starck M, Stewnius J, Nassberger L. Low prevalence of anti-neutrophil cytoplasmic antibodies in ulcerative colitis patients with long-term remission. *Eur J Gastroenterol Hepatol* 1995; **7**: 563-568
- Moran A**, Jones A, Asquith P. Laboratory markers of colonoscopic activity in ulcerative colitis and Crohn's colitis. *Scand J Gastroenterol* 1995; **30**: 356-360
- Ivanov AF**, Eroshkina TD, Fomin SA, Musin II, Chirkin VV. The effect of hemosorption on the glycoprotein concentration of the blood serum in inflammatory diseases of the large intestine. *Ter Arkh* 1995; **67**: 36-39
- Philipsen EK**, Bondesen S, Andersen J, Larsen S. Serum immunoglobulin G subclasses in patients with ulcerative colitis and Crohn's disease of different disease activity. *Scand J Gastroenterol* 1995; **30**: 50-53
- Sahmoud T**, Hocht-Boes G, Modigliani R, Bitoun A, Colombel JF, Soule JC, Florent C, Gendre JP, Lerebours E, Sylvester R, Mary JY. Identifying patients with a high risk of relapse in quiescent Crohn's disease. The GETAID groups. The groupe d' Etudes therapeutiques des affections inflammatoires digestives. *Gut* 1995; **37**: 811-818