

Microalbuminuria in inflammatory bowel diseases using immunoturbidimetry and high-performance liquid chromatography

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Abstract

Background and study aims : To measure urinary albumin excretion using immunoturbidimetry (IT) and high-performance liquid chromatography (HPLC) in inflammatory bowel diseases.

Patients and methods : A cross-sectional study was carried out on 60 selected patients with Crohn's disease (CD), 57 with ulcerative colitis (UC) and 22 healthy volunteers, as controls. Urinary albumin excretion was measured by IT and HPLC, and albumin-creatinine ratio was calculated. This ratio was compared in patients with active and inactive CD and UC and in healthy volunteers.

Results : Patients with CD and UC had higher albumin-creatinine ratio compared to controls using both IT and HPLC ($p < 0.05$). We measured higher albumin-creatinine ratio in patients with active compared to inactive CD ($p < 0.05$). Albuminuria did not correlate with disease duration of CD or UC, but patients with more extended CD according to the Montreal classification had higher HPLC-albumin-creatinine ratio. In CD, we found a significant correlation between HPLC-albumin-creatinine ratio and some inflammatory markers i.e. white blood cells ($p < 0.05$) and erythrocyte sedimentation rate ($p < 0.05$). In UC, there was no significant correlation between HPLC-albumin-creatinine ratio and the above markers of systemic inflammation or activity of UC. Albumin-creatinine ratio measured by HPLC was higher in both active and inactive CD and UC groups than albumin-creatinine ratio measured by IT. Using a receiver operating characteristics curve analysis, in case of HPLC-albumin-creatinine ratio cut-off values of the activity of CD were 2.46 mg/mmol for men, 5.30 mg/mmol for women.

Conclusions : HPLC-urinary albumin-creatinine ratio is associated with the clinical and laboratory disease activity indices in CD, but not in UC. Using HPLC we found a more sensitive method compared to IT to measure albuminuria that would be a sensitive activity marker in CD. (*Acta gastroenterol. belg.*, 2009, 72, 394-401).

Introduction

The aetiology of inflammatory bowel diseases (IBD) is not fully understood and their course is unpredictable with remissions and exacerbation phases (1,2). For clinicians, estimation of activity in IBD represents a difficult problem. Some activity indices are based on clinical scores and they are often subjective (2,3). The Crohn's Disease Activity Index (CDAI) with clinical and laboratory parameters can be usefully employed to quantify the symptoms of patients with Crohn's disease (CD). Patients having a CDAI score ≥ 150 are defined as active (4). The Harvey-Bradshaw (H-B) index is devised as a simplified version of the CDAI. It consists exclusively of clinical parameters, and may be used for quantification of CD activity (2-5). The modified H-B Activity Index is useful to identify not only the activity in CD patients but also the disease activity in ulcerative

colitis (UC) (6,7). Patients having a modified H-B Activity Index score ≥ 4 are defined as active (6,7). In UC, the colitis activity index (CAI) is useful, consisting of clinical parameters only. Patients having CAI score ≥ 6 are defined as active (8). Objective measures of disease activity are acute phase proteins [like C reactive protein (CRP)] (9-15), erythrocyte sedimentation rate (ESR) (16,17), but in many patients in whom the disease displays a high activity, these markers do not increase in line with the activity (2). White blood cell count (WBC) and some biomarkers have also been studied in relation to IBD, including neopterin, eicasonoids, β -2 microglobulin, antineutrophil cytoplasmic antibodies (ANCA), anti-*Saccharomyces cerevisiae* antibodies (ASCA), and some cytokine and cytokine receptor levels [interleukin (IL)-1, IL-2R α , IL-6, IL-8, tumor necrosis factor α (TNF- α) and TNF- α receptor, ICAM-1, VCAM, and E-selectin]. None of these biomarkers has been found to be sensitive enough to indicate disease activity in IBD (18). Fecal biomarkers (calprotectin, lactoferrin, dimeric M2-pyruvate kinase) are useful tools to differentiate between organic and functional intestinal disorders and they can predict disease activity both in CD and also in UC. Among them, calprotectin is the most investigated and most sensitive form (19-21). Endoscopic and radiological evaluations are still the gold standards, but they are invasive and thus are only carried out in highly active disease. Indium-labelled leukocyte scanning shows a very high correlation with disease activity, but this method has been found expensive and time-consuming, with significant exposure to radioactivity.

In previous studies urinary albumin determined by immunoturbidimetry (IT) has been found a simple but useful marker of IBD activity (2,3). In healthy humans, urinary albumin excretion does not exceed 30 mg/day. Microalbuminuria (MAU) means the urinary excretion of 30-300 mg/day albumin (22). Urinary albumin may exist in multiple forms (23). Conventional immunoassays (such as IT) detect intact albumin (immunochemically

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Submission date : 04/02/2009

Acceptance date : 25/07/2009

reactive albumin), albumin-derived fragments greater than 12 kD and polymer albumin aggregates (22). Immunochemically non-reactive albumin seems to have a limited number of polypeptide chain scissions and is held together by non-covalent intra-chain bonding disulfide bridges (22,23). In 2003 a high-performance liquid chromatography (HPLC) method, based on size-exclusion was introduced by Comper *et al.* (24) who reported that by means of this new method not only the immunochemically reactive albumin but also the immunochemically non-reactive form (total intact urinary albumin) is detectable. The first human study to compare immunological methods with HPLC found a significant 2 to 3-fold higher urinary albumin concentration measured with HPLC in diabetic normoalbuminuric and microalbuminuric subjects but not in non-diabetic controls (24). In the Australian Diabetes, Obesity and Lifestyle (AusDiab) study a significantly higher urinary albumin was measured even in the healthy controls by means of HPLC compared to IT (25). In the Heart Outcomes Prevention Evaluation (HOPE) study urine samples of subjects at high cardiovascular risk were reanalyzed by using HPLC to measure albuminuria (26). The “gold standard” to assess albuminuria is 24-hour urinary albumin excretion. Because 24-hour urine collection is cumbersome, guidelines suggest that measuring albuminuria in first morning urine adjusted for urinary creatinine concentration [albumin-creatinine ratio (ACR)] is a good and precise alternative (23,27).

Until now no studies examined the HPLC method in measurement of urinary albumin in IBD patients. We aimed to measure urinary albumin excretion by means of IT and HPLC in both groups of IBD patients (CD and UC) separately and to find associations of HPLC- and IT-determined ACR (HPLC-ACR, IT-ACR) with clinical activity of the diseases and with clinical parameters.

Patients and methods

Patients and laboratory parameters

Two hundred and seven outpatients with IBD (107 with CD, 100 with UC) who attended the 2nd Department of Medicine and Nephrological Center and the 3rd Department of Medicine and Family Medicine Institute between August 2007 and September 2008 were studied. The diagnosis of IBD was confirmed on the basis of endoscopy and histology. We excluded patients suffering from diabetes mellitus (DM), hypertension, renal disease, end-stage liver disease, malignancy, autoimmune disease, infection, acute illnesses such as sepsis, trauma, burns, ischemic injuries such as myocardial infarction, stroke and peripheral vascular disease, as well as pregnant and menstruating women, smokers, patients older than 80 years of age. We also excluded those patients who were using non-steroidal anti-inflammatory agents [except 5-aminosalicylic acid (5-ASA)], diuretics, angiotensin-converting enzyme inhibitors or

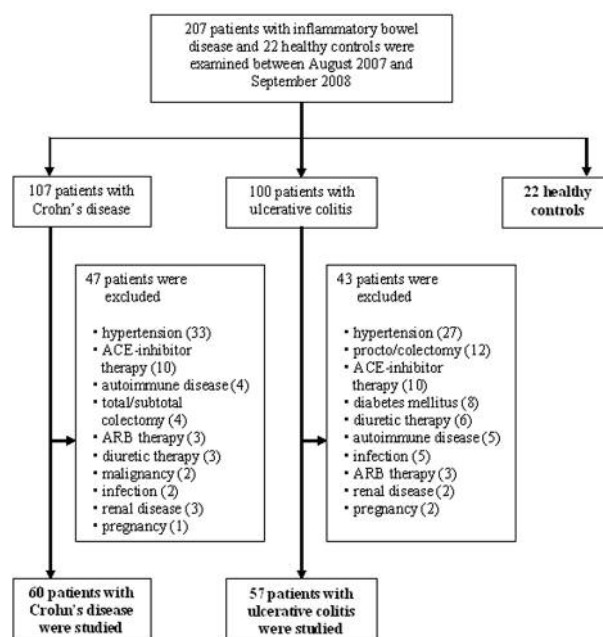


Fig. 1. — Enrollment.

ACE, angiotensin-converting enzyme ; ARB, angiotensin II receptor blockers ; (), number of patients. Some patients had more exclusion criteria.

angiotensin II receptor blockers, because the abovementioned diseases, conditions and medications can modify the urinary albumin excretion (2,3,18). Patients who underwent a total or subtotal colectomy were also excluded. Finally a cross-sectional study with 60 CD patients and with 57 UC patients was performed who were treated by usual treatment in IBD [5-ASA preparations (mesalamine, sulfasalazine), budesonide, methylprednisolone, azathioprine]. As controls, urine specimens were obtained from 22 healthy medical students and health care professional volunteers (Fig. 1). Disease activity was measured by means of the modified H-B Activity Index (6,7) and CDAI or CAI (4,8). At their clinical visit, samples were taken for routine biochemistry (serum sodium, potassium, calcium, phosphate, bilirubin, urea, creatinine, total cholesterol, LDL-, HDL-cholesterol, triglyceride, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, gamma-glutamyl transpeptidase, amylase, total protein, albumin, high-sensitive CRP, iron, transferrin, transferrin saturation, ferritin, vitamin B12, folic acid, prothrombin, blood count, ESR, ANCA, ASCA) and urine analysis. All routine laboratory measurements were carried out at the Department of Laboratory Medicine of the University of Pécs. All urinary creatinine levels were measured using the Jaffé method (28). The study was approved by the Ethical Committee of the Medical Faculty of the University of Pécs, Hungary and informed consent was obtained from all participants.

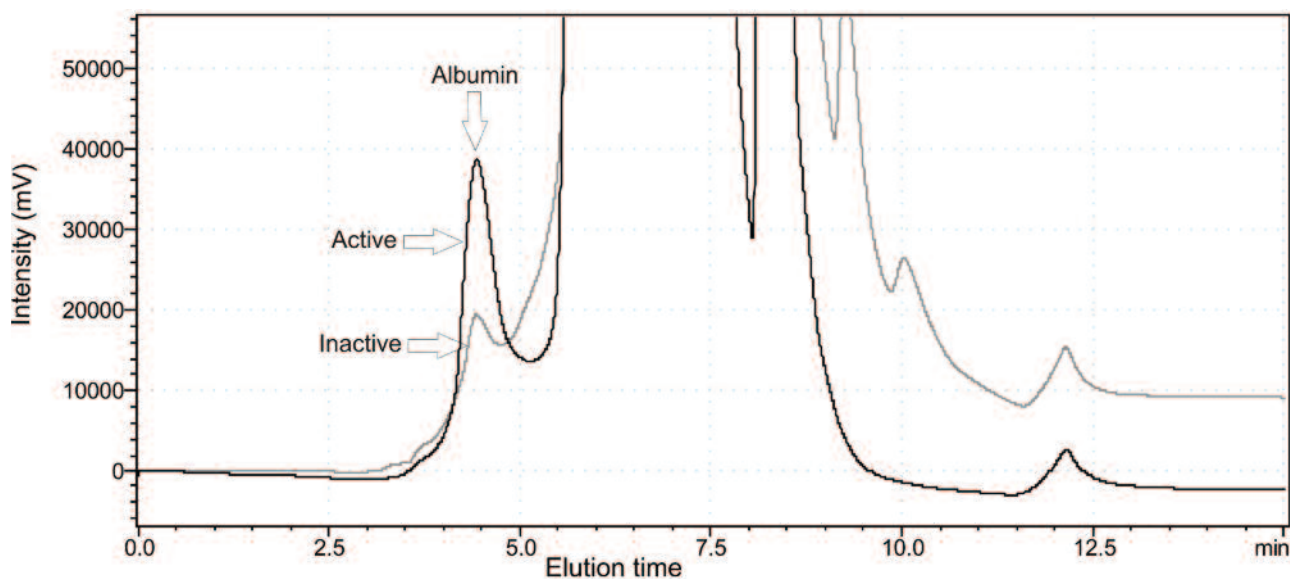


Fig. 2. — Original HPLC chromatograms of urine samples of the same patient with active and inactive Crohn's disease stage. Black line (—), chromatogram of active Crohn's disease patient (CDAI, 210 ; Modified Harvey-Bradshaw (H-B) Activity Index, 7 ; HPLC-ACR, 19.5 mg/mmol ; IT-ACR, 5.4 mg/mmol) ; grey line (—), chromatogram of an inactive Crohn's disease patient (CDAI, 30 ; H-B index, 0 ; HPLC-ACR, 3.4 mg/mmol ; IT-ACR, 0.4 mg/mmol).

Analysis of urinary albumin

First morning urine samples were obtained, they were vortexed and centrifuged (2500 G for 10 min), after which they were stored at -80°C for a maximum of 2 weeks before measurement. Urinary albumin levels were measured by means of IT (2,3) (Roche/Hitachi Roche Diagnostics GmbH, Mannheim, Germany) method using Roche/Hitachi 812 Modular P analyser (sensitivity : 3 mg/l, intra-assay precision 1.3% and 1.7%, inter-assay precision 4.3% and 2.6% respectively, linearity : 3-3000 mg/L) and by means of HPLC (Shimadzu SPD 10 AVvp, Shimadzu Corp., Japan) method using Accumin™ kit (29) (Accumin Diagnostics Inc., New York, NY, USA, sensitivity : 3 mg/L, linearity : 3-2000 mg/L, inter-assay and intra-assay precision 5.8% and 2.5% respectively). The Accumin™ kit is based on size-exclusion chromatography and contained a Zorbax Bio-Series GF 250 column and Zorbax Diol guard column (both from Agilent Technologies Inc., Santa Clara, CA, USA). The mobile phase was phosphate buffered saline (pH = 6.93). Twenty-five μL of centrifuged urine was used in duplicate. Time program included 6 min at flow rate of 0.5 mL/min, then ramp up to 2 mL/min and wash between 6.5-11.5 min. Then ramp down to 0.5 mL/min in 0.5 min and wash until straight baseline observed. Peak retention time of albumin was between 4.5 and 5.2 min under the circumstances recommended by the manufacturer, and was verified daily by run of an albumin standard. Concentration of albumin was measured upon UV absorption at 214 nm using the UV detector of the HPLC system (Fig. 2).

Statistical analysis

Statistical analysis was performed using the SPSS 13.0 (SPSS Inc., IE, USA) software. Data were tested for normality. Data of active and inactive groups with normal distribution were compared using t-test. Non-normally distributed data of active and inactive groups were analyzed using Mann-Whitney u test. Multiple comparisons were carried out using ANOVA and post-hoc tests (normal distribution) or Kruskal-Wallis test and Mann-Whitney u tests (non-normal distribution). Receiver operating characteristics (ROC) curves and Youden index [maximum (specificity + sensitivity)-1] were used to select appropriate cut-off values. Non-parametric correlation and linear regression were used to discover any connection between data of albumin excretion and clinical parameters.

Results

Clinical characteristics of patients and controls

There was no significant difference between CD and UC in duration of disease, or in disease activity as determined by the modified H-B Activity Index, which is the only comparable activity score in both diseases. Patients with CD had higher ESR than patients with UC ($p < 0.05$) and CD patients had a higher platelet count (PLT) than the UC group ($p < 0.05$) (Table 1).

Microalbuminuria in IBD

One hundred seven outpatients with CD and 100 with UC were examined at the beginning of the study, but

Table 1. — Clinical characteristics of patients and controls

	Controls (n = 22)	Crohn's disease (n = 60)	Ulcerative colitis (n = 57)
Age (years)	38.4 ± 2.8	37.3 ± 1.65	42.0 ± 1.7
Gender (male/female)	13/9	26/34	27/30
Duration of disease (years)	—	7 (3-11)	7 (4-12)
Estimated glomerular filtration rate [†] (ml/min)	n.a.	96.5 ± 3.0	94.1 ± 2.6
Serum ferritin (µg/l)	n.a.	56 (21-111)	65 (32-96)
High-sensitive C reactive protein (mg/l)	n.a.	4.80 (1.45-8.48)	3.60 (1.50-9.20)
Erythrocyte sedimentation rate (mm/h)	n.a.	21 (10-36)	13 (6-24) ^a
White cell count (G/l)	n.a.	6.98 (5.63-9.36)	6.36 (5.06-7.95)
Haemoglobin (g/l)	n.a.	135 ± 2	139 ± 2
Platelet count	n.a.	327.0 ± 12.7	274.4 ± 10.5 ^a
Activity indices			
– CDAI	—	108 ± 13	—
– CAI	—	—	2.8 ± 0.4
– Modified Harvey-Bradshaw Activity Index	—	3 (1-7)	2 (0-5)
Montreal classification			
– Location at diagnosis			
L1	—	13 (22%)	—
L1+L4	—	0	—
L2	—	11 (18%)	—
L2+L4	—	2 (3%)	—
L3	—	31 (52%)	—
L3+L4	—	3 (5%)	—
E1	—	—	7 (12%)
E2	—	—	20 (35%)
E3	—	—	30 (53%)
– Behaviour at diagnosis			
B1	—	43 (72%)	—
B1+p	—	2 (3%)	—
B2	—	8 (13%)	—
B2+p	—	0	—
B3	—	7 (12%)	—
B3+p	—	0	—
Treatment*			
– Mesalamine	—	43 (72%)	24 (42%)
– Sulfasalazine	—	4 (7%)	23 (40%)
– Methylprednisolone	—	8 (13%)	8 (14%)
– Budesonide	—	20 (33%)	3 (5%)
– Azathioprine	—	17 (28%)	12 (21%)

^a $p < 0.05$ vs. Crohn; n.a., data not available; [†] calculated using the Modification of Diet in Renal Disease (MDRD) formula; CDAI, Crohn's disease activity index; CAI, colitis activity index. L1, ileal; L2, colonic; L3, ileocolonic; L4, upper gastrointestinal; B1, non-stricturing, non-penetrating; B2, stricturing; B3, penetrating; p, perianal; E1, proctitis; E2, left-sided; E3, extensive, *, some patients received more than one medication.

Data are presented as mean ± SEM or median (25-75 percentile) as appropriate.

patients with the above mentioned exclusion criteria were excluded, finally 60 selected CD patients and 57 selected UC patients were studied (Fig. 1). Patients with CD and UC had significantly higher IT/HPLC-ACR than did the controls (Fig. 3).

Microalbuminuria and clinical disease activity in CD

CD patients were grouped according to activity upon the modified H-B Activity Index (Fig. 4A) and CDAI (Fig. 4B). We found 24 active and 36 inactive patients by means of the modified H-B Activity Index and 17 active and 43 inactive patients by means of CDAI. HPLC-ACR was significantly higher than IT-ACR in the active and inactive groups as well (Fig. 4A, Fig. 4B). Using both IT and HPLC, higher ACR was measured in patients with active CD compared with patients with inactive CD (Fig. 4A, Fig. 4B). In addition, inactive patients (classi-

fied according to either activity indices) had higher albuminuria (measured either with HPLC or IT) than controls ($p < 0.001$ for all).

ROC curve analysis was carried out to select an appropriate cut-off value for IT-ACR and also for HPLC-ACR to predict likelihood of the clinical disease activity among patients with CD, i.e. patients with ACR higher than the calculated cut-off value will be likely to have an active disease while those with ACR lower than the cut-off value are likely to have an inactive disease. The results are summarized in Table 2. In addition, there were two patients who had two measurements. One patient turned from clinically inactive to active, and parallel to that, HPLC-ACR became positive regarding the above cut-off criterion. The other patient became clinically inactive, and parallel to that, HPLC-ACR became negative.

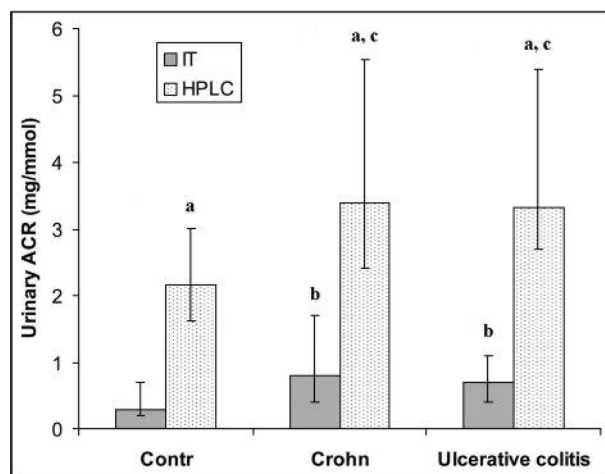


Fig. 3. — Urinary IT- and HPLC-ACR in controls and in patients with Crohn's disease and ulcerative colitis.

Filled bars, IT ; dotted bars, HPLC ; IT, immunoturbidimetry ; HPLC, high-performance liquid chromatography ; ACR, albumin-creatinine ratio ^a $p < 0.01$; ^b $p < 0.05$ vs. contr (IT) ; ^c $p < 0.05$ vs. contr (HPLC) ; multiple group comparison using Kruskal-Wallis test was significant for both HPLC and IT values.

Microalbuminuria and clinical disease activity in UC

We found 19 active and 38 inactive patients by means of the modified H-B Activity Index and 9 active and 48 inactive patients by means of CAI. In patients with UC,

only IT-ACR was significantly higher in patients with active UC compared to inactive UC diseases as judged by the modified H-B Activity Index. HPLC-ACR did not differ between active and inactive cases. When grouping UC patients according to activity upon the CAI index, neither IT- nor HPLC-ACR differ between active and inactive cases, and by using the HPLC method significantly higher ACR could be detected compared to IT-ACR in both disease activity scores (Fig. 5A, Fig. 5B).

Association between microalbuminuria, clinical characteristics and clinical disease activity

The correlation between HPLC-ACR and disease duration was not significant neither in CD ($r = 0.247$; $p = 0.061$) nor in UC ($r = 0.263$; $p = 0.071$).

We tested the connection between albuminuria and disease extent according to the Montreal classification (30, 31, 32) (see Table 1 legend). Patients with Montreal L3 had higher HPLC-ACR [8.51 (4.29-12.58) mg/mmol] compared to Montreal L1 [3.59 (3.09-4.30) mg/mmol] or L2 [1.90 (1.63-2.36) mg/mmol] [$p < 0.01$ for both, data given as median (25-75 percentile)]. Only five patients had L4 i.e. upper gastrointestinal tract (GIT) manifestation, these patients tended to have higher albuminuria than patients without upper GIT manifestation, but the difference was not statistically significant. Analysis according to Montreal B (behaviour) was not investigated, as in most cases (55 out of 60) current clinical status of the patients did not indicate endoscopic investigation at the time when albuminuria was measured.

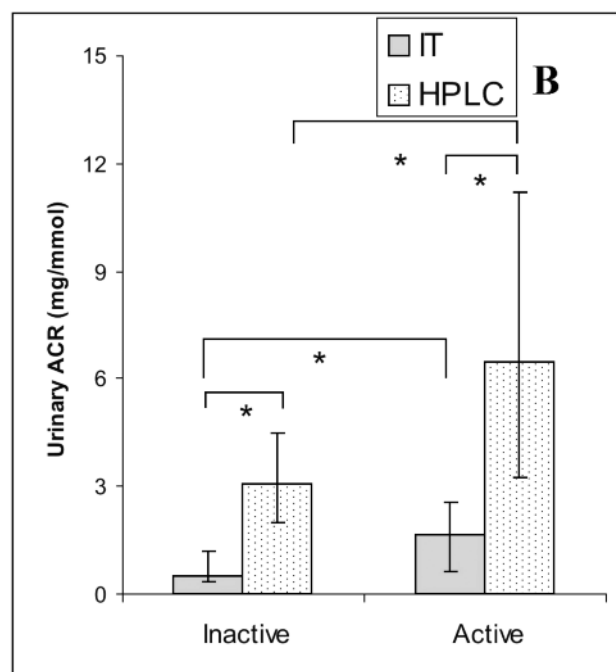
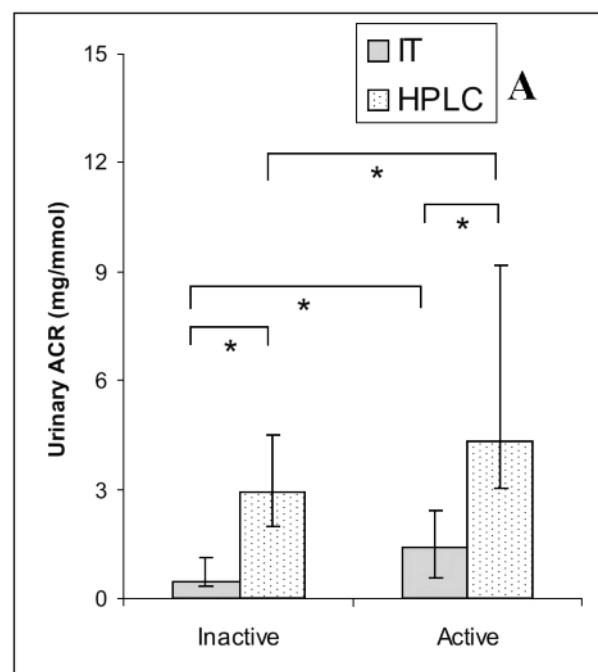


Fig. 4. — IT- and HPLC-ACR in inactive and active Crohn's disease categorized upon the modified H-B Activity Index (A) or upon the CDAI (B).

Filled bars, IT ; dotted bars, HPLC ; IT, immunoturbidimetry ; HPLC, high-performance liquid chromatography ; ACR, albumin-creatinine ratio ; * $p < 0.01$.

Table 2. — Cut-off values for IT- or HPLC-ACR for activity indices in men and in women with Crohn's disease

	ACR cut-off values (mg/mmol)	Modified Harvey-Bradshaw Activity Index	CDAI
IT used in MEN	0.37	sensitivity : 75% specificity : 59%	sensitivity : 100% specificity : 61.5%
HPLC used in MEN	2.46	sensitivity : 81.8% specificity : 66.7%	sensitivity : 100% specificity : 60%
IT used in WOMEN	1.62	sensitivity : 78% specificity : 85%	sensitivity : 83% specificity : 75%
HPLC used in WOMEN	5.30	sensitivity : 66.7% specificity : 78.9%	sensitivity : 75.0% specificity : 77.3%

IT, immunoturbidimetry ; HPLC, high-performance liquid chromatography ; ACR, albumin-creatinine ratio ; CDAI, Crohn's disease activity index.

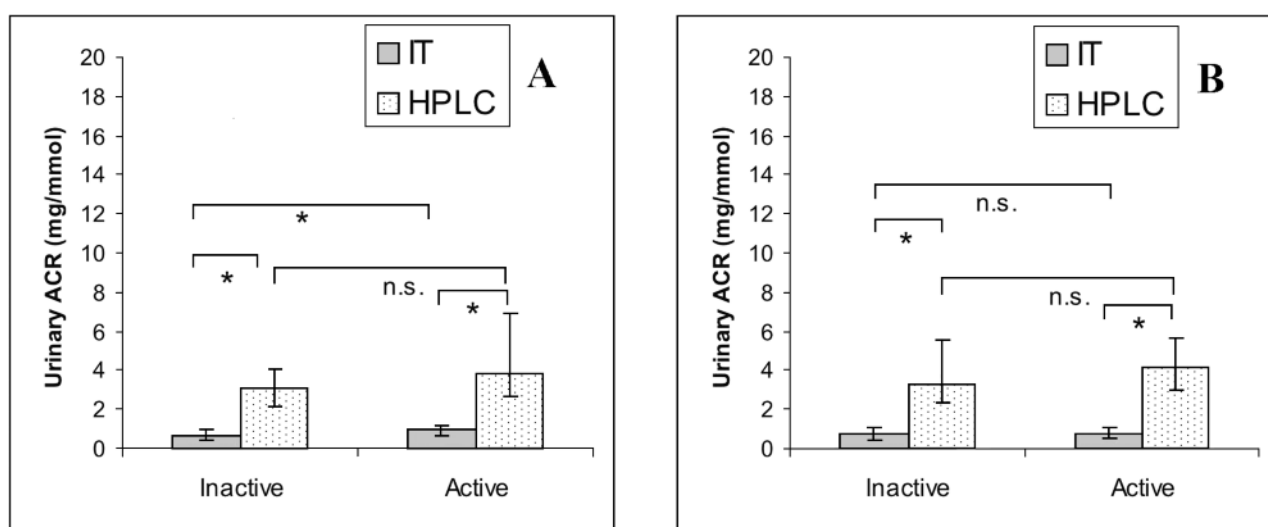


Fig. 5. — IT- and HPLC-ACR in active and inactive ulcerative colitis categorized upon the modified H-B Activity Index (A) or upon the CAI (B).

Filled bars, IT ; dotted bars, HPLC ; IT, immunoturbidimetry ; HPLC, high-performance liquid chromatography ; ACR, albumin-creatinine ratio ; * $p < 0.01$; n.s., non-significant..

In CD, we found a significant correlation between HPLC-ACR and some inflammatory markers i.e. WBC ($r = 0.257$; $p = 0.047$) and ESR ($r = 0.262$; $p = 0.047$). The correlation between HPLC-ACR and CRP in CD was not significant ($r = 0.036$; $p = 0.785$). In UC, there was no significant correlation between HPLC-ACR and the above markers of systemic inflammation.

Stepwise linear regression analysis was carried out in patients with CD to find predictors of clinical activity scores (the modified H-B Activity Index and CDAI). No similar analysis was carried out in UC patients as we found no significant correlation between urinary HPLC-ACR, IT-ACR and clinical scores. Similarly, no correlation was found between MAU and disease extent (Montreal E), treatment or disease duration in UC. In patients with CD, we tested a model including duration of disease, PLT, WBC, ESR, CRP, urinary IT-ACR, and urinary HPLC-ACR as independent variables and the modified H-B Activity Index as a dependent variable.

Independent predictors of the modified H-B Activity Index were duration of disease, CRP and urinary HPLC-ACR (adjusted R^2 , 0.562 ; duration of disease : $\beta = 0.542$, $p < 0.001$; CRP : $\beta = 0.423$, $p = 0.001$; urinary HPLC-ACR : $\beta = 0.269$, $p = 0.036$).

In patients with CD, we also tested the same linear regression model using CDAI as a dependent variable. Independent predictors of the CDAI were duration of disease, CRP and urinary HPLC-ACR (adjusted R^2 , 0.511 ; duration of disease : $\beta = 0.536$, $p < 0.001$; CRP : $\beta = 0.341$, $p = 0.010$; urinary HPLC-ACR : $\beta = 0.284$, $p = 0.036$).

Discussion

In our study MAU was measured in IBD-patients, where CD and UC patients were handled separately. In IBD, inflammatory cells such as neutrophils, lymphocytes, and macrophages invade the colonic mucosa, and

peripheral mononuclear leukocytes also produce cytokines (IL-1, TNF- α , and IL-6), which contribute to damaging the inflamed bowel wall. Increased circulating cytokine levels may lead to systemic inflammation and are responsible for increased glomerular permeability of albumin by increasing superoxide production in the glomerular epithelial cells, and by affecting the metabolism of glycosaminoglycan. Glycosaminoglycans play an important role in the barrier function of glomerular basement membrane and their loss may lead to MAU (3, 18).

There are many studies about MAU as an early marker of diabetic nephropathy and cardiovascular complications (23,29,33-35). MAU detected by HPLC can predict the development of diabetic nephropathy 2.4-3.9 years earlier than the immunoassay-detected MAU (36). On long term, the glomerular damage may lead to decreased glomerular filtration rate and lead to compensatory mechanisms, such as activation of the renin-angiotensin-aldosterone system, which may subsequently affect the cardiovascular system (32).

We found that HPLC-ACR is a good marker of activity in CD but not in UC. The explanation for this finding may be that MAU in IBD develops on the basis of systemic inflammation, which is more pronounced in CD than in UC. This is also supported by the fact that HPLC-ACR correlated well with inflammatory markers in CD but not in UC.

In patients with DM the generally accepted cut-off values using conventional methods to measure activity of diabetic nephropathy are 2.5 mg/mmol ACR for men and 3.5 mg/mmol ACR for women (23). Using the data of the HOPE study, cut-off value of urinary ACR was calculated for the prediction of cardiovascular events in the general population. In patients with DM the value was 1.4 mg/mmol and in persons without DM the value was 0.7 mg/mmol for immunological method. In patients with DM the value was 5.2 mg/mmol and in persons without DM the value was 3.1 mg/mmol for HPLC (26). In our study of non-hypertensive, non-diabetic IBD patients, cut-off values for CD activity were calculated. For men the cut-off value measured by HPLC was 2.46 mg/mmol [with an excellent sensitivity for disease activity (100%) upon CDAI].

We found that albuminuria did not correlate with disease duration. Furthermore, albuminuria was higher in inactive CD patients than in controls. A further finding was that in case of two patients, HPLC-ACR followed the changes of clinical activity. Considering all these findings, we believe that albuminuria mainly represents actual clinical activity of the disease in CD, but it is even in remission not totally reversible, not decreasing to the level of controls. In a previous study, inactive CD patients had higher MAU than controls, supporting our data (2,3). We also found a correlation between disease extent and albuminuria, patients with more extended CD had higher HPLC-ACR, which is consistent with previous findings (2,3).

Our patients were treated by the conventional treatment of IBD. The medical treatment in IBD (especially 5-ASA preparations) seems to be without any effects to urinary albumin (2,3,37,38). We have also found that there was no difference between patients receiving or not receiving a particular medication (data not shown) regarding HPLC-ACR. However, we have to bear in mind that most patients are receiving a combination therapy, not only one medication.

In summary, ACR can be a marker of disease activity in IBD. Measurement of albuminuria using HPLC is a novel and more sensitive method detecting the urinary albumin that adds a fast and non-invasive measure of disease activity in CD to existing clinical and laboratory markers.

Acknowledgement

The technical assistance of Mrs. Sámikné Ilona Varga is highly appreciated.

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