# Microalbuminuria in inflammatory bowel disease.

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**Abstract**

**Microalbuminuria independently predicts the development of nephropathy and increased cardiovascular morbidity and mortality in diabetic patients, but it may be an indicator of the acute phase response. This study examined microalbuminuria as a marker of the acute phase response in patients with inflammatory bowel disease and correlated it with the disease activity in 95 patients with inflammatory bowel disease (ulcerative colitis (n=52), Crohn's disease (n=43)) determined by the simple index of Harvey and Bradshaw. Fifty patients were in complete clinical remission and 45 patients had active disease. Microalbuminuria was detected in all patients with inflammatory bowel disease (147 (17) *v* 18 (2) μg/min, inflammatory bowel disease *v* controls mean (SEM), p<0·007). Patients with active inflammatory bowel disease had higher concentrations of microalbuminuria compared with patients in remission (206 (19) *v* 65 (8) μg/min, mean (SEM), p<0·0001). Eight patients with active inflammatory bowel disease who were sequentially followed up with measurements of microalbuminuria had significantly lower values, when the disease was inactive (active inflammatory bowel disease 192 (44) *v* inactive inflammatory bowel disease 64 (14) μg/min, p<0·03). There was a significant correlation with the simple index of Harvey and Bradshaw (*r*=0·818, p<0·0001). Microalbuminuria values were significantly lower in inflammatory bowel disease patients in remission, maintained with olsalazine compared with those patients maintained with mesalazine and salazopyrine, but no significant difference was seen in values of microalbuminuria in active inflammatory bowel disease patients receiving different salicylates. This study also measured serum amyloid-A as an indicator of the acute phase response in the same patients. Serum amyloid-A was significantly increased in active disease compared with inactive disease (151 (43) *v* 33 (7) or controls 11 (2) μg/ml, p<0·05). In conclusion microalbuminuria is present in abnormal amounts in all patients with active inflammatory bowel disease, and values fall when the disease is quiescent. Microalbuminuria is probably a consequence of an acute phase response and provides a simple, rapid, and inexpensive test, which has the potential to monitor inflammatory bowel disease activity and response to treatment.**

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Persistently increased excretion of albumin above normal, which is not detected by the conventional semiquantitative strips test is defined as microalbuminuria. Albuminuria excretion greater than 20 micrograms per minute in a timed overnight urine specimen is diagnostic of microalbuminuria. Microalbuminuria when present in abnormal amounts in diabetic patients predicts diabetic nephropathy and increased incidence of cardiovascular related morbidity and mortality.[1–7]. Microalbuminuria in patients with hypertension, non-diabetic patients, and the elderly is also indicative of increased morbidity and mortality resulting from cardiovascular events.[2 8 9] Microalbuminuria is also described, however, as a non-specific marker for acute illness[10] such as sepsis, trauma, burns[11] and ischaemic injuries such as myocardial infarction, most probably as a result of the acute phase response to inflammatory mediators and in this respect it may provide a more longlasting and easily measurable indicator than other acute phase parameters. Microalbuminuria in malignancy may reflect the microvascular response to tumour related mediators and is of prognostic value.[12]

The aetiology of inflammatory bowel disease is unknown and its course is unpredictable with high recurrence rates. It has been suggested, however, that inflammatory bowel disease, in particular Crohn's disease, has a vascular aetiology.[13 14] Evaluation of disease activity in chronic inflammatory bowel disease is fraught with difficulty. Many indices are based on clinical scores that are subjective and may be significantly changed by psychological factors. Evaluation of acute phase response by erythrocyte sedimentation rate and C reactive proteins does provide an objective measure of disease activity.[15–17] Many patients do not show increases in these indices, however, even when highly active. Indium labelled leucocyte scanning has been found to correlate well with clinical activity in inflammatory bowel disease.[18–20] This is expensive and time consuming and results in significant exposure to radioactivity. The response to treatment in chronic inflammatory bowel disease is currently monitored by clinical parameters, which are not entirely ideal for the monitoring of clinical trials. Endoscopic and radiological evaluations of inflammatory bowel disease activity are invasive and their use is limited in acutely ill patients with severe disease. Thus there is a critical need for a simple objective test, suitable for serial measurements in evaluation of the response to treatment.

The aim of our study was to find out if microalbuminuria was detectable in patients

with inflammatory bowel disease and to determine the role of microalbuminuria as a disease activity marker in inflammatory bowel disease. We have correlated microalbuminuria with clinical disease activity[21] and with the markers of the acute phase response including C reactive protein[15 22–24] and serum amyloid A protein.[24]

## Methods

### SUBJECTS

Ninety five consecutive patients with inflammatory bowel disease were studied prospectively. These consisted of patients attending the outpatient department at St James's Hospital with ulcerative colitis or Crohn's disease confirmed on the basis of endoscopy, histology, and radiology. Fifty two patients had ulcerative colitis and 43 patients had Crohn's disease. Their median age was 42 (range 16–69) years, and they received conventional medical and surgical treatment. All patients recruited in this study who were receiving medical treatment with 5-ASA compounds had been maintained with these drugs for a duration of at least 12 months (sulphasalazine (n=35), olsalazine (n=33), and mesalazine (n=15)). A group of patients (n=12) receiving no drugs was studied as drug free disease controls. The non-treatment disease control group included newly diagnosed patients with inflammatory bowel disease and patients with small bowel Crohn's disease who were not receiving any drugs. At their hospital visit clinical disease activity was assessed using the simple index of Harvey and Bradshaw. The disease was considered active if the simple index of Harvey and Bradshaw score was greater than 4. At the same visit samples were taken for routine biochemistry and 10 ml of serum was obtained and saved at −70°C for later estimation of amyloid A protein and C reactive protein. Twenty healthy controls also had 12 hours overnight urine collection for microalbuminuria and venesection for serum amyloid-A and C reactive protein estimation. All patients and controls received instructions for 12 hour overnight urine collection starting from 9 pm to 9 am. After completing collection, the urine was received within two hours, the volume was measured, and 30 ml of urine was stored at −20°C for measurement of microalbuminuria. In addition routine urine analysis was performed and estimation of urinary urea, electrolytes, protein, and creatinine clearance was performed. Blood samples were taken within two hours of completing the 12 hour overnight urine collection.

*Exclusion criteria*

Patients aged more than 70 years or with diabetes mellitus, hypertension, renal disease, urinary tract infection, and patients using non-steroidal anti-inflammatory agents were not recruited in the study. Ten patients were excluded from the study, and four patients failed to complete the protocol.

### MEASUREMENT OF SERUM AMYLOID-A AND C REACTIVE PROTEIN

Serum amyloid-A was measured in all patients and controls with a commercially available enzyme linked immunosorbent assay (ELISA) (Biosource International, Camarillo, California). The sensitivity of the assay is 10 μg/ml. The intra-assay and interassay coefficients of variation are 8·5% and 9·8%. C reactive protein was measured by the nephelometry method.

### MICROALBUMINURIA ASSAY

Microalbuminuria was measured in all patients and 20 normal controls using an immunoturbiditimetric method (Microalbs, Ames, Bucks, UK). In this method human albumin reacts with a specific antibody in the presence of polyethylene glycol. As the antibody was present in large excess the precipitated complex produced a turbidity related to the concentration of albumin in the sample. The turbidity was photometrically measured at wavelength 340 nm. Coefficient of variance was less than 3%.

### GLUTATHIONE S TRANSFERASE pi

The urinary concentrations of glutathione S transferase pi was measured using a radioimmunoassay supplied by Immundiagnostik GmbH Bensheim, Germany.

### STATISTICAL ANALYSIS

Statistical comparison was carried out using the Student *t* test. Simple regression and analysis of variance (ANOVA) were used to calculate correlation coefficients. A probability value of $<0·05$ was considered to be statistically significant. The results are expressed as mean (SEM).

## Results

### MICROALBUMINURIA IN CHRONIC INFLAMMATORY BOWEL DISEASE

There was no significant difference in urinary volume between active (708 (92) ml/12 h) and inactive (780 (107) ml/12 h) patients. Similarly there was no significant difference in urinary volumes between patients receiving mesalazine, olsalazine, sulphasalazine or disease controls (720, 696, 705, 682 ml/12 h respectively). In our study significantly abnormal concentrations of microalbuminuria were detected in ulcerative colitis and Crohn's disease patients compared with normal controls (147 (17) *v* controls mean 18 (2) μg/min, mean (SEM), $p<0·0001$). Patients with active inflammatory bowel disease had significantly higher values compared with patients in remission ((mean 206 (19) *v* 65 (8) μg/min, $p<0·001$), Fig 1). Although the mean values of microalbuminuria were higher in Crohn's disease patients (152 μg/min) compared with ulcerative colitis patients (121 μg/min), these values did not reach statistical difference

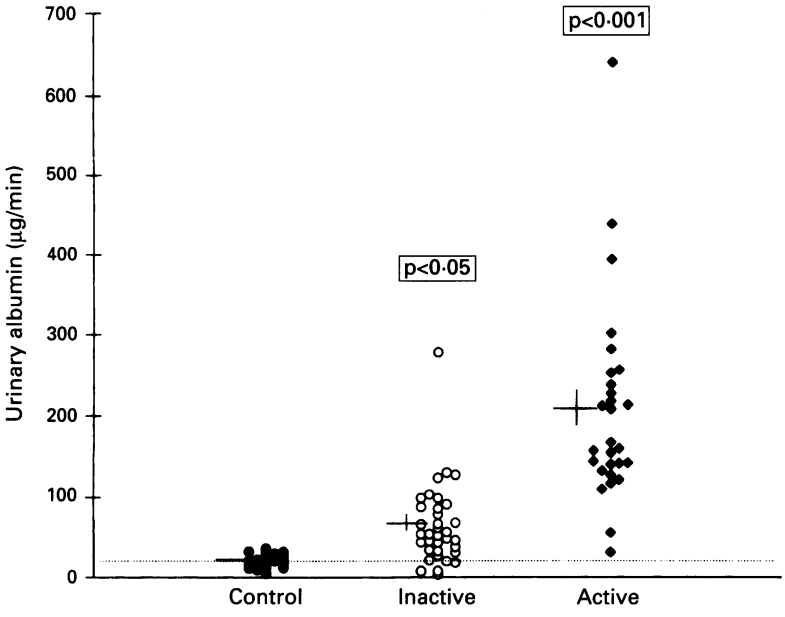


*Figure 1: Urinary albumin in inflammatory bowel disease. Each point represents a single patient and the mean of a duplicate measurement. The mean is indicated by a horizontal line, the vertical line represents mean (SEM). The upper normal range for urinary albumin is shown by the dotted line. p Values, calculated using the unpaired Student's* t *test, refer to comparison with the control group.*

(Fig 2). Microalbuminuria concentrations were also higher in patients with extensive inflammatory bowel disease (mean 164 (20) µg/min) compared with limited rectosigmoid disease (90 (12) µg/min), $p<0·006$. Eight patients with inflammatory bowel disease were studied prospectively from relapse into remission with sequential measurement of microalbuminuria. Significantly lower values of microalbuminuria were detected, when the disease was inactive ((active inflammatory bowel disease mean 192 (44) *v* remission inflammatory bowel disease 64 (14) µg/min, $p<0·003$), Fig 3). Microalbuminuria correlated strongly with clinical disease activity monitored by simple index of Harvey and Bradshaw ($r=0·818$, $p<0·0001$). This correlation was roughly equal for both ulcerative colitis ($r=0·73$) and Crohn's disease ($r=0·76$). Urine analysis, urinary urea/electrolytes, creatinine clearance, and estimation of urinary glutathione S transferase pi, were normal in all patients.

SERUM AMYLOID-A, C REACTIVE PROTEIN, AND MICROALBUMINURIA

Considering microalbuminuria as a disease activity marker, we measured serum amyloid A and C reactive protein in the same patients as indicators of acute phase response. Serum amyloid-A concentrations were significantly increased in inflammatory bowel disease patients (111 (28) *v* controls mean 11 (2) µg/ml, $p<0·05$). In patients with active inflammatory bowel disease, serum amyloid-A values were statistically raised compared with those with inactive disease (151 (43) *v* 33 (7) µg/ml, $p<0·03$). Microalbuminuria, however, did not correlate with serum amyloid-A ($r=0·329$, $p=0·52$). Serum C reactive protein concentrations were significantly raised in patients with active disease compared with those patients with quiescent disease (mean (SEM) 42 (15) *v* 6·5 (3·2) mg/l, $p<0·02$). Furthermore, a significant correlation was found between C reactive protein and clinical disease activity index ($r=0·477$, $p<0·004$) as well as with microalbuminuria ($r=0·80$, $p<0·001$).

EFFECT OF DRUGS ON MICROALBUMINURIA

The effect of drugs on microalbuminuria was evaluated in patients with active and inactive inflammatory bowel disease. Microalbuminuria in patients with active disease receiving no salicylates did not differ significantly from those receiving salicylates (232 (54) *v* 177 (22) µg/min; $p=0·28$). There was also no significant difference between different salicylates in the active group. Microalbuminuria concentrations were significantly lower, however, in patients with inactive disease maintained with olsalazine compared with those who were maintained with mesalazine or sulphasalazine (olsalazine mean (SEM) 43 (5) µg/min *v* mesalazine 103 (35); $p<0·05$, sulphasalazine 112 (19); $p<0·001$,

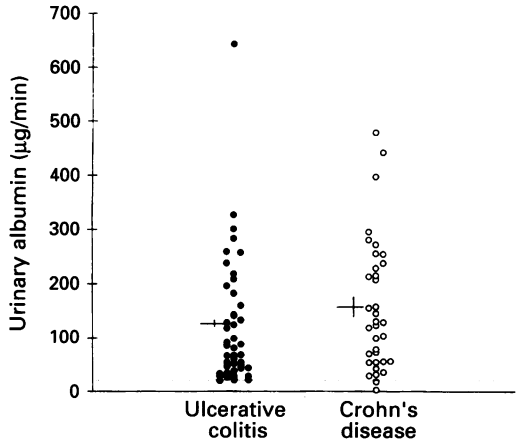


*Figure 2: Comparison of urinary albumin in ulcerative colitis and Crohn's disease. Each point represents a single patient and the mean of a duplicate measurement. The mean is indicated by a horizontal line, the vertical line represents mean (SEM). The upper normal range for urinary albumin is shown by the dotted line. Using unpaired Student's* t *test, p=NS.*

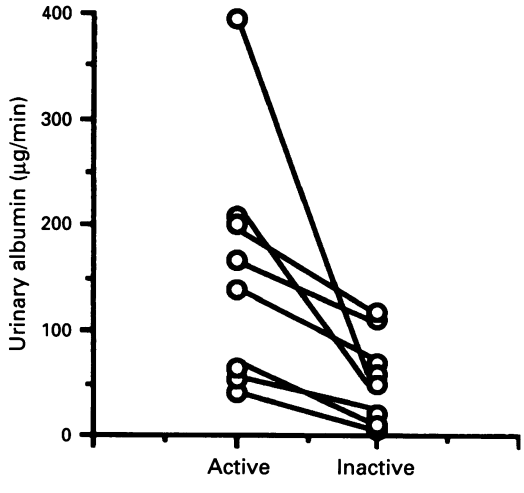


*Figure 3: Urinary albumin in inflammatory bowel disease. Two point comparison of urinary albumin in patients with active inflammatory bowel disease and when the disease was quiescent. Using paired Student's* t *test, p<0·003.*

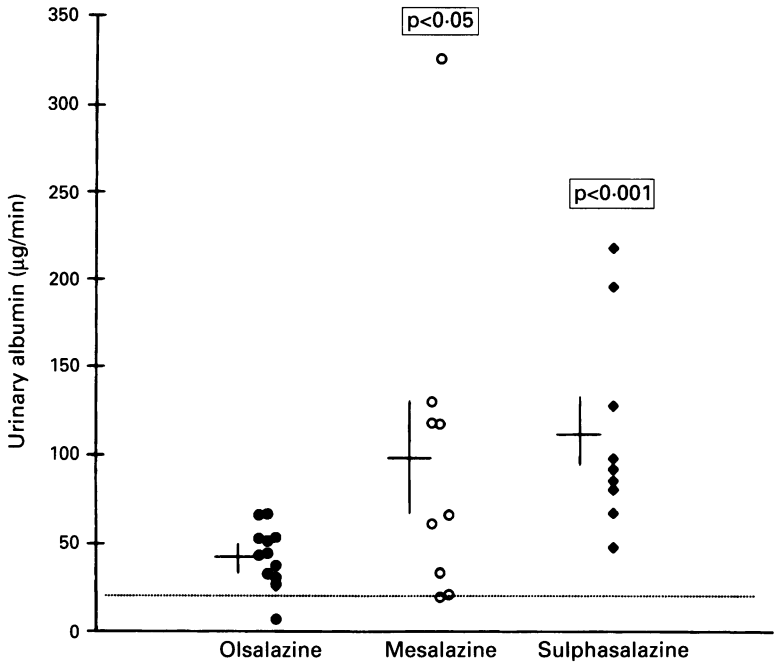


*Figure 4: Comparison of urinary albumin in inactive chronic inflammatory bowel disease with different drug treatments. Each point represents a single patient and the mean of a duplicate measurement. the mean is indicated by a horizontal line, the vertical line represents mean (SEM). The upper normal range for urinary albumin is shown by the dotted line. p Values, calculated using Student's unpaired* t *test, refer to the comparison with the olsalazine group.*

Fig 4). There was no significant difference, however, in the disease activity indices or C reactive protein concentrations in these groups (olsalazine *v* mesalazine, or sulphasalazine; simple index of Harvey and Bradshaw score 0·5 (0·2) *v* 0·8 (0·4); 1·0 (0·3); p=NS; C reactive protein 2·8 (1·1) *v* 3·0 (1·2); 3·1 (1·2) mg/l; p=NS, respectively).

## Discussion

Monitoring of treatment of patients with chronic inflammatory bowel disease requires measurement of objective indices of disease activity. A large number of disease activity indices are available largely based on the measurement of the acute phase response. In addition a number of clinical disease activity indices available at present[21 25 26] are dependent on subjective features and are of limited use in day to day treatment of patients, however, they are still in practice. Laboratory tests like erythrocyte sedimentation rate, C reactive protein, and other acute phase reactants are objective, but it is well known that these tests can be normal in clinically active disease.[27] Best *et al* introduced an index for inflammatory activity in Crohn's disease, which has been used in a number of prospective studies.[25] This index, however, incorporates a number of variables based on subjective symptoms (general well being, abdominal pain), which may significantly skew the results. The Van Hees activity index uses both subjective and objective variables including erythrocyte sedimentation rate and C reactive protein and serum albumin.[26] This has the disadvantage of inter-laboratory variation, however, and also that the values can be spuriously normal in dehydrated patients. This index has 18 variables including stool weight (mean of five consecutive days), and neither patients nor evaluating physicians find it convenient. Furthermore, this index requires patients to use a diary card to record symptoms over seven days. This is obviated in the simple index of Crohn's disease activity designed by Harvey and Bradshaw, which is also dependent on subjective variables.[21] The platelet count is often increased in active inflammatory bowel disease,[28] but difficulty arises when these patients are receiving corticosteroid treatment or other immunosuppressive agents (azathioprine), which cause bone marrow depression and decrease in platelet count. Indium-111 labelled leucocyte scan is the most commonly used radioisotope technique[18–20] and permits faecal excretion studies for the assessment of disease activity. Technical difficulty, expense, the need for collection of stools over four days, and use of radioisotopes are the main disadvantages of faecal indium excretion studies.

Our study has shown that microalbuminuria is a useful disease activity marker for inflammatory bowel disease. Large increases in the concentrations of microalbuminuria were detected in patients with active Crohn's disease and those with active ulcerative colitis, which fell with clinical improvement. A significant correlation was found between microalbuminuria and the clinical disease activity index. Nevertheless, microalbuminuria continues even in remission in patients with inflammatory bowel disease. This may suggest that microalbuminuria could be a highly sensitive indicator of a low level of ongoing inflammation in these patients. Further studies correlating microalbuminuria with histological activity will be necessary to find out if this is the case and these studies are ongoing. Microalbuminuria in inflammatory bowel disease does not result from glomerular or tubular damage as a result of drugs (sulphasalazine, olsalazine, mesalazine), as there is no correlation with drug therapy in patients with active disease. Furthermore, there is no indication of drug induced tubular damage; a sensitive index of tubular dysfunction glutathione S transferase pi[29] was measured and found to be negative in all cases tested (data not shown). Using microalbuminuria, however, as an objective disease activity marker in patients in remission maintained with olsalazine, we found significantly lower concentrations compared with those patients in remission and maintained with sulphasalazine and mesalazine. This could possibly suggest that olsalazine treatment results in a remission with lower levels of background inflammation, although further prospective studies are required to confirm this finding. Patients receiving olsalazine for maintenance treatment received a similar dose of 5-ASA (1 g daily) to those receiving mesalazine (1·2 g daily).

Eight patients with active disease who were followed up with sequential analysis of microalbuminuria showed that when the disease became quiescent concentrations of microalbuminuria decreased significantly. In

our study four patients with active disease (ulcerative colitis n=2 and Crohn's colitis n=2, one patient in each group had toxic megacolon) who did not respond to medical treatment and who needed a surgical colectomy had the highest recorded values of microalbuminuria with persistently high concentrations of microalbuminuria (all greater than 312 μg/min). Thus microalbuminuria may have a prognostic value and its serial analysis may be helpful in the early recognition of non-responders to medical treatment.

Microalbuminuria has also been reported in acute illness, sepsis, post-trauma, and burns. The underlying mechanism of protein leakage through the kidney in these conditions and inflammatory bowel disease probably results from the increased renal microvasculature permeability in response to increased circulating inflammatory mediators. In recent years there has been considerable interest in the role of inflammatory mediators including interleukin 1, interleukin 2, interleukin 6, and tumour necrosis factor α in inflammatory bowel disease.[30–33] An important part of the acute phase reaction is the modulation of the synthesis of acute phase proteins in the liver by systemic cytokines.[34 35] In our study serum amyloid-A correlated with clinical disease activity, however, microalbuminuria correlated poorly with serum amyloid-A.

It has been suggested that the intestinal microvasculature may play an early part in the pathogenesis of inflammatory bowel disease, and Crohn's disease in particular. This hypothesis suggests that an initial vascular insult results in the formation of granulomatous vasculitis, accelerated thrombogenesis, and multifocal gastrointestinal infarction.[13 14] We found no evidence of renal damage to suggest that renal vasculitis may have been responsible for microalbuminuria in inflammatory bowel disease patients. All our patients had normal urine analysis, microscopy, creatinine clearance, and estimation of urinary glutathione S transferase pi; a sensitive marker for early renal tubular damage.[29]

We believe that microalbuminuria may result from the increased renal microvascular permeability in response to increased circulating cytokines. It has been shown in animal models that the cytokines interleukin 1 and tumour necrosis factor α cause microvascular permeability, which may be either direct or mediated by their effects on neutrophil migration.[36] The local tissue damage in the intestine leads to the activation of leucocytes and fibroblasts with resultant release of cytokines such as interleukin 1, interleukin 6, and tumour necrosis factor α, which may induce a receptor mediated systemic reaction. Microalbuminuria may reflect either the direct effect of the cytokines on the renal matrix molecules or indirect effects mediated through an inflammatory cell infiltrate into the affected kidney. Sulphated glycosaminoglycans have been reported to regulate albumin flux in the glomerulus basement membrane.[37] Patients with chronic inflammatory bowel disease have substantial loss of glycosaminoglycans from the intestine,[38] although renal tissue has not been examined. It has been suggested that the loss of glycosaminoglycans is mediated by cytokines including interleukin 1 and tumour necrosis factor α.[39] High concentrations of these cytokines have been reported in chronic inflammatory bowel disease and might contribute to potential loss of glycosaminoglycans in the renal microvasculature and glomerulus basement membrane.

In summary, microalbuminuria is a feature of chronic inflammatory bowel disease. Development of microalbuminuria may reflect increased circulating cytokines with effects on renal microvasculature. Concentrations of microalbuminuria correlate with the clinical and laboratory indices of disease activity and may prove to be an effective objective measurement in the monitoring of response to treatment in chronic inflammatory bowel disease.

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