

PMO-246 URINARY MATRIX METALLOPROTEINASES DO NOT CORRELATE WITH ENDOSCOPIC OR HISTOLOGICAL DISEASE ACTIVITY IN ULCERATIVE COLITIS

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¹D S Pearl,* ²S Edgar, ³M Whittaker, ³H Nitch-Smith, ²J F Brown, ²J K Shute, ¹T M Trebble. ¹Department of Gastroenterology, Portsmouth Hospitals NHS Trust, Portsmouth, UK; ²Institute of Biomedicine and Biomolecular Sciences, University of Portsmouth, Portsmouth, UK; ³Histopathology, Portsmouth Hospitals NHS Trust, Portsmouth, UK

Introduction Ulcerative colitis (UC) is a colonic inflammatory disorder of unconfirmed aetiology. Clinical assessment involves invasive endoscopic examination with a small yet significant procedural risk, on which therapeutic decisions are made. Non-invasive biomarkers may be better tolerated and reduce procedural costs and risks. Matrix metalloproteinases (MMP) are enzymes involved in tissue remodelling; MMP are elevated in mucosa and urine of children with active UC. We measured urinary MMP activity in adult patients with UC, matched controls investigated for functional symptoms and normal healthy volunteers to evaluate as a potential biomarker of disease activity.

Methods Ethical approval and informed consent were obtained. Patients with UC and age-sex matched controls, identified during outpatient assessment, were prospectively recruited and flexible sigmoidoscopy (FS) performed. Endoscopic (Sutherland) and histological (Gomes) appearance in patients with UC was graded. A group of healthy volunteers were recruited at a local University. Urine samples, snap frozen at collection in liquid nitrogen, were thawed, centrifuged, and assayed using commercially obtained fluorescein-labelled gelatinase activity kits. MMP activity was corrected for creatinine concentration. Results were expressed as median \pm IQR. Statistical tests included Kruskal–Wallis analysis and Spearman's correlation.

Results 80 active and 16 quiescent UC patients, 77 age-sex matched controls and 22 normal healthy volunteers were compared. Urinary MMP activity in active compared with quiescent UC ($p=0.185$) and in each compared with age-sex matched controls ($p=0.237$, $p=0.525$ respectively) was not significantly different. Exclusion of patients taking 5-aminosalicylates and corticosteroids did not alter significance. There was no correlation between urinary MMP activity and UC disease activity measured endoscopically ($r=0.09$, $p=0.425$) or histologically ($r=0.178$, $p=0.127$). Urinary MMP activity in healthy volunteers was significantly lower than patients with active UC ($p<0.0001$), quiescent UC ($p<0.002$), and controls ($p<0.0001$).

Conclusion Contrary to previously published work, our findings suggest that urinary MMP activity, measured using fluorescein-labelled gelatinase assay, does not discriminate between quiescent and active UC and does not correlate with UC disease activity. Significant differences noted between healthy volunteers and patients with UC were unexpected, but may reflect difference in group demographics. MMP gelatinase assays are therefore a poor non-invasive biomarker of disease activity in UC.

Competing interests None declared.

PMO-247 MUCOSAL CYTOKINE EXPRESSION IN ULCERATIVE COLITIS: ELEVATED IL-8 BUT NOT TNF- α AND REDUCED TGF- β IN INFLAMED COMPARED TO NON-INFLAMED MUCOSA

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¹D S Pearl,* ²K Shah, ³M Whittaker, ³H Nitch-Smith, ²J F Brown, ²J K Shute, ¹T M Trebble. ¹Department of Gastroenterology, Portsmouth Hospitals NHS Trust, Portsmouth, UK; ²Institute of Biomedicine and Biomolecular Sciences, University of

Portsmouth, Portsmouth, UK; ³Histopathology, Portsmouth Hospitals NHS Trust, Portsmouth, UK

Introduction Ulcerative colitis (UC) is a chronic inflammatory condition of the colon of unconfirmed aetiology. Microscopic examination of inflamed biopsies is characterised by progressive neutrophil infiltration and tissue destruction. There is conflicting evidence from studies on the relative roles of TNF α , IL-8, TGF β and other cytokines in UC; however, current therapy includes cytokine targeted interventions. We compared cytokine profiles of inflamed and non-inflamed mucosa in patients with distal UC, and age-sex matched controls.

Methods Ethical approval was obtained. Patients were prospectively recruited from outpatients' clinics. Mucosal biopsies at flexible sigmoidoscopy (FS) were taken from UC patients within macroscopically inflamed and non-inflamed proximal mucosa, and from age-sex matched controls undergoing FS. Severity of endoscopic (Sutherland) and histological (Gomes) inflammation were recorded. Quantitative cytokine analysis for IL-4, TNF α , IL-17A, IL-8, IL-10, TGF β and IFN- γ were carried out using commercially available assays on tissue homogenates prepared with protease inhibitors, corrected for total protein. Statistical comparison was by Wilcoxon signed rank pair analysis and Spearman's correlation.

Results 69 active UC patients (54 paired normal/inflamed mucosa) and 69 controls were compared. Significant elevation in IL-8 ($p<0.001$; $p<0.001$) and reduction in TGF β ($p<0.02$; $p<0.0002$) with significant positive correlation of IL-8 ($r_2=+0.46$; $p<0.01$) and negative correlation of TGF β ($r_2=-0.46$; $p<0.01$) to severity of inflammation was detected in inflamed compared with non-inflamed mucosa from the same patient and compared to age-sex matched control mucosa; however, TNF α concentration was not significantly different. Comparisons of macroscopically inflamed mucosa compared with non-inflamed mucosa from the same patients also demonstrated significant reduction in concentration of IFN γ ($p<0.001$), IL-4 ($p<0.005$) and IL-17A ($p<0.002$). No significant differences were noted between normal tissue from UC patients and external controls.

Conclusion Our findings suggest that IL-8 (a neutrophil chemo-attractant) is elevated and TGF β (involved in cell repair) is reduced with no change demonstrated for TNF α . Significantly lower concentration of IFN γ , IL-4 and IL-17A suggests downregulation of Th1, Th2 and Th17 adaptive immune response. These findings suggest that the inflammatory response in UC may predominantly involve IL-8 mediated neutrophil infiltration and failure of TGF β mediated tissue healing, with limited evidence for the role of TNF α in mild-moderate distal UC.

Competing interests None declared.

PMO-248 MUCOSAL UPREGULATION OF ARACHIDONIC ACID PRODUCTION IN ACTIVE ULCERATIVE COLITIS: DELIVERY OF PRO-INFLAMMATORY EICOSANOID PRECURSORS

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¹D S Pearl,* ²M Masoodi, ²M Eiden, ³J Brummer, ³D Gullick, ⁴M A Whittaker, ⁴H Nitch-Smith, ³J F Brown, ³J K Shute, ³G Mills, ⁵C C Philip, ¹T M Trebble. ¹Gastroenterology Department, Portsmouth Hospitals NHS Trust, Portsmouth, UK; ²Elsie Widdowson Laboratory, Medical Research Council, Cambridge, UK; ³Institute of Biomedicine and Biomolecular Sciences, University of Portsmouth, Portsmouth, UK; ⁴Histopathology Department, Portsmouth Hospitals NHS Trust, Portsmouth, UK; ⁵Institute of Human Nutrition, School of Medicine, University of Southampton, Southampton, UK

Introduction Ulcerative colitis (UC) is a colonic inflammatory disorder of unconfirmed aetiology. Eicosanoids, inflammatory mediators involved in UC pathogenesis, are enzymatically converted from dietary polyunsaturated fatty acids (PUFA), arachidonic acid (AA) and eicosapentaenoic acid (EPA), themselves competitive

substrates also generated via a fatty acid (FA) biosynthetic cascade. Dietary studies using fish oil-derived EPA have been disappointing in UC; we hypothesised that the PUFA biosynthetic pathway in inflamed tissue is altered. This study evaluated PUFA profile in inflamed and non-inflamed mucosa from UC patients and compared to matched controls.

Methods Ethical approval was obtained. Patients were prospectively recruited from outpatients' clinics. Mucosal biopsies at flexible sigmoidoscopy (FS) were taken from UC patients within inflamed and normal proximal mucosa. Age-sex matched control patients undergoing FS for functional symptoms were compared. Inflammation was scored endoscopically and histologically. Membrane bound FA (MBFA): Biopsies were spiked with deuterated internal standard, followed by liquid-liquid extraction and quantitative gas chromatography mass spectrometry (MS). Free Fatty Acid (FFA): Biopsies were homogenised, followed by solid phase extraction and liquid chromatography orbitrap MS. Data were expressed as percentage abundance. Dietary fatty acid analysis was undertaken. Wilcoxon signed rank pair and Spearman's correlation analysis were employed.

Results 69 active UC patients (54 paired normal/inflamed mucosa) and 69 controls were compared. No biologically significant differences were noted between endoscopically normal mucosa from UC patients and controls other than DPA ($p < 0.0025$). Inflamed mucosa compared to non-inflamed mucosa demonstrated highly significant reduction in LA and α LNA ($p < 0.0001$) and increased AA, DPA, and DHA ($p < 0.0001$); EPA was reduced ($p < 0.005$). The ratio of AA/EPA was increased in inflamed mucosa ($p < 0.0001$). These findings are consistent between MBFA and FFA and correlate with severity of inflammation.

Conclusion Mucosal PUFA bioavailability is altered in active UC, with significant elevation of AA and reduction of LA, α LNA and EPA. This suggests modification of the FA biosynthetic pathway with elevated delivery of AA as a precursor of pro-inflammatory eicosanoids in active UC. These findings may explain the lack of efficacy of supplemental fish oil and suggests new alternative therapeutic targets.

Competing interests None declared.

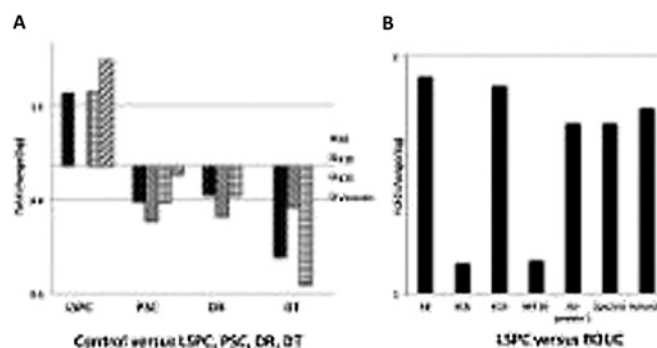
PMO-249 **QUANTITATIVE PROTEOMIC ANALYSIS OF INTERMEDIATE FILAMENT PROFILE IN ULCERATIVE COLITIS REVEALS INCREASED LEVELS OF KERATINS 8, 18 AND 19 IN PATIENTS WITH LONGSTANDING PAN COLITIS WHICH ARE REDUCED WITH DEVELOPMENT OF DYSPLASIA**

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^{1,2}D Majumdar, * ²B M Corfe, ³C Evans, ¹A J Lobo. ¹Gastroenterology, Royal Hallamshire Hospital, Sheffield, UK; ²Department of Oncology, University of Sheffield, Sheffield, UK; ³Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, UK

Introduction Intermediate filaments (IF), principally keratins (K), are key components of epithelial cytoskeleton. K8, 18 and 19 are expressed in intestinal epithelial cells and play a role in cell-death signalling pathways, in particular apoptosis mediated by tumour necrosis factor- α . Reduced K8 and K20 expression is linked to epithelial-to-mesenchymal transition indicative of increased tumour aggressiveness. We investigated the change in levels of insoluble IF proteins in well-characterised groups of patients at differing risk of UC-associated cancer.

Methods Rectal biopsies were obtained from patients with inactive UC with: (1) Long-standing (20–40 years) pancolitis (LSPC) (n=10); (2) Recent onset (<5 years) UC (ROUC) (n=8); (3) UC with primary sclerosing cholangitis (PSC) (n=7); (4) pancolitis with dysplasia (n=4) and 10 controls, with additional biopsies from dysplastic/neoplastic lesions and snap frozen. An iTRAQ (isobaric tagging for relative and absolute quantification)-compatible extrac-



Abstract PMO-249 Figure 1 Tandem mass spectrometry results showing significant log fold changes ($p < 0.05$) in IF levels.

tion and solubilisation protocol for IF proteins was developed. Labelled peptides from pooled patients were analysed by SCX-LC-MS/MS (strong cation exchange-reverse phase HPLC tandem mass spectrometry) and data reconstituted in GeneBio Phenyx. Inter-group comparisons were made using in-house algorithms based on t-testing with multiple test correction.

Results Tandem mass spectrometry (MS/MS) identified 52 proteins; 32 (61.5%) were matched by two or more peptides. Abstract PMO-249 figure 1A shows the log fold change in IF levels compared to control, with significant increase in levels of K8, K19 and vimentin in those with LSPC, but marked reduction in IF levels in areas of dysplasia (DT) and rectal mucosa distant from this (DR). Marked increase in levels of keratins was noted in patients with LSPC compared to those with ROUC (Abstract PMO-249 figure 1B), suggesting an effect of disease duration on IF levels.

Conclusion This is the first study using a quantitative proteomic approach with an iTRAQ based proteomic workflow to analyse changes in IF levels in patients with UC with differing colon cancer risk. LSPC is associated with enhanced mucosal levels of keratins, spectrin and vimentin, which is reduced in dysplasia and in distant rectal mucosa of those with dysplasia—suggesting a field change. These changes need further characterisation including of post-translational modifications, which may help better understanding of the pathogenesis of colitis associated cancer.

Competing interests None declared.

PMO-250 **QUANTITATIVE PROTEOMICS IN ULCERATIVE COLITIS REVEALS MUCOSAL INFLAMMATION REDUCES LEVELS OF KERATINS IN THE INSOLUBLE FRACTION OF THE INTERMEDIATE FILAMENT PROTEOME**

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^{1,2}D Majumdar, * ³C Evans, ²B M Corfe, ¹A J Lobo. ¹Department of Gastroenterology, Royal Hallamshire Hospital, Sheffield, UK; ²Department of Oncology, University of Sheffield, Sheffield, UK; ³Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, UK

Introduction Keratins (K) are a key component of intermediate filaments (IF), primarily composed of K8, 18 and 19 in the intestinal epithelia. Apart from a structural role, they may play a role in moderating TNF effects, including cytotoxicity. K8-null mice develop colitis, a subset of patients with IBD have missense mutation in K8 gene. Colonic expression of K8/K18 has been shown to be regulated by IL-6. In order to examine the relationship between acute inflammation and alteration in levels of insoluble IFs in mucosa of patients with ulcerative colitis (UC), we undertook a quantitative proteomic approach using an iTRAQ (isobaric tagging for relative and absolute quantification)-based proteomic workflow.

Methods Endoscopic biopsies were obtained from patients with UC, from actively inflamed rectum and from non-inflamed proximal