tract. It has been suggested that faecal calprotectin might be a useful screening tool in distinguishing organic from functional bowel disease, especially in children. We report, for the first time, elevated faecal calprotectin in patients with diarrhoea due to pancreatic insufficiency.

Method: A total of 115 patients (77 women: 38 men; age 19–85) with chronic non bloody diarrhoea were screened in this study. Both pancreatic elastase and calprotectin were assayed by ELISA in faecal samples (normal : calprotectin < 30 µg/g, elastase >150 µg/g.) Pancreatic imaging in those with low faecal elastase was carried out by a combination of CT scanning, endoscopic pancreatograms and endoscopic ultrasonography.

Results: Twenty six (21%) patients had low faecal elastase. Of these, 5 were confirmed radiologically to have chronic pancreatitis. All 5 patients were shown to have abnormally high faecal calprotectin levels (90–534 µg/g). 14 patients had suspected pancreatic insufficiency in the absence of other causative pathology. 13 (93%) of these patients had elevated faecal calprotectin (73–799 µg/g). 7 patients with low faecal elastase and 'watery' faecal samples were subsequently shown to have probably normal pancreatic function and an alternative explanation for symptoms (adenoma, Clostridial colitis, small bowel bacterial overgrowth, diverticular disease). Of these patients, 2 (29%) had abnormally high faecal calprotectin (176 µg/g=adenoma, 2670 µg/g=C.diff colitis) (table 1).

Abstract 223, Table 1

	Total patients	Low elastase	High calprotectin
Confirmed pancreatic disease	5	5 (100%)	5 (100%)
Possible pancreatic disease	14	14 (100%)	13 (93%)
No pancreatic disease	96	7 (7%)	42 (44%)

Conclusion: Pancreatic insufficiency may be associated with high faecal calprotectin in the absence of neoplastic or inflammatory intestinal disease. Low faecal elastase may also be associated with watery diarrhoea secondary to inflammatory intestinal disease, itself associated with an elevated faecal calprotectin concentration.

Symposium: Guts, Liver and Bones: 224–225

224 GUIDELINES FOR OSTEOPOROSIS PREVENTION: HOW FAR HAVE WE YET TO GO?

E. Seward, T. Quigley, M. Smith. *Dept of Gastroenterology, Havering Hospitals' NHS Trust, Romford, Essex RM7 0BE, UK*

Background: The British Society of Gastroenterology has published guidelines for the prevention and treatment of osteoporosis in patients with coeliac disease and inflammatory bowel disease (IBD) (*Gut* 2000;46(suppl 1):i1-i8). This has important implications for the planning of clinical services, especially with the limited availability of DEXA scanning. We have undertaken an assessment of the need for DEXA scans in our gastroenterology out-patient population.

Methods: In a district general hospital, population 460,000, we identified all patients with coeliac disease and IBD attending all gastroenterology out-patient clinics (3.5 consultant gastroenterologists, total 6 clinics per week) over a one month period. We assessed the number of these patients who required DEXA scanning and further investigation according to the BSG guidelines.

Results: Over a four week period 124 of a total 204 patients with either coeliac disease or IBD were found to require a DEXA scan according to the guidelines. The mean age of those requiring a scan was greater than those who did not (54.1 (18.6)y vs 38.6 (12.3)y (age (sd)). All 21 patients with coeliac disease required a scan. Of those with Crohn's disease 43/72 required a scan as did 60/111 patients with ulcerative colitis. Reasons for scans in patients with IBD are given in the table. DEXA scans had already been requested in 12 patients and 5 patients were on treatment (table 1).

Conclusions: Currently we are achieving BSG guidelines in less than 10% of our patient population. Introducing guidelines will mean that almost 2/3 of patients with Crohn's disease and more than half our ulcerative colitis patients will require a DEXA scan. Subsequently scanning will be required for patients with a new diagnosis of IBD,

Abstract 224, Table 1

	On steroids	Post-menopausal or male >55y	Both steroids and age criteria
Crohn's disease	21	21	1
Ulcerative colitis	14	32	14

those requiring long courses of steroids and those reaching the age thresholds, as well as those on bone protection treatment. Patients with coeliac disease who require a scan at diagnosis place less pressure on resources.

225 PREVALENCE OF FRAGILITY FRACTURE IN COELIAC DISEASE AND INFLAMMATORY BOWEL DISEASE

W.E. Fickling, A. Holdoway, D.A. Robertson, A.K. Bhalla¹. *Royal United Hospital; ¹Royal National Hospital for Rheumatic Diseases, Bath, UK*

Background: Osteoporosis is a common complication of both inflammatory bowel disease and coeliac disease, but there is little information on the prevalence of fragility fracture in these patients.

Methods: Ambulant patients with coeliac disease (102), Crohn's disease (88) and ulcerative colitis (130) completed a questionnaire detailing their fracture history. Fractures involving major trauma e.g. Road Traffic Accidents, falls from greater than three feet and sports injuries were excluded from analysis.

Results: 42% of patients with coeliac disease had experienced a previous fracture. Commonest fractures involved the distal radius (32%) and ankle (16%). Prevalence was higher in females than males (46% vs 33%). Females were younger than males (52.3yrs vs 59.1yrs) and were diagnosed at an earlier age (45.9yrs vs 52.3 yrs). In neither sex was age at diagnosis related to history of fracture. 99 patients were diagnosed after the age of 25, when peak bone mass is achieved. 130 patients with ulcerative colitis (47 male, 83 female, mean age 45.7yrs) and 88 patients with Crohn's disease (31 male, 57 female, mean age 38.7yrs) completed the questionnaire. 29% of patients with ulcerative colitis and 30% of patients with Crohn's disease had suffered a fracture. Patients incurring fractures after diagnosis of ulcerative colitis had earlier onset of disease (mean 28.6yrs vs 37.9yrs) and longer duration (14.0yrs vs 7.7yrs) than patients without fractures. Fracture risk was unrelated to disease extent, use of systemic corticosteroids, smoking and family history of fragility fracture.

Conclusions: Fractures are very common in patients with coeliac disease and inflammatory bowel disease. Efforts to prevent, detect and treat low bone mineral density may reduce this prevalence.

Cell Biology/Neoplasia/Coeliac Free Papers: 226–239

226 IMMUNE RESPONSES IN BARRETT'S METAPLASIA

J.A. Eksteen¹, J.L. Smith², C. Tselepis¹, I. Perry¹, R.F. Harrison³. *Depts of ¹Medicine and ²Pathology, University of Birmingham; Dept of ³Immunology, Southampton General Hospital, UK*

Introduction: Barrett's metaplasia (BM) is a chronic inflammatory disorder of the oesophagus in response to persistent gastro-oesophageal reflux and is associated with two distinct complications: benign oesophageal strictures and a 125-fold increased risk of developing an oesophageal adenocarcinoma. Benign strictures usually form in proximal areas of BM whereas adenocarcinomas arise distally. The characteristics of the inflammation and its role in the development of these complications have however been unclear.

Hypothesis: We propose that different immune responses are involved in the pathogenesis of these complications and that inflammation in BM is not a homogenous condition.

Methods: Endoscopic biopsies were obtained from normal oesophageal mucosa (n=10), oesophagitis (n=10) and paired biopsies from proximal and distal BM (n=20). The samples were assessed using immunohistochemistry, immunofluorescence, western blotting, and PCR amplification of DNA isolates from paraffin-embedded biopsies with consensus primers TCRB, TCRG and IgH.

Results: We have shown that two distinct areas exist within BM based on cytokine production and inflammatory cell phenotype. Proximal segments of BM were associated with more intense inflammation and a polyclonal population of CD4 lymphocytes, CD8 lymphocytes and macrophages (CD68). Distal segments of BM were however significantly less inflamed and were characterised by a predominance of monoclonal CD4 lymphocytes. Proximal segments were associated with increased production of interferon γ , interleukin 1 β , interleukin 2 and interleukin 12 suggesting a TH1-type immune response whereas distal segments were associated with production of interleukin 4, interleukin 5 and interleukin 10 suggesting a TH2-type immune response. In both areas the majority of inflammatory cells were located around the bases of the crypts and deep glands with significantly fewer cells at the luminal surface.

Conclusions: Our data suggest that BM is not a homogenous condition but that benign stricture and adenocarcinoma formation are associated with distinct immune responses and different pathogenesis.

227 MORPHOGENIC EFFECTS OF GASTRIN ON GASTRIC EPITHELIAL CELLS

A. Pagliocca, A. Varro. *Physiological Laboratory, University of Liverpool, Liverpool, UK*

Background: The gastric epithelium is characteristically invaginated to form tubular glands, and tubule-like structures are a feature of some gastric tumours. Other epithelial cells eg mammary and kidney, form tubules when cultured on basement membrane.

Aims: To determine the factors regulating formation of tubular structures by gastric epithelial cells.

Methods: Gastric cancer cell lines (AGS, MKN-45, HGT-1), non-transformed cells (RGM-1), and AGS cells stably transfected with the gastrin-CKK $_B$ receptor (AGS-G $_R$), were cultured on plastic or artificial basement membrane (Matrigel).

Results: None of the cell lines formed tubule-like structures when cultured on plastic. However, AGS and RGM-1 cells (but not HGT-1 or MKN-45 cells) assembled within 3–6 hours into linear, branching, tubule-like arrays when cultured on Matrigel in medium containing 5–10% fetal calf serum. Gastrin (30 to 300pM), a known gastric morphogen, stimulated the formation of tubule-like arrays in AGS-G $_R$ cells cultured on Matrigel in serum-free conditions. The response to gastrin was blocked by the gastrin-CKK $_B$ antagonist, L-740,093 (100nM), and partially inhibited by the protein kinase C inhibitor, Ro-32-0432 (1 μ M) but not by PI-3-kinase (LY-29042, 20 μ M) or MEK (PD-98059, 20 μ M) inhibitors. Phorbol 12-myristate-13-acetate (PMA, 100nM), which stimulates PKC, had a similar effect to gastrin. The effect of PMA was fully reversed by Ro-32-0432. Similar cellular assemblies were also formed in serum free-media in response to L- α -lysophosphatidic acid (2 μ M) which is a potential mediator of the effect of serum and is tubulogenic for other epithelial cells.

Conclusions: The assembly of gastric epithelial cells into tubule-like structures is regulated by PKC and stimulated by gastrin. Similar signalling systems may control gastric epithelial organisation *in vivo*.

228 HIGH-THROUGHPUT MOLECULAR PROFILING USING TISSUE MICROARRAYS—ASSESSING ACCURACY & PRACTICALITY

B.C. Oates^{1,2}, J.R. Dunn², F. Campbell¹, P. Fielding², A.I. Morris¹, A. Ellis¹, J.K. Field², A.J.M. Watson¹. ¹Royal Liverpool University Hospital; ²Molecular Genetic & Oncology Group, Clinical Dental Sciences; ^{1,2}University of Liverpool, UK

Background and aims: Interest has developed in trying to identify early molecular markers of malignant potential for many neoplasms. Gene expression microarrays allow screening of thousands of genes, a tumour at a time. However, in order to assess the importance of any emerging cancer gene candidate, it is necessary to look at hundreds of specimens at different stages of tumour development. Our aim was to see if tissue microarrays provide an accurate and practical method for undertaking such high-throughput genetic profiling in oesophageal adenocarcinoma.

Patients and methods: Microarrays were constructed using paraffin embedded tissue at different stages of cancer development (normal epithelium, intestinal metaplasia, dysplasia, adenocarcinoma and positive lymph nodes where present) in 114 patients (95 men, 19 women) who had undergone resection for Barrett's oesophageal

adenocarcinoma. The age range at resection was 39–83 years (mean age 63 years). Resection staging was 17.5% T1, 14.1% T2, 68.4% T3, 58.8% N1 and 6.1% M1. Sections taken from the microarrays were then stained using 10 immunohistochemistry antibodies representing markers of cell cycle, proliferation and some known tumour suppressor genes.

Results: Successful implantation of the tissue core was achieved in 96.7% of cores taken (1145 of 1184) and the intended histology was sampled in 82.2%. The histology has remained the same with depth in 89.5% of the cores sectioned. The immunohistochemistry experiments for all 114 patients took 3 days to perform. Tumour cores stained positive in 43.1% p53 (44 of 102), 88.9% cyclin D1 (88 of 99), 91.9% Rb (91 of 99), 22.8% p16 (23 of 101), 59.4% p21 (60 of 101), 28.3% Ki67 (28 of 99), 40.6% PCNA (41 of 101), 43.9% c-myc (43 of 98), 61.6% MLH1 (61 of 99) and 41.0% MSH2 (41 of 100).

Conclusions: The accuracy of microarray construction in our study is largely consistent with published results. Tissue microarrays do appear to be a realistic and practical way of screening for expression of proteins, which may be important in looking for markers of malignant potential.

229 ACUTE PANCREATITIS AND GENETIC POLYMORPHISM OF A GLUTATHIONE REDOX REGULATING ENZYME

S.H. Rahman, Larvin, M.J. McMahon. *Academic Unit of Surgery, The General Infirmary, Great George Street, Leeds LS1 3EX, UK*

Alterations in the glutathione redox ratio (GSSH/GSSG) contributes to the early lysosomal distortion and premature enzyme activation observed in acute pancreatitis (AP). This regulation may be disturbed by altered genetic polymorphic activity of glutathione-S-transferase, thereby conferring the cell to an increased susceptibility to oxidative stress during AP. This may play a pivotal role in the progression of acute pancreatitis (AP) in humans. The prevalence of genetic polymorphisms of glutathione-S-transferase μ (GST-M1), τ (GST-T1) and π (GST-P1) was investigated in patients with mild and severe AP in relation to the magnitude of the systemic inflammatory response (SIR). DNA extracted from peripheral leukocytes was genotyped by PCR. The peak C-reactive protein level (CRP) at 0–96 hours was used as a marker of the severity of the SIR. In total, 127 patients with AP (34 severe) and 200 healthy controls were studied. The prevalence of the GST-T1*O (null) genotype was significantly under-expressed in patients with severe AP (3 %) compared to mild (24.4 %, $p = 0.003$) and healthy controls (21.7 %, $p < 0.01$). In all AP patients, the GST-T1*A genotype was associated with higher CRP levels (median 191 g/dl, IQR: 90–286) than patients with the null genotype (median 104 g/dl, IQR: 45–144, $p < 0.05$). Dichotomised variable analysis suggested the GST-T1*A genotype conferred increased susceptibility to severe disease independent of the GST-M1 genotype, however the mechanism remains unclear.

230 KNOCKOUT MOUSE AND GENE ARRAY ANALYSIS OF ETOPOSIDE INDUCED APOPTOSIS IN THE MURINE SMALL INTESTINE

D.P. McMichael, A. Davies, E. Marshman, P.D. Ottewill, J.R. Jenkins, A.J.M. Watson. *Dept of Medicine, University of Liverpool, The Duncan Building, Liverpool, UK*

Introduction: As the epithelial cells of the small intestine move up the crypt to the villi they become progressively resistant to apoptosis relative to their position along the crypt/villus axis. The genetic regulation of etoposide induced apoptosis in the small intestine is currently unknown.

Hypothesis: Etoposide induces apoptosis via a mechanism dependent on the tumour suppressor p53, the cyclin dependent kinase inhibitor p21 and the pro-apoptotic protein Bax.

Methods: BDF wildtype mice, p53, p21 and Bax knockout mice and their wildtype littermates were dosed with 10mg/kg etoposide by intraperitoneal injection ($n=4$ for each). The small intestines were isolated over a series of time points and scored for apoptotic and mitotic bodies relative to position along the crypt/villus axis by histological examination. Clontech Atlas Mouse 1.2 Gene array analysis was performed three times on mRNA extracted from small intestinal epithelial cells isolated 4.5 hours after etoposide treatment from p53 knockout mice and wildtype littermates.

Results: At 4.5 hours after administration, etoposide induced apoptosis is p53 dependent and occurs maximally between positions 3 and 9 along the crypt/villus axis. Furthermore, etoposide induced

apoptosis is independent of p21 and Bax, both thought to lie downstream of p53 in the DNA damage induced pro-apoptotic pathway. Gene array analysis of wildtype and p53 knockout mice dosed with etoposide shows 42 genes exhibiting a p53 dependent increase in expression, including MAP kinase kinase 3 (4 fold increase ± 0.8), and 13 genes exhibiting a p53 dependent decrease in expression, of particular note is the c-abl protooncogene (2 fold change ± 0.44).

Conclusions: Etoposide induces apoptosis via a p53 dependent p21/Bax independent pathway, possibly involving a range of genes identified by the gene array analysis.

231 INHIBITION OF TUMOUR FORMATION IN A MODEL OF HUMAN DIETARY COLORECTAL TUMORIGENESIS BY THERAPEUTIC TARGETING OF NF KAPPA B DEPENDENT APOPTOSIS

G.P. Collett, C.N. Robson, L. Jonker, J.C. Mathers, F.C. Campbell. *Dept of Surgery, Queen's University of Belfast and University of Newcastle, UK*

Curcumin is a potent inhibitor of NF kappa B which may influence apoptosis. This study investigates Curcumin effects on (i) regulatory pathways of apoptosis in human intestinal epithelium in vitro and (ii) apoptosis and tumorigenesis in a model for human diet related colon cancer, comprising APC^{Min} mice treated by the human dietary carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP).

Curcumin inhibited TNF α induced NF κ B activation and promoted p53 expression in HCT116 and INT407 cells. PhIP induced small intestinal epithelial apoptosis although levels were lower in APC^{Min} than in wild type C57BL/6 APC^{+/+} mice (1.9% APC^{Min} ^{+/+} vs 3.7% (APC^{+/+} [p<0.001]). PhIP promoted tumor formation in APC^{Min} proximal small intestine (4.6 PhIP alone vs 2.1 untreated; p<0.05). Curcumin enhanced PhIP induced apoptosis (4.0% PhIP + Curcumin vs 2.1% PhIP alone; p<0.01) and inhibited PhIP tumorigenesis in the proximal small intestine of APC^{Min} mice (4.6 PhIP alone vs 2.2 Curcumin + PhIP; p<0.05).

APC mutation is associated with resistance to carcinogen induced apoptosis and increased tumour formation. Curcumin reverses this apoptosis resistant phenotype through the NF kappa B pathway and inhibits PhIP tumorigenesis in proximal APC^{Min} mouse small intestine.

232 EFFECT OF PPAR α ACTIVATION ON CELLULAR PROLIFERATION AND APOPTOSIS IN HCA7 COLON CANCER CELL LINE

L.M. Jackson¹, A.J. Bennett², C.J. Hawkey¹. ¹Division of Gastroenterology; ²Dept of Biomedical Sciences, University Hospital, Nottingham, UK

Introduction: PPARs have been proposed to act as a critical link between fatty acids, gene expression and colon carcinogenesis. We have previously shown that treatment with a selective PPAR α ligand methylclofenapate reduces tumour bulk in the *Min* mice model of Familial Adenomatous Polyposis. In this study we investigated the effects of PPAR α selective ligand on DNA synthesis and cell death by apoptosis and necrosis in HCA7 colon cancer cell line.

Methods: To confirm in vitro potency and selectivity of methylclofenapate (MCP) for PPAR α activation, HCA7 cells were transiently transfected with mPPAR α , xPPAR β or xPPAR γ expression vectors and PPRE-luciferase reporter gene (PPRE-tk-luc), exposed to MCP and harvested for luciferase estimation 48 hours later. Rates of proliferation were assessed using [³H] thymidine incorporation assay. Subconfluent cultures (70%) of HCA7 colon cancer cell lines were washed and then incubated in DMEM containing 0.1% FCS for 18 hours to partially growth arrest cells. PPAR pharmacological ligands were added and cell growth restimulated by exposure to 10% FCS for 16–20 hours as appropriate. Apoptosis and necrosis was assessed by flow cytometric analysis of cells after staining with Annexin V FITC and propidium iodide.

Results: MCP caused a significant increase in PPRE-tk-luc stimulation over range of concentrations 1 μ M to 100 μ M with maximum activation of (4.20 $\pm$ 0.6) times control when PPAR α was overexpressed but caused no significant activation of PPAR- β or PPAR- γ at concentration <100 μ M. MCP did not stimulate quiescent cells at concentrations up to 100 μ M. MCP 1 μ M had a significant inhibitory effect on serum stimulated DNA synthesis (DNA synthesis reduced to 68 $\pm$ 5%, p=0.001 when ligand was added in late G1). Rates of apoptosis and necrosis were not significantly altered by exposure to PPAR α ligands MCP 100 μ M or Wy14643 100 μ M, or by PPAR γ ligand ciglitazone 5 μ M.

Conclusions: At concentrations selective for PPAR α the potent PPAR α ligand methylclofenapate significantly inhibits cellular proliferation but does not affect apoptosis. This inhibition may be the mechanism by which methylclofenapate reduces polyp number in *Min* mice.

233 CRYPT FISSION AND ITS SUBTYPES IN COLONIC ADAPTATION AND CARCINOGENESIS

N. Mandir, N.A. Wright, R.A. Goodlad. *Imperial Cancer Research Fund, Histopathology Unit, 44 Lincoln's Inn Fields, London WC2A 3PX, UK*

Background: Crypt fission involves the longitudinal splitting of branched intestinal crypts to produce two new crypts. Fission is essential for intestinal development and increased fission is seen after damage, following dietary changes and after administration of growth factors. Crypt fission is significantly increased in carcinogenesis (in the *Min* mouse, in FAP and in hyperplastic and adenomatous polyps in man). Crypt fission is thus likely to be intimately involved in the multistep process of carcinogenesis. Moreover, the prominence of different subtypes of crypt fission appears to differ in various models of intestinal adaptation and pathology.

Aims: To quantify crypt branching and subtypes of branched crypts in animal and human models of altered mucosal proliferation and of carcinogenesis.

Methods: Carnoy's fixed rat and human tissue was stained with the Feulgen reaction and carefully microdissected to reveal individual crypts. The percentage of crypts in fission and the different subtypes of crypts seen (symmetrical or asymmetrical) were scored under the microscope.

Results: Reduced fission and a higher proportion of symmetrical crypts were seen in rats after infusion of KGF or EGF. Approximately 50% of the branched crypts were symmetrical, 25% asymmetrical, 13% of the budding subtype and approximately 5% were multiple. Branching also increased in hyperplastic human tissue and to a greater extent in adenomatous polyps. All of these neoplastic tissues had significantly more asymmetrical crypts. There was progression from symmetrical to asymmetrical from control tissue to hyperplastic to adenomatous polyps (4.0%, 69% and 88% p<0.001 and p< 0.017 respectively).

Conclusion: Crypt fission is significantly increased in carcinogenesis in both animals and in man. The different forms of crypt branching may reflect different fission dynamics and contribute to the development and amplification of carcinogenesis. Symmetrical subtypes are higher in growth factor treatments and asymmetrical subtypes are dominant in carcinogenic processes.

234 RELATIONSHIP BETWEEN CYCLOOXYGENASE-2 TUMOUR EXPRESSION AND SURVIVAL IN COLORECTAL CANCER PATIENTS TREATED WITH CHEMOTHERAPY

C. Steele¹, K. Sheehan², E. Kay², D. Fitzgerald³, F.E. Murray^{1,3}. ¹Gastrointestinal Unit, Beaumont Hospital Dublin; ²Dept of Pathology, Beaumont Hospital Dublin; ³Dept of Pharmacology, Royal College of Surgeons, Dublin, Ireland

Background: Expression of cyclooxygenase COX-2 is a poor prognostic marker in colorectal cancer and is linked with both lymph node and haematogenous metastases. Recent evidence suggests a potential action of aspirin and NSAIDs in chemoprophylaxis of colorectal cancer. The use of these drugs as adjuvant chemotherapy has been suggested. The aim of this study was to determine if COX-2 expression in colorectal cancer influenced survival in patients receiving standard 5-FU chemotherapy regimes.

Methods: 60 unselected patients with previous tumour resection and who had received chemotherapy over the period 1990–1999 with 5FU based regimens were evaluated retrospectively. Paraffin embedded sections of primary tumour were cut and stained by immunohistochemistry for COX-2 protein using the avidin biotin technique. Extent of staining was graded by the percentage staining of epithelial cells positive for COX-2 by two blinded independent pathologists. Patient data were retrieved by chart review and communication with patients' primary care physicians if necessary.

Results: Of the 60 patients, mean age was 60.8 years at diagnosis (M:F 2:1). All tumours were regraded for Dukes, TNM and Jass staging. Twenty four were Dukes B, 25 Dukes C and 11 Dukes D. All patients received 5-FU and folinic acid except two who received 5-FU

alone and one 5-FU and levamisole. COX-2 was expressed in 100% of the tumours. Staining was present in tumour epithelial cells, fibroblasts, vascular endothelium and inflammatory mononuclear cells. Percentage staining and intensity of staining varied between tumours. COX-2 expression was divided into <50% (n=30) or >50% (n=30) (Grades 1-3 vs. 4). Survival analysis demonstrated that patients whose tumours expressed higher grades of COX-2 had an improved survival compared to patients with lower grades of staining. Relative risk 2.65, log rank chi-square 6.49 (p=0.0109).

Conclusion: Although COX-2 expression correlates with a poor prognosis in colorectal cancer in-patients not receiving chemotherapy. We have found in this study that increased COX-2 expression is associated with a better survival in colorectal cancer patients treated with chemotherapy. The mechanism of this is unclear but warrants further evaluation with a larger population and longer-term follow up.

235 A-GLIADIN 51-70 TOXICITY ASSESSED IN AN ORGAN CULTURE SYSTEM

S. Martucci^{1,2}, F. Biagi^{1,2}, G.R. Corazza², P.J. Ciclitira¹, H.J. Ellis¹. ¹Gastroenterology Dept, Rayne Institute (KCL), St Thomas' Hospital, London SE1 7EH, UK; ²Cattedra di Gastroenterologia, University of Pavia, Italy

Background: Three related DQ2-restricted immunodominant epitopes, which reside within the region of A-gliadin 51-75 have been recently identified. Their immunological activity has been indirectly assessed using peripheral blood mononuclear cell secretion of IFN- γ , while small intestinal T-cell clones from coeliac patients have been shown to be selectively stimulated, tTG deamidation at Q65 being crucial for T-cell recognition and DQ2 binding.

Aims: To investigate using an organ culture system in vitro toxicity of region 51-70 of A-gliadin, a similar N-terminal peptide, overlapping the above mentioned sequences.

Methods: Jejunal biopsies obtained from each of 9 treated coeliac patients were cultured in vitro for 18 hours in presence of A-gliadin 51-70 (200 μ g/ml), organ culture medium only, peptic-tryptic digested gliadin (1mg/ml) or ovalbumin (1mg/ml), the last two acting as positive and negative controls respectively. Morphometric analysis involved measuring the cell height of 30 enterocytes, randomly selected from the middle third of different villi for each section. Mean enterocyte cell heights (ECH) were compared with values for specimens cultured in medium alone.

Results: In 7/9 patients (78%) A-gliadin 51-70 was significantly toxic, p<0.0001 (Kruskal-Wallis analysis of variance) causing a 25% decrease in ECH if compared to medium alone. In 2/9 subjects (22%) the peptide did not show any toxic effect. In all cases we found that both positive and negative controls worked as expected.

Conclusions: We showed that sequence 51-70 is characterized by in vitro toxicity to the coeliac jejunal mucosa, correlating with recent findings of an immunological role of similar peptides within A-gliadin.

236 IGA-ANTITISSUE TRANSGLUTAMINASE: SENSITIVITY AND SPECIFICITY FOR DIAGNOSING COELIAC DISEASE

J. West¹, C.A. Lloyd², P.G. Hill², G.K.T. Holmes³. ¹Division of Public Health Medicine and Epidemiology, Queen's Medical Centre, Nottingham, NG7 2UH; ²Depts of Chemical Pathology and ³Medicine, Derbyshire Royal Infirmary, London Road, Derby DE1 2QY, UK

Introduction: Tissue transglutaminase (tTG) is probably the main endomysial antigen in coeliac disease (CD). Our aims were to define a reference range for IgA antibodies to tTG (tTGA), to compare sensitivity and specificity for the diagnosis of CD with IgA anti-gliadin (AGA) and endomysial (EMA) antibodies and to confirm that the assay can detect subjects with selective IgA deficiency.

Methods: AGA: quantitative in-house ELISA; EMA: immunofluorescence (monkey oesophagus, Binding Site, Birmingham, UK); tTGA: quantitative ELISA (Bindazyme MK038, Binding Site, Birmingham, UK), samples were diluted 1 in 25 to improve precision. Sera from subjects with CD were collected whilst on a normal diet.

Results: The 97.5th percentile for tTGA of 409 control subjects (normal AGA, negative EMA) was 4.0 U/ml. Eight of 100 subjects investigated for small bowel disease in whom CD was excluded by histology had tTGA>4.0 U/ml (5.5-9.6 U/ml). Sensitivity (Sens) and specificity (Spec) for AGA, EMA and tTGA in all patients with CD (n=130) and patients with EMA positive CD (n=122) are compared

in the table. The 2.5th percentile for tTGA in the 409 control subjects was 0.2 U/ml. The assay identified 17 of 18 subjects with selective IgA deficiency.

Abstract 236, Table 1

Subjects with CD (histology proven)	AGA		EMA		tTGA (>4.0 U/ml)	
	Sens	Spec	Sens	Spec	Sens	Spec
All CD cases (n=130)	76.2	95	93.8	>99.5	86.2	97.5
EMA positive (n=122)	74.6	95	100	>99.5	90.2	97.5

Conclusions: This study has defined the normal range for tTGA for this assay and confirmed the high sensitivity and specificity of this marker for the diagnosis of CD. However tTGA is not as sensitive and specific as EMA for diagnosing CD. These data support the use of tTGA instead of AGA as the first line screening test for CD.

237 PREVALENCE OF UNDIAGNOSED COELIAC DISEASE IN THE GENERAL POPULATION OF ENGLAND

J. West¹, C.A. Lloyd², R. Reader³, P.G. Hill², G.K.T. Holmes⁴, K.T. Khaw³, R.F.A. Logan¹. ¹Division of Public Health and Epidemiology, University of Nottingham, Nottingham NG7 2UH; ²Depts of Chemical Pathology and ³Medicine, Derbyshire Royal Infirmary, London Road, Derby DE1 2QY; ⁴Clinical Gerontology Unit, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK

Introduction: Recent estimates from several European countries suggest that the prevalence of Coeliac disease (CD) is between 0.5 and 1%. No data have previously been reported from mainland UK. We aimed to estimate the prevalence of CD in the general population based on subjects aged 45-74 recruited from General Practice age-sex registers in Cambridge for a UK health survey.

Methods: Stored serum collected during the Cambridge General Practice Bone Health Study 1990-94 were tested for total IgA and anti-endomysial antibody (EMA: immuno-fluorescence-monkey oesophagus, Binding Site, Birmingham, UK). The specificity of EMA is at least as high as 99.9%. 3354 of approximately 8000 samples have been tested to date. IgA antitissue transglutaminase assays are currently being conducted (Bindazyme MK038, Binding Site, Birmingham, UK).

Results: The distribution of age, sex and proportion of positive EMA results are shown in the table.

Abstract 237, Table 4

EMA result	44-54 years		55-64 years		65-74 years		Total
	M	F	M	F	M	F	
Pos	10	8	10	7	2	5	42
Neg	862	660	619	499	281	391	3312
Total	872	668	629	506	283	396	3354
%	1.1	1.2	1.6	1.4	0.7	1.3	1.3

None of the 42 EMA positive subjects were known at recruitment to have CD.

Conclusions: This study found that the estimated prevalence of undiagnosed CD in this general population sample is 1.3% (95% CI 0.9-1.6%). Prevalence is similar in men and women. This is the largest general population sample studied and the prevalence of CD is higher than that of any previous study in the UK or elsewhere. CD may therefore affect up to 1 in 80 people aged 45-74 years in England.

238 MANAGEMENT OF ADULT COELIAC DISEASE: IMPLEMENTATION OF CURRENT BSG GUIDELINES THROUGH A SPECIALIST CLINIC

M. Priest¹, A. Galloway, A.J. Morris¹. ¹Dept of Dietetics, Glasgow Royal Infirmary; ²Dept of Gastroenterology, Glasgow Royal Infirmary, UK

Introduction: An audit of the management of patients with coeliac disease (CD), attending our Gastroenterology clinic, was undertaken to compare management with current British Society of Gastroenterology Guidelines. Subsequently a CD review clinic has been established to continue patient care.

Results: 222 patients were identified and 192 sets of case notes reviewed. Age and mode of presentation, sex distribution and co-morbidity did not differ significantly from other published series. Small bowel biopsy (SBB) had been performed on 181 patients (95.6%), however SBB had only been repeated in 91 (48%) of cases to assess response to gluten free diet (GFD). 32 patients were responsive, 42 showed no histological response, and 7 had serious complications of CD. Compliance with GFD was considered to be good in 118 (61.5%) of patients, and poor in the remainder. IgA antiendomysial antibodies (AEA) had never been checked in the majority (57.8%) of cases. DEXA scans had been performed in 34 patients (17.7%), although of those performed 27 (79.4%) show osteoporosis (BMD >2.5SD below mean for young adults). The CD review clinic has been established to allow review of all patients by a clinician and dietician. To date 125 patients have been reviewed (43 men, 82 women). Compliance with GFD was considered to be good in 82 cases (65.6%), of which 59 (72%) were AEA negative, 10 (12.2%) were AEA positive and equivocal in 13 (15.9%). Only 51.2% of patients were members of the Coeliac Society. Nutritional deficiencies were detected in 28.2% (19 patients folate deficient, 12 iron deficient, 3 B₁₂ deficient, 2 vitamin D deficient). DEXA scans were requested on 91 patients, 65 have been examined to date, of which 25 (38.5%) show osteoporosis and 10 (15.4%) show osteopenia.

Conclusions: Management of CD in our unit did not meet the standards suggested in current guidelines. As a consequence complications, particularly osteoporosis and ongoing nutritional deficiencies, were not being detected at routine follow up. The establishment of a specialist clinic has enabled a co-ordinated approach between clinician and dietician, and enabled compliance with current guidelines.

239 PRIMARY SMALL BOWEL MALIGNANCY IN THE UNITED KINGDOM—FINAL REPORT OF THE BSG NATIONAL SURVEY

P.K. Jalal¹, G.K.T. Holmes², R.S. Houlston³, P.D. Howdle¹. ¹St James's University Hospital, Leeds; ²Derbyshire Royal Infirmary; ³Institute for Cancer Research, Surrey; and The BSG Research Unit, UK

Objective: Primary small bowel malignancy is rare and little is known about its prevalence in the UK. This prospective survey aimed to obtain information regarding its presentation and prevalence and, in particular, its association with coeliac disease.

Study design: Cases were collected nationally over 24 months (June 98—May 2000) using the BSG monthly reporting system followed up by a preliminary form for confirmation. Further details of confirmed cases were obtained via a detailed questionnaire.

Results: A mean of 1400 cards was sent out each month, the mean response rate of returned cards was 44.5%. A total of 552 cases were reported (mean, 23 per month). The preliminary form was returned in 424 cases (77%) confirming primary small bowel malignancy in 373 (88%). Of these, 163 were adenocarcinoma (44%), 99 lymphoma (26%), 79 carcinoid (21%) and 32 leiomyosarcoma (9%). Detailed clinical information was obtained in 282 (83%) of the 341 cases of adeno-carcinoma, lymphoma and carcinoid. These data will be presented. Coeliac disease was present in 38 cases (38%) of lymphoma and in 17 cases (10%) of adenocarcinoma. Patients with lymphoma associated with coeliac disease had a high mortality at the time of reporting (64%); the mean survival from onset of symptoms was 12.9 months and from diagnosis was 3 months. The lymphoma in 85% of these cases was an EATL. Patients with adenocarcinoma, carcinoid and lymphoma without coeliac disease had better survival rates at the time of reporting (85%, 85% and 74% respectively). Comparison with the observed incidence of small bowel malignancies in the coeliac population of Derby over 28 years suggests that these nationally reported incidence figures, while allowing for expected under-reporting, are similar to those that are predicted.

Conclusions: Primary small bowel malignancy is rare, as is lymphoma, in coeliac disease. When small intestinal lymphoma does occur, it is commonly associated with coeliac disease and still carries a poor prognosis. The BSG data collection system, despite the modest response rate, has been useful in providing information about these rare tumours.

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240 FAS-LIGAND PROTECTS ISLET CELLS FROM APOPTOSIS IN CHRONIC PANCREATITIS

A.C. Bateman¹, K.S.A. Thomas¹, D.R. Fine², P.A. Johnson², C.D. Johnson³, J.P. Iredale². ¹Pancreatic & Liver Fibrosis Research Groups, Dept of Histopathology, ²Division of Cell and Molecular Medicine, and ³University Dept of Surgery, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK

Background: Exocrine (acinar) destruction and endocrine (islet) preservation in chronic pancreatitis (CP) is related to differential apoptotic indices within these cellular compartments.

Aim: To define the role of cell cycle regulatory proteins in the control of pancreatic epithelial cell apoptosis.

Methods: Formalin-fixed, paraffin-embedded tissue from six cases of CP and eight normal controls (N) were studied using immunohistochemistry for BCL-2, Bax, retinoblastoma protein (Rb) and Fas ligand (Fas-L). Labelling patterns were assessed using semi-quantitative (intensity-proportion [I-P]) or fully quantitative (labelling index [LI]) analysis.

Results: Rb protein was expressed at a higher level by acinar cells in CP than N (mean acinar cell Rb LI 1.45% in CP and 0.437% in N; $p < 0.05$) but this difference was not observed within islet cells. BCL-2 was more strongly expressed by acinar than islet cells in CP and N (e.g. N mean BCL-2 I-P score 4.88 in acini and 0.375 in islets; $p < 0.05$) while Bax was strongly expressed by a subset of islet cells and weakly by centroacinar cells (e.g. N mean Bax I-P score 1.75 in acini and 4.31 in islets; $p < 0.05$). Fas-L was more strongly expressed by islet than acinar cells in CP and N (e.g. CP mean Fas-L I-P score 5.67 in islets and 2.83 in acini; $p < 0.01$).

Conclusion: Strong BCL-2 expression by acinar cells and of Bax by islet cells was an unexpected result and indicates complex control of apoptosis within these cell populations. Increased Rb protein expression by acinar cells in CP may differentially promote apoptosis within these cells. Fas-L expression by islet cells may protect islets from apoptosis by promoting apoptosis of cytotoxic T-lymphocytes.

241 THE EFFECT OF SELECTIVE INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE ON THE OUTCOME OF SEVERE ACUTE PANCREATITIS

R.A. Al-Mufti, R.C.N. Williamson, R.T. Mathie. Division of Surgery, Anaesthetics & Intensive Care, Hammersmith Hospital, Imperial College School of Medicine, London W12 0NN, UK

Background: Nitric oxide (NO), overproduced by inducible NO synthase (iNOS), has been established as a key inflammatory mediator in systemic inflammatory response syndrome leading to multiple organ failure.

Aim: To examine the effect of treatment with the selective iNOS inhibitor L-N6-iminoethyl-lysine (L-NIL) on the course of severe acute pancreatitis (AP) in the rat, both local pancreatic and systemic organ failure.

Methods: Acute severe necrotising pancreatitis was induced in 10 adult male Wistar rats by a combination of biliopancreatic duct infusion of glycodeoxycholic acid and arterial infusion of caerulein. Data were compared to those in sham operated controls (n=10). The effect of L-NIL treatment was examined in a disease group (n=10) and in a control group (n=10). L-NIL was administered two hours after induction of pancreatitis. Blood pressure, PaO₂, and serum creatinine were measured at 7 h. Pancreatic sections were examined histologically and morphometric measurement of areas of acinar cell necrosis as a percentage of total acinar tissue area was performed.

Results: Compared with controls at 7 h, the pancreatitis group displayed reduced mean arterial blood pressure [mean (s.e.m.) 77.8 (3.7) versus 108.2 (2.2) mmHg, $P < 0.0001$], reduced PaO₂ [66.7 (1.9) versus 115.2 (3.6) mmHg, $P < 0.0001$], and raised serum creatinine [132.5 (20.6) versus 41.8 (2.5) µmol/L, $P < 0.0001$]. Treatment with L-NIL corrected the reduction in blood pressure [mean (s.e.m.) 113.5 (3.4) mmHg, $P < 0.0001$], and ameliorated the reduction of