Conclusions: In-patients with diarrhoea usually have a non-infectious cause for their condition. In the most of these, the cause is iatrogenic. When the cause is enteric infection, by far the commonest cause is *C. difficile* (not routinely tested for), with all other pathogens (routinely tested for) being very uncommon. All in-patients with hospital-acquired diarrhoea should be tested for *Clostridium difficile* infection routinely.

307 ABSTRACT WITHDRAWN

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308 THE EFFECT OF SULPHASALAZINE, 5-AMINOSALICYLIC ACID AND SULPHAPYRIDINE ON SULPHIDE PRODUCTION BY SULPHATE REDUCING AND AMINO ACID FERMENTING BACTERIA

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Background: Reduced sulphur compounds, such as sulphide, have been implicated in ulcerative colitis (UC).¹ Sulphide may be produced either from fermentation of sulphur amino acids or reduction of sulphate by sulphate reducing bacteria (SRB). In faecal slurries ASA and sulphasalazine (SAS) have been shown to reduce sulphide production from sulphate² and from methionine.³

Aims: To look at the effects of SAS, ASA and sulphapyridine (SP) in pure cultures on sulphide production from SO₄ by SRB and sulphur amino acids by an amino acid fermenter.

Methods: An amino acid fermenter producing sulphide was isolated from a human faecal sample and provisionally identified as a fusobacterium. Desulfovibrio desulphuricans was the SRB used. The SRB was grown in Postgate C in universals and the fusobacterium was grown in Wilkins Chalgren anaerobe broth with 0.5g/l cysteine and 0.5g/l methionine. Concentrations of SAS, ASA or SP from 0 to 40mM were added to the universals prior to autoclaving. 20mM ASA is considered physiological. After cooling 0.5ml fusobacterium or SRB stock was injected into each universal and the culture was incubated at 37°C for 18hr. 1ml of solution was used for sulphide determination by microdistillation and HPLC.

Results: SAS achieved a 90% inhibition of sulphide production from SRB and fusobacterium at 1mM and 5mM respectively. ASA 90% inhibited SRB at 20mM but only achieved about 50% inhibition from fusobacterium over the target range. SP had no significant effect on sulphide production from either bacterium.

Conclusion: In pure bacterial cultures SAS is more effective than ASA at inhibiting sulphide production from SO₄ and sulphur amino acids.

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3. Roediger WEW, Duncan A. *Med Sci Res* 1996;24:27-9.

309 PLATELET ACTIVATION MAY INITIATE LEUCOCYTE-PLATELET AGGREGATION IN INFLAMMATORY BOWEL DISEASE

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Background: We have recently shown that the formation of leucocyte-platelet aggregates is increased in the peripheral blood of patients with IBD (Irving, UEGW 2000). Platelets are known to have proinflammatory as well as thrombotic effects and their activation is increased in IBD (Collins, *Gastroenterology* 1994;106:840-5).

Aims: To assess whether platelet and/or leucocyte activation are correlated with, and might therefore induce formation of leucocyte-platelet aggregates in IBD.

Methods: 56 patients with IBD (29 Crohn's disease, 27 ulcerative colitis) and 19 healthy controls had venous blood drawn into EDTA and CTAD (citrate, theophylline, dipyridamole and adenosine). Samples were immediately mixed and then analysed by flow cytometry for P-selectin (CD62P—for platelet activation), L-selectin (CD62L—for neutrophil activation) and CD45/CD42a (leucocyte-platelet aggregates). Platelet activation was also assessed by analysis of mean platelet mass, an inverse composite measure of platelet degranulation and volume, using an ADVIA Haematology System (Bayer).

Results: We confirmed that leucocyte-platelet aggregation was increased in IBD (median 4.3 (range 1.2-17.3)) compared with controls (3.3 (1.5-7.4), $p<0.05$). The mean platelet mass was lower in IBD (1.9 (1.6-2.2)) than in controls (2.0 (1.7-2.3), $p<0.05$), but activation of platelets as measured by CD62P and of leucocytes (CD62L) were similar in the two groups. Platelet (CD62P), but not leucocyte (CD62L), activation correlated positively with leucocyte-platelet aggregates in IBD ($R=+0.44$, $P<0.01$).

Conclusions: Leucocyte-platelet aggregation is increased in patients with IBD, and the correlation with platelet activation measured by P-selectin, supports the hypothesis that platelets may contribute to the pathogenesis of the disease by aggregating with circulating neutrophils. The formation of aggregates does not appear to be related to the expression of CD62L on neutrophils.

310 PERTURBATION OF CLASSICAL CADHERINS IN THE LYMPHOID ASSOCIATED EPITHELIUM; FURTHER INSIGHT INTO THE PATHOGENESIS OF EARLY CROHN'S LESIONS

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Background: The pathogenesis of early Crohn's lesions is poorly understood but probably involves focal alteration of the integrity of the mucosal epithelium with a host inflammatory response to gut luminal antigens. Histologically early pre-ulcerating and aphthoid lesions are often characterised by mucosal lymphoid aggregates. Cadherins have a major adhesive role in maintenance of mucosal integrity and their expression may be altered by inflammatory cytokines.

Aims: We aimed to investigate a putative link between inflammation and epithelial cadherin expression, which may give further insight into the pathogenesis of early lesions in Crohn's disease.

Methods: Endoscopic and surgical resection material was collected from patients with proven Crohn's disease. Examples of pre-ulcerating lesions, early/aphthoid ulcers and non-involved bowel were serially sectioned and stained by routine immunohistochemistry for E- and P-cadherin (E- and P-cad), β - and γ catenin, the proliferation marker Ki-67 (MIB-1) and lymphocyte markers CD 20 and CD3.

Results: Membranous upregulation of P-cad, co-expression with E-cad, and increased mucosal proliferation was seen exclusively in crypt and surface epithelium immediately adjacent to lymphoid aggregates and reactive follicles in pre-ulcerating Crohn's lesions (8/10), adjacent to lymphoid follicles in non-involved bowel (5/10), and in crypts adjacent to aphthoid ulcers (10/10). No alteration in catenin expression was noted.

Discussion: Upregulation of P-cad and co-expression with E-cad is a phenotype associated with cell proliferation, migration, repair and neoplasia in the GI tract. Preliminary experimental data suggests cadherin expression may be influenced by inflammatory cytokines such as TNF α . Our current data shows that perturbation of the main epithelial adhesion molecules, the cadherins, is intimately associated with patchy focal accumulation and activation of lymphocytes in Crohn's disease, which may influence mucosal permeability and hence integrity, initiating patchy superficial aphthoid ulceration.

311 A DISTINCT PROFILE OF SOLUBLE SELECTIN DURING ACTIVE AND INACTIVE PHASES OF INFLAMMATORY BOWEL DISEASE (IBD)

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Introduction: Data concerning selectin measurements in the serum of patients with IBD and their correlation with disease activity is limited and somewhat contradictory.

Aims: To determine the levels of soluble (s) selectin (sE, sP, sL) in the serum of patients during active and inactive phases of IBD and examine their correlation with disease activity and laboratory markers (ESR, CRP, platelet count).

Methods: The levels of selectin in the serum of 14 patients with active Crohn's disease (CD), 30 with active ulcerative colitis (UC) and 22 healthy controls were studied. A second blood sample was taken from IBD patients one month after remission was achieved. Disease activity in CD was evaluated by the CDAI score and in UC by the activity index as described by Walmsley et al. Selectin levels are expressed as mean $\pm$ SD ng/ml.

Results: Both sE-selectin and sP-selectin levels were significantly higher ($p<0.01$) during the active phase of CD [(65.1 $\pm$ 19.9) and (227.2 $\pm$ 78.1), respectively] and active phase of UC [(57.3 $\pm$ 23.5) and (227.6 $\pm$ 173.5)] compared to those of inactive phase of CD [(40.7 $\pm$ 12 and 167.3 $\pm$ 68.6)] and of UC [(38.2 $\pm$ 18.5 and 154.1 $\pm$ 96.9)] or healthy controls [(38.2 $\pm$ 15.7) and (156.8 $\pm$ 65)]. sL-selectin levels were significantly higher ($p<0.01$) during active phase of UC (1241.3 $\pm$ 240.6) compared to inactive phase (1021.6 $\pm$ 245.6) or controls (915.3 $\pm$ 195.8). No significant differences were found for sL-selectin levels between active and inactive phases of CD and the controls. No significant correlation between selectin levels and disease activity or laboratory markers was found.

Conclusions: Our data show that serum levels of sP and sE selectin are significantly different between active and inactive phases of IBD in the same patients but they do not correlate with disease activity. This is also true for sL-selectin levels, but only for UC patients.

312 THICKNESS AND CONTINUITY OF THE HUMAN COLONIC MUCUS LAYER IS DECREASED IN ACTIVE UC BUT REMAINS NORMAL IN QUIESCENT UC

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Introduction: The colonic epithelium is protected from luminal aggressors such as bacterial enzymes, toxins and shear stress by the adherent mucus layer. It has previously been shown that the colonic mucus layer is reduced in thickness in ulcerative colitis (UC). Here we present a blinded investigation into the effect of UC, active and quiescent, on the thickness and continuity of the rectal mucus layer using an improved method of visualising the mucus gel.

Methods: Rectal biopsies were taken from patients with either histologically normal bowel or with UC. Patients gave written informed consent. Biopsies were immediately snap frozen and cryostat sections were stained with periodic acid-Schiff's/Alcian Blue under conditions which preserve the mucus layer. Mucus thickness and continuity were measured by light microscopy by an observer blinded to patient diagnosis.

Results: There was no difference in the thickness of the rectal mucus layer in normals ($n=11$) and patients with quiescent UC ($n=21$) both with median (IQR) thickness of 25 (10–50) μ m, which was fully continuous. Median (IQR) mucus thickness in active UC ($n=19$) was 10 (5–35) μ m, significantly thinner than the mucus layer in both the normal and quiescent UC groups ($p<0.0001$). The mucus layer became discontinuous in active UC with a median percentage discontinuity of 19%, 8% and 43% in mild, moderate and severe UC respectively. As disease severity increased (Powell-Tuck symptom score) there was a significant correlation ($p=0.003$) with decreased continuity of the mucus layer.

Conclusions: The colonic mucus layer is compromised, in terms of both thickness and continuity, in active UC. This will allow luminal aggressors to gain access to and damage the underlying epithelium and exacerbate the disease. The mucus layer appears to be restored to normal thickness and continuity when UC enters remission.

313 ALTERED EXPRESSION OF FUCOSYL-TRANSFERASES IN INFLAMMATORY BOWEL DISEASE

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Background: We have previously hypothesised that alterations in glycosyltransferases may be a key event in the pathogenesis of IBD. As support for this concept, mice transfected with human H fucosyltransferase spontaneously develop colitis (*Gastroenterology*

2000;118:A3769). Fucosyltransferases attach fucose to oligosaccharides forming structures such as Lewis and H blood groups. We have examined the expression of four fucosyltransferase genes (*FUTs*) in IBD epithelium.

Methods: With informed consent colonoscopic biopsies were taken from patients with normal colon (NC) ($n=9$), ulcerative colitis (UC) ($n=9$) and Crohn's disease (CD) ($n=5$) and epithelial cells isolated, to exclude contamination by fucosyltransferases from other cell populations. Semiquantitative RT PCR was then carried out for *FUT 1*, 2, 5 and 6. Products were electrophoresed and band intensity measured. The results were calculated as the ratio of each *FUT* to cytokeratin 20 (an epithelial cell marker gene) and expressed as a percentage of normal colon expression. Statistical analysis was by ANOVA.

Results: Mean (SEM) abundance of *FUT 1*, 2, 5 and 6 expressed as a percentage of normal colon expression. * $p<0.05$, ** $p<0.001$. Sialyl Lewis^x (SLe^x).

Abstract 313, Table 1

	Epitope formed	NC ($n=9$)	UC ($n=9$)	CD ($n=5$)
<i>FUT 1</i>	H antigen	100 (8.9)	77 (5.0)	87 (20.4)
<i>FUT 2</i>	Secretor (Se)	100 (14.8)	47 (1.4)**	56 (7.1)**
<i>FUT 5</i>	Lewis ^x , SLe ^x	100 (16.4)	168 (18.4)*	199 (41.9)*
<i>FUT 6</i>	Lewis ^x , SLe ^x	100 (18.9)	108 (6.7)	88 (23.4)

Discussion: There was a significant decrease in *FUT 2* and significant increase in *FUT 5* expression in both UC and CD. These enzymes are involved in the production of the secretor and Lewis antigens respectively. These data suggest that changes in the abundance of fucosyltransferases may be important in the alteration in glycosylation seen in inflammatory bowel disease.

314 TNF α GENE POLYMORPHISMS AND EXTRAINTestinal MANIFESTATIONS OF IBD: FURTHER EVIDENCE FOR PHENOTYPE DETERMINING GENES IN THE MHC REGION

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Background: Type 1 and 2 peripheral arthritis (PeA), erythema nodosum (EN) and uveitis are distinct, but overlapping extraintestinal manifestations (EIMs) of inflammatory bowel disease. We have previously described the clinical association of Type 1 PeA with both EN and uveitis, and Type 2 PeA with uveitis only. In addition we have described associations with distinct HLA-B and HLA-DR alleles, which suggest that EIMs may be determined by genes in this region, in linkage disequilibrium with each other. To investigate this further this study was undertaken to examine polymorphisms of the TNF α gene in these EIMs. TNF α is an important cytokine in mediating inflammation, and the gene lies on chromosome 6 between HLA-B and HLA-DR.

Methods: EDTA blood was collected from 28 patients with Type 1 PeA, 24 patients with Type 2 PeA, 29 patients with EN and 29 patients with uveitis. DNA was extracted and the presence of four polymorphisms in the TNF α gene, at positions -1031, -380, -308 and -238 was assessed by a pcr based technique using sequence specific primers. The results of the different groups were compared with each other and with 261 healthy controls, using 2x2 contingency tables and Fisher's exact test.

Results: There was a significant association between EN and possession of the -1031C polymorphism - 20/29 (69%) patients vs 96/261 (37%) of controls ($p=0.001$, $pc=0.016$). There was no significant difference in the prevalence of this polymorphism between EN and Type 1 PeA patients (69% vs 46%), but there was a significant difference between EN and Type 2 PeA (69% vs 25%, $p=0.002$, $pc=0.024$), and between EN and uveitis (69% vs 34% $p=0.006$), although the latter did not withstand correction for multiple comparisons ($pc=0.07$). No associations were seen at positions, -380, -308 or -238 with any EIMs.

Conclusions: These data demonstrate a strong association between EN and polymorphism -1031 of the TNF α gene. In addition they support the hypothesis that the clinical phenotype of extraintestinal manifestations may be determined by genes in linkage disequilibrium within the MHC region. This may determine the distinct yet overlapping clinical syndromes of arthritis, EN and uveitis.

315 NOVEL CHEMOKINE RECEPTOR 9 POLYMORPHISMS: CANDIDATE GENES FOR CROHN'S DISEASE (CD)?

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Introduction: Chemokines are chemoattractant cytokines that attract and activate leukocytes. Three independent linkage studies have implicated 3p21 as a susceptibility locus for IBD. The chemokine receptor cluster (3p21.3) is located within this region of linkage. Chemokine receptor 9 (CCR-9) is expressed in high levels on all small bowel CD4+ and CD8+ T lymphocytes (but only on a small subset of colonic lymphocytes). Thymus expressed chemokine (TECK) is the only known agonist for CCR-9 and is selectively chemotactic for small bowel, but not colonic, lamina propria mononuclear cells. Thus, functionally and positionally, CCR-9 is an attractive candidate gene for small bowel CD. No CCR-9 polymorphisms have been previously described.

Aims: To identify polymorphisms in CCR-9 and to test their association with small bowel CD.

Methods: We screened the exonic and 500 bp of 5' promoter sequence of CCR-9 in 12 healthy individuals using DHPLC (transgenic wave). Heterozygote traces were verified by direct sequencing. PCR-RFLP assays were developed. Allele counts were compared in 2x2 tables. More than 97.5% of reactions were successful.

Results: The 6 SNPs identified were genotyped in 116 patients with small bowel CD and 242 healthy controls.

Abstract 315, Table 1

	Allele frequency		
	Controls	Small bowel CD	
SNP 1	15.3%	19.4%	p = 0.17*
SNP 2	10.2%	8.6%	p = 0.40*
SNP 3	2.1%	0.9%	p = 0.35**
SNP 4	8.0%	6.6%	p = 0.54**
SNP 5	0.4%	0.4%	p = 1.0**
SNP 6	1.7%	0.0%	p = 0.06**

*chi-squared test, **Fisher's exact test

Conclusions: No significant association was found between these novel CCR-9 polymorphisms and small bowel CD. SNP 6 warrants further investigation in a larger cohort.

316 ANALYSIS OF CANDIDATE GENES WITHIN THE CHROMOSOME 16 REGION ASSOCIATED WITH SUSCEPTIBILITY TO IBD

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Background: Inflammatory bowel disease (IBD) is a complex disorder influenced by both environmental and genetic factors. Genome-wide linkage screens have identified numerous chromosomal regions associated with an increased risk of IBD, with a region on chromosome 16 (≤ 30 Mb) being the most widely implicated in influencing susceptibility to Crohn's disease.

Aim: We aim to identify the gene(s) conferring susceptibility to IBD within the chromosome 16 region. Our approach involves identifying single nucleotide polymorphisms (SNPs) in candidate genes and then testing these SNPs for association with the IBD phenotypes using the transmission disequilibrium test.

Methods/results: Candidate genes implicated in the inflammatory response, and mapping to chromosome 16, are identified from published literature, mined from sequence databases or inferred using gene prediction programs, and subjected to SNP analysis. SNPs are identified either *in silico*, or experimentally using denaturing high performance liquid chromatography. Initial studies on a chemokine gene cluster have identified 14 SNPs across a 57 kb region incorporating 3 chemokines (SCYA22, SCYD1 and SCYA17) with the frequency of the least common allele ranging from 0.016 to 0.251. Within these 14 SNPs 6 distinct linkage disequilibrium groups have been identified. One SNP from each LD group are being genotyped in a large cohort

of British and German families using a biallelic discrimination assay and the association of each allele with IBD phenotypes tested. Systematic application of this approach should lead to the identification of candidate susceptibility gene(s).

317 IS THERE A ROLE FOR MICA AND MICB IN DETERMINING SUSCEPTIBILITY AND PHENOTYPE IN ULCERATIVE COLITIS?

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Background: The non-classical MHC class I like genes MICA and MICB are located within IBD3, a replicated area of linkage on chromosome 6. These genes encode stress inducible membrane-anchored glycoproteins, expressed by epithelial cells of the gastrointestinal tract that interact with NK and $\gamma\delta$ T-cells through the receptor NKG2D. Exons 2-4 encode the extracellular domain and are highly polymorphic. A previous study examining a limited number of alleles of MICA only, in a small population of patients with ulcerative colitis (UC) found an association with MICA*007. This study examined all known MICA/B alleles in a larger cohort of UC patients to determine whether disease susceptibility and phenotype may be determined by MICA/B alleles.

Methods: A PCR-SSP system was developed to identify the 45 known MICA and 9 MICB alleles. This typing technique was applied to 287 UC patients divided into 3 phenotypic subgroups (116 total colitis + no surgery; 68 total colitis + proctocolectomy / ileo-anal pouch formation; 103 proctitis + no surgery) and 296 healthy Oxfordshire controls recruited from General practice health screening clinics.

Results: Of the 45 previously reported MICA alleles 19 and 18 were identified in the UC and control populations respectively. 5 of the previously reported MICB alleles were identified in both the control and UC groups. 10 new alleles (frequency less than 1%) were identified (4 controls, 6 UC). No significant difference was found in allele frequencies between controls and UC patients when analysed by phenotypic subgroup or as a combined group.

Conclusion: Polymorphisms in exons 2-4 of the MICA/B genes appear not determine susceptibility or phenotype in patients with UC. Further work is now being carried out to evaluate the role of polymorphisms in exon 5 that encodes the transmembrane portion of the MIC molecule.

318 ASSOCIATION OF INTERLEUKIN-1 α AND INTERLEUKIN-1 β GENE ALLELES WITH ULCERATIVE COLITIS: EVIDENCE FOR GENETIC SUSCEPTIBILITY

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Background: Case-control studies in ulcerative colitis (UC) assessing single nucleotide polymorphisms (SNP) within the interleukin-1 genes have not demonstrated significant associations. However, these studies had limited power for the detection of genes of modest effect on disease susceptibility.

Aims: To further assess the IL-1 α (IL1A) and IL-1 β (IL1B) genes as candidate loci for disease susceptibility and severity in UC in a large well characterised cohort of patients.

Patients and methods: 319 UC patients whose disease diagnosis and extent had been confirmed by endoscopic and histological criteria were studied. 84 of these patients had undergone colectomy. 1116 anonymous blood donors were used as controls. All individuals were genotyped for the -511 and +3954 SNPs in IL1B and the +4845 SNP in IL1A using the Taqman allelic discrimination system. Chi-squared statistics were used to compare allele carriage rates and odds ratios were calculated.

Results: Homozygous carriage of +3954 allele 1 was associated with UC (P=0.002; OR 1.5(1.2-1.9) but not surgery (P>0.05). The -511 allele 2 was not associated with disease (p>0.05) but was increased in surgical compared to non-surgical patients (p=0.07; OR 1.6(0.96-2.7). Carriage of +4845 allele 1 was associated with disease (p = 0.007; OR 1.41(1.1-1.8).

Conclusions: Genes in the IL-1 gene cluster other than the IL-1 receptor antagonist gene may have a role in determining disease susceptibility and behaviour.

Abstract 318, Table 1

	+3954 IL1B			-511 IL1B			+4845 IL1A		
	11	12	22	11	12	22	11	12	22
Controls	609	435	72	490	486	140	524	487	105
UC	204	102	12	129	154	36	177	122	20
Surgical	148	77	9	102	110	23	132	87	16
Non-Surgical	56	25	3	27	44	13	45	35	4

319 TRANSMISSION DISEQUILIBRIUM TESTING CONFIRMS THE ASSOCIATION OF THE TNF α -1031C ALLELE WITH CROHN'S DISEASE

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Introduction: Increased tumour necrosis factor (TNF) expression in the TNF ARE mouse model results in a Crohn's disease (CD) like phenotype, and anti-TNF therapy is effective in treating CD. Linkage studies from five groups worldwide have confirmed an inflammatory bowel disease (IBD) susceptibility locus on chromosome 6p. We have previously reported a significant association with the TNF α promoter -1031 C allele and CD in both Japanese and European Caucasian case-control populations. In order to overcome potential false positive results from a case control study, IBD families were genotyped and the transmission disequilibrium test (TDT) was used to assess association.

Aims: To confirm the association seen in the case-control studies in an independent family based population.

Methods: We obtained complete genotypes for the TNF α -1031 C/T polymorphism using PCR-SSP in 515 parent-child trios from 434 European Caucasian nuclear families (349 simplex, 85 multiply affected). A permutation based probability test was used to calculate association independent of linkage (Aspex).

Results: We observed significantly increased transmission of the TNF α -1031 C allele for the CD but not IBD or UC phenotypes.

Abstract 319, Table 1

	n genotyped trios	C allele transmission	Significance
IBD	515	TR 197 : NT 179	n.s.
CD	273	TR 118 : NT 81	p=0.01
UC	233	TR 77 : NT 96	n.s.

Under a multiplicative model the genotype relative risk of the C allele is 1.5, and the population attributable risk approximately 20%.

Conclusion: We have confirmed the association of the tnf -1031 polymorphism with Crohn's disease by the TDT. This common allele (22% frequency), or one in linkage disequilibrium with it, influences nearly a fifth of the cases of Crohn's disease in a Caucasian population.

320 TWO NOVEL MUCIN CORE GENES MUC 11 AND MUC 12 ARE NORMALLY EXPRESSED IN ULCERATIVE COLITIS

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Background: Two novel mucin core genes have recently been described, MUC 11 and MUC 12, both of which are downregulated in colon cancer (*Cancer Research* 1999;59:4083). Both are located on chromosome 7q22 which has been identified as a candidate IBD locus. Mucins are abnormal in ulcerative colitis (UC) where the mucus layer is thinner, less glycosylated and less sulphated. MUC 11 or MUC 12 may therefore represent potential candidate genes for UC. The aim of this study was to assess mRNA abundance of MUC 11 and MUC 12 in UC using Northern analysis and semi-quantitative RT-PCR.

Methods: With informed consent, colonic biopsies were taken from patients with ulcerative colitis and patients with normal colon. Twenty micrograms of total RNA was electrophoresed and transferred onto nylon membranes. Membranes were then sequentially hybridised with ³²P labelled PCR generated cDNA probes for MUC 11, MUC 12 and 28S ribosomal RNA. mRNA abundance of MUC 11

and MUC 12 was expressed as a ratio of 28S RNA expression by phosphorimage analysis. For semi-quantitative RT-PCR 5 μ g of total RNA was subjected to first strand cDNA synthesis and then PCR was performed using specific oligonucleotide primers for MUC 11, MUC 12 and cytokeratin 20 (a marker of epithelial abundance). PCR band intensity was quantified and expressed as a ratio of cytokeratin 20 abundance. All results were expressed as a percentage of control normal colon (100%).

Results: Northern analysis for MUC 11 and MUC 12 revealed a polydisperse signal typical of mucins. A wide variation in expression of both mucins was seen with no significant difference between groups: MUC 11 (mean $\pm$ SE) normals (n=10) 100 $\pm$ 35%, UC (n=18) 144 $\pm$ 30, MUC 12 normals 100 $\pm$ 20, UC 120 $\pm$ 17. Semiquantitative PCR likewise showed no significant differences between groups: MUC 11 normals (n=6) 100 $\pm$ 18%, UC (n=6) 90 $\pm$ 38% and MUC 12 normals (n=6) 100 $\pm$ 54%, UC (n=6) 76 $\pm$ 23.

Discussion: MUC 11 and MUC 12 are normally expressed in ulcerative colitis. It is unlikely that these are the candidate genes for IBD on the chromosome 7 locus.

321 GENETIC ASSOCIATION STUDIES IN IBD; NEGATIVE RESULTS FROM THREE CANDIDATE GENES

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Introduction: Candidate genes for susceptibility to inflammatory bowel disease include the chemokine receptor 5 (CCR5) gene, the tumour necrosis factor superfamily member 6 (TNFSF6) and the vitamin D receptor (VDR) gene. All three represent positional candidates indicated by whole genome scanning techniques and are located on chromosomes 3, 10 and 12 respectively.

CCR5 mediates activation of T lymphocytes and macrophages. TNFSF6 is a transmembrane protein which mediates apoptosis and plays an important role in the function and regulation of the immune response.

VDR is the cellular receptor for 1,25 (OH)₂ vitamin D₃ (calcitriol) which is expressed by monocytes and activated B and T lymphocytes.

Aim: To determine whether previously positive results with CCR5 and VDR gene can be reproduced, and whether initial novel positive result with TNFSF6 gene represents a genuine association.

Methods: A total of 351 IBD patients comprising; 251 UC and 100 CD were genotyped for the 32bp deletion (CCR5), and the single nucleotide polymorphisms *MvaI* (TNFSF6) and *TaqI* (VDR) by polymerase chain reaction with sequence specific primers and compared with 107 racially matched, healthy controls.

Results: There were no significant differences for both genotype or gene frequency between patients with UC or CD, when compared with controls. Gene frequencies were:

Abstract 321, Table 1

Populations (n = 351)	CCR5 (Delta 32)	TNFSF6 (MvaI)	VDR (TaqI)
UC1 (n = 107)	11%	64%	39%
UC2 (n = 144)	11%	50%	N/a
CD (n = 100)	10%	54%	40%
Controls (n=107)	11%	50%	40%

Conclusion: These three candidate gene association studies are negative. These results are important because previous positive associations have been reported. Our results make it unlikely that these polymorphisms have any influence on IBD susceptibility.

322 COELIAC SEROLOGY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE (IBD)

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Introduction: There is an association between IBD and coeliac disease (CD) though its frequency is not known. Endomysial (EmA) and anti gliadin (AGA) antibodies are useful serological markers of CD.

Aims: To determine frequency of co-existent CD and IBD in a large gastroenterology clinic by retrospective survey and prospective study.

Methods: A diagnostic register of patients attending a gastroenterology clinic between 1984 and 1999 was searched for patients known to have both CD and IBD. Consecutive IBD patients attending between April 1999 and May 2000 were screened for possible CD by IgA EmA (immunofluorescence) and AGA (fluorimetric enzyme labelled immunoassay). Total IgA levels were measured (immunoturbidity). Duodenal biopsies were taken if either EmA or AGA was detected or if IgA levels were low.

Results: Among 586 patients with IBD and 168 with CD, 7 were known to suffer both conditions. 239 consecutive IBD patients without known CD (UC 108, Crohn's disease 102, indeterminate colitis 29) have been screened for serological evidence of CD. 37 (15%) were positive for AGA and 21 (9%) positive for EmA (7 strong, 14 weak positive), 6 (2.5%) were IgA deficient. Correlation between AGA and EmA positivity was poor with only 9 patients having both antibodies. 47 of these 55 patients agreed to undergo duodenal biopsy: 27 were normal, 2 had partial villous atrophy, 4 showed mild increase in intra-epithelial lymphocytes, 3 had slight villous shortening, 10 showed mild and 1 moderate duodenitis.

Conclusions: Of 586 IBD patients and 168 CD patients attending a gastroenterology clinic, 7 were known to suffer both diseases. Screening of 239 consecutive IBD patients from the clinic revealed AGA in 15%, EmA in 9% and IgA deficiency in 2.5%. Only two were shown to have firm histological features of CD. Some may have mild CD as judged by minor histological abnormalities and others may have latent CD but this can only be determined by clinical follow-up. The alternative explanation is that false positive EmA and AGA may occur in IBD indicating a reduced specificity for CD of these antibodies among IBD patients.

323 IS PASSIVE SMOKING IN CHILDHOOD ASSOCIATED WITH INFLAMMATORY BOWEL DISEASE?

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Background: Cigarette smoking is recognised as a risk factor for Crohn's disease (CD) and appears protective against ulcerative colitis (UC). The relationship between passive exposure to smoke in childhood and inflammatory bowel disease (IBD) is controversial.

Aims: To investigate the association between passive smoking by age 16 years and IBD.

Methods: Subjects were from the 1970 British Cohort Study (BCS70), a national prospective birth cohort study. Prospectively collected data on smoking and potential confounding factors were collected at parental interviews at birth, 5, 10 and 16 years. Diagnosis of IBD by age 26yrs was confirmed by physicians. Cross-tabulation, chi-squared test and logistic regression analysis were used.

Results: 9757 subjects responded at age 26yrs. Of these, 30 had CD (23 with smoking data), 22 had UC (15 with smoking data). 6689 of 9757 responding subjects had complete data available for all variables. 71% subjects with CD and 92% subjects with UC were exposed to smoking in the family home by age 16yrs, compared with 91% of the remaining cohort. (Unadjusted OR 0.23, 95% CI 0.08 to 0.65 $p=0.01$, and OR 1.20 95% CI 0.15 to 8.88, $p=0.89$ respectively). Adjusting for sex, father's social class at birth, household crowding, ethnic origin and child's smoking habit did not affect these findings. Maternal smoking in pregnancy was not associated with IBD. Only Asian ethnic origin (as reported in a previous study) was an independent risk for UC.

Conclusions: In this national, population-based prospective study, passive exposure to cigarette smoke was not a risk for subsequent CD, and a significant protective effect was observed. Passive smoking may be a more sensitive proxy measure of childhood exposure to micro-organisms than other measures such as parental social class, household crowding, or availability of hot water, as these have become more homogeneous over the last 50 years. In contrast, improved childhood material circumstances, and atypical patterns of childhood infections have been found in several studies to be a risk for CD.

324 IS THE INCREASING INCIDENCE OF JUVENILE-ONSET IBD IN SCOTLAND RELATED TO SOCIO-ECONOMIC DEPRIVATION?

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Introduction: We have recently shown that the incidence of inflammatory bowel disease (IBD) in Scottish children of under 16 years of age, is increasing. Environmental factors, such as childhood hygiene and infections have been implicated in the aetiopathogenesis. Therefore, using data already collected, we investigated whether economic deprivation was important in juvenile-onset IBD.

Methods: Scottish children, of under 16 years old, with onset of IBD between 1981 and 1995 were identified. The methodology has been previously reported and the data included postcodes of addresses at onset of symptoms. 383 children had Crohn's disease (CD), 197 had ulcerative colitis (UC). The Carstairs Deprivation Score is a classification which takes into account levels of unemployment, car ownership and socio-economic status in each postcode sector. From this, a calculation can be made which gives a measure of deprivation (1 being most affluent, 7 being most deprived). The postcodes of addresses at onset of symptoms were classed for deprivation. The patient data were plotted according to their deprivation score and adjusted for the population aged under 16yrs in each area (1991 Census).

Results: The frequency of new cases of CD and UC appeared to be normally distributed in children from all backgrounds, 1 - 7. However, the age (<16yrs)- adjusted population incidence indicated that children with either CD or UC tended to live in the more affluent, as opposed to the more deprived, areas.

Conclusion: The incidence of juvenile-onset IBD in Scottish children is not related to economic deprivation.

325 A CASE-CONTROL STUDY OF CHILDHOOD ENVIRONMENTAL RISK: FACTORS FOR THE DEVELOPMENT OF INFLAMMATORY BOWEL DISEASE

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Background: There is increasing suspicion that environmental factors encountered in childhood may have an important influence on the risk of IBD in later life.

Aim: To clarify the relationship between childhood environment and the risk of developing Crohn's disease (CD) or ulcerative colitis (UC).

Subjects: The study groups comprised patients with CD (n=139) and UC (n=137) aged between 16 and 45, each matched for gender and age with an outpatient control.

Method: A case-control study, assessing the risk of developing IBD in relation to a series of historical and serological markers of childhood circumstance, analysed using the maximum likelihood form of conditional logistic regression.

Results: Helicobacter seroprevalence was substantially reduced in CD (OR 0.18 ; 0.06 to 0.53) but not in UC (OR 0.84 ; 0.35 to 2.01). In UC, a strong negative association with childhood appendectomy was confirmed (OR 0.06 ; 0.01 to 0.55). CD was associated with childhood eczema (OR 2.80 ; 1.23 to 6.40) and the frequent use of a public swimming pool (OR 2.82 ; 1.17 to 6.80). There was no significant association between hepatitis A seroprevalence and either disease.

Conclusion: The links identified for CD are consistent with the hypothesis that improved childhood living conditions are associated with increased risk of disease, and this may account for the markedly increased incidence of CD over the last century. The study confirms that the previously described negative association between appendectomy and UC relates primarily to events in childhood. Overall, the findings provide strong support for the assertion that childhood environmental factors are important determinants of the risk of IBD in later life.

326 MANAGED CARE FOR DISTAL ULCERATIVE COLITIS: PRELIMINARY RESULTS

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Background: The prevalence of refractory distal colitis is uncertain, because management of distal colitis varies. With the introduction of clinical governance, we adopted a staged management protocol.

Methods: Patients with active proctitis or proctosigmoiditis were entered, excluding those on prednisolone or thiopurines. Activity was assessed by Walmsley clinical and Baron sigmoidoscopic indices. Treatment stages [duration] were: 1. Topical steroid or mesalazine suppository [2 wk]; 2. Oral steroids [4 wk] 3. Mesalazine foam enemas [2 wk] 4. Treatment of proximal constipation [2 wk] 5. Intensive intravenous steroids [1 wk] 6. Cyclosporin, 'anecdotal therapy', or surgery. The end point was the stage at which remission (BO $\leq$ 3/day, no bleeding) was achieved.

Results: 40 patients have been treated (23 women, proctitis 24, proctosigmoiditis 16; median age 48 (23–86) years, disease duration 5 (0.2–44) years). 28% reported <75% compliance with maintenance therapy. At entry, duration of relapse was 8 (1–52) weeks, activity index 6 (3–10) and sigmoidoscopy index 2 (1–3). 12 (30%) had symptoms for >12 weeks before treatment. 39 patients entered remission and 1 came to colectomy after failing on cyclosporin. Remission was achieved in 22 (55%) at stage 1, 10 (25%) at stage 2, 3 (8%) at stage 3, 1 (3%) at stage 4, 3 at stage 5, and 1 at stage 6. None needed anecdotal therapy. Only the severity of relapse correlated with the stage of remission ($p = 0.008$, Spearman). There was no relation between duration of relapse, duration of disease, time since relapse, or extent of disease and refractoriness.

Discussion: 55% patients with active distal colitis achieve remission in <2 weeks with topical treatment. Refractory distal colitis (failure to respond to topical treatment or oral steroids) affects 20% and is less common than a clinical impression in clinic. Poor compliance with maintenance therapy is common and disease activity (but not treatment delay) is related to refractory disease.

328 AN ELECTRONIC PATIENT RECORD FOR INFLAMMATORY BOWEL DISEASE (IBD): HELPING PATIENTS AND DOCTORS

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The incidence of IBD in our outer London population is 125–200% greater than the UK national average. Most patients are in remission most of the time but require regular review by an informed professional. Outpatient clinics are very busy causing well patients to become frustrated waiting for routine follow-up, which might be performed by a succession of junior doctors with imperfect knowledge of each patient. To overcome this we have created a customised computer database (using MSAccess) that holds the salient details of each patient's disease. The database is used in each outpatient consulting room to provide immediate access to the details held in a prototype electronic patient record, thereby avoiding time wasted trawling through patients' notes for essential information. In the department, reports from the database facilitate automatic identification of patients awaiting surveillance colonoscopy or hospital re-assessment or who are suitable for clinical research projects. In addition, the database is an integral part of our shared care programme, with the patient's accustomed general practitioner, by facilitating the production of a printed extract to create an individualised patient held record. The record indicates the roles of general practitioner, hospital GI department, local patient support group and not least the patient in maintaining remission and managing relapses. The database provides the hospital doctor with an instantly available complete record of each patient's condition. Local general practitioners receive

an extract of the database providing fuller information about their patients than normally provided in hospital correspondence and our patients have felt empowered by 'owning' their own record, particularly when unwell or away from home.

329 GENERAL PRACTITIONER (GP) EVALUATION OF NURSE LED PHONE CLINIC (NLPC) FOR PATIENTS WITH INFLAMMATORY BOWEL DISEASE (IBD)

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A 12-month pilot study of an NLPC identified 99% patient satisfaction with the service and enabled those in relapse to be seen urgently in clinic.

Aims: To determine GP perceptions of an NLPC.

Method: Postal questionnaire distributed to all GPs whose patients had received a phone consultation.

Results: 72 questionnaires distributed. 37 (48%) returned. 97% expressed NLPC as an acceptable method of providing clinical supervision for patients with IBD in remission. Approximately one third (30%) very satisfied, over half (56%) satisfied, one tenth (13%) neither satisfied nor dissatisfied. 70% would find patient co-op card useful. GPs' views sought as to where long-term care for patients with various disease extents should receive follow-up.

Abstract 329, Table 1

	1	2	3	4	3+4	5
Distal UC	7%	7%	38%	24%	24%	
Extensive UC		41%	38%	7%		14%
Large bowel CD		14%	51%	14%		21%
CD other areas	3%	38%	31%	7%		21%

1) GP, 2) Outpatient clinic, 3) Phone clinic, 4) Shared care, 5) Combination. UC, ulcerative colitis; CD, Crohn's disease.

Some comments made by GPs on the service: "Good idea, enables problems to be picked up and an appointment arranged urgently when needed"; "All GPs know how many patients are followed up in the outpatients department too frequently"; "One of the ways forward"; "Shared care all very well, if it means dumping your workload on primary care ... NO!!".

Conclusion: Although the response rate was low, the GPs who completed the questionnaire felt that phone clinics are an acceptable way to provide follow-up for patients with IBD in remission, and suggested that this method would be suitable for other gastrointestinal conditions, namely, coeliac disease, irritable bowel syndrome and peptic ulcer disease.

330 AUDIT OF THE MANAGEMENT OF SEVERE ULCERATIVE COLITIS IN A DGH

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The outcome of severe ulcerative colitis (UC) has been reported to have improved since the advent of corticosteroids and guidelines for the timing of colectomy. The outcome of patients with severe UC had not been previously audited in our hospital. Our aim was to assess the investigation, treatment and outcome of these patients.

All patients coded as having UC been Jan 1994–Jan 2000 were identified, the notes were reviewed and data collected on a proforma. Patients were considered to have severe UC as defined by Truelove and Witt's criteria. 92% (107/117) of the identified notes were reviewed. There were 32 episodes of severe colitis in 25 patients (median 36 years, range 17–81). In these patient episodes, 75% had stool cultures and an abdominal x ray on admission. All patients not previously known to have colitis were started on antibiotics. All patients received iv steroids within 72 hours, no patient received cyclosporin. 50% were put on s/c heparin.

At day 10 there were 10 complete responses, 12 partial responses and 10 treatment failures. At final outcome, 17 patients left hospital without surgery, 9 patients had colectomies and there were a total of 6 deaths. Of the deaths, 3 died post operatively, 1 due to coexisting advanced PSC and 2 had post operative leaks. Of the others, 1 had a toxic reaction to azathioprine and 2 were considered not medical suitable for surgery.

The outcome at day 10 of our patients was in line with published results. But, the overall death rate was much higher than expected. On review, the management of these patients seemed appropriate other than in 2 of the deaths surgery was not performed till after day 10. In addition we feel it is relevant that half the deaths were in patients presenting with UC for the first time.

The high mortality has been addressed by a change in practice. All patients admitted with severe UC are now taken over by the gastroenterologists and in patient care is formally shared with the colorectal surgeons. It is hoped that this will help identify patients not responding to medical therapy and expedite surgery, if appropriate. This practice is now under continuous prospective audit.

331 BODY MASS INDEX AND BONE MINERAL DENSITY BY DEXA IN INFLAMMATORY BOWEL DISEASE

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Several factors underlie the low bone mineral densities (BMDs) reported in Crohn's disease (CD) and ulcerative colitis (UC) but their relative contribution remains unclear. In healthy populations, body mass index (BMI) is correlated with BMD and hence we measured these values in 57 male + 76 female CD patients age 48.5+/-15.8 yr (mean +/-SD) and 42 male + 57 female UC patients age 50.5+/-16 yr. Other possible risk factors were assessed by questionnaires, blood analysis and inspection of medical records. The table shows BMI and age/sex adjusted bmd results.

Abstract 331, Table 1

BMI	Crohn's disease			Ulcerative colitis		
	N (%)	FEM Z <-1	LUM Z <-1	N (%)	FEM Z <-1	LUM Z <-1
<20	21 (16)	13 (62)	9 (43)	11 (11)	6 (55)	7 (64)
20 - <25	54 (41)	11 (20)	9 (17)	42 (42)	9 (21)	15 (36)
25 - <30	41 (31)	2 (5)	8 (20)	31 (31)	2 (6)	5 (16)
30+	17 (13)	3 (18)	5 (29)	15 (15)	0 (0)	0 (0)

In CD, univariate analysis confirmed BMI/BMD correlations but BMI only accounted for 12.8% of BMD variance at the femoral neck and 4.3% at the lumbar spine (p values <0.001). In contrast, in UC, BMI accounted for 39.2% of BMD variance at the femoral neck and 22.5% at the lumbar spine (p values <0.001). Multivariate modelling confirmed that BMI had a far larger influence on BMD in UC patients although zero exposure to steroids was also influential. In CD patients, Vitamin D levels, alcohol intake and disease site influenced BMD as well as BMI. Activity levels, smoking habits and lifetime steroid usage had no apparent influence in either group. In conclusion, whereas BMI is by far the most important predictor of BMD in UC patients, in CD the situation is more complex.

332 THE IMPORTANCE OF PROCOAGULANT SCREENING IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE COMPLICATED BY VENOUS THROMBOSIS

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Introduction: Patients with inflammatory bowel disease (IBD) have an increased frequency of venous thromboembolism (VTE), and microvascular thrombosis has been postulated in the pathogenesis of IBD. The pathogenesis of thrombosis in inflammatory bowel disease is not well characterised. The aim of the present study was to evaluate the frequency of laboratory procoagulant risk factors in patients with IBD complicated by VTE.

Patients, materials, methods: 31 patients with IBD (11 Crohn's, 19 ulcerative colitis) complicated by VTE were studied. Assays for protein S, C, anti-thrombin III, anti-phospholipid antibodies, and factor VIII were performed. Genomic analysis for G20210A (Prothrombin gene mutation), and G1691A (Factor V Leiden) was also performed using standard techniques for PCR amplification and subsequent restriction analysis using HIND III or Mnl I respectively.

Results: 14 (45%) of the 31 patients had an identifiable coagulation defect predisposing to thrombosis. This comprised raised factor VIII levels (n=4), Factor V Leiden (n=2), Protein C deficiency (n=2), lupus anticoagulant (n=3), anti-thrombin III deficiency (n=2), and prothrombin gene mutation (n=1). One patient had multiple coagulation defects.

Conclusions: A significant proportion of patients with IBD and venous thrombosis have abnormal procoagulant profiles. A full thrombophilia screen should be measured in all patients with IBD complicated by venous thrombosis.

333 PREVALENCE OF USAGE AND TOLERANCE OF ORAL IRON THERAPY IN INFLAMMATORY BOWEL DISEASE: ARE WE USING IT APPROPRIATELY AND COULD WE DO BETTER?

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Background: Anecdotal evidence suggests that oral iron preparations are poorly tolerated and may even increase disease activity in patients with IBD, but there appears to be no formal support for this in the literature. However other evidence suggests that untreated anaemia may affect the quality of IBD patients' lives.

Aim: To assess the prevalence, indications, tolerance and outcome of oral iron therapy in IBD.

Methods: The case records of 156 consecutive patients with Crohn's disease, ulcerative colitis and indeterminate colitis attending this hospital were reviewed retrospectively using a standard proforma. Those attending the clinic for the whole period 1996-1999 and found to have been treated with oral iron therapy during that time were identified.

Results: During the 4-year period reviewed, 29/156 (19%) patients were treated with an oral iron preparation (ferrous sulphate 72%, ferrous fumarate 7%, sodium iron edetate 7%, unspecified 14%). Iron deficiency had been formally established (ferritin <20) in 11/29 (38%) of treated patients. 9/29 (31%) discontinued therapy due to intolerance (nausea, dyspepsia, abdominal pain, diarrhoea and / or constipation), and in 2/29 (7%) disease activity was shown to increase (as shown by measurements in ESR, CRP, albumin and platelet count) during treatment. In only 10/29 (34%) of patients treated was repletion of iron stores checked by laboratory testing within 6 months, and of these only 5 had an adequate response to therapy during this period.

Conclusions: Even in a specialist IBD clinic, oral iron therapy is often inappropriately prescribed and monitored, and is commonly ineffective. It is poorly tolerated in nearly 1/3 of IBD patients; in a minority of these, iron therapy is associated with an increase in disease activity. Prospective studies are needed to investigate further the mechanisms involved in iron intolerance, and the value of alternative preparations for the treatment of iron deficiency in IBD.

334 DO WHITE CELL COUNT (WCC) AND NEUTROPHIL COUNT AT 4 MONTHS PREDICT FUTURE COUNTS DURING AZATHIOPRINE THERAPY?

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Background: Severe neutropenia is potentially one of the most serious side effects of azathioprine. It is common practice to regularly monitor blood counts in patients taking azathioprine even when apparently stable. Time of maximal azathioprine effect is approximately 3 months, during which time the most frequent monitoring occurs. It is not known whether WCC and neutrophil counts after this period vary significantly from that at the initial treatment period.

Aim: To examine the nadir of WCC and neutrophil counts and correlate this with the respective WCC and neutrophil counts at 4 months into azathioprine therapy.

Methods: We constructed a database of IBD patients taking azathioprine for a minimum of 6 months and who had been brought into remission. Data that was included were the nadir WCC, nadir neutrophil count, azathioprine dose (mg/kg), WCC and neutrophil counts at 4 months into therapy. There were a total of 173 patients (95CD: 78UC) taking azathioprine for a median period of 3.9 years, range 0.7-21 years. Mean azathioprine dose was 1.8mg/kg (SD=0.45) and median duration of azathioprine therapy was 3.9 years, (range 0.7-21 years). A steady azathioprine dose had been reached by 4 months in all patients.

Results: In IBD patients, the nadir of WCC and neutrophil count correlated well with the respective blood count parameter at 4 months ($r=0.7$, $p<0.0005$). This was true for UC and CD groups when analysed separately (UC: $r=0.8$, $p<0.0005$; CD: $r=0.6$, $p<0.0005$). Neutrophil counts changed little after 4 months of azathioprine therapy.

Conclusion: These results suggest that WCC and neutrophil counts at 4 months may be a good predictor of the lowest WCC and neutrophil count that may occur during azathioprine therapy over a relatively long follow up period. Patients with normal neutrophil count at 4 months after initiation of azathioprine therapy are very unlikely to develop significant neutropenia at a later stage. Full blood count monitoring is essential during azathioprine therapy, as dangerous neutropenia may occur unexpectedly, but patients with normal neutrophil count 4 months into azathioprine therapy may be monitored less intensively, unless neutropenic symptoms occur.

335 RESPONSES TO LOW DOSE AZATHIOPRINE IN PATIENTS WITH HETEROZYGOUS THIOPURINE METHYL TRANSFERASE DEFICIENCY

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Introduction: Approximately one in ten Caucasians have heterozygous thiopurine methyl transferase (TPMT) enzyme deficiency. These individuals are at high risk of side effects on treatment with azathioprine but should, in theory, tolerate a lower dose of azathioprine.

Aim: To examine the outcome of low-dose azathioprine treatment in a group of patients with heterozygous TPMT deficiency.

Methods: From a database of TPMT-deficient patients, 24 (15 female, median age 37.5 yrs) were identified who had received azathioprine as a steroid-sparing agent at a reduced dose of 1mg/kg (initially 0.5mg/kg) according to departmental guidelines. Case records were examined to identify adverse effects and clinical response defined by ability to withdraw steroids (complete, partial (prednisolone dose <5 mg daily) or non-response).

Results: 17 patients were treated for inflammatory bowel disease (12 Crohn's disease, 5 ulcerative colitis), 2 autoimmune hepatitis and one intestinal vasculitis. 4 received azathioprine for chronic oral disease (2 lichen planus, 1 Bechet's disease and 1 oral ulceration). 5 of 24 (21%) patients had side effects requiring azathioprine withdrawal (2 pancreatitis, 3 nausea and malaise). 4 additional patients developed side-effects which resolved on dose reduction to 0.5mg/kg. Of the 19 patients who tolerated azathioprine, 17 responded favorably to azathioprine (89%). 15 (79%) withdrew steroids completely. 2 patients had no response.

Conclusion: Measurement of TPMT enzyme activity defines a group of patients with heterozygous TPMT deficiency that can be successfully treated with low-dose azathioprine.

336 ANTIMYCOBACTERIAL TREATMENT FOR CROHN'S DISEASE. DOES IT PREVENT SURGERY AND HOW FAST DOES IT ACT?

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Introduction: Despite a better understanding of the pathogenesis surrounding the inflammatory process in the Crohn's Disease, an overall cure remains illusive. Newer immuno-modulatory agents can produce rapid healing, but require continued treatment for long term effectiveness and are not without side effects or considerable costs. Unfortunately, surgery is often still required.

Paratuberculosis (pTB) has been suggested as a potential causative agent and with the improvement tissue sampling techniques, the detection rates have recently increased in CD specimens. Successful eradication therefore may represent a method of achieving a long-term CD cure.

We assessed the impact rifabutin, clofazamine, clarithromycin had upon patient with difficult to manage CD; with particular reference to speed of onset of symptomatic improvement, and 'surgery sparing effects'.

Results: 3 patients intolerant of treatment and (therefore excluded from analysis) a further 4 developed side effects after 4 - 6 months and needed their treatment modifying (1) or discontinuing (3). 28 patients

entered the study, 12 had previous surgery, 11 were intolerant of Azathioprine, 2 refused steroids and 5 were steroid dependant. 20 Patients showed a good initial improvement with a rapid onset of action (1week - 5 weeks) but there was subsequent relapsed in 10 patients (7 after the treatment was discontinued). 10 patients have maintained a longer response (mean 12 month follow up since commencing treatment). 5 patients were considered to require imminent surgery prior to therapy (3 extensive large bowel, 1 entero-vesical fistula, 1 small bowel) 2 have subsequently required surgery. This was for inactive disease (fistula patient) and a limited small bowel resection following healing of extensive colonic disease with limited small bowel recurrence). Irrespective of outcome, there is a perception that after Rx patients respond better to conventional treatment (3 patients).

Conclusions: When tolerated, this treatment produced a rapid initial improvement, with 50 % demonstrating longer remission. Surgical candidates responded and colectomy was avoided. Patients that needed resection had more limited and less active disease than at presentation.

337 THE SAFETY OF SULPHASALAZINE AND MESALAZINE REASSESSED ACCORDING TO DISEASE INDICATION AND NUMBER OF PRESCRIPTION ITEMS DISPENSED IN THE COMMUNITY FROM 1991 TO 1998

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Introduction: 5-ASA as mesalazine was developed to reduce adverse effects of sulphasalazine by removing sulphapyridine. The actual adverse effect profile of these medications has not been predictable and can be compared using voluntary reporting to the Committee of Safety of Medicines(CSM) but has not been published.

Aims: To evaluate the adverse effects of sulphasalazine and mesalazine between 1991 and 1998 with respect to number of prescription items dispensed and disease indication.

Methods: Total number and subcategories of adverse reactions reported using 'the yellow card' system of the CSM were obtained for 1991-1998. Number of prescriptions dispensed for 1991-1998 were provided.

Results: 4,672,000 prescriptions were dispensed for sulphasalazine and 2,798,000 for mesalazine with adverse events per million prescriptions summarised below and subdivided for sulphasalazine by disease indication.

Abstract 337, Table 1

	Mesalazine	Sulphasalazine	
	IBD	RA	
Blood dyscrasias	17.1	5.4	17.1
Serious skin reactions	4.3	0	3.2
Hepatitis and hepatic failure	2.6	1.9	3.2
Interstitial nephritis	10.4	0	0
Pancreatitis	6.4	0.4	0.4

Conclusions: Within the limitations of using spontaneous reporting interstitial nephritis appears particular to mesalazine and blood dyscrasias with sulphasalazine are largely limited to patients with rheumatoid arthritis.

338 AN OPEN TRIAL OF THALIDOMIDE IN REFRACTORY CROHN'S COLITIS

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Background: TNF α is a proinflammatory cytokine associated with the inflammatory response of Crohn's disease. Treatment with TNF α antibodies is efficacious in active disease. Thalidomide has recognised TNF α inhibitory properties and has demonstrated therapeutic efficacy in treating small bowel and fistulating Crohn's disease.

Aims: To assess efficacy, tolerability and safety of thalidomide in steroid resistant Crohn's colitis.

Methods: 6 patients, 3 female and 3 male, with a history of active Crohn's colitis (CDAI>150), resistant to corticosteroids, were recruited. After a 4 week observation period, they received 200mg of thalidomide per day for a total of 8 weeks. CDAI, ESR and CRP were measured at 0, 4 and 12 weeks. Endoscopic sigmoidoscopic assessment was performed at 4 and 12 weeks.

Results: One patient withdrew after developing a truncal rash, which resolved after discontinuation of treatment. The five remaining patients completed the full 8 week course without any significant side-effects. Median CDAI, CRP and ESR remained unchanged after the 4 week observational period, but demonstrated significant improvements after 8 weeks of treatment, see table 1.

Abstract 338, Table 1

	Median (range)			P value (baseline v. post treatment)
	Baseline	Post 4 weeks observation	Post 8 weeks treatment	
CDAI	175 (153-381)	170 (154-381)	54 (28-152)	(P< 0.05)
CRP	33 (9-62)	33 (9-62)	8 (4-18)	(P< 0.05)
ESR	36 (8-46)	24 (10-110)	12 (7-23)	(P< 0.05)

Endoscopic appearances improved over the 8 week treatment period with complete healing of ulceration in two patients and improvements in three.

Conclusion: Thalidomide at a dose of 200mg per day may be an effective treatment in steroid resistant Crohn's colitis, with improvements in symptom score, inflammatory markers and endoscopic appearances. Side-effects are few and transient.

339 IS THE DIAGNOSIS OF LOW-GRADE DYSPLASIA IN ULCERATIVE COLITIS CONSISTENT?

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Colonoscopic surveillance for long standing ulcerative colitis (UC) relies on the histological detection of dysplasia as a precursor lesion to cancer. Many gastroenterology centres would advocate prophylactic colectomy for any grade of dysplasia. AIM: To assess the agreement between 5 UK Gastrointestinal Pathologists on the diagnosis of low-grade dysplasia.

Method: 160 patients with long standing UC were recruited into a colonoscopic surveillance programme between 1978-1990. 40 patients were reported to have low-grade dysplasia (LGD) by both specialist and non-specialist pathologists at least once during this period. All the original histology slides diagnosed as LGD were retrieved. In total 87 LGD, 3 HGD and 50 control (non-dysplastic) slides from other UC patients on surveillance were re-stained (H&E) and randomly numbered. The 5 pathologists assessed the 140 slides independently and allocated them one of 3 categories: negative for dysplasia, LGD or HGD. We used a statistical program to calculate the chance-corrected inter-observer agreement (kappa coefficient). $\kappa < 0.5$ is considered as poor agreement or reproducibility, $\kappa = 0.5-0.75$ representing moderate to good agreement and $\kappa > 0.75$ taken as excellent agreement. We also calculated the κ value between the majority or consensus score and the original diagnosis.

Results: The frequency of each diagnosis ranged from 65.7% to 80% for negative, 16.4% to 32.1% for LGD, and 0.01% to 5% for HGD. The kappa coefficient for overall agreement between 5 Pathologists, $\kappa = 0.30$. (95% CI=0.253 - 0.347). Kappa for individual diagnoses: negative; $\kappa = 0.36$, LGD; $\kappa = 0.25$, HGD; $\kappa = 0.52$. Kappa coefficient between the original and consensus diagnosis, $\kappa = 0.23$. Only 14 patients out of the original 40 LGD (35%) had a majority diagnosis (at least 3/5 pathologists) of LGD.

Conclusion: The agreement between gastrointestinal pathologists on a diagnosis of LGD is poor. Therefore it is inappropriate to base a recommendation for surgery on a histological diagnosis of LGD in UC.

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340 FAMILY SIZE, SIBLINGS, SOCIOECONOMIC STATUS AND CHILDHOOD RISK OF *HELICOBACTER PYLORI*

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Background/aim: The vast majority of West African adults are colonised with *H. pylori* and most children acquire the infection in early life. There is an inverse relationship between the prevalence of *H. pylori* and socioeconomic development throughout the world. In Greater Glasgow 50% of postcodes belong to Deprivation Categories 5-7 (poorest socioeconomic status). Our aim was to study intrafamilial patterns of *H. pylori* infection in Glasgow and to identify possible modes of spread.

Methods: The families of 32 children, who presented at the RHSC Glasgow with suspected *H. pylori*-related disease, were studied. *H. pylori* status was determined by faecal antigen testing, a method that we have validated against the urea breath test in children.

Results: The families consisted of 57 parents, 47 siblings and 32 index children. Of these 136 subjects 93% provided stool samples. The prevalence of *H. pylori* in this sample was 35% (41% of parents and 30% of children). Those index children who were of Dep Cats 5-7 were 7 times more likely to be infected with *H. pylori* than those of Dep Cats 1-4 ($p=0.03$ Fisher's exact test). Index children whose siblings were *H. pylori* +ve were 9 times more likely to be *H. pylori* +ve themselves ($p=0.06$). Both the *H. pylori* status of parents ($p=0.3$, OR=2) and family size ($p=0.6$, OR=1) were not significantly related to the *H. pylori* status of the index child.

Conclusions: Poor socioeconomic status and having an *H. pylori* infected sibling are significant risk factors for *H. pylori* in children, suggesting that infection may be transmitted horizontally within families. We plan to extract DNA from the stool for genotyping in order to investigate these findings further.

341 IS DUAL CHANNEL pH MONITORING NECESSARY FOR INVESTIGATING CHILDREN WITH REFLUX-RELATED RESPIRATORY SYMPTOMS?

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Introduction: Children with gastro-oesophageal reflux related respiratory symptoms (GORRS) are often referred for evaluation by 24-hour pH study using dual pH sensors (in the lower and upper oesophagus). The aim was to determine the usefulness of dual sensor pH monitoring in this group of patients.

Patients and methods: Eighty children (48 males) with suspected GORRS (apnoeic attacks 38, recurrent chest infection 31, chronic cough and wheezing 11) aged 1 month-15 years (median 5.5 months) were studied. 24-hour ambulatory pH study was performed using dual channel antimony electrode (Synectics Medical, Sweden). The lower sensor position was located according to the child's length with the upper sensor 5cm proximal. Position confirmed by X-ray. Study was positive if the reflux index (RI = % time pH <4) was >10% in lower sensor.¹ Positive proximal reflux = RI > 3% in upper sensor. Total number of reflux episodes (TRE), longest reflux episode (LRE), number of episodes >5min (NLRE) in the lower oesophagus were compared for positive proximal reflux.

Results: Thirty-two children (40%) had a positive pH study result (21 children < 1 year of age). 24/80 children (30%) had positive proximal reflux, only 1 had no significant lower reflux. Children with proximal reflux were more likely than those with no proximal reflux to have had pathological reflux distally ($n=23$ (95.8%) versus 1 (4.2%)) ($\chi^2=44.53$, df=1, $p=0.000$). Patients with positive proximal oesophageal reflux had significantly greater LRE ($p=0.001$), NLRE ($p=0.007$) and RI ($p=0.000$) in the lower oesophagus.

Conclusion: Presence of acid in the upper oesophagus is determined by events in the lower oesophagus. Children presenting with possible GORRS can be adequately investigated with a single channel pH sensor.

1. Vandenplas Y, et al. *Pediatrics* 1991;88(4):834-40.