Hg; NS). The total number of impedence refluxes were significantly lower after ELF (22±19 vs 57±45, P= 0.01) with a lower number of acid refluxes (13±15 vs 20±11, P= 0.07). Conclusions: ELF with EsophyX device decreases number of gastroesophageal refluxes, as measured by means of esophageal impedance, and improves symptoms and quality of life of patients GERD responsive to PPI.

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A Sham-Controlled Study of Injection of Botulinum Toxin in Diffuse Esophageal Spasm

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Uncontrolled studies in limited numbers of patients suggest a benefit of injection of botulinum toxin (botox) in the distal esophagus for the treatment of diffuse esophageal spasm (DES). Improvement is reported within the first 4 weeks, but controlled data are lacking. The aim of the present study was to investigate the effect of botox on symptoms and esophageal motility in diffuse DES in a double-blind randomised cross-over design. Methods: Consecutive patients with a new diagnosis of DES were recruited for the study. They underwent two upper gastrointestinal endoscopies with 4 weeks interval, during which distal esophageal injection with saline or botox 8x12.5U was performed in a randomised double-blind fashion. Injections were prepared by a nurse who was otherwise not involved in the care of these patients. Before the start of the study and 4 weeks after each treatment, they underwent an esophageal manometry and filled out a dysphagia severity questionnaire (solid dysphagia, liquid dysphagia, regurgitation, chest pain, heartburn all scored 0-4). Both parallel treatment and pooled treatment data were compared using Student's t test. Results: 11 DES patients (10 women, mean age 62±4 years) were recruited; 5 received botox first and 6 saline first. After initial saline injection, solid and liquid dysphagia scores did not improve significantly (respectively 2.8±0.5 vs. 1.8±0.7 and 2.2±0.6 vs. 1.5±0.6, NS). After cross-over injection solid and liquid dysphagia scores decreased further to 1.5±0.7 (p=0.04) and 0.8±0.5 (NS) respectively. Initial botox injection was associated with significant improvement of solid (3.0±0.4 vs. 1.8±0.2, p=0.03), but not liquid dysphagia (2.4±0.4 vs. 1.4±0.4, NS). After the second injection with saline, solid and liquid dysphagia did not improve further (respectively 1.7±0.3 and 0.7±0.3, NS). When data for both treatment periods were pooled, significant improvement of solid and liquid dysphagia was seen after botox (respectively 2.4±0.5 to 1.6±0.4, p<0.01 and 1.9±0.4 to 1.1±0.3, p=0.04) but not after saline (respectively 2.4±0.3 to 1.9±0.5 and 1.8±0.4 to 1.1±0.4, both NS). No significant improvements were seen after botox in scores for regurgitation (1.0±0.4 to 0.9±0.4, NS), chest pain (1.6±0.5 to 1.7±0.5) or heartburn (0.8±0.4 to 0.9±0.4, NS). CONCLUSION: Distal esophageal injection of botulinum toxin is superior to placebo in improving dysphagia in DES.

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The Vexed Relationship Between *Clostridium difficile* and Inflammatory Bowel Disease (IBD) - a Prospective Assessment of Carriage in An Outpatient Setting in Patients in Remission

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Background & Aims The relationship between *Clostridium difficile* and Inflammatory Bowel Disease (IBD) has long been debated. Co-morbidity with *C. difficile* may cause diagnostic delay of IBD in newly presenting patients, trigger relapse in established IBD, confound therapy in all forms of IBD, and may be a marker of an underlying defect in innate immunity, particularly in Crohn's disease. Retrospective analyses have suggested a community source rather than nosocomial acquisition, in many instances. To address this, we conducted a prospective analysis of *C. difficile* in outpatients who were in clinical remission. **Methods** Participants had long-standing diagnoses of ulcerative colitis (n=64) and Crohn's disease (n=58), in clinical remission with no recent exposure (>3 months) to antibiotics, corticosteroids, immunomodulatory drugs or hospitalisation. *C. difficile* was cultured from stool using anaerobic conditions, antimicrobial susceptibility was tested (E-test), and isolates were characterised using PCR-ribotyping, 16SrDNA sequencing, pulsed-field gel electrophoresis (PFGE) and PCR-RFLP toxinotyping of the pathogenicity locus. **Results** The frequency of toxigenic *C. difficile* was higher in IBD patients than in healthy volunteers at 8.2% and 1.0% respectively (p = 0.02 Fisher's exact test). All strains belonged to the most common clinical toxinotype 0 sharing the same pathogenicity locus arrangement as *C. difficile* VPI10463. Patients harboured a diverse range of PCR-ribotypes including R015, R005 and R020 which are frequently associated with *C. difficile*-associated disease (CDAD) and rare types R003, R039, R131. Interestingly, common nosocomial PCR-ribotype groups were not observed here (R106, 001, 027). The considerable non-clonal distribution of distinct strains was further supported by genomic fingerprinting. **Conclusion** *C. difficile* detection is significantly higher in IBD outpatients in remission than in healthy adults regardless of therapeutic or environmental influences, and detailed strain information indicates community acquisition from a multitude of sources. The increasing frequency and severity of *Clostridium difficile*-associated disease (CDAD) poses a threat to the expression, diagnosis and treatment of IBD.

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Impact of *Clostridium Difficile* Infection On Pediatric Inflammatory Bowel Disease

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Background & Aims: An increased incidence and virulence of *Clostridium difficile* has recently been reported in adults with Inflammatory Bowel D isease (IBD). At the present time the impact of *C. difficile* on pediatric IBD is still unknown. The purposes of this retrospective, observational study were to determine the incidence and to explore possible differences in the risk for and symptoms of *C. difficile* infection between IBD and non-IBD pediatric patients. Methods: Stool specimens from IBD and non-IBD pediatric subjects, collected from January 2005 to June 2007, were evaluated for the presence of *C. difficile* toxin A and toxin B. Demographic information, diagnosis, anatomic location, symptoms, disease activity, IBD therapy, antibiotic exposures and hospitalizations were recorded. Results: A total of 284 consecutive specimens were collected from 100 IBD patients (UC: 70, CD 30; Mean age: 10,6 years, range of 1,6 to 18 years) and from 184 non-IBD patients (Mean age: 8,1 years range of 1,6 to 17,4 years). The prevalence of *C. difficile* infection was significantly higher in IBD patients (12%) than in non-IBD subjects (7.06%) (p=0.011; X2=0,02; OR=1,79). Ten of the 12 (83,3%) IBD patients with *C. difficile* infection were affected by active disease; in particular *C. difficile* infection was identified in 10 of the 30 (33%) IBD patients with symptomatic relapse. Colonic involvement was found in all IBD infected patients, and bloody diarrhea was significantly more present in infected IBD patients than in non-IBD subjects (p=0.003). We did not identify specific type of IBD, IBD therapy and antibiotic exposures that predisposed IBD patients to *C. difficile* infection (p=0,266; p=0.48; p=0.16, respectively), whereas hospitalizations were significantly more frequent in non-IBD than IBD subjects (p= 0.03). Conclusions: Our findings indicate that pediatric IBD is associated with increased risk for *C. difficile* infection. The presence of *C. difficile* in the stool is related to the activity of the disease. Differently from adults, we did not identify specific risk factors (such us IBD therapy, antibiotic exposures, hospitalization) in pediatric IBD patients except for underlying colonic disease, suggesting that other mechanisms for the acquisition of the pathogen might be involved.

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Clinical Course Following Autologous, Nonmyeloablative, Stem Cell Transplantation in Patients with Refractory Crohn's Disease, 2001-2007

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Objective: To report on the clinical course following autologous hematopoietic stem cell transplantation (HSCT) with lymphoablation in a cohort of refractory Crohn Disease (CD), in terms of clinical remissions, relapses, and requirement for surgery. Design: Open label, non-randomized, cohort study Setting: A tertiary care medical center. Patients: Patients with severe CD who have failed standard therapy including infliximab, whose Crohn's Disease Activity Index (CDAI) was > 250, and/or Crohn's Severity Index (CSI) was > 16 were accessed for therapy. Intervention: Stem cells were mobilized from the peripheral blood using cyclophosphamide (2.0 g/m2) and G-CSF (10 ug/kg/day), enriched ex vivo by CD34+ selection, and re-infused after immune conditioning with cyclophosphamide (200 mg/kg) and either equine anti-thymocyte globulin (ATG) (90 mg/kg) or rabbit ATG, 6 mg/kg. Main outcome measures: Primary outcomes of safety, clinical variables, small bowel radiography, and colonoscopy were followed annually post HSCT. The subjects were also analyzed in terms of relapses and remissions, and requirement for surgery. Results: 21 subjects have undergone the therapy successfully. HSCT had no treatment related mortality. Usually, diarrhea and abdominal pain resolved prior to hospital discharge. Perianal fistulae, colonic strictures, and perianal disease were slower to resolve. CDAI and Crohn's sefverity index (CSI) improved over baseline at each interval. 9 subjects have had relapse, 5 of whom required surgery. Three others required surgery for ileal stricture or colovesicle fistula, unrelated to relapse. 18 subjects are in clinical remission defined as having a CDAI < 150, a CSI < 12, and no corticosteroid therapy (1- 6yrs; 3- 5yrs; 4- 4yrs; 4- 3yrs; 4- 2yrs; 2- 6mos. 3 remain in relapse. Conclusion: The experience in the first 21 patients who received this radical therapy continues to be encouraging, although relapses have occurred.

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The Prevalence of Undiagnosed Disorders of Neutrophil Function Mimicking Crohn's Disease

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Introduction. Inherited disorders of neutrophil function, such as chronic granulomatous disease (CGD), are associated with gastrointestinal inflammation indistinguishable from Crohn's disease (CD) in up to 50% of cases.[1] Normal neutrophil microbicidal activity requires the NADPH oxidase enzyme complex to mount a respiratory burst - leading to the generation of superoxide (O_2^-) to optimise the action of digestive enzymes. This process is defective in a number of neutrophil disorders with disparate aetiologies[1-3], making its integrity a potentially useful screening test. The existence of defects in innate immunity in CD is of increasing interest, and it is possible that a proportion of patients possess an underlying disorder of neutrophil function. **Methods.** To determine the frequency of such defects, neutrophils were isolated from 50 healthy control subjects and 100 consecutive, unselected patients with quiescent CD attending a tertiary inflammatory bowel disease clinic. O_2^- production was calculated in an assay based on the reduction of cytochrome-c by neutrophils after stimulation with phorbolmyristylacetate. Patients with a defect were investigated further. **Results.** Mean O_2^- production in healthy controls (27M; mean age 31.7±7.2 yrs) was 10.96±1.9 nmolO_2/10^{6}cells/min. O_2^- production in three unrelated patients (table 1) in the CD group was abnormally low (<35% healthy control mean[2]). Common characteristics were: diagnosis of CD at a young age; granulomatous inflammation in colonic biopsies; a history of recurrent systemic bacterial infections. Subsequent analysis diagnosed p47-deficient CGD (CGD-p47) in one patient, glycogen-storage disease type-1b (GSD-1b) in another, whilst the third has an as yet uncharacterised defect. **Conclusion.** In this group of patients with CD, 3% were found to have neutrophil dysfunction. A high index of suspicion for such disorders is prudent, particularly for patients with CD presenting at a young age, with complex disease or a history of recurrent systemic bacterial infections. It is important to identify these patients to improve their prognosis by altering or augmenting the conventional treatment regimens employed for idiopathic CD. *BHH / FR equal contributors.* **References.** 1. *Gastroenterology* 2007; 4(S1): A156 2. *Blood* 1995; 85: 231-241 3. *Eur J Pediatr* 2005; 164: 501-8

	Patient 1	Patient 2	Patient 3
Gender	M	F	F
Age at diagnosis (yrs)	9.4	9.1	10.0
Disease location	Colonic; Perianal	Colonic	Oral; Ileocolonic
Current treatment	Methotrexate	Mesalazine	Mesalazine
O_2^- production (nmolO_2/10^6 cells/min	0.1	2.7	3.3
Final diagnosis	CGD-p47	GSD-1b	Uncharacterised

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Preoperative Wireless Capsule Endoscopy Does Not Predict Long-Term Outcome After Ileal Pouch-Anal Anastomosis

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PURPOSE: Pouchitis is a common complication following ileal pouch anal anastomosis (IPAA) for ulcerative colitis (UC) and indeterminate colitis (IC). The extent of preoperative small bowel mucosal inflammation may be an important predictor of outcome after IPAA. Wireless capsule endoscopy (WCE) appears to be more sensitive for the assessment of mucosal lesions than any radiological test. The purpose of this study was to investigate the value of preoperative WCE in predicting long-term outcome of IPAA in patients who carry a diagnosis of UC or IC. METHODS: 47 patients (32 UC patients and 15 IC patients) undergoing complete WCE before IPAA with at least a 3 month follow up over a 7-year period ending July 2007 were identified. IC features included atypical distribution of disease, presence or history of perianal disease, small bowel inflammation more than 3 cm proximal to the ileocecal valve, or by identification of transmural inflammation or granulomas in the resected colon. Findings on WCE were classified as positive (erosions, ulcers, erythema), negative, or incomplete. Long-term outcome was assessed prospectively and included no pouchitis (NP), acute pouchitis (antibiotic responsive), chronic pouchitis (antibiotic dependent/resistant), or the development of de novo Crohn's disease (CD). Patients with acute pouchitis (AP), chronic pouchitis (CP) or Crohn's disease (CD) were considered to have pouch inflammation (PI). RESULTS: The study group had a median age of 36 years (range, 9 to 73 years), and included 23 males and 24 females. Median follow up time after ileostomy closure was 9 months (range, 3 to 60 months). WCE was positive (WCE+) in 14 patients (30%) and negative (WCE-) in 33 patients (70%). Within WCE+ patients, 1 (7%) developed AP, 1 (7%) developed CP and 3 (21%) developed CD. Within WCE- patients, 6 (18%) developed AP, 2 (6%) developed CP and 2 (6%) developed CD. There was no significant difference in AP, CP, CD or PI between WCE+ patients and WCE- patients. Seven of the 32 UC patients (22%) were WCE+ compared to seven of the 15 IC patients (47%) (p= 0.10). There was no significant difference in PI between the WCE+ and WCE- groups in both the UC (29% vs.36%, p=ns) and IC (43% vs. 13%, p=0.28) patient subgroups. CONCLUSION: This study suggests that there is no significant advantage of using preoperative WCE to predict pouch inflammation after IPAA.

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Family History of Crohn's Disease Increases the Risk for the Development of Crohn's Disease of Ileal Pouch-Anal Anastomosis

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Background: Crohn's disease (CD) of the pouch can occur in patients with restorative proctocolectomy and ileal pouch-anal anastomosis originally performed for a pre-operative diagnosis of ulcerative colitis (UC). CD of the pouch was often observed in patients with a family history of CD. The aim of the study was to determine whether the family history of CD increased the risk for the development of CD of the pouch in patients who underwent restorative proctocolectomy. Methods: A total of 558 eligible patients seen in our subspecialty Pouchitis Clinic were prospectively enrolled from 2002- 2007, including 116 patients with CD of the pouch and 442 patients with a normal pouch or other pouch disorders (including pouchitis, cuffits, and irritable pouch syndrome). Demographic and clinical variables were included in the study. Multivariable logistic regression analyses were performed. Results: The adjusted multivariable logistic analyses revealed that the risk for the development of CD of the pouch was increased in patients with a family history of CD with odds ratio (OR) of 3.61 (95%CI 1.72-7.57), or with a first-degree relative with CD (OR=4.48, 95%CI 1.57-12.8), or with a greater number of family members with CD (OR=2.16 per family member, 95%CI 1.27-3.66), adjusting for age, gender, smoking status, duration of IBD, and duration of having a pouch. In addition, patients with a younger age and longer duration of having a pouch had a higher risk for the development of CD of the pouch. A diagnosis of CD of the pouch was associated with a poor outcome with a 7-fold increased risk for pouch failure (OR=7.08 and 95% CI, 3.21 -15.6). Conclusions: The presence of a family history of CD is associated with an increased risk for the development of CD of the pouch, which in turn has a high risk for pouch failure. The presence of a family history of CD in patients with underlying UC may have an impact on decision on whether restorative proctocolectomy should be performed.

Risk Factors for Crohn's Disease of the Pouch-Multivariable Analysis

Variables	Odds Ratio (95% CI)	P value
Age	0.87 (0.79 - 0.97)	0.011
Female Gender	1.39 (0.89 - 2.17)	0.14
Ex-smoker	0.95 (0.52 - 1.75)	0.88
Active smoker	1.31 (0.64 - 2.67)	0.46
Duration of Pouch	1.56 (1.24 - 1.95)	<0.001
Duration of IBD	1.05 (0.90 - 1.23)	0.54
Pouch Failure	7.08 (3.21 - 15.6)	<0.001
Family History of Crohn's	3.61 (1.72 - 7.57)	0.001

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Generation and Characterization of a Novel Nf-κB Reporter System to Study Bacteria-Host Interactions in the Zebrafish Intestine

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Introduction: The NF-κB family of transcription factors integrates microbial and physiological stimuli in the gut, and improper activation of this pathway is associated with inflammatory disorders including IBD. Our transgenic mouse expressing EGFP under transcriptional control of NF-κB has provided useful information on bacteria-host interactions in the gut, however this system is not suitable for chemical and genetic screens. We recently established a gnotobiotic zebrafish model to investigate bacteria-host relationships in the gut. **Aim:** Generate a zebrafish NF-κB reporter system to study bacteria-host interactions. **Methods:** We performed microarray-based functional genomic comparisons in zebrafish larvae raised germ-free versus those colonized with a zebrafish gut microbiota. We generated a reporter plasmid that permits expression of EGFP under control of consensus NF-κB binding sites (pNFKB:EGFP). Zebrafish PAC2 embryonic fibroblasts were transfected with pNFKB:EGFP, and stimulated with LPS or *Pseudomonas aeruginosa* lysate. Expression of the active NF-κB subunit RelA and the inhibitory protein IκBα was evaluated by Western blot analysis, and transcription of NF-κB target genes was monitored by quantitative RT-PCR. We used the Tol2 transposon system to create a transgenic zebrafish carrying the pNFKB:EGFP reporter (*Tg(NFKB:EGFP)*), and then monitored EGFP expression *In Vivo* as a function of microbial status. **Results:** We identified zebrafish genes that are transcriptionally regulated by the gut microbiota, many of which have mammalian homologs that are involved in innate immunity and regulated by NF-κB. Although the zebrafish genome encodes components of the NF-κB pathway, their functions have not been established. Treatment of PAC2 cells with LPS stimulated RelA nuclear translocation and expression of NF-κB targets such as IκBα. PAC2 cells transfected with pNFKB:EGFP showed enhanced EGFP expression following stimulation with LPS or bacterial lysate. Exposure of zebrafish larvae to the NF-κB inhibitor BAY11072 caused a reduction in IκBα mRNA levels. Having shown that the NF-κB system is functional in zebrafish, we next generated a *Tg(NFKB:EGFP)* zebrafish line. Colonization of germ-free *Tg(NFKB:EGFP)* zebrafish with a normal microbiota or *P. aeruginosa* resulted in elevated EGFP expression in the liver and a distinct population of intestinal cells. **Conclusions:** Our results indicate that the zebrafish NF-κB pathway is active and capable of responding to microbial stimuli. The optical transparency of the zebrafish provides new opportunities for investigating mechanisms underlying temporal and spatial control of the NF-κB response to gut bacteria.

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T Cell Signaling Via the Toll-Receptor Associated Activator of Interferon (TRIF) Pathway Protects from the Development of Acute and Chronic Murine Colitis

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Introduction: Abnormal T cell responses to commensal bacteria are involved in the pathogenesis of inflammatory bowel disease (IBD). Recognition of bacteria is dependent on toll-like receptors (TLRs) in the intestinal mucosa. Most TLRs use myeloid differentiation-88 (MyD88) as an adapter molecule for signaling whereas TLR3 and TLR4 use TRIF as an alternative activation pathway. We tested the hypothesis that signaling via TRIF protects against the development of colitis in both acute and chronic models. Methods: Acute colitis was induced by 2.5% dextran sodium sulfate (DSS) for 7 days. Chronic colitis was established by transfer of CD4+CD62L+ naïve T cells from TRIF mutant (LPS2) mice and WT spleens to RAG1-/- mice and followed for 9 weeks. TLR expression was examined by flow cytometry. Severity of colitis was assessed via colonoscopy, change in body weight, and histological score. T cell proliferation and cytokine expression were assessed via 3H-Thymidine and ELISA assays, respectively. Results: In the acute model, TRIF-/- mice showed significantly more weight loss at the end of 7 days of DSS treatment as compared to WT mice ($p<0.05$). Compared to the transfer of WT naïve T cells, transfer of TRIF-/- T cells resulted in significantly more weight loss in the RAG1-/- recipient mice within the first 3 weeks after transfer. TRIF-transferred mice showed increased inflammatory cell infiltration and crypt architectural distortion on histology as well as more severe inflammation endoscopically. Naïve TRIF mutant T cells exhibit increased proliferation compared to naïve WT T cells. Lamina propria T cells from RAG1-/- mice given TRIF mutant T cells showed increased expression of IL-17 as compared to WT T cells. Conclusion: TLR signaling via TRIF on T cells plays a role in the protection from colitis in both acute and chronic models of murine IBD. The absence of the TRIF signaling complex results in preferential Th17 T cell differentiation, which may be the mechanism for increased inflammation. These results suggest a role for TLR signaling by T cells in the regulation of mucosal T cell responses and their contribution to IBD.