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Falk Symposium 179

Revisiting IBD Management: Dogmas to be Challenged

September 30 – October 1, 2011
Sheraton Brussels Hotel
Belgium

Abstracts
Poster Abstracts
Falk Symposium 179

REVISITING IBD MANAGEMENT: DOGMAS TO BE CHALLENGED

Brussels (Belgium)
September 30 – October 1, 2011

Scientific Organization:
G. D’Haens, Amsterdam (The Netherlands)
A. Dignass, Frankfurt (Germany)
S. Vermeire, Leuven (Belgium)
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Dogma 1

**IBD is a disorder of defective autophagy and innate immunity**
Evidence from genetics for the role of autophagy and innate immunity in the pathogenesis of IBD

Dr. Miles Parkes
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A major contribution of genetics studies has been in highlighting the role that defects in innate immunity play in IBD pathogenesis. The power of hypothesis-free genome scans in investigating complex disease is the reproducibility of their results and their ability to highlight important but previously unsuspected pathogenic pathways. The genetic signals might be hard to explain based on existing knowledge, but we know that they are true and the challenge is to understand how they exert their effect at the functional immunological, cellular and whole-organism level.

In no other common disease have genetic studies so illuminated new pathogenic mechanisms as IBD. The early identification of NOD2 as a susceptibility gene for Crohn’s disease, in the era of genome-wide linkage studies, first catalysed major interest in the role of innate immunity in IBD \(^1\). This interest has been substantiated by GWAS with the identification of genetic association between Crohn’s disease and variants in two separate autophagy genes, ATG16L1 and IRGM \(^2,3\). Prior to the GWAS era this had not been considered relevant to Crohn’s disease pathogenesis; 5 years later and a Google search on ‘Crohn’s autophagy’ produces > 70,000 hits, underlining the interest in this pathway.

Since genetics studies first highlighted the contribution of NOD2, ATG16L1 and IRGM numerous studies have explored the functional impact of the Crohn’s disease-associated risk variants. Hugot’s original study identified 3 low frequency variants clustered in the part of the NOD2 gene encoding the leucine rich region, which binds muramyl dipeptide. Extensive resequencing of NOD2 has identified a number of additional rare variants and private mutations associated with Crohn’s disease – and interestingly has shown that NOD2 variants do not segregate with Crohn’s disease in Asian populations. A variety of disease-predisposing mechanisms have been proposed for the NOD2 mutations, ranging from defects in viral sensing and reduced mucosal defensin production to abnormal autophagy induction and antigen presentation. As more is understood of NOD2 biology, ever more putative IBD-predisposing mechanisms are revealed – and it may be precisely because of NOD2’s pleiotropic roles in innate immune responses that its mutation exerts such a powerful effect in predisposing to Crohn’s disease.

Recent work has also highlighted the complexity of the contribution made by genetic variation in the autophagy genes ATG16L1 and IRGM. Thus in an elegant study using ATG16 hypomorphic mice Cadwell et al showed the presence of major morphological change in Paneth cells – a finding that was then also observed in humans homozygous for the CD-associated ATG16L1 coding variant. Further these mice developed a Crohn’s-like phenotype – but only in the presence of an environmental stressor, an intact gut flora and a viral trigger in the form of a specific persisting strain of norovirus \(^4\). The complexity of this model is perhaps beginning to approach the complexity of human IBD, and is all the better for that.
IRGM has also been intensively studied. Here the risk alleles are non-coding and appear to affect mRNA transcription or stability. A number of mechanisms have been proposed including disruption of a transcription factor binding site in the IRGM promoter and, intriguingly, alteration of a microRNA binding site by a synonymous coding variant. The functional impact of the altered production of IRGM has been explored, and particularly correlated with impaired clearance by macrophages of CD-associated adherent-invasive E coli.

In addition to exploring the contribution of classical ‘innate immunity’ genes to IBD pathogenesis, there is also an intriguing literature developing on the impact of ‘adaptive immunity’ genes on innate immune mechanisms. For example, Buoncore et al recently reported the accumulation of IL23 responsive innate lymphoid cells and intestinal cells in the colon, the former capable of producing IL17 and interferon-γ and mediating innate colitis in mice. Production of Th17 cytokines by analogous cells in humans appeared higher in colons from IBD cases vs. controls. Thus, while intuitively the IBD-associated variants in IL23R might be predicted to exert their effect on adaptive immunity via Th17 pathways, they may also impact innate immunity. Thus some genetically-mediated mechanisms may bridge the divide between innate and adaptive immunity and an open mind is, as always, required.

Many other genes linked to various components of innate immunity are evident among the >> 100 confirmed IBD susceptibility loci highlighted by GWAS scans, recently published meta-analyses and on-going follow-up studies using Immunochip. An intriguing finding is that variants in genes linked to epithelial barrier function seem to be specifically associated with UC and not Crohn’s disease - the converse of NOD2 and the autophagy genes which are Crohn’s specific. While many complex explanations might exist, these observations correlate nicely with UC being confined to the superficial layers of the colon, while the transmural inflammation of Crohn’s disease is caused by defects in cellular innate immunity and bacterial handling in the deeper layers of the lamina propria and beyond.

References:


Bacterial interactions with innate mucosal immunity

Prof. Dr. Elke Cario
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Commensal microbiota play a key role in health and disease of the host\(^1\). The innate immune system comprises an essential functional component of the intestinal mucosal barrier, maintaining hyporesponsiveness to omnipresent harmless commensals in the lumen, but rapidly recognizing and combating invading bacteria through diverse antimicrobial mechanisms. Interactions between commensals and innate immune cells are constant and multidimensional. Accumulating evidence underscores the diversity of commensal-mediated effects and physiological functions of innate immune responses in the intestinal mucosal barrier.

Tight control of commensal composition and innate immunity is critical to mucosal homeostasis. Disruption of normal innate immune signalling by environmental factors, host mediators and/or gene defects may imbalance commensal-host interactions in a susceptible individual, thus initiating and perpetuating mucosal inflammation and tissue injury in the intestine. Perturbed homeostasis between the commensal microbiota and innate immunity serves as a critical determinant in the complex pathogenesis of inflammatory bowel diseases (IBD)\(^2,3\).

Genetic association studies have linked sensors (NLR, TLR) of the innate immune system with the development of chronic intestinal inflammation in IBD\(^4,5\). Mutations in innate immune sensors (NOD2, NLRP3, TLR2, TLR4) may mediate aberrant immune cell priming and bacterial handling. Variants in intestinal epithelial genes (e.g. HNF4a) may facilitate stress-induced barrier disruption, allowing bacterial translocation and subsequent persistent mucosal innate immune activation. Genetic defects in innate immune effector pathways can induce severe alterations in antimicrobial host defense, such as mucus dysintegrity (MUC1) and impaired autophagy (ATG16L1, IRGM). Deregulated autophagy has been linked to Paneth cell abnormalities, reducing defensin production. Cellular and metabolic imbalances, e.g. due to endoplasmatic reticulum damage (XBP1), can sensitize innate immune cells to auto-inflammatory effects. Furthermore, pro-inflammatory cytokine responses (STAT3, IL-23R) skew both innate and adaptive immune cells in IBD, thus critically disturbing commensal-mucosal interactions.

Selected references:


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Endoplasmatic reticulum stress and inflammation

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The presence of misfolded and hence potentially dysfunctional proteins in the endoplasmic reticulum elicits stress in this cellular locale, which – under physiological conditions – is resolved via the so-called unfolded protein response (UPR). The UPR is a coordinated adaptive response that senses ER stress and instigates mechanisms involving a temporary selective halt of translation and induction of a transcriptional programme that expands and improves the protein folding capacity of the ER. Highly secretory cells appear particularly dependent on a proper functioning UPR. The UPR transcription factor XBP1, part of the evolutionary most conserved branch of the UPR, has been identified to play an important role in the homeostasis of intestinal epithelial cells. Specifically, hypomorphic function of XBP1 leads to ER stress in the epithelium and consequently impaired Paneth and goblet cell function. Moreover, hypomorphic XBP1 function alters the responsiveness of the absorptive epithelium towards signals from the microbiota (e.g. TLR ligands) as well as host mediators (e.g. TNF). While Paneth cell dysfunction leads to impaired handling of orally infected model pathogens, hyperreactivity of the absorptive epithelium leads to activation of several classical pro-inflammatory signal transduction pathways. In their entirety, these alterations lead to the spontaneous development of intestinal inflammation resembling human IBD in a mouse model system with a conditional deletion of the Xbp1 gene specifically in the intestinal epithelium. Hence, cell type-confined stress in the ER of the epithelium is sufficient to induce organ-specific inflammation in the intestine. Notably, the XBP1 locus has been genetically associated with both forms of IBD, Crohn’s disease and ulcerative colitis. Indeed, deep sequencing of a large cohort of patients revealed that rare (‘private’) variants of non-synonymous coding XBP1 variants are found in IBD, and functional studies revealed hypomorphic induction of the UPR. In addition to XBP1, genetic studies have revealed further ER stress and UPR-related genes that are associated with IBD, like ORMDL3 and AGR2. Further studies have also shown that point mutations in Muc2, the major constituent of the mucin layer, leads to ER stress and consequently intestinal inflammation in a murine model system, with similar ultrastructural alterations present in patients with ulcerative colitis. Moreover, ER stress might also be induced by manifold environmental stimuli, which might be particularly relevant in hosts with a genetic impairment in their capacity to resolve ER stress. Studies of human tissue specimen from IBD patients have revealed that the presence of ER stress in the intestinal epithelium is a very common feature, suggesting that in addition to primary genetic factors, environmental factors (i.e. those found in the intestinal microbiota or the diet) as well as inflammation per se might lead to substantial stress in the ER. Primary genetic impairment in the capacity to resolve ER stress (e.g. hypomorphic XBP1 variants) as well as secondary ER stressors might thereby induce and amplify, respectively, intestinal inflammation via the mechanisms highlighted above.
How can innate immunity be stimulated or corrected?

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Inflammatory bowel diseases are characterised by chronic intestinal inflammation at different sites. Data from animal models as well as human patients including gene association studies suggest that different components of the innate barrier function are primarily defective. These recent advances support the evolving hypothesis that intestinal bacteria induce inflammation predominantly as a result of a weakened innate mucosal barrier (e.g. antimicrobial peptides including defensins) in genetically predisposed individuals. Together, these findings should result in new therapeutic avenues aimed to restore defective innate barrier function to prevent a bacterial triggered inflammatory response. Current standard treatments do not seem to have substantial effects on the expression of main antimicrobial defensins. However, antibiotic therapy is currently effectively used in inducing remission, treating fistula as well as maintenance therapy after surgery. Results from several clinical trials suggest that infection with helminths is protective in IBD. Oral therapeutic intervention with live ova from *Trichuris suis* has been shown to improve the clinical outcome in IBD. This provokes the testable question whether the stimulation of protective innate barrier function like mucins, Paneth cell defensins or other antimicrobials by parasitic worms might be an explanation for their therapeutic effect. In addition, probiotic bacteria like *E. coli* Nissle 1917, other therapeutic probiotic E. coli (Symbioflor) as well as *Lactobacilli* have been shown to strongly induce some specific antimicrobial peptides. In case of *E. coli* Nissle (Mutaflor), the oldest known (in use since World War I, 1917) probiotic, this induction is mediated by a specific Flagellin. Its efficacy in maintaining remission in ulcerative colitis has been shown to be as effective as standard treatment with mesalazine in 3 placebo controlled, double-blind studies. Possibly, a defective β-defensin induction due to different and partly unknown mechanisms contributes to the low efficacy of probiotic treatment in Crohn’s disease. Another very promising and so far effective therapeutic strategy is lecithine which stabilizes the mucus barrier. Confirmatory multicenter studies in Europe are currently underway. Probiotic bacteria are the first known therapeutic agents for IBD that induce the production of antimicrobial peptides and this might be an important mechanism to prevent bacterial invasion into the mucosa but it is likely that other therapeutic agents like worm eggs, vitamin D, specific bacterial, food, artificial components or possibly prebiotics could have similar effects. Understanding these mechanisms and identifying new targets which treat the primary – not secondary – problem of the disease will be the aim of current and future studies.
Dogma 2

No bacteria, no IBD
Lessons from diversion studies and antibacterial interventions

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If bacteria cause inflammatory bowel disease then it should be possible to target them with therapies and cure or at least treat the disease. Discovery of a successful intervention, unless found by chance, will depend on knowing more about which bacteria are involved, where they are (lumen, mucosa, intracellular for example), and how to remove them.

Diversion studies only give indirect evidence. The faecal stream not only contains bacteria but also other potentially harmful components – secondary bile acids, bacterial metabolites including hydrogen sulphide, food additives including emulsifiers and particles, phenols and others. Moreover results of diversion have not been clearcut. Although diversion “by-pass” surgery was often used in the early years to treat Crohn’s disease, exclusion of small or large intestine can itself lead to inflammation – e.g. diversion colitis. Small bowel diversion surgery for Crohn’s disease was abandoned when it was shown to produce results that were worse than for resection and also accompanied by a risk of cancer in the excluded segment. Defunctioning stoma and bowel rest has however been shown to be more successful for colonic Crohn’s disease. Research studies performed by the Oxford and Leuven groups showed convincingly that restoration of the faecal stream induced recurrence of Crohn’s diseases in excluded colon and ileum respectively.

The best evidence for the beneficial impact of “bowel rest” on Crohn’s disease comes from trials of enteral feeding. There is no doubt that replacement of the normal diet with a formula defined enteral feed (and not all enteral feeds are equally effective) can achieve mucosal healing, probably particularly in small bowel Crohn’s disease. It is unclear though whether this is because of an impact on the gut bacteria. In marked contrast there is strong negative evidence to show lack of effect of bowel rest on ulcerative colitis, whether surgically or nutritionally achieved.

The response to antibiotics is mixed with some evidence for short term efficacy in both Crohn’s disease and ulcerative colitis from a variety of antibiotics that includes ciprofloxacin, metronidazole and rifaximin. We are not likely to make substantial progress though until we have a better understanding of the bacterial target. Is it for example \textit{E. coli} replicating within macrophages? – in which case we will probably need combination therapies that are better at targeting intracellular bacteria. Intriguingly a combination of antibiotics plus hydroxychloroquine – an antimalarial that enhances intra-vesicular killing of bacteria in macrophages, has now become first line therapy for two conditions: Q fever and Whipple’s disease, that are characterised by intra-macrophage bacterial replication. We have shown synergy between hydroxychloroquine and antibiotics \textit{in vitro} in killing of Crohn’s \textit{E. coli} isolates within macrophages and are about to start a controlled trial to address this.

\textit{H. pylori} only became accepted as the cause of duodenal ulcers when its eradication was shown to prevent ulcer recurrence and bacteria will similarly only become accepted as the cause of IBD, whether Crohn’s or UC, once therapies targetted against bacteria are shown to produce consistent results.
References:


Microbiology in pouchitis

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Restorative proctocolectomy with ileal pouch-anal anastomosis has become the surgical treatment of choice for the majority of patients with ulcerative colitis who require the surgery. While the surgical procedure offers a cure in some patients, postoperative inflammatory and non-inflammatory complications are common. Pouchitis is the most common long-term complication of the procedure. Pouchitis represents a spectrum of disease processes with heterogeneous risk factors, clinical phenotypes, natural history, and prognosis. Clinical evidence strongly suggests that luminal bacteria play a key role in the initiation and development of pouchitis.

Introduction
Approximately 25% to 30% of patients with ulcerative colitis (UC) would eventually require colectomy despite advances in medical therapy.1 Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) has become the surgical treatment of choice for the majority of patients with UC who fail medical therapy or develop dysplasia and for the majority of patients with familial adenomatous polyposis (FAP). Advantages of IPAA surgery include reestablishment of gastrointestinal continuity and improvement of health-related quality of life. However, trade-off of this bowel-anatomy-altering procedure is its high risk for the development of inflammatory and non-inflammatory complications, with a cumulative pouch failure rates ranging from 4% to 10%.2,3,4,5,6 The most common causes for pouch failure are pelvic sepsis,7,8 followed by Crohn’s disease (CD) of the pouch and chronic pouchitis.9 Pouchitis is one of the most challenging disorders in IPAA.

Incidence and prevalence of pouchitis
Pouchitis significantly affects patients’ health-related quality of life and long-term surgical outcome.10 Reported cumulative frequencies of pouchitis after 10–11 years of IPAA surgery ranged from 23% to 46%.11,12,13,14 It is estimated that approximately 50% of patients with IPAA for UC would develop at least one episode of pouchitis.15 The incidence within the first 12 months after ileostomy closure was estimated as high as 40%.16 In patients with pouchitis, 70% of them had the initial episode during the first 12 months after ileostomy closure.17 As the incidence of inflammatory bowel disease (IBD), including UC, appeared to increase, we expect a growing number of patients with pouchitis or other pouch disorders in clinical practice.

Role of microbiota in the etiology and pathogenesis of pouchitis
Pouchitis occurs almost exclusively in patients with underlying UC, and is rarely seen in patients with FAP who undergo the same surgical procedure.18,19 It is generally believed that pouchitis results from alternations in luminal microflora (i.e. dysbiosis), leading to changes in abnormal mucosal immune response in genetically susceptible hosts. Alteration in the bowel anatomy with fecal stasis after pouch surgery may result in the creation of an “inflammation”-prone environment. Without doubt, bacteria play a critical role in the pathogenesis of pouchitis in the majority of patients. This notion is supported multiple layers of evidence. For example, pouchitis only develops
after ileostomy closure, when the pouch mucosa starts to expose fecal stream. Manipulation of microflora with antibiotic or probiotic agents in patients with pouchitis often achieves therapeutic effect. Therefore, it is believed that qualitative and quantitative changes in bacterial flora in the ileal pouch may be a triggering factor for the development of pouchitis.\textsuperscript{20,21,22,23}

**Pathogenic microbes in pouchitis**

Hunting has been going on for pathogenic microbes in pouchitis as well as in IBD. After several case reports of *Clostridium difficile* infection in patients with IPAA, the role of the pathogen was systematically evaluated in our specialty Pouchitis Clinic at the Cleveland Clinic. Eighteen percent of symptomatic patients with IPAA were tested positive for *C. difficile* toxins A and/or B with a single-test enzyme immunoassay (EIA).\textsuperscript{24} The EIA-based assays have inherent pitfalls of lacking sensitivity and having inter-laboratory variation.\textsuperscript{25} One of the interesting findings of the study was that men were 5.12 (95% confidence interval: 1.38–20.46) times more likely to have *C. difficile* infection than women.\textsuperscript{24} Our anecdotal experience with molecular microbiological assay in *C. difficile* infection in IPAA patients confirmed the prevalence and male predominance of the infection [author's unpublished data].

The pathogenesis of *C. difficile* infection leading to pouchitis is not clear and mechanism of *C. difficile* colonization in small-bowel type mucosa is not known. There appeared to be a wide range of clinical presentations in IPAA patients who were tested positive for *C. difficile*, ranging from being an asymptomatic carrier to having fatal outcome.\textsuperscript{26} The patients may present with pouchitis, irritable pouch syndrome, or CD of the pouch.\textsuperscript{24} *C. difficile* in the setting of IPAA may colonize the small bowel mucosa, ileal pouch or the retained rectal columnar cuff. Reasons for the variable clinical presentation ranging from simple colonization in some patients to a fatal outcome in others are unclear. One of the postulated reasons may be because of the virulence of the strain. The increase in the reports of the highly virulent NAP1/O27 strain may be a contributing factor to fatal outcomes in a subset of patients. The other reason may be variations in the protective antibodies.\textsuperscript{27,28} For example, patients who became asymptomatic carriers exhibited an early increase in serum IgG antibodies against toxin A after colonization; whereas patients with *C. difficile* infection did not have similarly increased levels.\textsuperscript{28} Secretory immunoglobulin A antitoxins which were found in colonic secretions may inhibit binding of toxin A to their receptors at the specific brush border, providing additional immune protection.\textsuperscript{29} Thus serum and intestinal secretory antitoxins may provide protection and may be associated with mild colitis or carriage, while patients with deficient response develop severe or recurrent *C. difficile* infection.\textsuperscript{30,31,32} With regards to clinical management, our experience suggests that the majority of patients with *C. difficile*-associated pouchitis had been on or was on oral metronidazole at the time of diagnosis. Therefore, it has been advocated that oral vancomycin be considered as the first-line agent for the treatment of symptomatic *C. difficile*-associated pouchitis.

Routine stool cultures may yield other pathogenic bacteria, such as *Clostridium perfringens*,\textsuperscript{33} *Campylobacter* spp.\textsuperscript{34} Group D Streptococci (*Enterococci*),\textsuperscript{35} hemolytic strains of *E. coli*,\textsuperscript{36} and *Salmonella typhoid* spp. (author’s unpublished data). Those patients typically had systemic symptoms such as fever, chills, and night sweats. The
main limitation to these culture-based, cross-sectional studies is the fact that approximately 50% of gut bacteria are not culturable.

There are case reports of cytomegalovirus (CMV) infection in immunocompetent patients with pouches.\textsuperscript{37,38} Patients with CMV pouchitis typically presented with fever and general malaise, which have not been common symptoms of conventional pouchitis. "Opportunistic" infection of fungi (such as \textit{Candida albicans}) has also been implicated in pouchitis, particularly in patients with chronic antibiotic-refractory pouchitis.\textsuperscript{39} Probiotic therapy for those patients may be beneficial.

While microbiological investigation of the bacterial communities in the gut failed to demonstrate consistently the existence of pathogens in pouchitis, a large body of evidence suggests that alteration in bacteria community, i.e. dysbiosis, of human gut likely plays a key role in the initiation and development of pouchitis.

\textbf{Dysbiosis in pouchitis}

While extensive investigation of the bacterial communities in the gut failed to demonstrate consistently the existence of etiologic pathogens in IBD as well as pouchitis, a large body of evidence suggests that alteration in luminal and/or mucosa-associated bacteria, i.e. dysbiosis of human gut likely plays a key role in the initiation and development of chronic inflammation in IBD and pouchitis. Phylogenetic composition of the fecal bacterial community has notable stability in healthy individuals. This may not be the case in patients with IPAA. The construction of an ileal pouch may give rise to changes in fecal bacterial composition. In a culture-based study of fecal specimens in patients with UC pouches or FAP pouches, plate enumerations did not show significant differences in \textit{Lactobacilli}, \textit{Clostridium perfringens}, \textit{Bacteroides}, \textit{Bifidobacterium} groups, \textit{Enterococci}, and \textit{Coliforms} between UC and FAP pouches.\textsuperscript{33} However, sulfate-reducing bacteria were detected in higher numbers in active pouchitis than in those without a history of pouchitis, past episode(s) of pouchitis, or on antibiotic therapy, and in patients with FAP.\textsuperscript{40} This particular group of bacteria was sensitive to antibiotic treatment.\textsuperscript{40} Extended spectrum beta-lactamase-producing bacteria were frequently found in patients with antibiotic-dependent or antibiotic-refractory pouchitis, particularly in those with a long-term use of fluoroquinolones.\textsuperscript{25}

Longitudinal studies monitoring fecal bacterial compositions may provide clues for the evolution of bacterial community before and after pouch construction and before and after therapy for pouchitis. Gosselink \textit{et al}.\textsuperscript{36} analyzed bacterial content at the episode of pouchitis before and after antibiotic treatment, and during pouchitis-free periods and found that in the absence of inflammation, pouch microbiota were featured with by the presence of \textit{Lactobacilli} and large numbers of anaerobes. During pouchitis episodes there was a decrease in anaerobes, increase in aerobes, a lower number in \textit{Lactobacilli}, and a higher number in \textit{Clostridium perfringens}.\textsuperscript{36} Administration of metronidazole helped to eradicate anaerobic microbiota including \textit{C. perfringens}, while treatment with ciprofloxacin inhibited the growth of \textit{C. perfringens} as well as \textit{Coliforms}, including hemolytic strains of \textit{E. coli}. Ruseler-van Embden \textit{et al}.\textsuperscript{41} sequentially analyzed the bacterial composition of the ileal reservoir from patients with UC or FAP pouches. Two fecal samples were collected from each subject with normal pouches every 2 months and plate counts showed large differences in the anaerobic bacterial compositions between the samples taken at
different times in a given individual patient. The results suggest that that the non-
inflamed pouch had a bacterial community with unstable composition. Patients with
active pouchitis had an increased number in aerobes, decreased ratio of anaerobes
to aerobes, less *Bifidobacteria* and *Lactobacilli*, and a large number of *C. perfringens*
than patients with normal pouches. In a separate clinical cohort study, mucosa-
associated bacteria of the pouch were assayed using tissue biopsy samples at the
time of colectomy, pouch construction, ileostomy closure, and post-operative routine
pouch examination at 1, 3, and 12 months after ileostomy closure. The pouch
microbiota were similar to that in the normal colon for the presence of clones with
sequences resembling those of the *C. perfringens* group and *Turicibacter*. The
bacterial composition differed between the two patients studied and the microbiota
changed with time, suggesting that the composition is not stable during the first year
of ileostomy closure. Almeida *et al.* collected mucus of UC patients during
colonoscopy from all segments of the colon and terminal ileum before surgery, and
from the ileal pouch 2 and 8 months after ileostomy closure. They found that *Veillonella sp.* was the most prevalent bacterium in UC patients and controls and
*Klebsiella sp.* was significantly more prevalent in the ileum of controls that patients
with UC. *Enterobacter sp.*, *Staphylococcus sp.*, *Bacteroides sp.*, *Lactobacillus sp.*, and *Veillonella sp.* had higher mean concentrations in the ileal pouch of patients after
surgery than in controls. Therefore, it appears that microbiota change over time
before and after colectomy.

Nucleic acid-based microbiology methods for assessing gut bacterial community
have revolutionized the field. Advance in molecular microbiology with 16S ribosomal
RNA techniques is a cornerstone of microbial taxonomy, which has made the assay
of bacteria composition in fecal and/or mucosal biopsy specimens possible. A variety of molecular microbiology techniques have been applied to identify, monitor,
and characterize mucosa-associated bacteria in patients with healthy or diseased
pouches. 16S rRNA-based technologies, such as fluorescence in situ
hybridization denaturing gradient gel electrophoresis may be less labor intensive and
more feasible. A broad range of 16S rRNA gene PCR coupled with pyrosequencing
may provide rapid and cost-effective approach for sampling gut microbiota with
species level resolution and deep sampling. Komanduri *et al.* studied pouch
biopsy specimens from 5 patients with active pouchitis and 15 patients with normal
pouches using a fingerprinting technique. The study showed mucosa-associated
microbiota patterns unique to each individual. Moreover, specific bacterial amplicons
were unique to active pouchitis mucosa: *Clostridial* cluster XIVa, *Enterobacteriaceae*,
and *Streptococci* were associated with control pouches. It was also shown the
perseverance of *Fusobacter* and enteric species associated with the disease state. It
appears that a time-dependent shift from "ileum-like" to a "colon-like" bacterial
community, including non-culturable bacteria, in the ileal pouch after total
proctocolectomy. Terminal restriction fragment length polymorphism (T-RFLP)
analysis with cluster analysis demonstrated that the ileal pouch was characterized by
a time-dependent decrease in "ileal" and increase in a part of "colonic" fragments,
which represented mainly non-culturable bacteria such as the *Clostridium coccoides*
group. While there may be difference in bacterial composition between healthy and
diseased pouches, the variation in the microbiota among individuals was great.
Another hot topic on gut microbiology is bacterial diversity. Changes in mucosa-associated bacteria in pouchitis are also reflected by therapeutic administration of probiotic agents. Bacterial diversity was increased and fungal diversity was reduced in patients in remission maintained. Kuhbacher et al. conducted a double-blind, placebo-controlled trial to study the impact of a probiotic agent containing viable lyophilized bacteria per gram, comprising Lactobacilli, Bifidobacteria, and Streptococci on the dominant mucosa-associated bacteria from chronic pouchitis patients in remission induced by antibiotics. It was found that the mucosal microbiota was mainly detected within the epithelium and nearly all bacteria were affiliated with the Enterobacteriaceae group. Compared with the placebo group, an increase in Enterobacteriaceae within the mucosa during the probiotic therapy was observed. In a concurrent modular microbiological study using Proteobacteria/Enterobacteriaceae group-specific primers, slight differences in phylotypic composition were observed between the placebo and probiotic groups. Enterobacter species and E. coli were mainly identified. Lactobacillus and Bifidobacterium clone libraries generated from the probiotic group displayed a diverse spectrum of species in comparison with the 2 other experimental groups (pretreatment remission and placebo group). Analysis of the mucosa-associated microbiota showed that the probiotic therapy increased the bacterial diversity in comparison with pretreatment remission and placebo administration. McLaughlin et al. analyzed mucosal pouch biopsies from 16 UC (pouchitis 8) and 8 FAP (pouchitis 3) patients and genotyped the species (or phylotype) level by cloning and sequencing of 3184 full-length bacterial 16S rRNA. They found a significant increase in Proteobacteria and a significant decrease in Bacteroidetes and Faecalibacterium prausnitzii in the UC-pouch patients as compared with the FAP-pouch controls, and a limited difference between the UC-normal pouch and UC-pouchitis patients and between the FAP-pouchitis and FAP-normal pouch groups. Bacterial diversity in the FAP-normal group was significantly greater than in UC-normal pouches and significantly greater in UC-normal pouches compared with UC pouchitis. However, no individual species or phylotype specifically associated with either UC or FAP pouchitis were found. Those findings were supported by a separate study. However, the theory on bacterial diversity in pouchitis has not been validated by the other study.

Summary and conclusions
Like in IBD, microbiological assays of bacteria communities of gut in pouchitis would need overcome great technical hurdles. For example, approximately 50% of gut bacteria are not culturable. Bacterial cloning of 16S rRNA is expensive and labor intensive. With the advance in molecular microbiology, conventional stool cultures may still play a role in the investigation of etiopathogenesis and management of pouchitis. For example, fecal coliform sensitivity test has been used for the detection of potentially drug-resistant strains and for directing therapy of antibiotic-refractory pouchitis.

Bacterial composition varies between individuals even among healthy hosts. This has created the largest challenge to identify bacterial profiles unique for pouchitis with cross-sectional study design. Even with molecular microbiology techniques, no individual species or phylotype specifically associated with either UC or FAP pouchitis were found and the role of bacterial diversity and dysbiosis in pouchitis is controversial. It is not clear which compartment of bacterial community (luminal bacteria vs. mucosa-associated bacteria) is more responsible for the
development of pouchitis and whether there are true biofilms present on the epithelia of the pouch. Finally, there have been wide discrepancies in the findings from various studies for IBD and pouchitis, which may attribute several factors, including 1) patient population studied, pouchitis as well as IBD, likely represents a heterogeneous disease process; 2) analytical reliance on a polluted databank of 16S rRNA gene sequences; 3) relatively shallow phylogenetic analyses due to the nature of the available analytical tools, and PCR bias, which results in preferential amplification of 16S rRNA gene sequences from some bacterial genomes compared to others; and 4) from clinical perspective, there has been no uniformed diagnostic criteria for pouchitis and the disease status of the pouch can be a moving target.

The natural course of pouchitis dictates that some patients with antibiotic-responsive pouchitis may later develop antibiotic-refractory entity which would require anti-inflammatory, immunosuppressive, or even biological therapy. The natural history of pouchitis in this subset of patients may mimic that in IBD. Pouch and pouchitis may provide one of ideal human models to study the evolution of bacterial communities and host-bacteria interactions in IBD by sequentially monitoring microbiological and corresponding mucosal immunological profiles before, during, and after pouch construction, and before and after development and treatment of pouchitis. The NIH Human Microbiome Project initiative which seeks to understand human microbial communities in the healthy and diseased may help provide a reliable and feasible tool for study of pouchitis as well as IBD.

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The role of anti(myco)bacterial interventions in the management of IBD: Is there evidence at all?

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The etiology of inflammatory bowel disease (IBD) is unknown but may relate to an unidentified bacterial pathogen or an immunological reaction to gut microbiota. There is now strong evidence to support the hypothesis that Crohn’s disease (CD) develops as a result of invasion of the mucosa by live bacteria with defective clearance by the innate immune system and consequent replication of bacteria within macrophages. In ulcerative colitis (UC) there is little evidence of invasion by whole bacteria and more to support the hypothesis that a defective mucosal barrier may lead to increased interaction between bacterial components and basolateral receptors with inflammation as a consequence. Antibiotics have therefore been proposed as a therapy for CD and UC to induce remission in active disease and to prevent relapse. Current data are conflicting but a recent systematic review of randomized controlled trials (RCTs) has shown a statistically significant effect of antibiotics being superior to placebo for active CD, perianal CD, quiescent CD and active UC. Yet, these results have been poorly translated in clinical practice and the place of antibiotics is restricted to some specific situations in the international guidelines. This is first linked to the difficulties in interpreting clinical trials with antibiotics in IBD. Indeed, in all trials of active UC and CD a variety of single antibiotics and antibiotic combinations were evaluated, so it is not possible to recommend a specific antibiotic therapy. The exception to this is the use of either ciprofloxacin or metronidazole for treating CD perianal fistulas. Various parameters were used to measure efficacy and there were variations in what authors choose to call success with scarce use of objective parameters such as endoscopy. The pathology of Crohn’s disease, the likely primary and known secondary pathogens in this disease, and the successful responses in animal models all plead for new trials of antibiotics in IBD. This is a call to select patients more carefully, and to continue antibiotics for longer than is customary. Patients need to be stratified according to pathological type, bacteriological and maybe genetic markers. Beside antibiotics, new therapeutic approaches that can balance gut dysbiosis should be tested. The use of probiotics and prebiotics has been disappointing so far. Alternative strategies targeting interaction between proinflammatory species such as adhesive invasive *E. coli* (AIEC) and the epithelium should be tested.
**Enterotypes of the human gut microbiome**

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The functioning of the human body constitutes a complex interplay of human processes and ‘services’ rendered to us by the 1000 trillion microbial cells we carry. Disruption of this natural microbial flora is linked to infection, autoimmune diseases and cancer, but detailed knowledge about our microbial component remains scarce.

Recent technological advances such as metagenomics and next-generation sequencing permit the study of the various microbiota of the human body at a previously unseen scale. These advances have allowed the initiation of the International Human Microbiome Project, aiming at genomically characterizing the totality of human-associated microorganisms (the “microbiome”).

Here, I will present our work on characterizing the human intestinal flora based upon the analysis of high-throughput meta-omics (metagenomics, metatranscriptomics, metaproteomics) data. I will show how the healthy gut flora can be classified into three major types (“enterotypes”) that are independent from host nationality, age, BMI and gender. The discovery of enterotypes, characterized by distinct constellations of co-occurring gut micro-organisms, indicates that intestinal microbiota variation is generally stratified and not continuous. These host-microbial symbiotic states might respond differently to diet and drug intake, and could be of great importance in early diagnosis of intestinal dysbiosis.

Finally, I will show how the combination of meta-omics and dedicated computational techniques can lead to the detection of diagnostic markers for host properties and disease (e.g. in IBD and obesity), and aid in further understanding on how the gut flora disturbances contribute to these pathologies.

**References:**


Dogma 3

**TNF plays a pivotal role in IBD**
Is efficacy of anti-TNF therapy really related to neutralisation of TNF and apoptosis?

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The introduction of the IgG1 anti-tumour necrosis factor alpha (anti-TNFα) antibodies infliximab and adalimumab has been an important breakthrough in the treatment of Crohn’s disease. However, not all anti-TNFα agents are effective. For example, both a chimeric IgG1-soluble TNF receptor molecule (Etanercept) and an IgG4 humanized anti-TNFα antibody (CDP571) have failed to show any efficacy in Crohn’s disease.

The speaker will discuss our recent data that show that anti-TNFα antibodies but not Etanercept bind to membrane bound TNFα on activated T cells and activate the Fc receptor on monocytes with their Fc portion. This activation induces differentiation towards a wound healing macrophage phenotype in mixed lymphocyte reactions in vitro and in anti-TNFα treated patients in vivo. These wound healing macrophages potently suppress T cell proliferation in vitro. A role for the Fc receptor in the mechanism of action of anti-TNFs is supported by the failure of CDP571 as IgG4 is characterized by a very weak interaction with Fc receptors.

It has recently been shown in the SONIC trial that the combination therapy of an anti-TNFα with azathioprine is superior to an anti-TNFα alone. Interestingly, we find that the wound healing macrophage differentiation is potentiated by azathioprine.

Thus we find that IgG1 anti-TNFα antibodies are capable of inducing wound healing macrophages. This novel mechanism of action may help to understand several clinical observations that have thus far not been explained.
Mechanisms of relapse and loss of response to anti-TNF treatment

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Secondary failures are frequent in IBD patients. In most cases, losses of response are related to an increased clearance of anti-TNF monoclonal antibodies (mAbs). Data in patients with IBD and with rheumatoid arthritis suggest that low infliximab trough serum concentration correlate with loss of response. It has been demonstrated that low infliximab trough levels are associated with shorter duration of response in CD and less mucosal healing. Similarly, low adalimumab trough levels are associated with early and late discontinuation.

Elimination of monoclonal antibodies varies between individuals and is most likely influenced by immunogenicity. Anti-drug antibodies are associated with infusion reactions and loss of response. Immune complexes containing mAbs (as those made of mAbs and anti-mAbs) can be eliminated through interactions with Fc γ receptors (FcγRs). Also, anti-drug antibodies can inhibit the binding to TNF.

A decrease in drug levels may be driven by mechanisms other than the induction of anti-drug antibodies. Clearance of mAbs is a multi-factorial process, involving different mechanisms that are either antibody-dependent or host-dependent. Specific binding sites on the Fc domain of the mAb that interact with the FcRn and the Fcγ receptors play a crucial role. The neonatal Fc receptor (FcRn) has a protective role regarding IgG catabolism. Also, the inflammatory load (quantity of inflamed tissue and of TNF) could affect mAbs elimination.

Finally, lack of response to anti-TNF agents could be due to the importance of other inflammatory pathways which are potentially TNF-independent. Events of paradoxical inflammation in patients with immune mediated inflammatory disorders receiving anti-TNF suggest that some inflammatory pathways could be induced or promoted by TNF blockade. Beside his pivotal role in IBD, TNF have a regulatory role in normal tissues.

TNF blockade can lead to shifts of inflammatory cell populations and differentially regulate cells secreting specific cytokines. Some pro-inflammatory cytokines may be up-regulated. Several co-stimulatory molecules, such as NK receptors or members of the TNF superfamily, can amplify the immune response and promote inflammation. These pathways play a critical role in mucosal inflammation and experimental colitis that could be TNF-independent.

In conclusion, relapses under anti-TNF therapy occur frequently in IBD patients. Anti-drug antibodies play a major role, increasing the clearance of mAbs and reducing its binding to TNF. Other mechanisms may participate to the increased clearance of mAbs. TNF plays a pivotal role in IBD, but may have also a regulatory role. Thus, we may consider that a complete blockade of TNF could induce inflammation. The role of TNF-independent inflammatory pathways should be investigated extensively.
Anti-TNF therapy in CD and UC: Similar diseases, similar efficacy

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Crohn’s disease (CD) and ulcerative colitis (UC) collectively comprise a group of inflammatory disorders of the gastrointestinal tract that can vary significantly in virtually all aspects, including severity of disease, anatomic extent of inflammation, the presence and nature of extra-intestinal manifestations and response to a variety of therapeutic approaches.1,2-5 This clinical heterogeneity has led to more refined attempts at classifying CD based on the location and behavior of disease.6 More recently, advances in our understanding of the genetic susceptibility to inflammatory bowel disease (IBD) has led to the recognition that CD and UC may well represent a continuum of over-lapping disorders.7,8 This has led to an attempt to better classify IBD based on clinical, molecular and serological grounds.9 Beyond classification, important differences in clinical, genetic and immunological profiles may guide more targeted, refined treatment approaches based on more than disease location and severity. This is an area of active interest in IBD research which will hopefully help clinicians who must make decisions regarding therapeutic approaches in the rapidly evolving area of recently introduced biologic agents.

The use of anti-TNF therapy in Crohn’s disease and ulcerative colitis

The introduction of anti-TNF therapy into our therapeutic armamentarium has significantly changed how we treat IBD. It has well established that infliximab and adalimumab are effective for induction and maintenance of remission and lead to important endpoints such as mucosal healing and decrease in hospitalization and surgery.10-13 More recently, the discussion on how to use these agents has turned to the timing of the introduction of these agents, their uses as monotherapy or combination therapy and proper patient identification to insure that we are optimizing the benefit that patients may achieve. With respect to anti-TNF therapy, infliximab, adalimumab and induction therapy is associated with response rates of approximately 40–80% at 4 to 12 weeks in patients who have failed other standard therapies.10-13 However, earlier introduction of these agents may allow for better performance and improved outcomes. In a randomized control trial of patients with newly diagnosed CD who were naïve to corticosteroids, immunomodulators, and anti-TNFα agents, treatment was initiated either as a top-down approach with infliximab 5 mg/kg at weeks 0, 2 and 6 in addition to AZA 2.5 mg/kg day with infliximab being delivered subsequently on an episodic, as needed schedule or a more traditional step-up approach with corticosteroids followed by the initiation of AZA upon relapse or in cases of corticosteroid dependence, only then to be followed by infliximab in cases of ongoing disease activity.14 The primary endpoint was clinical remission without corticosteroids and without surgery at weeks 26, and 52. At weeks 26 and 52, 60% and 62%, respectively of patients treated with early infliximab met this stringent endpoint versus 36% and 42%, respectively in the step-up group. Moreover, the trial demonstrated that active CD could theoretically be treated without using corticosteroids. At 104 weeks, there was no longer a difference between the two groups in terms of clinical remission. However, in patients treated with early...
infliximab, the rate of mucosal healing at 104 weeks was 71% versus only 30% in patients treated in a conventional step-up manner. In what is perhaps the most important finding of this trial, the achievement of mucosal healing at 2 years was a strong predictor of remission off of steroids, absence of subsequent relapse or even need for further anti-TNF therapy out to follow up of 4 years implying that early, aggressive therapy could have long-term benefits.\textsuperscript{15}

Although the SONIC trial is not top-down in the strictest sense, it provides similar insight into the advantage of earlier use of an anti-TNF agent. The median disease duration in the SONIC trial was just over 2 years.\textsuperscript{16} Patients with active CD requiring corticosteroids were randomized to either AZA 2.5 mg/kg/day or infliximab 5 mg/kg induction and maintenance or the combination of both agents. The primary end point was remission off of corticosteroids at week 26, but important secondary endpoints included mucosal healing at week 26 as well as pharmacokinetic data on infliximab levels and antibodies to infliximab. At 26-weeks, 30.6% of patients on AZA were in remission off of corticosteroids compared to 44.4% with infliximab monotherapy and 56.8% for those on combination therapy with both agents. Mucosal healing at the same time point was even more striking at 16.5% with AZA, 30.1% with infliximab monotherapy and 43.9% with combination therapy.

Although we do not have the bounty of clinical trials in UC as we do in CD utilizing anti-TNF therapy, anti-TNF therapy has also proven to be a valuable addition to our therapeutic strategy. Infliximab has been approved for the treatment of moderate to severe UC in multiple jurisdictions and there are available data for adalimumab. In the ACT 1 and ACT 2 trials for moderate to severe UC, the use of 5mg/kg of infliximab induction therapy was associated with a 67% response rate and a 36% remission rate at 8-weeks in patients who had active disease despite standard therapies (aminosalicylates, or corticosteroids, or 6-MP/AZA).\textsuperscript{17} There is evidence for reduction in colectomy rates with infliximab. A clinical trial evaluating AZA versus infliximab versus combination therapy with both agents in patients naïve to these therapies for induction and maintenance of UC has recently been presented and results are strikingly similar to the SONIC trial in CD demonstrating superior efficacy of combination therapy.\textsuperscript{18} Adalimumab is also effective for the induction and maintenance of remission in moderate to severe UC.\textsuperscript{19} It is under review for this indication at the EMEA and FDA.

Taken together, these various lines of evidence unequivocally demonstrate a number of key points. Anti-TNF therapy is effective in both CD and UC. Combination therapy with infliximab and azathioprine is superior to infliximab alone in both CD and UC. Intervention earlier in the course of disease with effective therapy is likely to be more successful. This type of early intervention may decrease the likelihood of disease progression and need for surgical intervention. The next frontier to conquer is to accurately establish which patients would benefit from introduction of these agents at diagnosis and which patients may be able to stop these agents without losing longterm benefit.
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Therapeutic alternatives after failure of anti-TNF treatment for inflammatory bowel disease

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With the increasing use of biological agents over the last decade, failure of anti-TNF therapies has emerged as a key problem in inflammatory bowel disease management.

The key issues to address include the definition of failure of anti-TNF therapies, with an important distinction being that between loss of efficacy rather than unacceptable toxicity. Early clinical reassessment is necessary to determine the extent and activity of disease.

In the presence of active refractory disease, one needs to explore whether conventional medical therapies – thiopurines, or methotrexate – merit re-explanation, either as monotherapy, or in conjunction with anti-TNF therapy. It is of great importance to reassess whether surgical intervention provides the best alternative to continuing medical therapy – the arguments in ulcerative colitis for this approach are often compelling.

With the success of anti-TNF agents, there has been an almost exponential increase in the excitement associated with the development of novel biological therapies or immunomodulatory treatments. However, the sobering reality is that the lack of long term efficacy or safety data for these interventions needs to be considered carefully, and discussed fully, before use, even in the group of unfortunate patients with refractory Crohn's disease, whose quality of life is markedly impaired by disease and complications.

The unmet need in inflammatory bowel disease remains an intervention that will reliably alter disease progression with acceptable toxicity. The hope that haematopoietic stem cell transplantation in Crohn’s disease may provide this continue, but are tempered by recent data showing that even this intervention is associated with significant disease relapse over relatively short follow up periods. The future must involve a more basic understanding of disease pathogenesis, with the hope being either of these disease prevention, or the development of tailored therapies for individual patients at an early stage of disease.
Dogma 4

Steroids are useful for the management of IBD
How to guide therapeutic decisions in a patient-tailored approach?

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Individualized medicine is slowly entering care of patients with IBD, but the evidence to guide a patient-tailored is still scant. Some algorithms are however, backed by evidence from randomized trials and cohort studies and can be used in a tailored approach.

1. At diagnosis stratification of a patient with Crohn's disease should be based on the best estimate of the risk of a complicated disease course. Patients at risk such as: young patients with extensive small bowel disease, patients with severe rectal disease and those with complex perianal fistulas should be treated with early combined immunosuppression involving an anti TNF agent. On the contrary those with limited ileal disease and a predominant fibrostenotic phenotype may be eligible for surgery prior to any medical intervention.

2. In ulcerative colitis initial management should be tailored to the severity and the extend of the disease. Acute severe colitis at onset requires IV steroids and early progression to medical rescue or colectomy in case of none-response. Patients with mild to moderate disease should always be treated with mesalamine orally and rectally before moving to other treatment options.

3. Tailored management is also of paramount importance for patients on immunosuppressives and biologicals. Methotrexate is not the best choice for young female patients who may want to become pregnant. Although there is no clear evidence to prefer one of the anti-TNF agents available to treat Crohn's disease, a tailored approach is essential when dose adjustments are considered for loss of response. Reasons other than active inflammation should always be excluded and drug serum level and anti drug antibody levels may assist the clinician faced with secondary loss of response to an anti TNF agent.

4. Combined infliximab and azathioprine therapy is the best strategy for efficacy in patients naïve to immunosuppressives, but at least a proportion of patients may be able to continue with infliximab or azathioprine in monotherapy. The decision to stop any two should be based on a full re-assessment including infliximab trough levels and endoscopy to ascertain mucosal healing. For adalimumab no data are available to support combined therapy with azathioprine.

5. Post operative medical prophylaxis may be tailored to the risk of in a given patient of early endoscopic and clinical relapse. Patients with abdominal fistulizing disease, active smokers, and patients with a second or third intervention may be at increased risk but the data supporting this all stem from retrospective observations. The choice between surgical resection or surgical/endoscopic strictureplasty should also be tailored to the length and the complexity of the affected bowel segment.
Pathophysiological changes during steroid treatment for IBD (or mechanisms of response or resistance to steroids)

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Glucocorticoids (GC) exert their physiological effects predominantly through the GC receptor (GR) α. The GR is comprised of C terminal ligand binding domain (LBD) which is involved nuclear localization, transactivation, chaperon interaction and cofactor interaction, a hing region (HR), a DNA binding domain (DNB), important for DNA binding, transcription factor interaction and dimerization and an N terminal domain (NTD) important for cofactor and transcription factor interaction. A GR β form is also present and comprises up to 1% of cellular GR protein. This GR form is incapable of hormone binding and is thought to play mainly an inhibitory function, although some nuclear responsive elements for this protein have been identified. The GR is mainly present in the cellular cytoplasm bound to chaperons. Following binding of the LBD to the ligand, the GR undergoes a conformational change which allows its nuclear translocation and exertion of its biologic effects. The GR may exert its biologic functions via several cellular mechanisms. Binding of a GR dimer to GC responsive elements (GREs) leads to transactivation of the corresponding promoter. Alternatively, GRs may interfere with the binding of other transcription factors (transrepression), thereby inhibiting the activation of their respective products (such as NF-κB and AP-1). Additionally, GC may act via nongenomic pathways by affecting the interaction of the GR protein with other signaling pathways.

The immune-suppressant effect of GC in IBD has been traditionally attributed to induction of apoptosis and inhibition of cytokine production. However, GC variably affect different cells and inflammatory pathways. Activation of the innate immune system, antigen presentation and activation of the adapted immune system are thought to play an important role in IBD pathogenesis. GC were shown to suppress MHC class II expression and cytokine secretion from dendritic cells and to induce secretion of the suppressor cytokine IL-10. Similar effects were noted in macrophages including interference with activation of NF-κB in these cells. While GC increase the migration of polymorphonuclear cells from the bone marrow to the peripheral blood, they inhibit their tissue migration via down regulation of adhesion molecules. Variable effects have been shown for the effects of GC on T cells. While thymic and naïve T cells appear to be sensitive to GC-induced apoptosis, mature T cells are more resistant to this mechanism and in these cells GC effectively inhibit cytokine secretion, cellular proliferation and blastic transformation.

Not all patients respond equally to GC therapy. Increased expression of the drug efflux pump P-glycoprotein 170 encoded by the multidrug resistance (MDR) gene and of GR β were shown to be associated with a reduced clinical response to GC therapy. Furthermore, recent studies have shown that DNA structure affects response to GR binding and that different GR isoforms affect GR-regulated cellular function differentially. These observations combined with experiments showing that non steroid GR ligands variably regulate specific cytokine expression, offer the potential for development of improved drugs with increased specificity, better response rate and reduced side effects.
Steroids with biologics: Allies or enemies?

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Since their development, over 60 years ago, glucocorticoids continue to be the foundation of treatment for such diverse immune disorders as rheumatoid arthritis (RA), asthma and IBD. These agents are characterized by high potency, rapid onset of action, and the convenience of either oral or parenteral administration. Furthermore, in distinction to biologic therapy, they are very inexpensive.

Glucocorticoids have multiple effects on immune function including blockade of leukocyte trafficking, suppression of cytokine production and reduction of antibody formation. Although these pleiotropic actions provide the basis of the drugs high potency, additional off-target effects that result in the adverse effects of Cushing’s syndrome are an important limitation to long-term use. Of particular importance to patients with IBD are osteoporosis and an increased risk of infection. It is noteworthy that both retrospective and prospective cohort studies have documented an independent increased risk of mortality. Thus corticosteroid therapy presents clinicians and patients with both opportunities and challenges.

The role of corticosteroid monotherapy is well established in Crohn’s disease. Large scale clinical trials have shown prednisone (NCCDS) and prednisolone (ECCDS) to be highly effective for induction therapy, with rates of symptomatic remission of approximately 60%. Nevertheless, both of these trials demonstrated no durable benefit of maintenance treatment. In contrast TNF antagonists have shown efficacy for both induction and maintenance of remission in both Crohn’s disease and UC. The lack of long-term efficacy of corticosteroid therapy and the high incidence of adverse events has led some experts to advocate glucocorticoid-free treatment algorithms. Preliminary support for this concept was provided by the “Top-Down” trial that showed that initiation of early combined immunesuppression with infliximab and an antimetabolite was a superior strategy to a conventional “Step-Care” approach featuring initial treatment with glucocorticoids. Minimal exposure to prednisolone was achieved in the early combined immunesuppression arm. An important and unanticipated finding of this trial was that the higher rate of clinical remission observed in the experimental arm of the trial was associated with better long term outcomes.

Despite these results more data are required before glucocorticoid therapy for IBD is abandoned. In RA clinical trials have demonstrated that these agents are highly effective when combined with other disease modifying drugs. No trials in IBD have specifically examined the efficacy and safety of glucocorticoids as an adjunct to TNF antagonist therapy, however the strikingly high rates of clinical remission observed in the COMMIT and Leman trials, where these agents were combined for the treatment of high risk patients, holds out the possibility of the additive benefits of this approach. The concept of combination therapy (infliximab with an antimetabolite) has recently been validated by the SONIC and UC SUCCESS trials, however the optimum combination of drugs remains unknown. Given that glucocorticoids and TNF antagonists are the most powerful inductive drugs it is not unreasonable to speculate that their combined use would result in additive or synergistic efficacy and the potential for disease modification. This possibility should be tested in future trials.
Novel steroid formulations: Higher efficacy, less toxicity?

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Steroids have been used for many years in the treatment of both Crohn's disease and ulcerative colitis. However, beside being highly effective, the toxicity profile of systemic steroids is impacting their use in the long term. In addition, steroids are not effective in most of the patients in maintaining long term remission.

Significant concerns exist regarding their risk for adverse events, particularly when used for long treatment courses. Therefore several new steroids have been developed, with a better safety profile.

Budesonide is a glucocorticoid with limited systemic bioavailability due to extensive first-pass hepatic metabolism. Budesonide has been shown to be effective for induction of remission in Crohn's disease. In addition, budesonide has been recently delivered to the colon using the multimatrix system (MMX) and has been recently proven to be effective in mild-moderate ulcerative colitis. Also, beclomethasone dipropionate has been also been used in ulcerative colitis as a new steroid with a better safety profile. Finally, Erythrocyte-mediated delivery of dexamethasone in mild-moderate ulcerative patients is under development.
Dogma 5

Early and late Crohn’s disease are distinct entities
Phenotypically, the transition from early to late Crohn’s disease is characterized by the occurrence of complications including strictures, intra-abdominal fistulas and perianal fistulas, all of them leading to various types of surgeries and currently non reversible tissue damage. It must however be kept in mind that this transition is absolutely not a uniform and linear process. According to these simple phenotypic criteria, Crohn’s disease can already be a late disease at diagnosis while in other patients, it can still be an early disease after 20 years of evolution. This simply highlights the relativity of time in this field, actually reflecting the nature, the location and the severity of the inflammatory process. The risk over time of the development of these complications has been described, first in cohort studies⁠¹,² and then in population-based studies³. Globally, at diagnosis, only between 19 and 38% of Crohn’s disease patients have complicated Crohn’s disease. After 10 years, between 56 and 65% have developed either stricturing or penetrating complications. After 20 years, these numbers are between 61 and 88%. Beside these general figures, some particular aspects must be emphasized and some pending questions remain. First, the development of these complications is highly depending on the disease location, stricturing and internal fistulizing disease being much more frequent in small bowel Crohn’s disease (up to 90% over 20 years in ileal disease) while perianal penetrating disease is much more frequent in colonic and particularly rectal disease (up to 90% over 20 years for rectal disease). Second, we don’t know anything about the real timing of development of these complications mainly because there is a time-lag between their anatomical development and its clinical diagnosis. Particularly, the speed at which a stricture may develop and the causative and mechanistic relationship between intestinal stricture and internal fistula development would require dedicated studies. Third, we don’t know what are the main driving forces of these changes: genetic background or environmental triggers?

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Markers that differentiate early from late IBD?

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Inflammatory bowel diseases (IBD) are life long conditions with an onset that can occur at any age, but peaking in the late teens and early twenties, the age where childhood transcends from puberty into adulthood. Although no hard scientific evidence exists about differing aetiology, pediatric onset IBD does ‘differ’ from adult IBD in many aspects. In fact, there is growing evidence from clinical observations, as well as, epidemiologic and natural history studies that pediatric onset IBD represents a distinct disease with differences in disease type, disease location, disease behavior and gender preponderance. Recent data suggest that genetically attributable risk and serological markers against microbial antigens may also differ compared to its ‘adult’ counterpart. While the pathogenesis of IBD is thought to be multifactorial, disease expression commonly is characterized by the production of immune responses to a variety of microbial antigens. Detection of the serologic responses associated with CD has the potential to identify subgroups of patients at risk for aggressive or complicated disease. Identifying differences in immune response among large populations of patients at the time of initial diagnosis may also offer clues to the pathogenesis of the disease. In this lecture, first we will examine the genetic determinants and serological markers of IBD by demonstrating how pediatric onset IBD differs from adult disease including evidence from family and population studies, as well as highlighting differences in disease demographics and phenotype. Particularly, anti-CBir1 assay can identify a subgroup of children with Crohn’s disease (CD) who would otherwise not be characterized serologically. The age-associated differences in the patterns of antimicrobial seropositivity will be highlighted. In addition, we will review the current knowledge of molecular genetics in pediatric IBD emphasizing the similarities and differences with respect to adult IBD. Lastly, we will highlight possible future directions of genetics and serology in the field of pediatric IBD that differ from adult IBD.

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Can fibrosis be prevented in Crohn’s disease?

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Crohn’s disease (CD) is a very heterogeneous condition and more than one-third of CD patients will develop a fibrostenosing phenotype, characterized by progressive narrowing of the intestinal lumen. In these patients abnormal bowel fibrogenesis is due to chronic transmural inflammation and impaired wound healing, which result in massive fibroblast proliferation and an excessive deposition of ECM in the bowel wall. Ultimately, abnormal contraction of the ECM will also contribute to tissue distortion and intestinal obstruction. Relatively minor progress has been made into the molecular mechanisms that lead to bowel fibrosis, as compared to liver, lung, kidney, or skin fibrosis. Several molecules have been shown to be involved in the abnormal bowel fibrogenesis that takes place in stenosing CD patients. Among them, basic fibroblast growth factor (bFGF) and insulin-like growth factor 1 (IGF-1) seem to play a key role in that process. They are up-regulated in bowel strictures of CD patients where they promote both fibroblast proliferation and ECM production.

It is well known that an exquisite equilibrium between cell proliferation and programmed cell death is required to maintain physiological homeostasis in any tissue. In CD, enhanced proliferation along with defective apoptosis of immune cells are considered key pathogenic elements. This notion is supported by the histological observation that a large number of inflammatory cells infiltrate the bowel wall in patients with active IBD. Such leukocyte infiltration must result from either enhanced leukocyte recruitment from the blood stream, an abnormal expansion of these cells within the bowel wall, or a combination of both events.

Of note, no medical treatment for bowel fibrosis has become available to date, in spite of the remarkable success of the new, anti-inflammatory therapies recently developed for inflammatory bowel disease (IBD). Due to the lack of medical therapies for bowel fibrosis, most CD patients with a stenosing phenotype will require surgical resection of the involved bowel segment, either once or, often, more times during their lives.

Taking the previous into account, and from a conceptual point of view, prevention of bowel fibrosis in CD patients might be accomplished by either acting at an earlier phase, this is reducing the bowel inflammation and damage or at a later stage, influencing the molecular mechanisms responsible for the abnormal tissue repair and fibrogenesis. In respect to the first option, while the notion that any reduction of bowel inflammation and damage should have, by itself, an anti-fibrogenic effect seems logical, we still lack a direct corroboration of this hypothesis. Moreover, some evidences point towards a pro-fibrogenic effect of some of the most powerful anti-inflammatory drugs for CD, such as infliximab. In respect to specific anti-fibrogenic strategies, several very different approaches, incluging the use of the angiotensin converting enzyme inhibitors captopril, losartan and others, IL-13, tocotrienols, and the NFkB p65 antisense oligonucleotides, have proven their antifibrogenic capacity, not only in the bowel but also in other tissues, in the setting of experimental, animal models of fibrosis. It’s clearly time to translate these pre-clinic observations into pilot studies in human CD.
Can we slow down evolution from early to late CD?

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Crohn’s disease represents a life-long condition that is characterized by chronic progression with development of non-inflammatory complications (e.g. stenoses, fistula, abscesses). Only few patients present with uncomplicated courses in which phases of symptomatic activity intervene with a complete remission. Most patients develop an ongoing, chronic inflammatory activity (as defined on the level of the immune system) which persists even if a symptomatic remission can be induced and maintained by therapy.

The concept that chronic inflammation as the main initial causation of symptoms leads to non-reversible damages during the course of disease (and hence develops into a symptomatic state that can no longer be influenced by anti-inflammatory therapies) calls for development of disease modifying interventions. Anti-TNF therapies have been demonstrated to alter the course of disease (i.e. induce mucosal healing and reduce CD-specific hospitalizations and surgeries). However, these agents are often been used late in the disease career and therefore in patients who have already developed some non-inflammatory complications.

The use of anti-TNF therapies in early disease (e.g. before azathioprine or after the first course of steroids, respectively) has shown a high efficacy of these drugs to induce symptomatic remission, prompt mucosal healing and allow the discontinuation of glucocorticoid therapy. The next step in the evolution of CD therapies will be therefore the use of early interventions with agents like the anti-TNFs in patients who carry a risk profile for fast progression of disease and development of complications.
Dogma 6

Innovative treatments will offer a better outcome for patients with IBD
Biologic therapies: Lessons from multiple sclerosis

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New evidence points to possible association of multiple sclerosis (MS) with inflammatory bowel disease (IBD) (1). Tumour Necrosis factor (TNF) is involved in the pathogenesis of MS. Paradoxically administration of anti-TNF monoclonal antibodies to IBD patients have led to exacerbation of MS or precipitation of demyelination. TNFR1 mediates demyelination and TNFR2 mediates remyelination (2) suggesting that a more selective approach to TNF antagonism may be required for an anti-TNF strategy to be effective in MS. Conversely interferon treatment for MS may worsen IBD. Anti-lymphocyte trafficking strategies such as alpha 4-integrin blockers are effective in both MS and IBD. Recent advances in small molecule development in this area may provide further effective therapies. Common inflammatory cytokine and signalling pathways may be shared between IBD and MS, such as TNF, IL-12/23, IL-17, CD40, STAT3. However ustekinumab did not show efficacy in MS in contrast to Crohn’s disease. Vitamin D deficiency is a hot topic in both diseases. Several drugs developed for MS are also being studied in IBD such as glatiramer acetate. In addition to rheumatoid arthritis, MS also provides an example of a chronic relapsing inflammatory disease where disease modification is the goal of treatment based on objective evidence derived from imaging. These provide examples of how to conduct IBD studies in future.

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Current status of mesenchymal stem cell and bone marrow transplantation in IBD

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Cellular therapy is a promising new approach to address unmet medical needs in patients with inflammatory bowel disease, mainly Crohn’s disease (CD). The interest on the stem cell field is based on the unique biological properties of these cells and their capacity to self-renew and regenerate tissue and organ systems, and also on the immunomodulatory ability of stem cell therapy.

Apart from case records, two series have reported autologous hematopoietic stem cell (HSCs) transplantation for CD as the primary indication. The first is a phase I study from Chicago including 24 patients with active moderate-severe CD refractory to conventional therapies. All patients went into remission with a CDAI less than 150. The percentage of clinical relapse-free survival defined as the percent free of restarting CD medical therapy after transplantation was 91% at 1 year, 63% at 2 years, 57% at 3 years, 39% at 4 years, and 19% at 5 years. The percentage of patients in remission (CDAI < 150), steroid-free, or medication-free at any post-transplantation evaluation interval more than 5 years after transplantation remained at or greater than 70%, 80%, and 60%, respectively. A second is a phase I–II study from Milan including 4 patients with similar characteristics, and showing clinical remission at 3 months in all cases, and sustained remission after a median follow-up of 16.5 months in 3 patients. No mortality was observed in the two series of patients.

In Europe and Canada, a randomized trial on autologous HSCT (ASTIC) is currently ongoing to answer the question of whether autologous HSCT adds any benefit to the effect of immunosupression used during mobilization.

Although promising, HSCT for CD is still experimental and its toxicity leaves this option for a considerably reduced number of refractory patients in whom the disease is not amenable to surgical resection. A more recently developed less aggressive approach to stem cell-based therapy involves the use of mesenchymal stem cells (MSCs). Successful pre-clinical studies using MSCs in models of autoimmunity, inflammation or tissue damage have cemented the way for clinical trials. A recent phase I study showed that intravenous application of autologous MSCs was feasible and well tolerated in 10 patients with refractory Crohn’s disease and that might produce clinical benefits.
Resetting the mucosal immune system: Dream or reality?

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In Crohn's disease at least, tissue injury appears to be driven by Th1/Th17 cells responding to luminal bacterial antigens, although the evidence for this occurring via a TCR/peptide/MHC interaction is in fact extremely sparse. The cascade of cytokines, mediators and proteases produced downstream of T cell activation have been targets for therapeutic intervention, and in the case of TNF-α, with spectacular success. Nonetheless, the ultimate goal of therapy should be to either reprogramme pathogenic T cells to a more benign phenotype (including memory T cells), or to replace the immune system. The latter is underway with autologous stem cell transplantation. Strategies are also underway with the former notion. Although visiluzimab, a tolerising anti-CD3 antibody, failed in ulcerative colitis, this may because UC is not a T cell-mediated disease.

We have been investigating the function of another tolerising anti-CD3 antibody, otelixizumab. Otelix does not induce Crohn's disease or healthy mucosal T cells to divide, undergo apoptosis, or secrete IFN-γ or IL-17A. Instead, when added to mucosal explants or lamina propria mononuclear cells from Crohn’s patients, it reduces pro-inflammatory cytokine production and increases IL-10 production. This effect appears to be TGF-β and Smad7-independent. Global phosphor-protein pathways associated with Crohn’s disease are also dampened. The reduction in pro-inflammatory cytokines can be blocked by anti-IL-10 antibody. Otelix therefore appears to signal to pathogenic T cells in Crohn’s disease to induce immune-deviation towards a more benign cytokine pattern. This is also the first example of therapeutic antibodies inducing immune deviation in human tissues.
Old wine in new bags: 5-ASA revisited

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5-Aminosalicylates (5-ASAs) have been successfully used for the treatment of Crohn’s disease (CD) and ulcerative colitis (UC) for several decades. While treatment with 5-ASA remains a fundamental strategy for the induction and maintenance of remission for mild to moderate ulcerative colitis, several controversies have evolved since the original trials with sulfasalazine and mesalazine in CD. While the original trials with sulfasalazine in CD demonstrated benefits for sulfasalazine in colonic involvement, controlled trial evidence for the role of sulfasalazine and other 5-ASAs as an induction and maintenance therapy for both small bowel and colonic CD is still controversial. Oral mesalazine has been demonstrated in controlled trials to be superior to placebo in mild–moderate CD. In a recent trial with mesalazine granules in patients with mild to moderate CD, mesalazine was not inferior to the comparator budesonide and remission rates over 60% were observed. However, it seems to be less efficacious than corticosteroids in inducing remissions in CD. The maintenance benefits of 5-ASAs in CD appear to be limited to patients that have been induced into remission with 5-ASA and in some postoperative settings.

It is generally accepted that topical 5-ASA is the most efficacious approach to distal UC. In addition, oral 5-ASAs have been shown to be highly effective in inducing and maintaining remission in mild-moderate UC. Interestingly, combined treatment with oral and rectal application of 5-ASA improves the therapeutic responses in both distal and extended UC.

Current interest focuses on optimal use of 5-ASAs. Recent studies assess new dosing schedules, new formulations with different release kinetics and combination of oral and rectal 5-ASAs or combination of 5-ASAs with other medications (probiotics, immunomodulators, antibiotics). New dosing schedules with new 5-ASA formulations have been demonstrated to improve patients’ adherence to 5-ASA therapy and have turned out to be at least as effective as 2-4 times daily dosing of 5-ASAs in clinical trials. Recent trials with various slow-release mesalazine formulations in patients with active and quiescent UC have demonstrated that OD dosing of retarded mesalazine was more efficacious than the previously widely accepted and recommended BID or TID dosing of this 5-ASA-formulations.

Nevertheless, a number of unsolved questions need to be addressed in the future regarding optimal dosing of oral and rectal 5-ASAs as an induction and maintenance agent for both UC and CD. Additional clinical data from controlled trials are needed to assess whether oral 5-ASA can maintain remissions after rectal 5-ASA induction in UC. The dose-response and efficacy of 5-ASAs after steroid-or immunomodulator-induced remissions in UC has also not been studied systematically. The role of 5-ASA for induction and maintenance treatment of CD needs to be addressed again, as better characterization of patients may help to select subgroup of patients that may benefit from treatment with 5-ASAs.
Dogma 7

Patient tailored therapies are superior to algorithmic approaches in IBD
Predicting the disease course from diagnosis

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[de Romberg-Camps, 2009] The disease course in IBD is highly variable, from acute illness leading to colectomy or even death, to a single episode of disease symptoms in the course of a lifetime. Disease course seems to be difficult to predict on the basis of information acquired at the moment of diagnosis. However, for clinical purposes, it would be useful to categorize patients at the onset of disease into low- or high-risk groups. In that way, specific therapeutic and preventive strategies might be chosen earlier in the disease course, and patients could be informed more precisely about their prognosis. Even more because recent data suggest that treating severe IBD "top-down" might change natural history of the disease. In Crohn's disease, cohort studies found that a relatively small proportion of patients remain free of disease recurrence for prolonged periods of time; this proportion has been estimated to be 14% at 5 years and 12% at 12 years. In a study from Copenhagen, 22% of patients had no relapse after a mean follow-up duration of 8.5 years, and a European cohort study showed that half (57%) of all patients had two recurrences or less during a 10 year observation period.

Around 10% of the CD patients had need resective surgery at the moment of diagnosis. Out of all patients with resective surgery, 21% were operated on during the first year; after 12 years of follow-up, reoperation rate is around 10% for all types of surgical intervention and 2% for resective surgery.

In CD, the correlation between disease phenotype at diagnosis and postsurgical recurrence has been reported in several referral-center-based studies, indicating penetrating disease as a risk factor. Other reported phenotypic risk factors in CD for postsurgical disease recurrence were young age at diagnosis, upper gastrointestinal disease localization, small bowel involvement, perforating disease and perianal disease. In a European study, patients from the North of Europe had a higher risk for surgery, with colonic disease localization at diagnosis as well as nonsmoking during the disease course were found to be protective. CD patients with disease localization in the terminal ileum, ileocolonic, and upper gastrointestinal, as well as patients with stricturing and combined stricturing-penetrating behavior have a relatively higher surgery risk than other presentations.

Phenotype at diagnosis has also been found to influence the need of immune-suppressive and steroid medication. Patients under 40 years of age at the time of diagnosis used these medications more often, and experience more recurrences, most probably reflecting a worse disease course. With regard to environmental factors, various studies have shown a deleterious effects of continuing smoking on disease course, although also this concept has been recently challenged.

There have been numerous attempts to provide predictive tools based on the analyses of genetic and serological immune markers at the time of diagnosis. Only recently the prognostic ability of a group of serologic markers has been demonstrated, although this findings need to be validated and its real value in clinical management carefully studied.

In UC the proportion of patients remaining relapse free have been reported to be 20% after 5 years of follow-up, and 22% after 10 years in another study. Other studies providing estimations of total relapse rate at 10 years after diagnosis in UC...
showed that between 67% and 97% of patients have suffered recurrence of symptoms during this period\textsuperscript{9,17,18}. Phentotypic characteristics of the disease at diagnosis influencing disease recurrence have not been well established, and contradictory reports on the influence of age and disease extension have been published\textsuperscript{9,16}. No serologic or genetic marker has been found to have a predictive ability of clinical relevance in UC.

References:


How to guide therapeutic decisions in a patient-tailored approach to treatment of IBD?

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Therapeutic decisions in the treatment of IBD involve the initial choice of therapy(ies) and designing a long-term strategy for the individual patient. Putting forward clear therapeutic aims therefore is critical in order to assess treatment success and to guide sequential use of therapies. Although the eventual goal of therapy is to achieve steroid-free remission and avoid complications and surgeries these aims will be met in a minority of patients only with the first therapeutic intervention. Depending on the needs and the successes of each therapeutic step interim goals are pursued which may be small steps towards the total control of the disease. For instance when we start steroids the aim still is to achieve remission which will be complete only if successful tapering and weaning at a later stage is possible. Resection of a bowel segment in Crohn’s disease eventually should allow long-term control of the disease without steroids and avoidance of repeated surgeries although the initial medical therapy was unsuccessful.

A patient-tailored approach does not necessarily conflict with algorithm based decision making. They are rather complementary. The former allows to skip parts of the steps in the algorithm based on the individual patient characteristics. The latter supplies a basis for rational sequential use of drugs. Now already many physicians use an accelerated step-up approach in the treatment of IBD, mainly Crohn’s disease although it is not well established whether this is associated with a better outcome.

We strongly feel that the therapeutic approach should be based on mucosal activity and the location and extent of the disease whether it is assessed by endoscopy or (and) CT or MRI. These assessments are very useful at times where important decisions have to be made, e.g. at start of biological therapy, before surgery and also to monitor the effect of therapy. Treatments that do not heal (or at least improve) ulcers are not to be continued if they have been given a reasonable time to work. Biomarkers like CRP and calprotectin can be useful surrogates in this setting. So we need to treat lesions instead of mere symptoms. Optimization of the treatment effects involves the measurement of drug levels and metabolite levels.

There are specific situations which need always an individualized approach such as perianal fistulizing disease, severe extra-intestinal disease, growth retardation where the luminal load of disease is not the most important determining factor.

Eventually we will probably only be successful if we use therapies that heal the mucosa as soon as possible after diagnosis.

Reference:

Pharmacogenetics and prediction of therapy success

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The ability to not only predict the natural history of disease, but also response to, and side effects from, therapies in their patients has long been a goal for clinicians managing patients with inflammatory bowel disease. A number of non-genetic ‘biomarkers’ have been associated with response to and lack of response to IBD therapies. For example a number of studies have demonstrated that both Crohn’s disease (CD) and ulcerative colitis (UC) patients who are perinuclearantineutrophil cytoplasmic antibody (pANCA) ‘positive’ are less likely to respond to anti-TNF therapy. Other studies have suggested that CD patients with raised CRP are more likely to respond both to mono- (anti-TNF alone) and combination (anti-TNF and thiopurine) therapy. These data and the heterogenous nature of the IBDs strongly suggest that there will be subgroups of patients who are more likely to respond to different therapies and that the potential benefits of identifying the patients that fall into these groups would be a significant step towards a more personalised approach to managing IBD patients. Of course many clinicians already utilise pharmacogenetics in the form of either measuring genotype or enzyme activity of thiopurine methyltransferase (TPMT) prior to commencing azathioprine or 6-mercaptopurine in the hope of minimising the risk of bone-marrow toxicity. One of the hopes of the recent significant advances in the genetics of IBD is that these advances will enable the identification of genetic markers that predict additional outcomes related to therapies. There are a number of ways that this may conceivably be achieved/attempted. The first methodology follows the hypothesis that different susceptibility genes define different sub-phenotypes of IBD with different pathological mechanisms that therefore may respond differently to various categories of therapies. For example a recent small-study has demonstrated some association between a number of the known IBD genes and response to anti-TNF therapy in pediatric CD. In addition studies may examine specific genes as candidates for predicting outcomes based on the knowledge of how therapies are proposed to achieve clinical response. One example of this approach is the identification of polymorphisms within an Fc immunoglobulin receptor and response to anti-TNF therapies in CD. These hypothesis or candidate gene driven approaches can be complimented by the hypothesis-free or genome-wide approach that has shown spectacular success in identifying IBD susceptibility genes. This approach allows for the principle that the exact mechanisms of how drugs achieve clinical response in IBD are not fully understood. Another approach benefits from genetic associations with particularly severe forms of IBD thereby identifying novel therapeutic targets for treating these extreme phenotypes. We recently published a study examining genetic markers associated with the need for colectomy in UC for failing to respond to medical therapy which demonstrated strong association with this phenotype and the HLA region and also with the known IBD locus incorporating TNFSF15 (see below). Finally, the identification of pathways relevant to IBD pathogenesis may help, through an understanding of an individual’s genomic profile, classify which pathways are important in any given individual. This may allow pathway specific therapies to be targeted to the correct individual. One example of where this approach may be pursued in the future is through the development of therapies targeted at TNFSF15,
a pivotal pro-inflammatory transcription factor. Polymorphisms at TNFSF15 are associated with IBD and have a particularly significant role in Asian IBD pathogenesis as well as potentially being a gene of particularly severe disease. One hope is that if trials of anti-TNFSF15 therapy are successful then individuals who carry TNFSF15 disease associated polymorphisms may particularly benefit from these therapies.

A cautionary note is that the majority of studies to date have been retrospective, with the inherit difficulties that are consequences of these approaches and large prospective studies examining the roles of genomics (including gene expression), clinical factors and other biomarkers are necessary for the full benefits of pharmacogenetics to be realised in IBD.
Approach to IBD: Treating the disease or the patient? How I do it

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Until very recently the therapies for ulcerative colitis and Crohn’s disease have been based on a symptomatic response and, hence, have been aimed at treating the patient according to both the clinical severity and prior clinical responses to conventional agents. Indeed, our assessment of “disease” was really an assessment of symptomatic responses. Disease activity indices for UC [1] have been shown to correlate well with simple assessments of symptoms without the need for endoscopy (or biopsy) [2]. Similarly, the primary disease index used in clinical trials for Crohn’s disease, Crohn’s Disease Activity Index (CDAI), is also a symptom-based index [3]. In both diseases, these clinical assessments have been validated in clinical trials and demonstrate reproducibility and responsiveness; but have not correlated well with endoscopic or histological activity indices, and have not predicted disease progression or complications. Nevertheless, the sine qua non question: “whether to treat patients who are completely asymptomatic with residual endoscopic (or histological) disease should be treated to ‘deeper’ mucosal remissions” is usually negative if it requires advancing the current level of therapy (i.e. from aminosalicylates to corticosteroids). Hence, with conventional (non biological) therapies treatment has been targeted to induce clinical remissions…hence treating the patient.

However, utilizing the aforementioned approach has not altered the natural history of Crohn’s disease progression towards transmural complications of strictures and fistulae leading to surgical resections [4], or the long-term course of ulcerative colitis [5]. Hence, as we begin to expand our outcomes and end-points for clinical trials, observations, and expectations, the ability to impact on structural damage in Crohn’s disease and mucosal healing in both diseases offers the potential to not only treat symptoms, but to alter the course of these chronic disorders [4].

Recent observational [6] and clinical trial series [7–10] have demonstrated that mucosal healing in ulcerative colitis and Crohn’s disease can impact on the long-term course of disease by reducing the need for surgical resections, colectomies and hospitalizations[11]. Furthermore, there is increasing evidence that biological responses, such as C-reactive protein, also correlate with response to anti-TNF therapy and mucosal healing [12]. Ultimately, disease modification will require a measure of structural damage that can be measured with reproducibility and responsiveness such as the Sharpe score in rheumatoid arthritis [13].

In the past, the question of “whether to treat the patient or the disease?” may have been more philosophical, than practical. However, with the advent of more effective therapies and the potential to alter the long-term course of IBD a consilience is evolving that will incorporate both patient-reported outcomes with objective biological criteria to end points in clinical trials and clinical practice.
References:


Immunosuppression puts IBD patients at risk for severe complications
Combined immunosuppression is more dangerous than monotherapy: What is fact and what is rumor?

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In spite of the fact that Crohn’s disease (CD) is meanwhile considered at least in part the consequence of a disturbed innate immunity exacerbations and chronic activity are still treated with immunosuppressants. About 20% of patients in a Norwegian population based cohort needed immunosuppression during each 5 year period assessed. Numbers were smaller in ulcerative colitis (UC) and it is estimated that in a population based long term about 30% with CD and 15% of UC patients will need immunosuppression.

Several immunosuppressants are available, among them azathioprine, methotrexate (MTX), calcineurin antagonists and TNF-antibodies as the major players. The mechanism of action of all of those is different – they affect different cell populations in different combinations. As expected all immunosuppressants lead to an increased number of infections, in particular opportunistic infections.

They also lead to an increased number of malignoma, in particular lymphoma. This has been studied extensively in other disciplines, in particular in transplant medicine. Interestingly data on toxicity in different diseases causing immunosuppressive treatment are quite variable at least for azathioprine. A number of studies assessed the effects of the different immunosuppressants on natural killer cells, macrophage function, number of T helper cells and many other functions of the immune system. The effects observed vary widely and do not allow to suggest risks for specific infections associated to specific drugs. Tuberculosis in anti-TNF treated patients may be one exception.

Beside infections and malignoma several other side effects may occur, in particular liver damage with methotrexate, renal damage with cyclosporine and several others. The number of side effects in controlled trials seems to be lower than in daily practice probably due to a much more stringent patient supervision and patient selection. Population based data indicate significant numbers of infectious complications and an increased risk of malignoma as the main problems.

Combined immunosuppression has been used since the advent of azathioprine and 6-mercaptopurine as treatment for IBD. These drugs were mostly used in addition to steroids in patients with steroid dependency or steroid refractoriness. More recently the TNF blockers have been used in combination with immunosuppressants such as MTX in rheumatoid arthritis and azathioprine in IBD although not all studies showed that combination is necessary.

We have recently shown in patients with rheumatoid arthritis and other immune disorders that combinations of steroids with other immunosuppressants often lead to T-helper cells below 250, i.e. a status comparable to HIV infection. A dose of more than 10 mg prednisolone equivalent was the cutoff for an increased risk of infectious complications. Interestingly the number of opportunistic infections in patients with IBD
has been increasing significantly in recent years concomitant with the increased use of immunosuppression. Furthermore it is of importance that malnutrition which often occurs in chronic active CD patients and increased age are associated with an increased risk of such infectious complications.

Very few studies analysed effects of immunosuppressants on complications in a systematic way. Recently it was shown that the use of two or three immunosuppressants increased the odds ratio for an infectious complication significantly versus the use of only one. This was confirmed in another study for several infectious complications such as tuberculosis, candida infection, varicella zoster and sepsis. Interestingly recently the combination of immunosuppressants and antibiotics worsened the prognosis of IBD patients with Clostridium difficile infections.

Thus the question cannot completely be answered looking at the data from IBD patients. Recent studies suggest that combined immunosuppression is more dangerous. This suggestion will never be tested in a prospective controlled way. The overall risk of such events is too small to assess it in a prospective randomised trial in an appropriate time frame – we depend therefore on cohort studies and other concepts. Even meta-analysis will not really solve the problem mainly due to the fact that the randomized controlled trials used for meta-analysis have much lower complication rates as is observed in daily routine.

We might conclude that immunosuppression causes immune deficiencies. Immunosuppressants affect different parts of the immune system. Steroids are the most pluripotent. They increase effects and side effects of all or most immune suppressants. In the future we might need to use immunosuppression only as initial part of IBD therapy which has to be replaced after the inflammation recedes by reconstitution of epithelial integrity and finally improvement of the mucosal barrier including the innate immune system.
In an era of increasing use of immunomodulator therapy and biologics, opportunistic infections have emerged as a pivotal safety issue in patients with inflammatory bowel disease (IBD). Clinical studies, registries and case reports warn for the increased risk for infections, particularly opportunistic infections. Today’s challenge to the physician is not only to manage IBD, but also to recognize, prevent and treat common and uncommon infections. The recent European Crohn’s and Colitis Organisation (ECCO) guidelines on the management and prevention of opportunistic infections in patients with IBD provide clinicians with guidance on the prevention, detection and management of opportunistic infections. Proposals may appear radical, potentially changing current practice, but we believe that the recommendations will help optimize patient outcomes by reducing morbidity and mortality related to opportunistic infections. In this ongoing process, prevention is by far the first and most important step. Prevention of opportunistic infections relies on recognition of risk factors for infection, the use of primary or secondary chemoprophylaxis, careful monitoring (clinical and laboratory work-up) before and during the use of immunomodulators, vaccination and education of the patient. Special recommendations should also be given to patients before and after travel.
Lymphoma and cancer in IBD: Can they be prevented?

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Intestinal lymphoma is a rare complication of chronic IBD-related intestinal inflammation, favoured by local EBV infection and immunosuppressive therapy. This IBD-related rare type of lymphoma cannot be prevented at the moment. Most of the excess lymphomas in IBD are due to a loss of control of chronic latent EBV infection under immunosuppressive therapy, particularly thiopurines. Monitoring of EBV systemic viral load and preemptive treatment of EBV infection and/or early lymphoproliferation are extensively used in the post-transplant setting, but these methods have not been yet evaluated in IBD patients and cannot therefore be recommended in this context. However, measurement of systemic EBV viral load should be performed in cases of unexplained fever, adenomegaly or haemophagocytic syndrome, in order to optimize the diagnostic of early EBV-related lymphoproliferations. Young male IBD patients seronegative for EBV are at risk for fatal forms of primary EBV infection, with post-mononucleosis lymphoproliferation. This accident could be limited by avoiding the use of thiopurines in EBV-seronegative males, and may be in the future by detecting in these patients before initiation of thiopurines genetic X-linked predisposition. The risk of hepatosplenic T-cell lymphoma could be limited by avoiding prolonged combination therapy with thiopurines and anti-TNF (beyond 2 years) in young males. There is a highly plausible excess risk of non-melanoma skin cancer in IBD patients currently or previously treated with thiopurines, that justifies from now strict sun protection and dermatological screening. The risk is still unclear for monotherapies with anti-TNF. An excess of HPV-related uterine cervix dysplasia and cancer has been reported in various populations of IBD women, but the proper role of immunosuppressive therapy remains to be quantified. Prevention of such cancers relies on strict periodical screening for cervix dysplasia in women with IBD.

Colorectal cancer is the most frequent inflammation-related excess cancer in IBD, due to chronic macroscopic and microscopic inflammation of colonic mucosa. It is plausible that emerging therapeutic strategies, aiming to obtain sustained mucosal healing from diagnosis, will reduce this risk. As an illustration, there is a growing evidence for a frank reduction in the risk of colorectal cancer in IBD patients at risk exposed to thiopurines. Such a feature should be evaluated for long-term exposure to anti-TNF alone. Carcinogenesis of IBD-related colorectal cancer is a specific multistep process, with growing evidence for early mutations in still normal-appearing cells, before dysplasia becomes detectable. There is a theoretical basis for various impacts of 5-aminosalicylates on inflammation-related carcinogenesis. The reality of the chemopreventive effect of 5-ASA is still matter of hot controversy but favourable signals are evidenced in the best characterized cohorts with optimal statistical methodology, justifying the European recommendation of chemoprevention with 5-ASA in ulcerative colitis, except in patients with proctitis. In Crohn’s colitis, evidence is weaker at the moment. Regarding other types of IBD inflammation-related cancers (small bowel adenocarcinoma, anal carcinoma, cholangiocarcinoma), we are still lacking efficient tools of screening and prevention.
How to manage IBD in patients with infections or malignancies?

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Infections and malignancies are a major issue for clinicians in the management of patients with Inflammatory Bowel Disease (IBD) because of concerns about the safety of drugs currently used in the treatment of IBD that includes immunosuppressive agents, steroids and Tumor Necrosis Factor (TNF) antagonists.

Infections are strongly associated with IBD both in their etiopathogenesis and in their clinical course. Although the role of infections in the onset of IBD is still hypothetical with contrasting evidences, it is nonetheless clear that infection can play an important role in the clinical course; particularly, infections can be responsible for the relapse of the disease, they can represent a complication of the disease itself or of its pharmacologic and surgical treatment. The most common problems for the management of IBD patients are due to infections with symptoms undistinguishable from active IBD, which eventually may complicate the intestinal disease, and infections that contraindicate immunosuppressive and biological treatments. Multiple organisms have been associated with relapse episodes including bacteria, viruses and parasites. The clinical importance of infections in the relapse of IBD requires prompt identification of the infectious agent, although the isolation from stools and biological fluids of a potential pathogen do not require always specific treatment. A number of viral infections, tuberculosis and other therapy-related infections create challenges for the successful management of the intestinal disease with immunosuppressive agents or TNF antagonists. Recently published guidelines offer a strong support in dealing with these issues.

Major concern about IBD patients with malignancies is related to the consequences of chemotherapy on the intestinal disease and the risk of maintaining immunosuppressant or anti-TNF therapy after the diagnosis of malignancy. No data are available to assess the specific treatment options in patients who have IBD and develop cancer, mainly intestinal cancer, even if IBD patients are known to be at higher risk for developing severe intestinal symptoms, due to the toxic effect of the cytotoxic drug or to a flare of the underlying bowel disease. The lymphoma risk in IBD patients is slightly increased although seems to be lesser than in other autoimmune diseases. However, whether the risk of developing a lymphoma is related to the immunosuppressive treatment, to disease severity or to the combination of these factors is not clear. In the literature the issue of treatment in IBD-associated lymphomas has received no attention. The available data from other autoimmune disorders, such as Rheumatoid Arthritis, show that standard chemotherapy alone or in association with the monoclonal anti-CD20 antibody is well tolerated and often induces a remission also of the underlying autoimmune disease. The use of high-dose myeloablative chemo-radiotherapy followed by autologous or allogeneic haematopoietic stem cells transplantation can induce a durable remission in Crohn’s disease patients also without an ongoing immunosuppressive treatment, but long term data are required.
Immunosuppressive drugs such as azathioprine, methotrexate, or TNF antagonists should be stopped during chemotherapy because they may lead to a higher incidence of haematological toxicity.

Despite recently published papers that undoubtedly are a significant addition to our understanding of the complex relationship between malignancies and anti-TNF therapy, there are a number of important questions that remain unanswered.

The possible effects of anti-TNF therapy on malignancy are difficult to predict accurately given the pleiotropic effects of TNF and the conflicting results from the existing literature.

There are no data regarding the beneficial or harmful effects of maintaining anti-TNF therapy in IBD patients with cancer. Most of the literature on the association between anti-TNF therapy and malignancy has focused on the incidence of cancer, but little is known about the influence of anti-TNF therapy on the progression and on the outcome of cancer or on the possible differential risk of cancer subtype.

Optimizing strategies for IBD patients with malignancies requires further research. At the moment all therapeutic choice is taken on an individual basis with an integrative multidisciplinary approach.
Wrap-up session

Leaving the dogmas behind and looking into the future
Towards a novel molecular classification of IBD

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University Hospitals, Leuven, Belgium

In general, classifying IBD patients is important for decisions on intensity of follow up, therapy and mode of delivery. The most recent classification of Crohn’s disease (CD) and ulcerative colitis (UC), the Montreal classification, is based on clinical grounds: for CD age at diagnosis, location and behaviour of disease and for UC age at diagnosis and extent of colitis. During the working party in 2005 in Montreal, it was judged by the panel of experts that a molecular reclassification using serology and or genetic markers, was too premature and not yet justified.

In the meanwhile, the number of genes for IBD has increased to > 100 and also more serological peptides have been associated with the disease. We recently showed that the genetic variants enable classification of CD patients in distinct clusters, which was different from clusters seen in healthy individuals. There was furthermore a poor relationship between the genetic-based subgroups and the used clinical subphenotypes.

The promising role for molecular markers even more lies in disease stratification and prediction of prognosis at the time of diagnosis. The behaviour of CD and UC varies between patients, and the characteristic transmural inflammation in CD – when untreated – will often progress and lead to stricture or fistula formation over time. Predicting which patients are at risk for progression to complications and at what speed could have therapeutic implications, as more aggressive treatment strategies could become defendable in the appropriate patient. Genetic factors are definitively more appealing for risk stratification on more solid grounds. Molecular markers may also better explain changes in disease behaviour from a pathogenic point, and by combining several markers, this strategy can approach clinical utility. A number of studies using genetic or serologic markers have correlated genes (NOD2/CARD15) or antimicrobial/anti-glycan antibodies (ASCA, Cbir1, anti-OmpC, anti-I2, ALCA...) with a more complicated disease. Before one can start applying molecular markers to predict these disease behaviours, tools should be made available to score disease progression. Only then, the value of molecular markers to predict the speed of progression can be explored fully in prospective studies.
Towards objective disease measurement

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Inflammatory Bowel Disease (IBD) follow an alternating disease course characterized by periods of remission and relapses. However, disease flare-ups occur in a random way and are often unpredictable. Biomarkers are important to gain an objective measurement of disease activity and severity, as well as prognostic indicator and outcome of therapy. Moreover they can be helpful to avoid invasive procedures.

A NIH working group defined in 2001 a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. They recognised two types: type 0 as marker of natural history of disease and a type 1 as marker of the effects of a therapeutic intervention.

The ideal biomarker is:
- easy to quantify in accessible tissue or fluid
- quickly measured in a reliable, reproducible, standardized way
- easy to interpret by the physician
- closely related with established clinical-pathological parameters of disease
- acceptable for the patient
- cost-effective.

Moreover those biomarkers must change in a almost linear way with disease progression and/or therapeutic intervention.

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<td>- CRP</td>
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<td>- Faecal calprotectin/lactoferrin</td>
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<th>More investigational biomarkers are:</th>
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<tr>
<td>- markers of bacteriemia: ASCA, pANCA, Omp-C antibodies, lipopolysaccharide binding protein, soluble CD14, serum MPO level</td>
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<tr>
<td>- markers of inflammation: IL6 – IL8 – fractional exhaled NO</td>
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<td>- anti-glycan antibodies: anti-laminarin IgA, anti-chitin IgA</td>
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<th>Future: molecular biologic tools</th>
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**CRP level**

CRP is still a very valuable index of activity in IBD with a sensitivity of 70–100% in CD and 50–60% in UC. In general, levels of CRP tend to be higher in CD than in UC reflecting the fact that CD is more a systemic inflammation than UC.
CRP is useful as predictor of short term relapse in CD although one third of patients with quiescent disease have an elevated CRP and up to 10% of patients with active disease have persistent low CRP (predominantly patients with low BMI). CRP is not useful as predictor of relapse in UC.

CRP is a good predictor of remission and response to treatment. In most clinical trials patients with elevated CRP had a better response than those with normal basal levels.

**Faecal calprotectin**

Faecal calprotectin levels correlate with clinical and endoscopic activity in UC but less in CD. In general a sensitivity of 76–100% and specificity of 83–95% is reported. In CD a good correlation is found with endoscopic lesions reflecting mucosal inflammation, but correlation with clinical scoring systems like Harvey Bradshaw index is much less good reflecting the systemic disease activity which could rely on the more transmural disease phenotype (Ricanek et al. 2011). In this cohort of treatment-naive IBD patients a high median concentration of fecal calprotectin (172 mg/kg [1–10825]) seemed to identify patients with definite IBD, especially in the case of UC, where there was also a short median symptom duration (2 months, 0–20). A low fecal calprotectin concentration seems to indicate a low risk of IBD.

Clinical application is hindered by an apparent lack of consensus with respect to normal values and units of measurement in published reports. Calprotectin is very useful for distinction between IBD and IBS but not for distinction IBD and infectious colitis.


Faecal calprotectin seems to be useful as marker of remission after therapeutic intervention. In adults with severe acute colitis calprotectin levels were higher in those requiring colectomy and were able to predict response to first and second line medication (Ho et al. 2009).

Recently we performed a prospective study in UC patients where we demonstrated in patients with active disease a very sharp decrease in levels after the initiation of Infliximab (De Vos et al. 2010). Moreover in the longterm we showed that the maintenance of calprotectin levels to less than 50 mg/kg was associated with a deep remission during one year (no symptoms and no inflammation) Flare (clinical and/or endoscopic) was associated in more than the half of the patients with an increase in calpro levels to > 150 mg/kg already the month before the flare.

**Markers of bacteriemia**

Atypical perinuclear antineutrophil cytoplasmic antibodies (atypical pANCAs) are associated with UC (present in 40–80% of patients), CD patients are known to exert a serum immune response to a variety of antimicrobial components. Best studied are ASCA present in 50–70% of CD patients. Other antibodies are directed against *Pseudomonas*-associated sequence I2 (anti-I2), outer membrane porin C (anti-OmpC) of *Escherichia coli*, and against the bacterial flagelin cBir1 (Anti-cBir1) and present in CD patients.
pANCA and ASCA have a limited value as predictor of disease relapse or response to therapy. In a Retrospective study pANCA/ASCA status have been reported to predict responders (Ferrante et al. 2007).

LBP and sCD14 are endotoxin related markers associated with bacterial translocation in the inflamed gut. A study demonstrated that LBP and soluble CD14 are markers of disease activity in CD with a similar accuracy as hs-CRP. Moreover they were independent predictors of clinical relapse in one year follow up (Lakatos et al. 2011).

**Anti-glycan antibodies**

Glycans are predominant cell surface oligosaccharides, which can be found on microorganisms, immune cells, erythrocytes, and tissue matrices. Anti-glycan antibodies have been found including anti-mannobioside carbohydrate antibodies (AMCAs), anti-laminaribioside carbohydrate antibodies (ALCAs), anti-chitobioside carbohydrate antibodies (ACCAs), anti-laminarin (anti-L), and anti-chitin (anti-C)carbohydrate antibodies.

Detection of anti-glycan antibodies in serum seems to be useful in differentiation CD vs. UC. Although specificity is very high (85–97%) sensitivity is low (10–25%) (Lakatos et al. 2010). A large body of information is available about seroreactivity to anti-glycan markers and complicated disease behavior in CD. Most information is derived from crosssectional studies with samples taken at various random points during the disease course, combining serum taken before, at the time of, and after complications occur and are a promising tool for identification of CD patients at risk for rapid progression and need for surgical intervention. The accuracy of the markers remains constant over time (Rieder et al. 2011).

Glycan markers do not seem to be associated with disease activity at the time of sample procurement, whereas it is unclear if active disease might lead to a later (and then not detected) increase in marker expression. These antibodies are not useful for prediction of relapse or remission to therapy.

**Fractional exhaled nitric oxide**

Gut inflammation in IBD is associated with increased activity of inducible nitric oxide synthase. Increased mucosal, plasma and fractional exhaled nitric oxide (FENO) levels have been described in IBD patients with active disease, but their correlation with endoscopy and faecal calprotectin, an established disease marker of IBD, has never been investigated. Nowadays, hand-held FENO measurement devices are available, which allow fast in-office FENO analysis. We found a good correlation with both the CDAI ($R^2 = 0.709; P < 0.001$) and a fair correlation with faecal calprotectin levels ($R^2 = 0.358; P = 0.01$). Moreover FENO measurement is very useful for differentiation with infectious colitis.

Value as predictor of relapse or treatment response has to be investigated in a long-term prospective study.

In conclusion, in daily practice CRP and calprotectin are still most reliable type 1 biomarkers available in every office and useful as objective markers of disease activity in IBD and markers of response to therapy (CRP in CD – calprotectin in UC). In CD we can expect a role for fractional exhaled NO. Evidence suggests that anti-
glycan antibodies are promising as type 0 biomarker. In the next future we can hope that molecular biologic tools will lead to the identification of more specific and sensitive biomarkers.

References:


Towards individualized therapeutic approaches in IBD

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The choice of therapies for IBD has largely been based upon the activity and the extent of the inflammation. Guidelines and recommendations have been developed for mild, moderate and severe disease activity and with regard to location for limited versus extensive colitis in ulcerative colitis and for ileal, ileocolonic and colonic disease in Crohn’s disease. Based on that algorithm we have been moderately successful in treating patients to remission. Clearly many more components of the disease need to be taken into account including age at diagnosis, disease behaviour and/or complications, disease duration, and potentially the genetic and immunological/serological background of the individual patient. Taking all these elements into account would certainly require a major paradigm shift in physicians’ attitudes and have them embark on highly personalized medicine.

At this point a set of phenotypic, genetic and immunological markers allows us to speculate about the further disease course in an individual patient. This could lead to more aggressive treatment in an earlier phase of the disease in certain patients, potentially reducing the need for debilitating surgical intervention and functional loss of the gastrointestinal function.

Thinking along those lines we are facing several limitations, however. The first limitation is our therapeutic armamentarium. Even when used in the most optimal conditions, the most potent therapies can induce bowel healing and long standing remission in only 50–70% of the patients. Patients that do not tolerate combined immunomodulatory-biologic treatment often run into complications quite soon. The second limitation is the observation that even at diagnosis a significant proportion of patient already presents with complications. What appears to be ‘early disease’ is already a result of longstanding inflammation and fibrosis. In this regard, making the diagnosis of IBD sooner after the beginning of the symptoms (as it being done in rheumatoid arthritis) may be of benefit.

In conclusion, individualized treatment will certainly become the standard of care. In order to be most successful, however, we need to expand our therapeutic options and we need to make efforts to diagnose IBD sooner after the beginning of symptoms.
Towards a “cure” for inflammatory bowel disease

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To properly address the issue of a “cure” for inflammatory bowel disease (IBD) one should first define what is intended as cure, and this can be tricky and not entirely objective. If cure is intended as a general restoration of health, this is possible even today, as many current therapies do a pretty good job in inducing long periods of remission in Crohn’s disease, and colectomy can technically cure ulcerative colitis. If cure is more strictly defined as the complete and permanent elimination of the cause, predisposing and permissive factors, restoration of gut ecology, and control of the inflammatory mechanisms – all of which should lead to permanent discontinuation of medications, full restoration of personal, social and professional activities, and achievement of optimal quality of life – cure for IBD seems to be out of reach at least for now. Regardless of the definition, major strides have been made in attempting to cure IBD, and these have addressed what we presently believe to be the key components of its pathogenesis: the environment, the genetic make up, the gut microbiota and the immune system. There are good reasons to believe that the isolated correction of each single component is insufficient to achieve a cure, that different requirements may be needed depending on the stage of the disease (early vs. late), and that therapy should be tailored to each individual patient (personalized medicine).

In regard to correction of individual component of IBD pathogenesis, we cannot change the environment from birth to adulthood, we are still unable to implement genetic therapies, we do not know how to reinstate a protective gut microbiota, and current methods of immunomodulation are heavily geared towards suppression rather than selectively boosting immunity and restoring immune homeostasis. Moreover, we do not know how to use these approaches in a comprehensive fashion, and what we can do routinely, e.g., microbial modulation or immunosuppression, is usually carried out in a functionally disjointed and temporally uncoordinated way. However, since this imperfect approach can actually provide satisfactory results, this is to some degree surprising and leads to the conclusion that even suboptimal therapy is a practical and valid option.

In regard to addressing therapeutic needs according to the stage of IBD, the notion of early vs. late disease, each with distinct pathogenic mechanisms, is becoming established primarily through studies of pediatric and adult human IBD and animal models of IBD that can be followed up from the beginning of gut inflammation onwards. Although these approaches may not be ideal because IBD can present in a chronic form in children and early disease probably occurs in adults, and animal models do not really recapitulate human IBD, they do constitute a step in the right direction towards a better understanding of how the disease evolves over time and what types of interventions are required at each stage of evolution.
Finally, as far as the much touted “personalized medicine” approach, this makes scientific and clinical sense, but we need more information from each component and stage of IBD pathogenesis to implement it rationally and meaningfully. Nevertheless, tailored therapies will likely become the routine approach in a not too distant future.

From the above one can conclude that we are certainly on the way towards achieving a cure for IBD, but one obvious element is missing or not implemented to any significant degree: integration of the components of IBD pathophysiology. We continue to make progress in each one of them using traditional “canonical” systems that allow us to accumulate more and more data. On the other hand, we are still not paying attention to how the data obtained from one pathogenic component can be used to better understand the other components, and only merging of past, present and future data may explain how all components reciprocally influence each other, generate a global picture of IBD and identify specific targets of intervention. In other fields the “system biology” approach is being increasingly adopted, and it is time for this approach to be seriously implemented by the IBD research community if a future “true” cure of IBD is the final goal.
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POSTER ABSTRACTS

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Anemia in pediatric ulcerative colitis and Crohn's disease: Role of hepcidin and interleukines

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Background and aims: Anemia is one of the often symptoms of chronic inflammatory bowel diseases (ulcerative colitis [UC] and Crohn's disease [CD]) and may develop due to intestinal bleeding, iron malabsorption, or high levels of inflammatory cytokines. Aim of this study were to evaluate mechanisms of anemia in children with UC and CD.

Patients and methods: We assessed red blood cells parameters and iron metabolism, including serum ferritin and transferrin, in 27 children with UC and 12 children with CD (5–14 years old). Serum hepcidin concentrations, interleukines-6 (IL6) and interleukines-2 (IL-2) levels were also measured.

Results: In both groups serum iron levels were low. Serum ferritin concentration in both group is elevated, but the serum transferrin/ferritin ratio were slightly decreased in UC group and increased in CD group. We found significantly low serum hepcidin levels (17 ± 3 ng/ml) in patients with active UC and high levels (36 ± 12 ng/ml) in active CD. The IL-6 level was higher (3.2 ± 1.0 pg/ml) in group of CD patients, compared with UC children (2.3 ± 0.5 pg/ml). The IL2 level were high in both UC and CD groups without significant differences (6.9 ± 2.1 pg/ml vs. 2.5 ± 2.2 pg/ml). The IL-6 levels correlated positively (Pearson's correlation coefficient) with serum IL2 level, serum transferrin levels, total iron-binding capacity, and negatively with serum iron concentration, serum ferritin levels, transferrin saturation. A significant negative correlation was present between IL-6 and serum iron concentration (R = -0.342; p < 0.001), but not between IL-6 and hepcidin. Treatment with mesalazine (Salofalk©), corticosteroids (incl. Budenofalk©) and (in some cases) cytostatics leads to diminishing of clinic and laboratory activity, decreases the anemia rate but increases the hepcidin level in CD group.

Conclusion: We supposed the different mechanisms of anemia development in UC and CD children: the prevalence of iron deficiency in UC and significant role of anemia of chronic disease in CD. Moreover, our results showed that iron metabolism is under control of IL-6 not only via hepcidin synthesis. We purpose the model of iron metabolism regulation in inflammatory bowel diseases with direct IL-6-transferrin synthesis interaction and multiple-factor hepcidin control.
Autophagy and mTORC1 activity are dysregulated in Crohn’s disease and ulcerative colitis

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Introduction: Major CD susceptibility pathways implicated through recent genome wide association scanning include the innate immune response (NOD2), the more specific, acquired T cell response (IL23) and autophagy (ATG16L1, IRGM). Autophagy is a conserved degradation pathway that plays a key role in maintaining cellular energy homeostasis and recent studies have directly linked NOD2 with autophagy signaling (ATG16L1) in response to bacterial invasion. By directly inhibiting the activity of ATG1, a kinase essential for the initiation of autophagy, mammalian target of rapamycin complex 1 (mTORC1) acts as a master regulator of autophagy.

Methods: We examined the expression levels of the autophagy marker LC3, mTOR, phospho-mTOR (active), the mTORC1 substrate S6K and phospho-S6K (active) by immunohistochemistry using pinch biopsies isolated from the sigmoid colon (SC) or the terminal ileum (TI) from patients with the diagnosis of Crohn’s disease (CD), ulcerative colitis (UC) or unaffected patients (control).

Results: In the SC, the levels of phospho-mTOR and phospho-S6K are increased in CD and UC. In contrast S6K and LC3 levels are decreased in CD and UC samples. In the TI all the proteins exhibit lower level expression in CD compared with controls. Samples were scored using the Alred Immunohistochemistry score.

Discussion/Conclusion: We observe an altered mTORC1 and autophagy activity in patients with CD and UC compared with control patients. Autophagy levels are lower in CD and UC as measured by expression of LC3, while mTORC1 activity is increased in CD and UC as measured by phospho-mTOR and phospho-S6K.
Allelic variants of the multidrug resistance gene (MDR1/ABCB1) and response to corticosteroid therapy in patients with inflammatory bowel disease

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Background and aims: Azathioprine has a central place in maintenance treatment of IBD patients. Failure of treatment with azathioprine is an important problem in managing patients (pts) with inflammatory bowel disease (IBD). This study examined the association of single nucleotide polymorphisms in the MDR1 gene of 64 IBD pts (40 ulcerative colitis [UC] and 84 Crohn’s disease [CD] pts) with respect to response to azathioprine therapy.

Patients and methods: IBD patients in this study were characterized as good responders to azathioprine and azathioprine resistant patients (n = ). Analysis of G2677T polymorphisms in exon 21 and C3435T in exon 26 of MDR1 gene was performed by PCR-RFLP method.

Results: Test result for linkage disequilibrium between loci was found to be significant in good responders (p < 0.001), resistant patients (p < 0.001) and total sample (p < 0.001). Pair-wise comparisons of the allele and genotype frequency among different groups of responders revealed no statistical differences for both loci in total sample and CD and UC patients analysed separately. No statistical differences were found in distributions of the estimated haplotypes between good responders and resistant patients in the total sample, and CD and UC group analysed separately.

Conclusions: Results in this study indicate that G2677T polymorphisms in exon 21 and C3435T in exon 26 of MDR1 gene do not have an influence of the response to azathioprine therapy in IBD patients.
Transcription factor NFATc3 has tumor-suppressive effect in experimental tumor model of colorectal cancer

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Introduction: NFATc3 belongs to a transcription factor family of five members that is important for the activation Th2-mediates cytokines in T-lymphocytes. Due to involvement in IL-4 production NFATc3 can act as critical factor for regulation of inflammation process like IBD and subsequently for tumor development in chronical inflammatory bowel disease. Also NFATc3 is involved in modulation of apoptosis and cell proliferation we started to examine the role of this transcription factor in experimental model of inflammatory bowel disease and colorectal cancer.

Methods: NFATc3ko mice were treated with oxazolone to induce colitis or with AOM/DSS to induce tumors. Miniendoscopic analysis has been done to monitor the manifestation of the colitis and the tumors. The colon was isolated and histological sections were taken for staining of apoptotic cells as well as the immunohisto-fluorescent staining.

Results: In the oxazolone-colitis the NFATc3 ko mice were protected in contrast to the wildtype BJ/6. This has been noted in miniendoscopic analysis as well as in histological sections as there has been found erosions and ulcer in the wildtype BJ/6. Caspase 3 staining showed a decreased proapoptotic cell number in the ko mice. Whereas the number of apoptotic cells was increased in the TUNEL-staining. In contrast to the colitis model the NFATc3ko mice showed more tumors in the AOM/DSS model and bigger tumors concerning the size. Histological staining of FoxP3 cells showed increased cells in the tumor and the number of Treg cells in the normal tissue was increased in the ko.

Discussion/Conclusion: NFATc3 gene is important for induction of apoptosis in the gut cells as the increased numbers of death cells seems to protect the NFATc3ko mice in the colitis model. On the other hand NFATc3 can act as a tumor-suppressive factor. An explanation can be the increased number of regulatory T-cells, which could be found in lamina propria.
Intestinal permeability in Crohn’s disease and its relationship with the disease characteristics

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Introduction: To study the alterations in intestinal permeability (IP) and its relationship with the disease characteristics (location, behavior and clinical activity) in patients with Crohn’s disease (CD).

Methods: 32 patients with CD (16 males, 16 females, mean age 38.9 years) and 25 healthy subjects consented to participate in the study. The location and behavior of CD were estimated using Montreal classification and the clinical activity – using Crohn’s Disease Activity Index (CDAI). IP was assessed by using a contrast medium iohexol (Omnipaque), which was administrated orally (25 ml, 350 mg/ml) 2 hours after breakfast. Six hours later serum iohexol concentrations (SIC 6 mg/l) were determined by a validated HPLC technique.

Results: In comparison to the control group, IP (assessed by SIC 6 mg/l) was significantly higher in patients with CD (2.63 ± 2.18 vs. 1.11 ± 1.10 mg/l), (p < 0.05). Increased IP was established in 50% of CD patients vs. 8% of the controls (p < 0.05). The cases with ileo-colonic location had significantly higher median SIC (3.55 ± 3.01 mg/l) compared to the controls (1.11 ± 1.10 mg/l), (p < 0.05). SIC were also significantly higher in the subgroup with penetrating and stricturing CD in comparison to the inflammatory disease type (3.50 ± 2.67 vs. 1.96 ± 1.46 mg/l), (p < 0.05). A significant positive correlation was found between IP and disease activity (r = 0.74; p < 0.05).

Conclusion: Increased IP was found in 50% of patients with active CD. Serum levels of iohexol appear to be a reliable disease marker as they reflect increased IP in ileo-colonic location, also in penetrating and stricturing CD and are related to disease activity.
Role of β-catenin in ulcerative colitis

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Introduction: β-catenin, a component of the cadherin-catenin complex, possesses an armadillo repeat domain and thus is involved in protein-protein binds, which is important in the construction of the cytoskeleton. It also functions as a component of the Wnt signaling pathway and can be an oncogene.

Methods: The study was performed by using archived material of 26 patients with ulcerative colitis. The expression levels of β-catenin was identified by immunohistochemistry.

Results: Expression of β-catenin was observed in the cytoplasm of normal and dysplastic epithelial cells of ulcerative colitis and were classified as absent, weak and strong. Nuclear reaction of this protein was not observed. In normal epithelial cells, there was not observed a strong reaction of β-catenin, but in 46.2% (12/26) of cases had a poor one, and its absence was observed in 53.8% (14/26) of cases. In the case of dysplastic cells, β-catenin expression was weak in 46.2% (12/26) of cases and strong in 53.8% (14/26) of cases. The result of statistical analysis proved that the weak expression of β-catenin in dysplastic cells correlated with lack of this protein expression in normal epithelial cells. Whereas, a strong expression of β-catenin in dysplastic cells induce to appeared poor expression of this protein in normal epithelial cells (p = 0.000).

Conclusion: Activation of Wnt proteins causes the accumulation of β-catenin in the cytoplasm, which then moves to the nucleus and forms complexes with transcription factors Tcf/Lef. This process leads to the induction of gene expression dependent on Wnt, which is associated with the development of various types of cancer. In our study, we observed a significant increase in the accumulation of β-catenin in the cytoplasm of dysplastic cells in UC, which seems to be an important step in the progression of the disease. As it is known, ulcerative colitis may progress to cancer. Because of well documented and significant contribution of this pathway in the carcinogenesis it is searched inhibitors of Wnt ligands and their receptors. Elimination of β-catenin overexpression in UC seems to be a noteworthy factor in the pathogenesis of this disease and perhaps to be a target in the treatment of ulcerative colitis.
The roles of *Helicobacter hepaticus* in the initiation and the maintenance of colitis in the gnotobiotic system in mice

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**Backgrounds:** Although animal models of inflammatory bowel diseases (IBD) fail to develop colitis under germ-free (GF) condition, suggesting that intestinal microbiota play important roles in the pathogenesis of IBD, it remains unknown whether specific pathobionts or dysbiosis of microbiota are essential for the development of intestinal inflammation. In this regard, it is known that mice infected with Helicobacter hepaticus (Hh) develop more severe colitis in specific pathogen free (SPF) condition as compared to Hh-uninfected SPF mice. Here we assess the role of Hh using the gnotobiotic system.

**Methods:** To investigate colitogenicity of Hh and its ability to produce cytokines from innate immune cells, 1) SPF RAG-2^-/- (Group 1), GF RAG-2^-/- (Group 2) and gnotobiotic RAG-2^-/- mice mono-associated with Hh (Group 3) were transferred with CD4^+CD45RB^{high} T cells, and 2) SPF RAG-2^-/- mice (Group 1*) or Hh-gnotobiotic RAG-2^-/- mice (Group 2*) were transferred with colitogenic lamina propria memory CD4^+ T cells from the colon of SPF colitic RAG-2^-/- mice previously transferred with CD4^+CD45RB^{high} T cells.

**Results:** 1) Group 1 developed colitis, whereas Group 2 and Group 3 failed to develop colitis. Consistently, Group 2 and Group 3 showed decreased portion of IFN-γ^+IL-17A^- and IFN-γ^+IL-17A^+ CD4^+ T cells in LP of colon and cecum as compared to Group 1. 3) Group 1* mice developed colitis, while Group 2* kept healthy. Furthermore, the absolute cell number of LP CD4^+ T cells in Group 1* was significantly increased compared with that in Group 2*.

**Conclusions:** Hh mono-associated RAG-2^-/- mice transferred with CD4^+CD45RB^{high} T cells failed to develop colitis, indicating that other microbiota are indispensable to initiate the development of colitis. Furthermore, re-transfer of colitogenic LP CD4^+ T cells into Hh mono-associated RAG-2^-/- mice also failed to induce colitis, implying that other intestinal microbiota are also important during the maintenance of colitis. Our date demonstrated that Hh itself is not pathobiont and orchestration with some intestinal microbiota is essential for both the initiation and the maintenance of colitis.
Micronucleus evaluation in mitogen-stimulated lymphocytes of patients with ulcerative colitis

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Aim: Ulcerative colitis (UC) is a chronic inflammatory disease that predisposes to colorectal cancer. Chronic inflammation may contribute to cancer risk through the accumulation of specific products as a result of DNA damage. Micronucleus (MN) frequency is a biomarker of chromosomal damage, genome instability and cancer risk. Our study aimed to evaluate MN frequency by using the cytokinesis block MN assay to determine genetic damage in mitogen-stimulated lymphocytes of patients with UC.

Material and methods: The study was performed in 22 diagnosed patients with untreated UC and age/sex matched 22 healthy control subjects. MN values scored in binucleated cells obtained from mitogen-stimulated lymphocytes of patients and control subjects.

Results: We found significantly higher MN frequency in lymphocytes of the patients with UC than the control subjects (1.61 ± 0.75 vs. 0.89 ± 0.29, p = 0.001). While no significant relationship was found between age and MN frequency in patients with UC (r = 0.076; p = 0.735), the MN frequency in the lymphocytes of healthy control increased regularly and significantly with age (r = 0.476; p = 0.025).

Conclusion: Our results indicate that the increased MN frequency in lymphocytes of patients with UC may reflect the genomic instability or impairment of genomic repair of cells.
Immunomodulatory effect of the nitrogen-containing bisphosphonate pamidronate in the sodium dextran sulphate-induced colitis in rats

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Introduction: Bisphosphonates are drugs used as treatment for osteoporosis. Nitrogen-containing or second generation bisphosphonates act by inhibiting protein prenylation, thus affecting cell biology. We hypothesized nitrogen-containing bisphosphonates may modulate intestinal inflammation. We have previously shown that alendronate (Br J Pharmacol. 2007; 151: 206) and pamidronate (unpublished data) has intestinal antiinflammatory activity in a preclinical model of inflammatory bowel disease, the TNBS-induced colitis model. Here we report our results with pamidronate in another experimental model, sodium dextran-sulfate (DSS)-induced colitis.

Methods: Colitis was induced via DSS administration in beverage water. Concentrations of 3 and 5% (m V⁻¹) of DSS were used depending on the status of the experimental animals. We used 5% of DSS until animals showed signs of cachexia and diarrhea (day 5), then we decreased DSS to a 3% concentration until the end of the experiment (day 10). Oral daily treatment with pamidronate (80 mg kg⁻¹ day⁻¹) started two days before colitis induction (pretreatment). We observed changes in body weight and food and water consumption daily, and modification in the general status of the animals as well as consistency and presence of blood in stool. Taking into account these parameters, we established a score of the disease as described by Stucchi et al. (Stucchi et al. 2000). We administered DSS for a total period of 10 days, afterwards animals were sacrificed and probes from different organs and tissues were collected.

Results: During the experiment, pamidronate did not lead to any improvement of the body weight loss or disease activity index (DAI); actually, general evolution was similar to those animals included into the colitis group (untreated). Colonic damage did not show a pamidronate-related improvement, not at the macroscopic (score of colonic damage and weight/length ratio) nor biochemical (myeloperoxidase and alkaline phosphatase activities) level. Nevertheless, histology analysis (hematoxilin-eosine staining) shows a decreased microscopic damage of colonic tissues from animals treated with pamidronate. Colitic group shows epithelial destruction with crypts shortening and loss of epithelial architecture; moreover, colitis leads to a cellular infiltration into the mucosa and submucosa, specially. Pamidronate significantly improved epithelial damage, hence epithelium shows a quasi normal architecture with only localized zones where epithelium were destructed. Furthermore, we can observe a decreased of cell infiltration especially in the submucosa layer. A study of cell popoulat ions of spleen and mesenteric lymphoid nodes demonstrates the immunomodulatory effect of pamidronate in this colitis model, although this effect is not shown macroscopically, it can be connected with
the observed effect in the histology analysis. Pamidronate is able to decrease spleen weight, which was increased as a result of the colitis induction, as well as the total number of mononuclear cells of the spleen. In the gastrointestinal ambient, we could observe similar effects, even though these data did not reach statistical significance. Nevertheless, concanavalin A-induced production of cytokines by mononuclear cells was fully normalized in the case of cells isolated from mesenteric lymphoid nodes, i.e. pamidronate was able to decrease DSS-induced hyperproduction of IFN-γ, IL-2 and TNF-α. In the case of splenocyte production of cytokines, results did not reach statistical significance.

**Discussion/Conclusion:** Pamidronate exerts immunomodulatory effects in the DSS-induced colitis model in rats. These effects did not lead to an improvement of general status of animals nor colonic macroscopic damage, but it’s shown by an improvement of the epithelial damage and the cell infiltration in the histology study as well as a modulation of infiltration and biology of mononuclear cells from spleen and mesenteric lymphoid nodes. Pamidronate normalizes number of mononuclear cells and proinflammatory cytokine production (IFN-γ, IL-2 and TNF-α). These results are well connected with the anti-inflammatory effect of pamidronate that we have already observed in the TNBS-induced colitis, where we can also observe a decrease of body weight loss and colonic damage. Basing in our model, in which a contact between epithelium and pamidronate is required for its antiinflammatory effect, the loss of epithelial barrier due to DSS could be the key of the different response observed in both colitis model.
The flavonoid glycoside rutin exerts immunomodulatory effects on epithelial cells

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Introduction: Flavonoids are frequently consumed in the diet and they have been suggested to exert different beneficial actions in the organism, including intestinal antiinflammatory activity. These properties have been studied in many cell types but their effect on epithelial cells, which are known to be increasingly involved in inflammatory bowel disease, is poorly known. Here we report data on the effects of quercetin and quercetin 3-O-β-D-rutinoside (rutin) on cytokine production by IEC18 cells, used as a non-tumoral model epithelium.

Methods: Confluent IEC18 monolayers were exposed to different concentrations (0.01 to 100 μM) of either flavonoid or vehicle (DMSO) for 24 h and the release of MCP-1 (CCL-2) and GRO (CXCL-1), which act as chemoattractants for monocytes and neutrophils, measured by ELISA. Coculture of IEC18 and primary rat T lymphocytes obtained by negative magnetic separation was also used to examine indirect effects on T cells.

Results: Quercetin had no effect on cytokine secretion in basal conditions. On the other hand, rutin exhibited a marked stimulatory effect on both MCP-1 and GRO (IC50 ≈ 10 µM). However, when cells were also treated with lipopolysaccharide (LPS 1 μg/ml), cytokine secretion was comparable in the absence and presence of both flavonoids. On the other hand, in IEC18-T lymphocyte cocultures, apically added quercetin but not rutin downregulated IFN-γ secretion by T cells at micromolar concentrations. This effect requires the presence of enterocytes, since direct exposure of lymphocytes to the flavonoid had no effect.

Discussion/Conclusion: Quercetin and rutin appear to exert opposite immunomodulatory actions on the intestinal epithelium.
Inflammatory bowel disease patients failing anti-TNF therapy show activation of the Th9/Th17 pathway

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Introduction: A new subpopulation of CD4⁺ T cells has recently been described, that is able to produce the cytokine IL-9. The data show that IL-9 can be produced to a considerable extent by Th17 cells.

Methods: Biopsies (colon) were taken from patients with Crohn's disease (CD) and ulcerative colitis (UC), mRNA isolation, quantitative PCR of TNF, IL-6, IL-9, IL-17, IL-21, IL-22, CCL20, IL-18R1, IL-23R, CXCR3, IRF-4. Cross-sectional study: Comparison of patients failing anti-TNF therapy with patients who responded to the treatment. Prospective study: Acquisition of UC and CD patients before and 8–12 weeks after initiation of therapy with anti-TNF antibodies and biopsy sampling at the different time points, respectively.

Results: In the cross-sectional study UC (n = 27), CD (n = 21) showed an overexpression of the TH17 cytokines IL-17, IL-21 and IL-22 as compared to control patients (n = 9). The expression depended on the degree of inflammation. IL-9 was significantly increased in UC compared with control patients; no statistical significance was observed for CD. IL-17, IL-21, IL-22 and CCL20, which is expressed by TH17 cells, were significantly overexpressed as well. A positive correlation between IL-17 and CCL20 (r = 0.6) could be shown for CD and UC. A medium correlation was found between IL-17 and IL-23R (r = 0.56) for UC; however not for CD. Contrary, there was a positive correlation between IL-17 and IL-18R1 (r = 0.56) in CD. Expression of IL-17 was significantly higher for CD and UC patients failing anti-TNF therapy. IL-9, IL-21 and IL-22 were significantly overexpressed in patients failing anti-TNF therapy compared to patients who responded to the treatment. In prospective studies (n = 6) a significant increase of the IL-17 production was observed for patients failing anti-TNF therapy.

Discussion/Conclusion: The IL-17 signalling pathway presents a possible escape pathway for patients failing anti-TNF therapy treatment. Possibly the Th17 phenotypes differ between CD and UC.
Non-absorbable glucids (prebiotics) directly contribute to the intestinal immune defense, increasing the production of cytokines by enterocytes

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Introduction: Non-absorbable glucids (NAGs) have generally prebiotic effects. They have been widely used for the treatment of chronic intestinal inflammation, showing benefit in animal models of colitis. Here we test the hypothesis that these products have the ability to modulate the immune response directly, interacting with the intestinal epithelium. NAGs assayed are: Active Hexose Correlated Compound (AHCC), an extract from *Basidiomycete* fungi enriched (74%) in oligosaccharides; fructooligosaccharides (FOS); inulin; galactooligosaccharides; and goat’s milk purified oligosaccharides (GMOS).

Methods: Confluent IEC18 monolayers were exposed to GNAs (5 g/l) for 24 hours and the release of MCP1 (CCL-2) and GRO (CXCL-1) which act as chemoattractants for monocytes and neutrophils, measured by ELISA. NF-κB (Bay-7082), Akt (wortmannin), JNK (SP600125), p38 (SB203580) and ERK (PD98059) inhibitors were added 1 hour before GNAs addition. MyD88 and TLR4 knockdown IEC18 cells were achieved by means shRNA lentiviral gene silencers.

Results: NAGs stimulated the production of GRO 1 and MCP1. The addition of the NF-κB but not the AKT blocker resulted in a substantial inhibition of the stimulatory effect, indicating the involvement of the NF-κB pathway in NAGs effects. Results obtained with MAPK (JNK, p38, and ERK) inhibitors showed a minor role of these kinases. Moreover, MyD88 and TLR4 knockdown partially reduced NAGs effects.

Discussion/Conclusion: NAGs increase the immune response of intestinal epithelial cells stimulating TLR4 and activating NF-κB (and secondarily MAPK) in both a MyD88 dependent and independent fashion.
Antibiotic therapy counteracts the delay in healing of experimental colitis exhibited by NSAID and selective cyclooxygenase (COX)-2 inhibitor

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Introduction: Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s disease, is a complex process involving genetic predisposition, immunological properties of gastrointestinal (GI) mucosa and microbial population disorder. Several factors, such as bacterial flora, prostaglandins, bile acid were implicated in the pathomechanism of UC but the effect of non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors on the time course of healing of colonic damage in experimental colitis has been little studied.

Methods: We compared the effect of vehicle, classic NSAID such as aspirin (ASA; 40 mg/kg i.g.), COX-2 inhibitor, celecoxib (25 mg/kg i.g.) and their combination with or without treatment with ampicillin (800 mg/kg i.g.) on the severity of experimental colitis, induced by the intrarectal administration of TNBS (15 mg/kg) in rats. At two weeks after TNBS administration, the distal 8 cm of the colon was removed for the determination of the tissue weight of the colonic segment and the area of colonic lesions was measured by planimetry.

Results: Administration of TNBS resulted in macroscopic and microscopic lesions accompanied by the significant fall in the CBF, an increase in tissue weight and G-bacteria, the 4-5-fold rise in the MPO activity and a significant increase in the plasma IL-1β and TNF-α levels. ASA or celecoxib alone significantly increased the area of colonic lesions, enhanced MPO activity and the number of G-bacterial colonies and produced the marked increase in colonic tissue weight and plasma IL-1β and TNF-α levels, and these effects were further significantly enhanced in rats treated with the combination of ASA and celecoxib.

Discussion/Conclusion: We conclude that classic NSAIDS such as ASA or combination of ASA with celecoxib, selective COX-2 inhibitor, delay the healing of colonic damage in experimental colitis and this effect is accompanied by the fall in the CBF and enhancement of expression and release of IL-1β and TNF-α and up-regulation of mRNA for iNOS; 2) Ampicillin exerts a beneficial influence on healing of colonic damage due to its anti-bacterial activity associated with suppression of expression and release of proinflammatory cytokines IL-1β and TNF-α and down-regulation of COX-2 and iNOS expression, and 3) intestinal microflora monitoring by antibiotics seems to be important in prophylaxis of colitis exaggerated by NSAIDs.
Nitric oxide-releasing aspirin accelerates the healing process of experimental colitis. Comparison with conventional aspirin and cyclooxygenase (COX)-2-selective inhibitor

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Introduction: Ulcerative colitis is a chronic inflammatory disease of complex etiology. NSAIDs inhibit prostaglandin COX activity, but their influence on the course of healing of experimental colitis has been little investigated.

Methods: We compared the effect of vehicle, aspirin (ASA), SC-560, the selective COX-1 inhibitor, celecoxib, the selective COX-2 inhibitor and NO-releasing ASA (NO-ASA) on the intensity of inflammation, colonic blood flow (CBF), myeloperoxidase (MPO) activity and expression of proinflammatory markers COX-2, iNOS, IL-1β and TNF-α in rat model of TNBS-induced colitis. The effect of glyceryl trinitrate (GTN), the NO donor, and carboxy-PTIO, the NO scavenger and the involvement of capsaicin-sensitive afferent nerves in colitis treated with NO-ASA was also determined.

Results: TNBS lesions were accompanied by the significant fall in CBF, a significant rise of colonic weight, MPO, IL-1β and TNF-α levels, these effects being aggravated by ASA and SC-560 but not celecoxib and inhibited by exogenous PGE2. NO-ASA dose-dependently accelerated colonic healing followed by the rise in plasma NOx content and CBF, the suppression of MPO and downregulation of COX-2, iNOS, IL-1β and TNF-α mRNAs. GTN significantly decreased the ASA-induced lesions and raised CBF while carboxy-PTIO or capsaicin-denervation counteracted the NO-ASA-induced improvement of colonic healing and this was restored by cotreatment with CGRP and NO-ASA in capsaicin-denervated animals.

Conclusions: 1) classic NSAIDs and selective COX-1 and COX-2 inhibitors delay healing of colitis via mechanism involving inhibition of COX-1 and COX-2 activity, 2) beneficial effect of NO-ASA is due to the NO mediated increase in colonic microcirculation and the activation of sensory nerves releasing CGRP.
A shift in the composition of innate lymphoid cell populations in Crohn’s disease

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Introduction: The exact nature of chronic intestinal inflammation in Crohn’s disease (CD) is unknown. Polymorphisms in the IL-23 receptor pathway are associated with CD. The IL-23R is expressed not only on Th17 cells but also on recently discovered populations of innate lymphoid cells (ILCs). Two RORC,c-kit,CD161,IL-7Ra-expressing ILC populations can be distinguished by their cytokine production profile: IL-17 or IL-22. Recent data from animal models show that IL-17 producing ILCs mediate IL-23 driven colitis. With this background, we investigated mucosal ILC populations in CD inflamed ileum and compared these with non-inflamed ileum and fetal intestine.

Methods: Lamina propria cells were isolated from CD inflamed ileum, non-inflamed healthy ileum and fetal intestine and were phenotyped by flow cytometry. NKp44 was investigated as a possible marker to distinguish cytokine production profiles of ILCs in CD inflamed or healthy ileum and fetal intestine.

Results: Flow cytometric analysis of ILCs in various tissues indicated that these cells can be divided in two subpopulations one expressing NKp44 and the other not. The expression of NKp44 correlated with secretion of IL-22 whereas the absence of NKp44 correlated with IL-17 production. In healthy ileum and fetal intestine, the majority of mucosal ILCs expressed NKp44 and produced IL-22. Significantly lower percentages of NKp44+ ILCs were observed in CD inflamed ileum, indicating a shift to NKp44- ILCs expressing IL-17 in CD.

Discussion/Conclusion: The decrease in the ratio of IL-22 producing and IL-17-producing ILCs may contribute to intestinal inflammation in CD, since these cells are part of innate immunity in the intestine.
Differential effects of quercetin and rutin on rat macrophages

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Introduction: Flavonoids are polyphenolic compounds usually consumed in the diet as part of vegetables and fruits. Some flavonoids display immunomodulatory activity, but this is believed to be mostly lost by glycosylation. Here we describe the differential effects of quercetin and quercetin 3-O-β-D-rutinoside (rutin) on rat primary macrophages.

Methods: Macrophages were obtained from rat spleen by negative magnetic separation and cultured at 500,000 cells/ml. Quercetin or rutin were assayed at different concentrations (0.01 to 100 μM) and compared with the vehicle (DMSO), with or without lipopolysaccharide (LPS 1 μg/ml). IL-1β, TNF and IL-10 secretion were determined (ELISA). In addition, the involvement of the NF-κB and MAPK (ERK, p38 and JNK) signaling pathways was assessed pharmacologically.

Results: Quercetin had no effect in basal conditions, while rutin had a concentration dependent stimulatory effect on IL-1β and TNF secretion starting at 10 μM. IL-10 was inhibited by rutin at high concentrations (50 μM). On the other hand, quercetin decreased IL-1β, TNF and IL-10 induction in LPS treated macrophages in a concentration dependent fashion (IC50 = 50, 70, 30 μM respectively). The effects of rutin on basal cytokine secretion were largely inhibited by pretreatment with Bay 11-7805, suggesting involvement of the NF-κB pathway, and by SB-203580, a p38 MAPK blocker.

Discussion/Conclusion: Quercetin and rutin showed distinct effects in primary rat macrophages. As expected, quercetin downregulated the immune response, while rutin exhibited a surprising stimulatory activity.
Pathological angiogenesis as an integral morphological component of active inflammatory bowel disease. Immunohistochemical study of endothelial cell markers CD31 and CD34 in colonoscopic biopsies from adult patients

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Introduction: The morphogenesis of complex chronic diseases, such as inflammatory bowel disease (IBD), is still unclear. Apart from classical immune cells, which have exclusive control over the initiation and duration of the disease, a decisive role is also ascribed to non-immune cells – endothelial and mucosal mesenchymal cells. Increased vascular density has been suggested as a major part of IBD pathology. Microcirculation and its endothelial lining are thought to play a key role in mucosal immune homeostasis through tight regulation of the nature and magnitude of granulocyte migration from the intravascular to the interstitial space.

Aim: The current study was to assess mucosal vascularization and expression of endothelial cell markers CD31 and CD34 in the actively inflamed intestinal mucosa of adult patients with histopathologically diagnosed IBD as compared to non-inflamed mucosa of controls.

Methods: 38 patients with active IBD were included in the study (24 with ulcerative colitis and 14 with Crohn’s disease) and 7 controls. The colonoscopically obtained biopsies underwent routine staining with Mayer’s hematoxylin and eosin (H,E) and immunochistochemical stainings for the presence of endothelial markers CD31 (Clone JC 70A, Dako, Denmark) and CD34 (Clone QBEnd 10, Dako, Denmark).

Results: The immunohistochemical investigations for angiogenic factors CD31 and CD34, as well as routine histological staining with H, E showed that microvessel density was markedly increased in active IBD mucosa as compared to the controls. The intestinal endothelium in newly formed or inflamed vessels was particularly well seen in IHC stained preparations. Apart from the increased number of microvessels, also individual immunopositive cells were observed in the areas of inflammation. Inflammatory cells were frequently visible in the lumen of the newly formed vessels. Immunoreactivity of proliferating and showing expansion cells of the intestinal endothelium was significantly higher in both UC and in CD compared to the control mucosa.

Discussion/Conclusions: Our IHC results provide morphological evidence for potent angiogenic activity in both ulcerative colitis and Crohn’s disease. They indicate that pathological mucosal angiogenesis constitutes an integral component of the microscopic picture observed in active IBD. Angiogenesis should be considered to be an important index of progressive phases of the disease.
MicroRNAs as disease markers and therapeutic targets in inflammatory bowel diseases

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Introduction: The pivotal role of microRNAs in the regulation of gene expression, in particular genes involved in the immune response, indicates that they may play an important role in the pathogenesis of inflammatory bowel disease (IBD) as well. MicroRNAs by their mechanism of action, are promising new therapeutic agents or targets.

Methods: To study the role of miRNAs in experimental colitis in mice we have used a colitis model that resembles human IBD. This colitis is mediated by CD4\textsuperscript{+}CD45RB\textsuperscript{high} T cells that are injected i.p. in SCID mice. To study miRNA expression we collected colonic tissue from the mice at 3 different time points during colitis progression. After 3 weeks a chronic progressive colitis developed characterized by a progressing wasting disease that was terminated at 9 weeks. MicroRNA was isolated from colons of mice in different stages of colitis progression (3, 6 and 9 weeks) and control mice that do not induce colitis (n = 3 for each timepoint). From all mice we also processed a part of the colon for immunohistochemistry to determine disease progression at the various time points after induction of colitis. We used the miRXplore\textsuperscript{TM} Microarrays for microRNA expression profiling (Miltenyi Biotec GmbH, Bergisch Galdbach, Germany). To determine the role of the upregulated microRNAs in the development of colitis we administered locked-nucleic-acid-modified oligonucleotide (LNA-antimiR) at the moment the mice demonstrated the first signs of disease 3 weeks after the transfer of the CD4\textsuperscript{+}CD45RB\textsuperscript{high} T cells.

Results: In both experiments a chronic colitis developed characterized by a progressive wasting that was terminated after 60 days. From the first study we selected four microRNAs that are significantly upregulated during the development of experimental colitis. There expression was found to correlate with disease severity and weight loss of the mice. In the second experiment one of the antimiRs demonstrated a significant reduction of disease severity and a prolongation of survival after i.p. injection (twice a week 5 mg/kg in 0.9% NaCl) starting when the mice loose 5% of their initial weight [p = 0.0026]).

Discussion/Conclusion: These results indicate that we have found a microRNA with proof of efficacy in a preclinical model.
Interleukin-17 stimulates the secretion of chemokines by primary intestinal fibroblasts

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Introduction: IL-17 is a proinflammatory cytokine which responds to invasion of extracellular pathogens. It is known that fibroblasts are a target of IL-17. To study the response of intestinal fibroblasts, we performed microarray mRNA analysis. Several genes were up regulated after stimulating fibroblasts with IL-17. In the top 10 of highly expressed genes, two chemokines were found: CXCL1 and CXCL6. In the present experiment the levels of both chemokines are analysed in culture supernatant of primary fibroblasts after IL-17 stimulation to validate the microarray data and to determine if there is a difference in response of fibroblasts of healthy individuals and fibroblasts of IBD patients.

Methods: Fibroblasts were isolated from resected colon or ileum of 20 patients (5 healthy controls, 10 Crohn’s disease patients, 5 ulcerative colitis patients) using DTT, EDTA, collagenase and ficoll. The cells were grown to confluency in a t25-culture bottle and incubated for 24 hours in 5 ng/ml IL-17. Supernatant was collected and stored at -80 °C. CXCL1 and CXCL6 were measured using an ELISA (R&D systems). Data was analysed using the two way ANOVA test (GraphPath PRISM 5.01).

Results: In most fibroblast cultures the concentration of both chemokines was significantly higher after stimulation with IL-17 (CXCL1 p < 0.0001, CXCL6 p < 0.0001). Analysis of different patient groups revealed that both chemokines were induced by IL-17 in healthy controls and Crohn’s disease patients (CXCL1: in HC p = 0.0043 in CD p < 0.0001, CXCL6: in HC p = 0.0002, in CD: p < 0.0001). For ulcerative colitis patients, there was a difference in stimulation. CXCL6 secretion was induced (p = 0.0013), whereas CXCL1 secretion was not induced by IL-17 (p = 0.1006).

Discussion/Conclusion: This experiment shows that primary intestinal fibroblasts demonstrate an induction of secretion of CXCL1 and CXCL6 after IL-17 stimulation. There was no difference in chemokine secretion between Crohn’s disease patients and healthy controls. These data suggests that intestinal fibroblasts contribute to chemotactic inflammatory responses in the gut, in response to increased IL-17 levels.
Analysing the role of mucosal mast cells inducing colitis-associated colorectal cancer (CRC)

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Introduction: Colorectal cancer is one of the most malignancies. However, the molecular pathogenesis of colorectal cancer is poorly understood. In order to investigate the functional role of mast cells, which play a more prominent role in immunological processes, we used a previously established murine colon carcinoma model (DSS/Azoxymethan) with mast cell deficient mice.

Methods: Accordingly, mice were treated with AOM followed by three consecutive cycles of orally administrated dextran sulfate sodium (DSS) over a period of 7 days. To monitor tumorigenesis in mice in vivo, we used our mini-endoscopic system.

Results: By using this system together with methylene blue staining, we were able to detect aberrant crypt foci in DSS plus AOM-treated wild-type mice at early time point before macroscopically visible lesions were seen. First visible lesions associated with inflammation appeared in wildtype mice around day 45, which were followed by the development of more and growing tumors until day 90. In contrast, mast cell deficient mice are protected against tumor development and although they showed colitis-similar symptoms. The possibility, that mast cells play a tumorpromoting role in the development of colon tumors led us to perform a screening of the expression of involved cytokines in colons and tumors of treated mice vs. untreated mice. Even in long-term study, a marginal increase of the tumor prevalence concerning mast cell deficient mice could be observed.

Discussion/Conclusion: Our data contribute extensively the understanding of mast cells in colitis-associated colon cancer and encourage of rethinking the role of mast cells in colitis-associated colorectal cancer.
ATG16L1 regulates immune responses through destabilization of the immunological synapse

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Introduction: Various polymorphisms in the autophagy related genes ATG16L1 and IRGM have been associated with the development of Crohn's disease (CD). Although the link between decreased autophagy and an inflammatory disorder like IBD suggests a role for this process in the regulation of immune responses, no data has been available on this topic. Therefore, this study focused on the effects of decreased autophagy on the immunogenicity of dendritic cells (DC).

Methods: Methods Gene knockdown was achieved in monocyte derived (human) or bone marrow (mouse) DC using siRNA technology. DC-T cell interactions were induced in mixed lymphocytes reactions (MLR), and cytoskeletal changes and interaction times were studied by immunofluorescence and time-lapse microscopy. T cell reactivity was determined by \(^{3}\)H incorporation and cytokine production assays. For patient studies, monocytes were obtained from peripheral blood of CD patients genotyped for rs_2241880.

Results: ATG16L1-low and IRGM-low DC induced significantly more T cell proliferation in both an allogeneic MLR and an antigen specific proliferation assay. This finding was consistent in human and mouse cells, suggesting a conserved role for autophagy in the regulation of immune responses. No alterations in cytokine production or surface marker expression of autophagylow DC were seen, indicating the immunostimulatory phenotype is not due to increased maturation. However, clear aberrancies occurred at the site of contact between DC and T cells, the so-called immunological synapse (IS). Autophagy-low DC displayed increased cytoskeletal polarization towards interacting T cells, and interaction times between DC and lymphocytes were prolonged, pointing to IS hyperstability. To confirm the physiological role of this mechanism, we compared IS formation in CD patients carrying the ATG16L1 risk allele and patients carrying the wild type allele. Indeed, IS formation was increased significantly in homozygous risk allele carriers compared to wild type controls.

Discussion/Conclusion: Decreased levels of autophagy result in an increased pro-inflammatory capacity in both human and mouse DC. This effect is regulated through hyperstabilization of DC-T cell interactions, leading to increased T cell activation. This phenotype was confirmed in cells obtained from risk allele carriers and is therefore likely to contribute to the increased immune activation as seen in CD patients carrying autophagy-related SNP.
The importance of new serological markers of inflammatory bowel disease

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Introduction: The aim of this study, the Turkish adult inflammatory bowel disease (IBD) cohort, a new anti-glycan antibody panel of Crohn's disease (CD) and ulcerative colitis (UC) with the diagnosis of disease complications, disease type, location, clinical features and to evaluate its relationship with disease activity.

Methods: The research was performed in patients diagnosed with IBD admitted to our clinic between 2010–2011. The study population, clinical, endoscopic, histopathological and radiological findings of 100 patients diagnosed with IBD (136 CD and 122 UC) and 90 healthy controls. Serum samples, anti-laminar IgA (anti-L), the anti-chitin IgA (anti-C), anti-chitobioside IgA (ACCA), anti-laminaribioside IgG (ALCA), anti-mannobioside IgG (AMCA) and anti-Saccaromyces cerevisiae IgG (gASCA) by ELISA, while pANCA were detected by indirect immunofluorescence.

Results: The study, 136 patients with CD (M/F 65/71, median age 36.34 ± 11.92), 122 UC patients (F/M 55/67, median age 44.45 ± 14.08) and 90 healthy controls (F/M 43/47, median age 44.49 ± 10.66) were included. CD 31.6%, at least one of the tested antibodies were positive. CD, ACCA (p < 0.05), ALCA and ASCA (p < 0.001) were significantly higher than in UC. Differentiation of CD and UC, pANCA combination ASCA and found the most accurate. ASCA level, inflammatory CD, was lower than structuring and penetrating type. ASCA and ACCA levels higher than those operated on for CD complications were detected in those appendectomy was higher than the value of ALCA (p < 0.05). Crohn's patients with perianal fistula AMCA level was significantly higher than those without (p < 0.05).

Discussion/Conclusion: CD, a disease process is more complicated to play a role in predicting the detection of clinical markers, a more aggressive therapeutic interventions at an early stage will be a guide for making decisions.
Prodromal irritable bowel syndrome may be responsible for delays in diagnosis in patients presenting with unrecognised Crohn’s disease and coeliac disease, but not ulcerative colitis

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Crohn’s disease (CD), ulcerative colitis (UC) and coeliac disease patients may experience a latency (a period between the onset of symptoms and correct diagnosis). IBS is more prevalent in coeliac Disease and IBD. We determined the prevalence of prodromal IBS (P-IBS) during latency and any impact P-IBS has on latency duration.

Methods: 683 biopsy proven patients (coeliac n = 225, 26% male; UC n = 228, 43% male; CD n = 230, 28% male) completed a postal survey. Patients were asked to recall if they perceived a ‘latency period’ and whether they had been diagnosed with IBS this period. Responses validated with medical records.

Results: 100/225 (44%) of coeliac patients reported a latency (mean length 13.2 years.) Of these 100 patients 67 (67%) had P-IBS. Mean latency length in P-IBS coeliac disease was 10 years versus 7 years without ($p = 0.046$). In IBD overall 326/458 (71%) reported a latency (mean length 4.99 years) of which 106 (32%) had P-IBS. Mean latency length in P-IBS IBD was 3 years versus 1.5 years without ($p = 0.01$). In UC 110/228 (48%) reported a latency (mean length 2.89 years) of which 42 (38%) had P-IBS. There was no difference in mean latency length in P-IBS UC versus those without, both 1 year in length ($p = n.s.$). In CD 216/230 (94%) reported a latency (mean length 6.15 years) of which 64 (29%) had P-IBS CD. Mean latency length in P-IBS CD was 4 years versus 2 years without ($p = 0.018$).

Discussion/Conclusion: This is the first study to make direct comparisons of latency periods between coeliac disease and IBD. Latency duration in coeliac disease is significantly longer and more often characterised by P-IBS than IBD. In UC P-IBS has no impact on latency duration; possibly as UC symptoms are inconsistent with IBS. In CD and coeliac disease, however, P-IBS increases latency duration. This may represent a failure to understand the overlap between IBS and CD/coeliac disease.
Mind or mesentry? Do the type, frequency and severity of reflux symptoms in patients with inflammatory bowel disease and coeliac disease shed light on their cause?

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Introduction: Inflammatory bowel disease (IBD) and coeliac disease are associated with an increased prevalence of reflux possibly due to GI motor dysfunction and visceral hypersensitivity. We charted reflux symptoms in patients with coeliac disease, ulcerative colitis (UC) and Crohn’s disease (CD) to determine the likelihood of experiencing reflux more frequently and/or severely than controls.

Methods: Postal survey n = 1031: controls n = 348 (36% male). Biopsy proven patients: coeliac disease n = 225 (25% male), UC n = 228 (43% male), CD n = 230 (28% male). Questionnaire included disease activity measures, reflux screen (type: heartburn, retrosternal pain, regurgitation, belching, dysphagia; frequency: < 2 days/week, 3–5 days, > 5 days; severity: mild, moderate, severe) and The Hospital Anxiety & Depression Scale (HADS).

Results: Age and disease activity were not confounding factors. Reflux prevalence: controls 50%; coeliac disease 66%; UC 62% and CD 72% (p ≤ 0.0001). Dysphagia more likely than controls (coeliac disease OR = 3.9, 95% CI: 2.2–7.0, p ≤ 0.0001; IBD OR = 2.5, 95% CI: 1.4–4.3, p = 0.0004). Retrosternal pain more likely in coeliac disease (OR = 2.3, 95% CI: 1.5–3.5, p ≤ 0.001) and regurgitation more likely in IBD (OR = 2.0, 95% CI: 1.3–3.1, p = 0.0004). No difference in reflux frequency between patients and controls (p ≥ 0.05). Severe reflux more likely in coeliac disease (OR = 6.8, 95% CI: 3.6–12.7, p = 0.001) and moderate reflux more likely in IBD (OR = 2.2, 95% CI: 1.6–3.2, p ≤ 0.0001). Stepwise increases in HADS scores in association with increasing reflux severity was observed in all participants (p ≤ 0.0001).

Discussion/Conclusion: Reflux is common in coeliac disease and IBD. We provide evidence of GI motor disturbances (dysphagia, retrosternal pain, regurgitation). Patients are more likely to perceive reflux of greater severity suggesting visceral hypersensitivity. Psychological unease is associated with increasing severity of reflux in all participants implying that psychology may contribute to hypersensitivity regardless of gut pathology. Given our results, however, there may be interplay of motor and sensitivity disturbances in IBD and coeliac disease.
Characterization of colonic stricture and as a risk factor for colorectal carcinoma in patients with inflammatory bowel disease

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Introduction: One of the considered strong risk factors for colorectal carcinoma (CRC) development in patients with IBD is colonic stricture which has not characterized very well, so far.

Aims & Methods: This study aimed to characterize of IBD patients with colonic stricture, response to steroids and evaluate whether colonic stricture increases CRC by time. Of the 2220 patients with IBD in our IBD center, 16 patients with IBD who had colonic stricture were detected. Of the 16, 14 were Crohn’s disease (CD) and 2 was ulcerative colitis (UC). Colonic stricture diagnosis was performed by clinical, radiological, endoscopic and histologic examinations for each patient. Date of the stricture and IBD diagnosed, number and type (whether the stricture allows to passage) of the stricture, follow-up term after the stricture diagnosed, pathologic result of the stricture, therapy before and after the stricture diagnosed, and responce to therapy were all recorded for each.

Results: Of the 16, 7 were female. Stricture diagnose date were between 5 months and 14 years after the diagnosis. Localization of stricture was left colon in 2, right colon in 6, sigmoid in 1, rectum in 5, splenic flexura in 1, transvers colon in 1. Two pts had 3 stricture, 1 had 2 and the rest had 1. Histologic examination showed dysplasia or malignancy in none. All patients, except 3, partially recovered after steroid therapy. Mean sedimentation rate was normal, but CRP elevated 3 fold at the stricture diagnose date.

Conclusion: Colonic stricture was a very rare condition in Turkish IBD cohort. Distribution of the stricture varies in the different parts of the colon. Steroid response was partial. Only laboratory abnormality was elevated CRP in this population.

Reference:

Enterovesical fistula during Crohn’s disease

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Introduction: Crohn's disease is a chronic inflammatory disease of the bowel often associated with fistula formation. Enterovesical fistula is a rare condition, few cases are reported in the literature. The aim of this study was to investigate the clinical manifestations and treatment of enterovesical fistulas in Crohn's disease.

Methods: We conducted a retrospective study including all patients with enterovesical fistula complicating Crohn's disease treated between 1995 and 2010.

Results: During this period, 7 patients (1% of all Crohn’s disease inpatients), 5 men and 2 women, were recorded. The mean age was 30.5 years (20–45 years). Location of Crohn’s disease was ileocolonic in 5 cases, and ileal in 2 cases including 1 extensive ileal disease and 1 localised ileal disease. Clinical disease activity was severe in 2 cases. No patient had previously received immunosuppressive therapy. Mean delay between the onset of the disease and the discovery of the enterovesical fistula was 36 months (0–108 months). Clinical features included urinary symptoms in 6 patients: pneumaturia, fecaluria, haematuria and urinary tract infection. In 3 cases, a perivesical abscess was found. Six fistulas were resected and one patient refused to undergo surgery. The mean follow-up period after surgery was 52 months. No case of post operative recurrence of the fistula occurred.

Discussion/Conclusion: Enterovesical fistulas in Crohn’s disease should be suspected in all patients presenting urinary symptoms. Surgical treatment is generally safe and long-term effective.
A meta-analysis of the involvement of renin-angiotensin system in the management of inflammatory bowel disease; Future perspectives and implications

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Introduction: Inflammatory bowel disease, or IBD, is a collection of systemic diseases involving inflammation of the gastrointestinal (GI) tract of unknown etiology. The renin-angiotensin system (RAS) is strictly related to the kallikrein-kinin system and are involved in many physiological and disease conditions (Figure 1) and possibly in the pathogenesis of IBD. Recent data indicate that RAS is well expressed and active in the GI tract although exact physiological roles are to be settled. Of particular interest is the increasing amount of experimental support for the involvement of Ang II formation and actions via the Ang II subtype 1 (AT1) receptor in the pathogenesis and treatment of IBD. In the present meta-analysis, we aimed to analyze the relationship between RAS and IBD and the effect of RAS blockade on disease management.

Methods: A systematic search of electronic databases up to March 2011 was performed to identify all primary studies examining the role of RAS in IBD. All articles were critically appraised with regard to methodological quality and risk of bias. Fifteen clinical trials that fulfilled the inclusion criteria were further pooled into a meta-analysis.

Results: Fifteen studies met initial selection criteria but only 12 were eligible for inclusion in the meta-analysis. The majority of studies demonstrated a significant role of RAS in the pathophysiology of IBD. Table 1 summarizes the essential components of the RAS and their selected representative interrelationships with IBD pathophysiology and the effect of RAS blockade on disease management.

Discussion/Conclusion: A significant effect of RAS components on IBD pathogenesis were observed in this meta-analysis. Furthermore, a potential role for angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists in IBD treatment were also demonstrated. Further studies that will specifically focus on AT1a receptor are needed which may be a novel therapeutic target for the treatment of IBD.
Figure 1: Potential contribution of renin-angiotensin system (RAS) to the physiological and pathologic inflammatory conditions.

Table 1: Essential components of the renin-angiotensin system (RAS) and their selected representative interrelationships with IBS pathogenesis and management.

<table>
<thead>
<tr>
<th>Essential RAS component</th>
<th>Reference</th>
<th>Result</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Angiotensin Converting Enzyme (ACE)</td>
<td>Silverstein et al. (1981)</td>
<td>No increase in enzyme activity was observed in CD ileum or colon or in UC colon</td>
<td>The granulomatous inflammation in CD differs from that in sarcoidosis, in which striking elevation of ACE is present in granulomatous tissue and frequently in serum. It is suggested that intestinal ACE may play a regulatory role in intestinal inflammation of CD.</td>
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<tr>
<td>ACE</td>
<td>Takeuchi et al. (1992)</td>
<td>ACE was found to be decreased in CD compared with controls. Significant negative regression between ACE and CDAI was observed.</td>
<td>Serum ACE levels seem to differentiate patients with active CD from patients with UC.</td>
</tr>
<tr>
<td></td>
<td>Letizia et al. (1993)</td>
<td>The average levels of ACE in UC were significantly higher than those of healthy subjects and CD (p &lt; 0.005). Among the UC patients there was a trend for lower levels in the group treated with prednisone. Serum ACE levels in patients with CD and UC were significantly lower than in healthy controls, irrespective of the genotype of ACE.</td>
<td>Considering ACE gene polymorphism, serum ACE levels in patients with IBD are lower than in controls. Serum ACE levels reflect a part of the pathogenesis of IBD. The results of this study suggested that the assessment of the tissue ACE expression can be helpful for making the differential diagnosis between CD and IT.</td>
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<td></td>
<td>Matsuda et al. (2001)</td>
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<tr>
<td></td>
<td>Kwon et al. (2007)</td>
<td>ACE was present in the cytoplasm of the epithelioid cells in the granulomas from 13 of 15 patients with CD and in 14 of 15 patients with IT.</td>
<td></td>
</tr>
</tbody>
</table>
Saibeni et al. (2007) DD, ID and II genotypes distribution did not show significant differences between IBD patients and controls. No significant difference was observed between CD and UC patients.

The ACE I/D polymorphism is not associated with IBDs but the D allele appears to increase the risk of developing extraintestinal manifestations in UC patients.

Koga et al. (2008) Transanal administration of ACE-I/PEG dose-dependently decreased the severity of fibrosis and pro-inflammatory cytokine expression.

ACE-I/PEG is found to be effective in preventing colonic fibrosis and pro-inflammatory cytokine expression in a DSS colitis model, most likely by down-regulating the TGF-beta signaling pathway. ACE-I/PEG may be a potential new option for treating inflammatory bowel disease.

Angiotensinogen (Atg) Hume et al. (2006) Angiotensinogen-6 AA genotype was significantly associated with CD.

The association of the angiotensinogen-6 variant with CD supports a potential role for ACE inhibitors and angiotensin II receptor antagonists in disease treatment.

Angiotensin II (Ang-II) Jaszewski R et al. (1990) Colonic mucosal levels of angiotensin I and II were greater in patients with Crohn's colitis than in normal subjects. Mucosal levels of angiotensin I and II correlated well with the degree of macroscopic inflammation in Crohn's colitis. Although Atg−/− mice developed colitis, the degree was much milder than that in Atg+/− mice (p < 0.05). Colonic cytokine analysis showed that the production of proinflammatory cytokines (interleukin [IL]-1beta, interferon gamma [IFN-gamma]) was impaired in Atg−/− mice.

These study suggests that angiotensin I and II may have a role in the inflammation associated with Crohn's colitis.

Inokuchi et al. (2005) Although Atg−/− mice developed colitis, the degree was much milder than that in Atg+/− mice (p < 0.05). Colonic cytokine analysis showed that the production of proinflammatory cytokines (interleukin [IL]-1beta, interferon gamma [IFN-gamma]) was impaired in Atg−/− mice.

This study revealed that the RAS is involved in the immune system in the colon. Antagonism of the RAS is a potential prophylactic strategy for the treatment of human IBD.

Angiotensin II Type I Receptor (AT1) Okayada et al. (2011) Deschloro-losartan demonstrated near equal angiotensin II type la receptor blockade compared to losartan as well as another angiotensin II type la receptor antagonist, candesartan. In the DSS model, each compound significantly improved clinical and histologic scores and epithelial cell apoptosis.

This study demonstrated efficacy of high-dose angiotensin II type la receptor antagonists in the DSS colitis model.

Katada et al. (2008) Induction of DSS colitis resulted in up-regulation of Ang II and AT1a receptor in the colonic mucosa of WT mice.

RAS is involved in the pathophysiology of DSS-induced colitis and AT1a receptor may be a novel therapeutic target for the treatment of IBD.

ACE: angiotensin converting enzyme; CD: Crohn’s disease; IT: intestinal tuberculosis; UC: ulcerative colitis; IBD: inflammatory bowel disease; RAS: renin-angiotensin system; DSS: dextran sodium sulfate; Atg: angiotensinogen; AngII: angiotensin II
Primary sclerosing cholangitis is associated with pancolitis and not backwash ileitis


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Introduction: Inflammatory bowel disease associated with primary sclerosing cholangitis (PSC-IBD) is reported to represent a distinct phenotype of IBD characterized by colitis, rectal sparing and backwash ileitis, but this has so far not been confirmed in large well-phenotyped cohorts. The aim of this study was to assess the IBD phenotype associated with PSC in a large Dutch PSC cohort using endoscopic and histopathological criteria.

Methods: PSC cases were identified and ascertained, fulfilling well-established biochemical, histological and radiological criteria in 35 hospitals in The Netherlands. IBD location was recorded according to the Montreal classification. To assess the occurrence of backwash ileitis a subgroup analysis was performed in 72 cases and 80 age- and sex-matched IBD controls with at least one complete ileocolonoscopy including terminal ileum histology, reviewing all endoscopy and pathology reports filed between 2000 and 2010.

Results: 368 (66%) of a total of 558 PSC patients had coexistent IBD, mainly ulcerative colitis (UC, 75%). 182 (83%) of the PSC-UC patients had a pancolitis, 28 (13%) a left sided colitis and eight (4%) a proctitis. Sixty seven (96%) PSC-Crohn’s disease (CD) patients had an (ileo)colitis and three ileitis only (4%). In the subgroup analysis of 72 PSC-IBD patients 46 (64%) PSC-UC patients were identified, 23 (32%) PSC-CD patients, and 3 (4%) PSC-IBD-U patients. 523 colonoscopy reports with histology were reviewed. Fourty-one (89%) PSC-UC patients had a pancolitis, compared to 31 (59%) matched UC patients (p = 0.0021). Left sided colitis was seen in 16 (31%) UC controls and in none of the PSC-UC patients (p = 0.0001). Backwash ileitis was seen in only 3 (7%) PSC-UC patient and in one (2%) of the UC controls (p = 0.5220).

Conclusion: Inflammatory bowel disease in Dutch PSC patients represents a distinct phenotype in that: Pancolitis is observed in 89% of ulcerative colitis patients with PSC and colitis is observed in 96% of Crohn’s disease patients with PSC. Backwash ileitis is a rare finding in ulcerative colitis patients with PSC.
Factors associated with high dose radiation in Crohn's disease patients

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The disease of Crohn (MC) is a disease of the whole life. The need for radiological explorations is frequent with an aim of diagnosis, research of complications (abscess, fistulae, stricture …), or extraintestinal manifestation

Aim: The aims of our study were to examine patterns of use of imaging in Crohn’s disease, quantify the cumulative effective dose (CED) received by patients and identify predictive factors associated with high levels of radiation (> 35 mSv).

Methods: We included patients with Crohn’s disease diagnosis established between 1998 and 2002 (to have a minimum of 5 year’s follow-up). We noted the epidemiologic characteristics of the patients, the parameters related to the disease (date of beginning, phenotype, treatments used) and précised all the radiological exams done during the follow-up. For each patient, we calculated the cumulative effective dose (CED).

Results: We included 167 patients (92 men and 72 women), with a mean age of 40 years (13–83). The location of the CD was ileal in 46%, colonic in 35%, ileocolonic in 36.5 %, upper gastrointestinal disease in 3% and perianal in 24.6%. The phenotype of the disease was strictureing in 42.2%, penetrating in 24.6%. The mean follow up was 6.4 years. The mean CED was 18.81 mSv (0.02–120.02. It was 13.81 mSv during the first 5 years of the CD course of the disease and only 0.31 mSv during the following period. The CED exceeded 35 mSv in 27 patients (16.2%) and 75 mSv in 4 patients (2.4%). The CED was respectively of 12.9, 4.74, 3.35 and 0.2 mSv if the imaging exams were done for complication’s diagnosis, extension appreciation of CD, diagnosis and extra intestinal manifestation’s diagnosis. Factors associated with of high level of radiation (> 35 mSv) were: age < 24 years at diagnosis, structuring phenotype (Odds ratio [OR] = 2.24), penetrating phenotype (OR = 3.554), requirement for infliximab (OR = 6.31) or surgery (OR = 3.15), a number of flare up > 8 (OR = 9.45). No malignancies occurred in our patients.

Discussion/Conclusion: High exposure to radiation is frequent during the first five years of Crohn’s disease diagnosis. We should minimise radiation during this period of the disease, by doing a magnetic resonance exam as much as possible.
A Crohn’s smoking cessation programme

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**Introduction:** Smoking is a proven risk factor for more severe Crohn’s disease (CD) and need for surgery. Of all the disease modifying strategies available smoking cessation remains the most effective, however, many with CD continue to smoke. Cessation interventions are usually limited to brief advice and attempts to quit often fail. Smoking cessation should have a much higher priority. The programme aimed to assess the acceptability, feasibility and effectiveness of a smoking cessation programme offered to people with CD.

**Methods:** We ran 2 standard 7 week courses of behaviour change therapy with NRT (nicotine replacement therapy) or varenicline, together with additional input from an IBD Nurse aimed at addressing particular CD related issues. Places were offered to smokers with CD attending the outpatient clinic, together with close friends/partners.

**Results:** 25 people with CD were invited to join 2 courses. A total of 9 with CD and 5 without attended at least one session. All those who completed the courses – 6 with CD, 2 without – quit smoking and remained quit at 6 months. Partial results at 12 months showed one had relapsed. None of those who dropped out managed to quit.

**Discussion/Conclusion:** This is an effective smoking cessation method for people with Crohn’s. The benefits extend beyond quitting smoking to an increased knowledge of disease management and support gained from other group members. Each programme takes 15–20 hours of IBD Nurse time over the 7 week period. Increasing the numbers by offering the programme across neighbouring hospitals would make this more cost-effective. Also, knowing the Crohn’s population and judging the ‘readiness to quit’ of patients is important.
The effect of steroids on the expression of Wnt pathway inhibitors in patients with long-standing ulcerative colitis

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Introduction: Individuals with long-standing (greater than 10 years) ulcerative colitis (UC) have an increased risk of developing colorectal cancer. Epigenetic-associated silencing has been reported in the colorectal epithelium from patients with UC and has been shown to increase during progression from colitis to dysplasia to cancer. We have demonstrated that the Wnt antagonists (sFRP’s and WIF1) are down-regulated and methylated in UC-associated dysplasia and neoplasia. The presence of methylation in normal colonic tissue from patients with UC-associated neoplasia suggests this is an important, early event in tumourigenesis. Longitudinal cohort studies indicate that the use of anti-inflammatory agents (corticosteroids and 5-aminosalicylates) is chemoprotective, reducing cancer risk. The exact molecular mechanism of these effects is unknown.

Methods: We assessed the expression of wnt pathway inhibitor proteins both in vitro and in patient biopsies taken from UC patients undergoing surveillance colonoscopy. The effect of steroids on protein expression was examined in vitro. The effect of steroid treatment (Budenofalk® enema) on inhibitor protein expression was examined in an open label randomised study funded by an educational grant from the Falk Foundation. Laboratory analysis was blinded to the treatment received.

Results: We present in vitro data demonstrating that corticosteroids can specifically increase the expression of the key Wnt antagonist’s sFRP1, sFRP2 and sFRP4. To date with have recruited six UC patients the open label randomised study. Preliminary data has demonstrated that sFRP1 expression is restored in 3 out of 4 UC patients following 4 weeks of steroid treatment. Analysis has shown that sFRP1 upregulation in these treated patients coincides with increased expression of the related Wnt antagonist WIF1. We are currently investigating the effect that treatment has on the methylation status of these genes.

Discussion/Conclusion: This preliminary data suggests that topical steroid therapy may affect the expression of colonic epithelial wnt inhibitor proteins in patients with longstanding UC and so ameliorate cancer risk.
Efficacy and safety of thiopurines in the treatment of inflammatory bowel diseases (IBD)

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Introduction: Thiopurines (THIO) are widely used in the management of IBD and adverse events (AE) occur in 15–30% of patients. The aim of our study was to investigate efficacy and safety of treatment with THIO.

Methods: We analyzed retrospectively data of 837 IBD patients. Harvey-Bradshaw index (HBI) or partial Mayo score (pMS) before and after 6, 12, 24 and 36 months of treatment with THIO were registered. Indications for treatment, mean duration of treatment, and AE were recorded. AE were divided in early (within 4 weeks of start), intermediate (from 2 to 6 months) and late (> 6 months). Data are mean values ± SEM. Comparison was performed with the Wilcoxon signed rank-test.

Results: We identified 266 patients (157 Crohn’s disease [CD], 109 ulcerative colitis [UC]) treated with THIO. The most frequent indication for treatment was steroid dependency (71%); the mean duration of disease prior to THIO was 5.3 years ± 8.7; the mean duration of treatment was 2.8 years ± 6.0. HBI and pMS fell significantly compared to pretreatment values at 6 months up to 3 years (all p < 0.001 vs. pretreatment). 115 AE were identified in 87 patients (32.7%), 73 patients (27%) discontinued THIO because of AE. We observed 55 early AE (47.8%), 26 intermediate AE (22.6%) and 34 late AE (29.6%), the most frequent are represented respectively by flu-like syndrome (38.2%), hepatotoxicity (46.2%) and leucopenia (35.3%). One probably therapy-related death occurred in UC.

Discussion/Conclusion: THIO represent a valid treatment in IBD being equally effective in CD and UC, maintaining efficacy over time, but patients need continuous surveillance.
Clinical validation of Simple Endoscopic Score for Crohn's Disease (SES-CD) in children

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Background: Simple Endoscopic Score for Crohn's Disease (SES-CD), validated in 121 CD patients, is based on 4 endoscopic variables (ulcer size, ulcerated and affected surfaces, stenosis) in 5 ileocolonic segments and the endoscopic parameters are scored from 0–3. PCDAI is basic clinical index used to assess the severity of Crohn’s disease (CD) in children.

Aim: The aim of this study was to evaluate the correlation between SES-CD and PCDAI in various clinical situations in children with CD.

Material and methods: PCDAI and SES-CD were analyzed three times in group of children with Crohn’s disease receiving therapy with infliximab: 66 pts (aged 14.8; 12.9; 16.3 [median; Q1; Q3]) prior to the therapy – week 0; 66 pts after induction therapy (22 clinical remission, 26 clinical response, 18 no response) – week 10; 32 pts (23 clinical remission) who finished maintenance therapy – week 50. Spearman’s rank correlation was used as a statistical method.

Results: The results of PCDAI were as follows [median; Q1; Q3]: week 0 – 52,5; 45,0; 57,5; week 10 – 15,0; 10,0; 30,0; week 50 – 5,0; 0,0; 12,5. The results of SES-CD were as follows [median; Q1; Q3]: week 0 – 18; 12; 22 vs. week 10 – 7,5; 1; 15; week 50 – 0; 0; 7,5. No correlation was found between PCDAI and SES-CD in week 0 (R = 0.07). We found significant (p < 0.05) correlation between PCDAI and SES-CD in week 10 (R = 0.5) and week 50 (R = 0.45) as well as between PCDAI and SES-CD changes after and prior induction therapy (week 10 – week 0) (R = 0.24).

Conclusions: SES-CD reflects clinical status in children with CD as well as its improvement measured by PCDAI, but underestimates PCDAI variance in subgroup of patients with moderate to severe CD.

White cell apheresis induces clinical and IBD-Q remission in patients with chronic severe refractory ulcerative colitis

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Introduction: A significant proportion of patients with chronic severe ulcerative colitis will fail conventional treatments. White cell apheresis (WCA) has emerged over recent years as a therapeutic option for this group. A cohort of 26 patients with severe chronic refractory ulcerative colitis treated with WCA showed a dramatic response at 12 weeks with 73.1% achieving clinical remission, this response was paralleled in quality of life (QOL) indicators. Clinical remission was sustained at six months in the majority of patients.

Aims & Methods: A prospective study was conducted in 26 patients with severe steroid-dependent or steroid-refractory ulcerative colitis referred for WCA. Inclusion criteria were (i) High disease activity score (partial Mayo score ≥ 6) (ii) Intractable symptoms despite treatment with steroids and/or immunosuppressants (iii) Severe disease at endoscopy and histologically. The aim was to induce clinical and IBD-Q remission at 12 weeks. A Mayo score ≤ 3 defined clinical remission. The 32-item Inflammatory Bowel Disease Questionnaire (IBD-Q) was used to assess QOL prior to treatment and at 12 weeks, response was defined as an increase in IBDQ total score of > 6 points, remission a score of ≥ 170 points.

Results: Patient characteristics; prior to treatment 24 patients (92.3%) were prescribed 5-ASA compounds. 10 patients (38.5%) were prescribed topical therapies (5-ASA enemas or suppositories/steroids enemas). 23 patients (88.4%) were steroid dependent, (prednisolone mean dose 23 mg, median 20 mg). 3 patients (11.5%) were steroid refractory (no response to high dose oral steroids). 13 patients (50%) were prescribed azathioprine, of the remainder all had documented intolerance. 1 patient (3.8%) was prescribed 6 mercaptopurine. 4 patients had failed infliximab (19.2%) in 1 patient (3.8%) it was contraindicated. 1 patient (3.8%) had failed intramuscular methotrexate. Outcomes: At week 12 clinical remission (Mayo score ≤ 3) was achieved in 19 patients (73.1%). 15 patients (57.8%) were no longer prescribed oral steroids. IBD-Q remission was achieved in 14 of 19 patients interviewed (73.7%). Of the remainder, 3 patients (15.8%) achieved an IBD-Q response. Of 7 patients (26.9%) who failed to achieve clinical remission at 12 weeks, 1 achieved delayed remission at 20 weeks. Of the remaining 6 treatment failures, 4 underwent colectomy (15.4%). 18 of 19 patients (94.7%) who achieved clinical remission at 12 weeks remained in clinical remission at 6 months post treatment.

Conclusion: WCA is effective in inducing clinical and QOL remission in chronic severe refractory ulcerative colitis. This data series suggests WCA should be considered before colectomy in this patient group.
Hemophagocytic lymphohistiocytic (HLH) syndrome in IBD patients treated with thiopurines – A case series

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Introduction: Immunosuppressive therapy constitutes the backbone of therapy in inflammatory bowel diseases. The major therapy-induced adverse events are represented by the potential development of malignancies, demyelinating disorders, and infections. These latter complications may be complicated further by the life-threatening HLH syndrome.

Methods: We reviewed the charts of 864 IBD patients followed by our centre between 2001–2010. Two hundred sixty-six patients were treated with thiopurines (THIO), 134 with biologics. We identified 3 cases of HLH syndrome according to Henter JI, 2007.

Results: Case 1: A 38-year-old female with UC treated with azathioprine (AZA) monotherapy for 8 years (did not present to follow-up visits over the last 3 years) developed high grade fever, leucopenia, splenomegaly and died 3 weeks later because of acute liver failure. Bacteriologic, fungal, and virologic testing did not reveal any responsible agent.
Case 2: A 35-year-old female with Crohn’s colitis treated with AZA monotherapy for 8 months developed a CMV-related pneumonia complicated by the HLH syndrome. She responded well to ganciclovir.
Case 3: A 72-year-old female with newly diagnosed ileal Crohn’s developed CMV sepsis together with HHV coinfection 7 months after introducing AZA (on combined therapy with steroids). Antiviral treatment was successful and she was subjected to surgery 2 months later.
In case 2 and 3, blood cell counts were normal 3 weeks before presentation of complication.

Discussion/Conclusion: HLH syndrome represents a severe complication of infections with a high mortality in immunocompromised patients. Regular control of blood parameters cannot prevent this complication. In our series AZA represented the common denominator.
Experiences with long-term infliximab therapy in patients with Crohn’s disease: Treatment beyond a 100 months

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Introduction: The introduction of infliximab (IFX) revolutionized the treatment of moderate to severe Crohn’s disease (CD). The aim of this study was to provide preliminary data on the impact of long-term IFX treatment on the natural history of CD.

Methods: Data on twelve patients with CD who had been receiving IFX treatment for a mean (± SEM) of 112.8 ± 2.7 months were analyzed retrospectively (the minimum duration of IFX therapy was a 100 months). The following parameters were assessed: need for surgical procedures, need for hospitalization, presence of extraintestinal symptoms, intercurrent infections, steroid withdrawal and need for IFX dose increase or combination treatment.

Results: Nine patients underwent altogether 17 surgical procedures before IFX treatment, which was either due to perianal abscess or fistula or uncontrollable disease activity. During IFX treatment surgery was performed in four patients because of fibrostenotic complications (on one occasion) or perianal disease (on four occasions). Disease-related hospitalizations during IFX treatment were only due to the previously mentioned surgeries. None of the 4 patients with extraintestinal symptoms had recurrence of the extraintestinal symptoms during biological therapy. Intercurrent infection was experienced in only one case. Permanent steroid withdrawal was achieved in 9 patients. Azathioprin or methotrexate was administered simultaneously with IFX in 7 patients. In 5 patients IFX was either used in monotherapy or in combination therapy that had been gradually tapered to monotherapy. IFX dose increase was administered in one case. 4 of the 8 fistulizing patients achieved and maintained fistula healing, continuous remission was achieved in 6 patients.

Discussion/Conclusion: These retrospective results show that long-term IFX therapy can be effective in the long-term maintenance of clinical remission and fistula healing in CD, which appears to be associated with corticosteroid withdrawal and significant reduction in the need for surgery and hospitalization.
Safety of infliximab treatment in patients with stenosing forms of Crohn’s disease

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Introduction: While Infliximab (IFX) proved its efficacy in induction and maintenance of remission in inflammatory and fistulating Crohn’s disease (CD), its effect on stenosing forms of CD is not well established. Considering its cicatrizing properties, IFX has the potential to worsen the strictures. The aim of our study was to evaluate the effect of IFX treatment on CD patients with stenosing CD.

Methods: All CD patients from our department treated with IFX for moderate-severe flares of disease were evaluated. Patients with narrowing of small bowel at barium follow through, those with narrowing of colon at colonoscopy and those with previous surgery for intestinal occlusion were defined as having stenosing disease and were followed prospectively. Every 8 weeks clinical exam and every 24 weeks ileocolonoscopy were performed.

Results: 43 patients were evaluated, from which 13 had stenosing forms of CD. 6 patients had previous surgery for occlusion: right colectomy and ileocolic anastomosis in 4 cases at CD diagnosis and in one case after 3 years of disease evolution, one colonic resection with ileosigmoid anastomosis for stenosis of transverse colon. For seven patients without previous surgery, stenosis was located as follows: colonic in three patients, anal canal in one, one inferior rectal, one at ileocecal valve, one in the distal ileum. From these latter patients two needed balloon endoscopic dilation at initiation of IFX treatment (the rectal and ileocecal stenosis). During two years of maintenance IFX treatment no occlusion episodes were observed but in three cases the symptoms (for the anal canal stenosis) or the grade of stenosis seen at programmed endoscopic evaluation (for ileocecal valve and ileocolic anastomosis stenosis) mandated balloon dilatation.

Discussion/Conclusion: Infliximab treatment is safe in patients with stenosing CD providing patients are carefully followed. Periodic colonoscopies identify early potential severe stenosis and through endoscopic treatment help avoid surgery in this high risk group of patients.
Cutaneous complications in Crohn’s disease – A therapeutic challenge

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Introduction: Cutaneous complications of Crohn’s disease are mainly represented by erythema nodosum (EN) and pyoderma gangrenosum (PG). Their treatment remains a challenge even in the era of biological therapies.

Methods: From 119 patients with Crohn’s disease (CD) in our data base, we report 7 cases (5.88%) with PG or EN. Patients’ clinical features, biological and endoscopic findings, therapeutic management, and disease evolution were noted retrospectively.

Results: The patients are 5 females and 2 males, with a median age of 37 years (range 25–59), five with colonic and two with ileocolonic Crohn’s disease. The youngest patients (25 and 27 years old) presented severe flares at CD diagnosis with concomitant extensive lesions of PG on upper and lower limbs. Both responded poorly to corticosteroids and azathioprine, biological treatment with Infliximab (IFX) was initiated, with complete resolution of lesions after the first 3 applications. One case known for 7 years with CD, presented, on maintenance treatment with Infliximab, severe flare of activity associated with deep, extended, and invalidating PG leg ulcers. The time between IFX infusions was shortened with rapid response of intestinal symptoms but very slow improvement of the cutaneous lesions. In all of PG cases local treatment with hydrocolloid patches was applied. The other four cases presented EN, all during intestinal flares of the disease, all lesions rapidly resolved: two after IFX treatment, two after parenteral corticotherapy.

Discussion/Conclusion: Cutaneous complications of Crohn’s disease are rare, but invalidating and frequently require therapy with antiTNF agents. Erythema nodosum responds well to systemic treatment while pyoderma gangrenosum is difficult to treat, needs more aggressive not only systemic but also local treatment. An interdisciplinary approach (collaboration with an expert dermatologist) is important in the management of these conditions.
Infliximab data in Serbia. Multicentric prospective data from five referral centers

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Introduction: Infliximab is established, effective therapy for active luminal and/or fistulizing Crohn’s disease (CD), as well as for moderate to severe ulcerative colitis (UC). Since 2002, in Serbia, the only approved indication was fistulizing Crohn’s disease. In the last two years is approved for all indications as in the Europe.

Methods: This is retrospective, multicentric study. The analysis was obtained by retrospective data of patient with inflammatory bowel disease (IBD) treated by Infliximab. The average period of follow up was 7 years.

Results: A total of 183 adult IBD patients (144 CD and 39 UC) from five referral centers were included in this study. In CD group 62/144 patients received infliximab only as an induction therapy. 57 (78%) of them had perianal fistula(s) and 5 (7.7%) patients had active luminal disease. F/M ratio was 1:1. Median age at starting of infliximab was 30.8 years. Median duration of disease was 5.5 years. 49 (90%) were treated with immunosuppressant’s previously. Ten (18%) patients were steroid dependant. At week 10, clinical responses had 47 (71%) patients. Mean CDAI were decreased for more than 100 points (p < 0.0001). 31 (46%) patients achieved clinical remission (CDAI < 150). According to the results of CDEIS, 63% of patients had endoscopic improvement but not complete mucosal healing. In 24/62 patients with perianal CD previous to Infliximab the seton was placed. The number of drainage fistulas decreased in 21 (37%) patients. Complete cessation of fistula drainage was seen in 10 (16%).

Discussion/Conclusion: 62 patients with perianalal CD were treated only with induction IFX protocol. 71% of patients achieved clinical response and 46% had clinical remission. 37% of pts had reduction in the number of drainage fistula.
 Predictors of therapeutic response to prednisolone in patients with severe attack of ulcerative colitis

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Introduction: Therapy with intravenous glucocorticosteroids remains the main method for management of severe attack of ulcerative colitis. In Russian practice most commonly prednisolone 2 mg/kg/day is used. The aim of the study was determining clinical, endoscopic and laboratory parameters, that allow predicting of unsatisfactory response to 2 mg/kg intravenous prednisolone in patients with severe attack of ulcerative colitis.

Methods: Sixty-seven patients (35 male), admitted to State Scientific Center for Coloproctology with severe attack of ulcerative colitis, were included. Mean age was 35.3 years (range: 17–68). Most patients (79%) had extensive colitis (21% had a left-sided form). All patients received 2 mg/kg/day intravenous prednisolone, antibiotics and fluid maintenance.

Results: Forty-two (63%) of 67 patients had a satisfactory response (SR) – less than 4 stools/day without visible blood in feces. Mean time for establishment of SR was 9.5 days (3–22). Ninety percent of patients had SR by Day 15. Twenty-five percent of patients had unsatisfactory response (UR). Parameters, distinguishing in UR and SR groups in univariate analysis, were entered in multivariate procedure to determine factors predicting UR in severe attacks of ulcerative colitis. Risk of treatment failure reaches 71% if hemorrhagic stool frequency exceeds 5 times/day on day 6 of intravenous steroid therapy (sensitivity 80%, specificity 76%). This risk approaches 80% if serum albumin level is lower than 35 g/l (sensitivity 82%, specificity 94%).

Discussion/Conclusion: If on day 6 of intravenous treatment with 2 mg/kg/day prednisolone hemorrhagic stool frequency exceeds 5 times/day and serum albumin level if lower than 35 g/l, immunosuppressors, anticytokine therapy or colectomy are indicated. Maximal duration of steroid intravenous monotherapy should not exceed 14 days.
Patients with active Crohn’s disease have a low possibility to successfully complete capsule endoscopy

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Crohn’s disease (CD) and anaemia or bleeding (AB) are the main indications to perform capsule endoscopy.

**Aim**: To determine if there are any differences between AB and CD patients concerning gastric passing time (GPT), small bowel passing time (SBPT) and completeness of the study in capsule endoscopy.

**Patients and methods**: 100 consecutive AB patients (28 bleeders, 72 anaemics, mean age 67 ± 13 years, 64 men, 20 current smokers) and 42 CD patients (all with active disease, 22 involving the large bowel, mean age 44 ± 17 years, 28 men, 12 current smokers) were prospectively evaluated with capsule endoscopy. All patients successfully passed a test capsule and had a gastroscopy and colonoscopy performed. Valid questionnaires were completed before the endoscopy. Stat: t-test, X2, Cox-regression analysis.

**Results**: There was no difference in GPT and SBPT, between AB and CD patients successfully completed the study (GPT: AB 48 ± 39 min, CD 39 ± 32 min, p = 0.21; SBPT: AB 296 ± 72 min, CD 265 ± 75 min, p = 0.19). Nevertheless although 70 (70%) AB patients successfully passed the capsule to the cecum, only 21 (50%) of CD patients do so (p = 0.02). 7 (35%) patients with small bowel and 14 (63%) with small-large bowel CD successfully completed the study (p = 0.06). The capsule was successfully expelled in all CD patients in 2.1 ± 0.6 days, although in one patient extensive corticosteroid treatment was needed to pass the capsule in 5 days, despite successful passing of test capsule. When capsule endoscopy was repeated with patients in remission 14/42 (33%) presented no small bowel lesions. In Cox-regression analysis of pooled AB and CD patients only small bowel CD was significant determinant of SBPT (p = 0.02), while age, male gender active smoking and presence of diabetes were not.

**Conclusion**: Patients with CD especially small bowel CD are less likely than AB patients to successfully complete total small bowel evaluation with capsule endoscopy.
Influence of the frequency of outpatient examinations of patients with ulcerative colitis on the number and length of relapses

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Introduction: Ulcerative colitis (UC) is inflammatory bowel disease characterized by recurring relapses alternating with periods of disease inactivity. An important factor that influences the frequency of relapses is the consistent maintenance of remission.

Set of patients and methodology: In 2000–2005, we admitted in the outpatient ward 52 patients with recently found UC. As soon as the diagnosis was confirmed, the patients were, with no respect to the age, sex, or the extent of affection, alternatively assigned to two branches of the study. In the first branch (A), the interval of outpatient examinations was 4 weeks, while in the other branch (B), the interval was 12 weeks. At each examination, the frequency and length of UC relapse was defined by MAYO score.

Results: We observed 147 UC relapses in branch A, i.e. 5.65 attacks per patient on average, with a relapse length of 19–76 days, i.e. 29.8 days on average. The number of UC relapses in branch B was 168, i.e. 6.46 attacks per patient on average, with a relapse length of 8–102 days, i.e. 35.2 days on average. As soon as the results were statistically processed we found that the decreased number of outpatient examinations does not have any impact on the number of relapses UC (p = 0.11), but the modified number of outpatient examinations has an impact on the length of the UC relapse (p < 0.05).

Conclusion: The found results prove that the occurrence and frequency of relapses cannot be influenced by higher frequency of outpatient examinations. On the other hand, it is possible to shorten the length of the UC attack by more frequent examinations, which brings improvement of the quality of life of patients as well as contribution to the shortening of the period of incapacity of a patient for work due to sickness.
Ulcerative colitis in remission and irritable bowel syndrome – Is any relation?

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Introduction: Many patients with inflammatory bowel disease (IBD) display gastrointestinal clinical symptoms in the period of remission. Recent data have suggested that in some cases of irritable bowel syndrome (IBS) there are inflammatory and immune markers which share common features with the immune–mediated pathophysiology associated with IBD.

Aim: evaluation of patients with ulcerative colitis (UC) in remission and the correlation of clinical symptomatology with IBS.

Methods: 64 patients with UC in clinical, biological and endoscopic remission lasting for at least 6 months were evaluated in Institute of Gastroenterology and Hepatology between 1st January 2009 and 31st December 2010.

Results: Out of the 64 patients, 38 (59.37%) had digestive symptoms, which according to Roma III criteria could be included in IBS. Out of these, 26 were female and 12 male, aging between 24 and 65. 22 patients (34.37%) had associated neuropshychical, rheumathological and genito-urinary symptoms frequently met in IBS. 12 patients (31.57%) in patients with IBS symptoms and only 1 (3.8%) in patients without IBS symptoms had a previous episode of infectious gastroenteritis (p < 0.05). Comparing the group of symptomatic patients with that of the asymptomatic patients, no correlations were found between the presence of symptomatology and the duration and extension of the disease.

Discussion/Conclusion: Symptoms of IBS occur in a high number of patients with UC in remission. The presence of symptomatology is associated with the female sex, psychiatric past history, other diseases normally associated with IBS. There is no correlation between the presence of symptoms and the duration and extension of the disease. Infectious gastroenteritis may increase both IBS and UC risks. These data reflect a complex interaction in the pathophysiology of IBS and IBD.
A nurse led, gastroenterology telephone clinic is a cost-effective method of reducing outpatient follow-up appointments and reducing the time patients wait for results

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Background: Despite a drive to reduce the number of out-patients being seen in secondary care, referral rates continue to rise. Telephone clinics (TC) are a method of reducing the burden of conventional face to face outpatient clinics (FTFOP). The aim of this study was to establish whether a nurse led gastroenterology TC is an effective way of following up patients after investigations and compare outcomes with FTFOP.

Methods: A retrospective study of 150, 20 minute nurse led consultations was performed over a nine month period. Referral time, demographics, referral reason, attendance and outcome data was extracted. A sub-group of 40 patients were called at random and invited to give feedback. Waiting times and attendance rates for conventional FTFOP clinics was analyzed. Finally a cost-effectiveness analysis was performed.

Results: The mean age of the patients in the TC was 47.4 vs. 63.7 in FTFOP p < 0.001, there was no difference in gender between the two groups. The median waiting time for FU in a TC vs. FTFOP was 3.0 weeks vs. 8.9 weeks p < 0.001. The reasons for referral to the TC is depicted below.

Only 6.7% of patients in the TC went on to require FTFOP. Non-attendance rates were similar in each group (20% TC vs. 28% FTFOP). In the telephone feedback 95% of patients were happy or very happy with the service. A cost effectiveness analysis demonstrated that the mean cost of FU was £29 in the TC compared to £111 in FTFOP, representing a saving to the PCT of £12,300 for the patients in this study.

Conclusion: This study found that TC offer significantly reduced waiting times for patients at a substantially reduced cost. Non-attendance rates were comparable and feedback from patients was excellent.
Serum neutrophil gelatinase-associated lipocalin (NGAL) concentration is highly elevated in children with inflammatory bowel disease

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NGAL appears to be a useful marker and probably an active factor in pathophysiology of kidney injuries, cardiovascular diseases, cancer, and metabolic disorders. However, an involvement of NGAL in the gastrointestinal diseases is not fully recognized yet. The aim of our study was to evaluate NGAL level in circulation in children with ulcerative colitis and Crohn’s disease.

**Methods:** Blood samples were taken from children for the diagnostic examinations, it was possible to use a part of each sample for this study. NGAL was determined with ELISA kit from Bioporto, Denmark.

**Results:** Serum NGAL level was significantly elevated (p < 0.05) in children with IBD (146 ± 76.9, range: 84.9–405 ng/ml, n = 17) as compared with the level in healthy controls (63.0 ± 22.6 ng/ml, n = 20). Higher elevation was observed in children with ulcerative colitis (164 ± 102, range: 84.9–405 ng/ml, n = 9) than in those with Crohn’s disease (125 ± 27.6, range: 94.8–182 ng/ml, n = 8). In children with obesity and type-1 diabetes serum NGAL level (86.0 ± 30.8, range: 53.1–134 ng/ml, n = 5 and 47.7 ± 15.9, range: 28.2–84.3 ng/ml, respectively) did not differ from that seen in healthy controls. Interestingly, children with non-alcoholic fatty liver disease exhibited significant decrease (p < 0.05) of serum NGAL level (17.1 ± 9.80, range: 4.35–44.1 ng/ml, n = 21), whereas in allergic non-IBD children (t-IgE 300–2000 kIU/l, n = 13) NGAL remained at normal level.

**Discussion/Conclusion:** We have shown, probably for the first time, that IBD in children is accompanied by significant elevation of NGAL level in circulation. Whether serum NGAL may serve as a marker of IBD progression or remission is now under study in our laboratory using numerous group of patients.
Real-life experience with adalimumab in 427 Crohn’s patients in The Netherlands

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Introduction: The aim of this study is to assess the real-life experience with adalimumab treatment since its introduction as treatment for Crohn’s disease (CD) in the Netherlands. This is a prospective cohort of CD patients from both tertiary referral centres and peripheral hospitals. Few real-life experience cohorts of the efficacy of adalimumab in CD have been described. Data from clinical trials, using subjective disease activity scores or mucosal healing do not reflect daily practice and the global physician’s assessment (GPA).

Methods: We included 427 Crohn’s patients who started adalimumab treatment before August 2010. Primary endpoint was treatment efficacy. Treatment success was classified as ongoing treatment at the time of inclusion and improvement in GPA. Patients, who stopped adalimumab within the first 3 months of treatment, were classified as primary non-responders. According to the GPA, loss of response was defined as discontinuation of adalimumab after more than 3 months of treatment.

Results: Medical charts of 427 Crohn’s patients (66% female) receiving adalimumab were investigated. Median age at diagnosis was 39.0 (IQR: 30.0–48.0) and 37.0 (28.0–46.0) at time of start with adalimumab. Median length of follow-up since the start of adalimumab treatment was 21 months (IQR: 11–31). In 67% of patients treatment was successful. 8.4% of patients showed a primary non-response. Loss of response occurred in 8.9% of patients. Off 212 patients previously failing infliximab treatment 64.1% still had a successful adalimumab treatment. In the total cohort, the indication to start adalimumab was disease activity in 60.3% (67.3% luminal, 12.6% fistulising, 13.3% both luminal and fistulising and 3.9% extraintestinal); non- or loss of response to infliximab in 19.1%; side effects of infliximab in 12.3% and patient’s wish in 3.6%.

Discussion/Conclusion: Adalimumab treatment was beneficial in 67% of Crohn’s patients in our cohort. Moreover, previous treatment with infliximab did not influence the outcome of adalimumab efficacy.
Prevalence of gastric pathology in Crohn's disease patients

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Introduction: It has been recognized that different abnormalities of the upper gastrointestinal (GI) tract, like chronic gastritis, are common in Crohn's disease (CD) patients (pts). The aim of the study was to compare CD phenotype with the frequency of histologic abnormalities in the gastric mucosa.

Methods: Biopsies from gastric mucosa were examined histologically in 66 hospitalized CD patients with no previously registered upper GI CD lesions. The presence of epithelial dysplasia, chronic gastritis and granulomas was evaluated. According to the Vienna classification pts were divided in the following groups: L1 – 22 pts, L2 – 10 pts, L3 – 34 pts; B1 – 35 pts, B2 – 18 pts, B3 – 13 pts.

Results: The prevalence of chronic gastritis was 74.24% (49/66) with 7.58% (5/66) H. pylori-positive biopsy specimens. Ten of our patients had macroscopically evident lesions of gastric mucosa, but only three of them had granulomas (4.55%, 3/66). Likewise, only three patients had epithelial dysplasia (4.55%, 3/66). Ileocolonic disease was associated with the higher frequency of the chronic gastritis (27/34, 79.41%) than ileal disease (15/22, 68.18%), although this association was not significant (p = 0.609). Penetrating (11/13, 84.62%) and stricturing (15/18, 83.33%) disease behaviour showed significantly higher rates of chronic gastritis than inflammatory behaviour (23/35, 65.71%, p < 0.001).

Discussion/Conclusion: Penetrating and stricturing forms of Crohn's disease are associated with significantly higher prevalence of gastric pathology than inflammatory phenotype. Association of Crohn's disease localization and behaviour with the abnormalities in the gastric mucosa could be a result of more severe disease course and intensive therapy regimens, but this remains to be elucidated.
Pancreatic insufficiency in ulcerative colitis; Assessment by fecal elastase-1

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Introduction: Clinically significant disease of the pancreas in association with inflammatory bowel disease (IBD) is unusual but reported. IBD patients have a risk of developing pancreatitis as well as pancreatic insufficiency (PI). Pancreatic elastase-1 is a human and pancreas specific enzyme that is not degraded during intestinal transport and correlates with exocrine pancreatic function tests. We aimed to investigate the association of ulcerative colitis (UC) and pancreatic insufficiency as an extraintestinal manifestation of the disease.

Methods: A total of 48 UC patients and 39 healthy controls were evaluated elastase-1 levels in the feces. In all UC patients disease severity was graded according to the modified Truelove and Witts criteria. All UC patients underwent endoscopy and severity of endoscopic findings was graded according to the Baron score. In all UC patients and controls level of elastase-1 in the feces was determined using commercially available ELISA kit (SheBoTech GmbH, Giessen, Germany). SPSS ver 15.0 was used for statistical analysis. Significance was set at p < 0.05.

Results: A significantly lower concentrations of elastase-1 were found in the feces of UC patients compared to controls (median 223.25 interquartile range [IQR] [85.92–333.85] vs. median 1171.38 IQR [1007.26–1292.25]; p = 0.0001). No significant difference in elastase levels was found in patients with inflammation on endoscopy (n = 38) compared to patients with no inflammation on endoscopy. However, significantly lower levels of elastase-1 were found in the feces of UC patients with no inflammation on endoscopy (n = 10) compared to healthy controls (median 250.28 IQR [87.25–381.34] vs. median 1171.38 IQR [1007.26–1292.25; p = 0.001]. No correlation was found between concentration of elastase-1 in the feces and serum CRP levels.

Discussion/Conclusion: Finding of significantly diminished levels of elastase-1 in feces of UC patients suggest that there might be a connection between UC and pancreatic insufficiency as an extraintestinal manifestation of the disease.
Evaluation of thymosin α-1, immunoglobulin E and adiponectin in children with inflammatory bowel disease

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Pathogenesis of inflammatory bowel disease (IBD) involves many factors including immune reactions. The IBD patients may have activated innate, acquired and humoral immune responses. We aimed to evaluate levels of three elements of the immune system: immunomodulatory thymosin α-1 (Th1), total immunoglobulin E (t-IgE) and anti-inflammatory adiponectin (Adn) in blood in children with ulcerative colitis (UC, n = 9) and Crohn’s disease (CD, n = 8). Adn level was also determined in the intestinal mucosa extracts.

Methods: Blood samples and duodenum, small and large intestine biopsy specimens were taken for the diagnostic examinations, it was possible to use a part of each sample for this study. Th1, t-IgE, and Adn were analyzed with the ELISA kits.

Results: Serum Th1 level in UC (4.26 ± 1.53 ng/ml) and CD children (3.24 ± 1.49 ng/ml) was not significantly different from that in healthy controls. However, two children with UC and one with CD had the Th1 level extremely low: < 0.50 ng/ml. In the IBD children, t-IgE level remained within the normal range (1.50–40.0 kIU/l) excepting four UC (62.5–820 kIU/l) and two CD children (85.4–146 kIU/l) where it was highly elevated. Serum Adn concentration was slightly lower in patients with UC (9.21 ± 2.68 µg/ml) than it was in those with CD (11.7 ± 4.21 µg/ml) but both levels were significantly higher as compared to the healthy controls (6.25 ± 3.01 µg/ml). Adn content in the duodenal and intestinal mucosa extracts was similar (20.8 ± 6.84, range: 9.55–35.0 ng/mg protein) in the UC, CD patients and controls.

Discussion/Conclusion: Thymosin α-1 was decreased and t-IgE was increased but only in a part of the IBD children. Serum, but not mucosal, adiponectin level was significantly elevated in our patients. These parameters may be useful for detailed clinical evaluation of particular IBD patients. Further studies are required to estimate clinical importance of our observations.
Pathological diagnosis of pediatric inflammatory bowel diseases. Ten years activity in a referral center experience

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Aim of the study: This study was performed in order to present a long-standing experience in histo-pathological diagnosis of inflammatory bowel diseases (IBD) from a third level centre for paediatric gastrointestinal in a northern Italy metropolitan area.

Material and methods: From January 1, 2000 to December 31, 2010 147 paediatric patients were referred to out Centre for investigation of inflammatory bowel disease. The area served by Centre is about 1 million inhabitants with a paediatric population (0–14 years) of about 180,000 people. Referred children reported symptoms and signs of chronic bowel inflammation for a medium duration of at least 3 months and all of them underwent total colonoscopy and multiple sites biopsies (terminal ileum, cecum, ascending, transverse, sigmoid colon and rectum) were systematically obtained in order to perform a histological diagnosis. Infections were ruled out by stool cultures. Further clinical investigation included standard laboratory tests, autoantibodies and search for signs of autoimmunity. Confidential morphological diagnosis relayed on clear cut features (mucosal limited ulcerative acute inflammation decreasing from distal to proximal large bowel segment with eosinophils and plasma cells, cryptic abscesses, glandular atrophy and distortion for ulcerative colitis, UC) (transmural inflammation with granuloma formation for Crohn's disease, CD); cases with histological picture not satisfying criteria for UC or CD were diagnosis as indeterminate colitis (IC).

Results: 135 out of 147 patients who underwent total colonoscopy for suspected IBD (85 males, 71 females; medium age 6.7 years; range 1.2–14 years, medium time of signs and symptoms 4.5 months) received an histological diagnosis of IBD (97 UC, 29 CD and 9 IC). 6/29 children diagnosed CD showed clinico-histological features of sclerosing cholangitis.

12 children had a final diagnosis different from IBD: 4 cases of B-NHL, 2 cases of parasitosis, 2 cases of multiple adenomatous polyposis, 3 cases of parasitosis, and 1 case of autoimmune colitis).

Among 135 patients with a final diagnosis of IBD, 13 have a first inconclusive histological examination and further diagnostic follow-up biopsies were needed to achieve a final diagnosis (10 UC, 3 CD).

Most of them (126; 93.3%) had histological follow-up (median time 5.5 years; range 1.1–10.5 years.) showing in 95 (70.3%) a morpholgical complete remission of active inflammation signs. None developed epithelial dysplasia or cancer during follow-up. Conventional IBD therapy (mesalamine, steroids, antibiotics) was able to induce clinical remission in 131/135 (99%). Infliximab was successfully adopted in 3 CD children unresponsive to usual treatment. No patients need total or partial colectomy; only two CD children had small bowel segmental resection due to intractable lumen stenosis.


Conclusion: IBD are common diseases in a third level referral centre experience and should be adequately managed. Colonoscopy and extensive histological investigation of bowel are mandatory in order to establish a clear cut diagnosis and consequential treatment: In about 10% of children with symptoms mimicking to IBD, a quite different histological diagnosis merged from microscopy. Epithelial dysplasia and cancer were not found out in this large series of paediatric patients.
Iron deficiency anemia versus anemia of chronic diseases in patients with ulcerative colitis

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Introduction: Differentiation of two most frequent types of anemia in IBD, iron deficiency anemia (IDA) and anemia of chronic diseases (ACD) may be a diagnostic problem, because the inflammation affects laboratory data including ferritin level as acute phase reactant.

Material and methods: In the present study we evaluate laboratory profile of iron pattern and complete blood count including red blood cell distribution width (RDW) in 74 patients with ulcerative colitis (UC); 36 with active disease and 38 with inactive disease, in relation to interleukin-6 (IL-6), transforming growth factor-beta (TGFβ) and C-reactive protein (CRP) levels. Clinical UC activity index was measured according to a slight modification of Tuelove and Witts criteria.

Results: Anemia was diagnosed in 30% of examined UC patients; 55% of which had IDA, 32% had ACD, and 13% had both types of anemia IDA/ACD. Among patients with anemia 69% UC patients were in active stage, and 31% UC patients with inactive stage. In UC patients with IDA, the following parameters were statistically lower as compared with those in ACD patients: ferritin concentration (Z = -3.22, p < 0.005), hemoglobin (Z = -2.60, p < 0.01), RBC (Z = -2.71, p < 0.01), hematocrit (t = -3.98, p < 0.005) and CRP (Z = 3.06, p < 0.05). In contrast, red blood cell distribution width RDW (Z = 2.7, p < 0.05) as well as total and unsaturated iron binding capacity (TIBC, t = 2.48, p < 0.05, and UIBC, t = 2.27, p < 0.05) were significantly higher in UC patients with IDA as compared with those with ACD. The mean serum levels of IL-6 and TGFβ as well as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were not statistically different in UC patients with IDA as compared with UC patients with ACD.

Conclusions: Introducing of red blood cell distribution width (RDW) value to the standard examinations panel (ferritin concentration and TIBC) seems to be diagnostically useful to discriminate between IDA and ACD in UC patients. Our data indicate that ACD and IDA profile in patients with UC is not involved in IL-6 and TGFβ serum levels.
Serum adenosine deaminase as a predictor of disease activity in patients with ulcerative colitis

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Introduction: Ulcerative colitis (UC) is a chronic inflammatory disease characterized by recurrent inflammation and ulcerations of colonic mucosa and an inappropriate and delayed healing. Adenosine deaminase (ADA) is a cytoplasmic enzyme involved in the catabolism of purine bases, capable of catalyzing the deamination of adenosine, forming inosine in the result process. ADA is widely distributed in human tissues and body fluid and its activity is higher in the lymphoid tissues, with the principal biological activity of ADA being found in T lymphocytes. Although ADA has been shown to increase in several inflammatory conditions, there is no literature data indicating an alteration in UC. In this study we aimed to explore whether the levels of ADA alter in UC patients and its correlation with inflammation markers of UC.

Methods: The present study was carried out in our tertiary reference center (Ankara Education and Research Hospital, Department of Gastroenterology) between January 2010 and March 2011. Serum levels of ADA were investigated in 44 patients with UC (24 in active state, 20 in remission). ADA levels were compared in patients with UC and in healthy controls. Correlation analysis was also performed between ADA and other inflammation markers of UC (white blood cell, hsCRP, sedimentation rate).

Results: Serum mean ADA levels were 11.12 ± 2.03 and 7.99 ± 2.04 U/l for patients with UC in active state and in remission and 8.55 ± 2.26 U/l in the healthy control group (Figure 1). Correlation analysis was also performed between ADA and other inflammation markers of UC. Mean serum ADA levels were significantly elevated in active UC patients compared with patients with UC in remission and control group. Overall accuracy of ADA in determination of active UC was 83.7% (with sensitivity 83.3%, specificity 84.2%).

Discussion/Conclusion: Serum ADA levels was found to elevated in UC patients in active state suggesting a partial role of activated T-cell response in the disease pathophysiology. ADA can be used as a supportive diagnostic marker in patients with UC. Further randomised controlled studies are warranted to demonstrate the role of ADA in UC patients with a special interest in specific targeted therapies against ADA for achieving disease remission.
Figure 1. Serum ADA levels in patients with UC with active state and in remission with control group.
Long-term safety and efficacy of H1N1 vaccine in a single-center cohort of IBD patients treated with immunomodulators and/or anti-TNFα biologics

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Introduction: Long-term efficacy and safety data are lacking on H1N1 virus vaccination in patients with Crohn’s disease (CD) and ulcerative colitis (UC) receiving immunomodulators such as azathioprine (AZA) and/or anti-TNFα biologic therapy such as infliximab (IFX) and adalimumab (ADA). This study summarizes our prospectively collected data on the long-term safety and efficacy of influenza H1N1 vaccination in IBD patients.

Methods: Patients with quiescent IBD on immunomodulators (IMM) and/or anti-TNFα therapy who received the H1N1 vaccine (Focetria®) between November 2009 and April 2010 were followed for one year to assess efficacy and safety of H1N1 vaccine and its effects on the course of IBD. The Harvey-Bradshaw Index for CD and the Partial Mayo Score for UC were used to assess disease activity. Patients were scheduled to receive 2 doses of Focetria® with an interval of 4 weeks.

Results: We enrolled 26 patients, 19 CD, 7 UC, 42.3% female, median age 34 years, 7/26 (26.9%) on AZA, 5/26 (19.2%) on ADA and 14/26 (53.9%) on IFX (3 on combo therapy IFX+AZA). Local pain was reported by 8/26 (30.7%) patients and systemic symptoms by 5/26 (19.2%) patients (headache 1; fatigue 2; fever 3) following the 1st dose. Patients who developed fever did not receive the 2nd dose of vaccination. These adverse events were unrelated to patient and/or disease characteristics but were numerically higher in patients on anti-TNFα biologics compared to IMM [4/19 (21%) vs. 1/7 (14.3%)]. Vaccination did not trigger a flare of either UC or CD. Furthermore, none of the patients reported flu-like symptoms or had documented H1N1 infection for 12–15 months following vaccination although the cases of H1N1 reported between September 2010 and March 2011 far outnumbered and were more severe than in the previous year.

Discussion/Conclusion: H1N1 vaccination was well tolerated by IBD patients on IMM or biologics and offered protection from the virus for at least one year. The risk of IBD flare was not increased after H1N1 vaccination.
Maintenance vs. intermittent therapy in clinical and endoscopic remission in non-extensive ulcerative colitis

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Introduction: Maintenance therapy is considered as obligatory recommendation for all patients with ulcerative colitis (UC) after achieving remission. The duration of maintenance therapy is not determined in the case of persistent complete remission.

The aim of the study was to compare the effectiveness of long-term maintenance and intermittent 3-month therapy with 5-ASA in patients with non-extensive UC after achieving complete (clinical and endoscopic) remission.

Methods: 59 patients (44% women, age range 29–65, mean age 37.4 ± 12.6) who achieved clinical and endoscopic remission after mild-to-moderate relapse with non-extensive distribution were included in the study. Previous proctitis was diagnosed in 8.5% (5), proctosigmoiditis – in 71.2% (42) and left-side colitis – in 20.3% of cases (12). Mild relapse had 39% (23) and moderate activity was diagnosed in 61% (36). Patients were randomized into two groups: first group (27 patients) received maintenance therapy with mesalazine 1.5–2 g/day and second group (32 patients) received intermittent treatment with mesalazine 1.5–2 g/day for three months. Monitoring the course of the disease was carried out over two years.

Results: Remission persisted in 81.5% (22/27) in the first group and in 78.1% (25/32) in the second group at 6 months, in 51.9% (14/27) and 46.9% (15/32) respectively – at 12 months, in 33.3% (9/27) and 31.3% (10/32) – at 24 months. Differences were not statistically significant. The severity and extent of lesion in case of subsequent relapses were not statistically different in the first and in the second group.

Discussion/Conclusion: Continuous maintenance therapy with mesalazine (1.5–2 g/day) showed no advantages over 3-month intermittent therapy in the same doses in patients with complete clinical and endoscopic remission after non-extensive mild-to-moderate relapses of UC. In this pattern of disease long-term maintenance therapy, probably, is not necessary. However, further observation is necessary to evaluate the long-term outcomes.
Dysplasia and the risk for colorectal cancer in ulcerative colitis

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Introduction: With respect to increasing colorectal cancer risk, the most important factor which has been reproducibly found in many studies, is the duration of disease in ulcerative colitis patients. The aim of our study was to evaluate the results of surveillance colonoscopy in patients with ulcerative colitis in order to estimate the occurrence of dysplasias or colorectal cancer.

Methods: 45 consecutive patients diagnosed with ulcerative colitis 7 years ago enter the study. All patients were under medical treatment and followed-up during 12 years. Colonoscopies with biopsies were performed every 12 months. Biopsies have been taken every 10 cm from normal appearing mucosa and from macroscopically suspicious areas. In some cases (low – grade dysplasia, high – grade dysplasia) the microscopic lesions were confirmed by a second expert pathologist. Dysplasia was estimated according to Riddel’s classification.

Results: In 11 patients (22.4%) low – grade dysplasia was present, and high – grade dysplasia was found in 5 cases (11.1%). After 12 years of evolution, 3 patients developed colonic cancer. Of the 5 cases with high – grade dysplasia found preoperatively, 1 had cancer found in the surgical specimen. In 2 of 11 patients with low – grade dysplasia an advanced lesion was detected after 3 years of colonoscopic follow – up (cancer or high – grade dysplasia). In total, 20% of cases underwent surgical treatment.

Discussion/Conclusion: The goal of surveillance colonoscopy is to detect preneoplastic lesions before they become dangerous. Thus, the detection and interpretation of dysplasia is crucial to decrease the mortality by colorectal cancer. Our study confirms that long-standing ulcerative colitis has a high risk for colorectal cancer and advocates intensive colonoscopic surveillance or yearly colectomy when dysplasia is discovered.
Ileostomy effectiveness in the treatment of the patients with the severe form of perianal Crohn's disease

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Introduction: The aim of the study is to determine the effectiveness of ileostomy in patients with complicated forms of Crohn’s disease of the colon.

Methods: 43 patients with Crohn’s disease of the colon and perianal lesions, in the period from January 1998 to July 2009 have undergone the fecal diversion by ileostoma formation, as their first stage of surgical treatment. The effectiveness was estimated on the base of three criteria: the general somatic state dynamics (index SAI – Severity – Activity – Index [Goebell, 1992] in the clinic’s modification – without counting the amount of stool per day); the dynamics of endoscopic inflammation signs changes; the dynamics of perianal lesions healing (ulcer-fissures, anal fistulas, anal canal’s strictures).

Results: Out of 43 patients, 41 had progressive improvement of somatic state during the first 2 weeks after the operation. In 2 (4.1%) patients no improvement of somatic state was demonstrated. Endoscopic effectiveness was estimated in 35 (81.3%) out of 43 patients. The full endoscopic remission, without colon resection, was achieved in 13 (37.1%). Dynamics of perianal lesions healing was also observed in 35 (81.3%) patients. Among them, in 20 (57.1%) cases complete healing was marked. Out of these patients 11 did not need surgical intervention and in 9 patients operative correction was necessary. By now, in 20 (57.1%) patients the treatment has been completed, in 15 (42.9%) – it is going on. Out of 43 patients, 8 have been lost for the study.

Discussion/Conclusion: Thus the study proves that a temporary fecal diversion in patients with Crohn’s disease of the colon allows: to normalize the somatic state of the patients in 95.4%; to achieve the remission of the inflammation process, without colon resection, according to the endoscopic data in 37.1%; to heal perianal lesions in 57.1%.
Improvement in biomarkers of bone formation during 54-week infliximab therapy in pediatric patients with Crohn's disease

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Introduction: Treatment with infliximab (IFX) may improve growth and disturbed bone metabolism in pediatric patients with Crohn's disease (CD), but the characteristics of bone formation and resorption factors under IFX treatment are not well known. This study examined changes in bone formation (osteocalcin/OC, bone-specific alkaline phosphatase/bALP) and resorption (beta-crosslaps/bCL) under IFX treatment. Moreover, associations between bone biomarkers, and CRP, vitamin D level, disease activity index (PCDAI), and dual energy x-ray absorptiometry (DEXA) after 54 weeks of IFX therapy were analyzed.

Methods: Twenty-eight subjects (male, 15, mean age, 15.4 years) with moderate-to-severe CD received IFX induction (5 mg/kg/dose) and maintenance therapy. Serum OC, bCL, bALP, and vitamin D were collected at baseline, 6 weeks, 30 weeks and 54 weeks, in addition CRP and PCDAI were determined. DEXA z-scores were assessed at baseline, 30 weeks and 54 weeks.

Results: Serum levels of bone formation OC increased significantly after IFX induction treatment. Mean OC concentrations were 31.3 ng/ml vs. 51.7 ng/ml, 61.6 ng/ml, and 64.3 ng/ml at week 0, weeks 6, weeks 30, and 54, respectively (p < 0.005). bALP increased significantly between baseline and weeks 6 (mean, 110 U/l, 161 U/l, respectively, p = 0.002). There were no significant differences concerning bCL and vitamin D at different time points. Nevertheless, both z-score of the lumbar spine and femoral neck improved after 54 weeks when compared with baseline (lumbar spine, -0.65 (-2.9–0.9), -2 (-3.5–1.7), femoral neck, -0.9 (-3.6–1.1), -1.6 (-3.5–2.1), respectively. Increment of bone forming OC correlated negatively with decrement of CRP and PCDAI.

Discussion/Conclusion: Clinical response to IFX therapy was associated with an increased level of bone forming osteocalcin in pediatric patients with 54-week treatment of IFX. In contrast to a previous study, bone resorption marker (bCL) was not increased suggesting a bone forming effect of IFX treatment.
Infliximab induces regulatory macrophages in responders but not in non-responders

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Introduction: Regulatory macrophages play an important role in wound healing and gut homeostasis and have anti-inflammatory properties. We have previously shown that anti-TNF antibodies induce regulatory macrophages in an Fc region dependent manner in vitro (Vos et al, Gastroenterology 2010). The aim was to examine the induction of regulatory macrophages in vivo, and to study a possible association between response and induction of regulatory macrophages in patients treated with infliximab.

Methods: Colonic mucosal biopsies were obtained during endoscopy from CD (n = 6) and UC (n = 4) patients before and 4-6 weeks after first infliximab therapy. Response to infliximab was defined as endoscopic and histologic healing. Regulatory macrophages were defined as the proportion of CD206+ (regulatory macrophage marker) to CD68+ macrophages (CD206+/CD68+) on immunohistochemistry.

Results: A significant (p = 0.0051) induction of regulatory macrophages was observed after treatment in responders to infliximab (n = 5: 2 UC, 3 CD). Strikingly, this induction was absent in non-responders (n = 5: 2 UC, 3 CD). The association between response and induction of regulatory macrophages was found in UC patients as well as in CD patients. Furthermore, non-responders had lower amounts of regulatory macrophages at baseline (week 0), suggesting a defect in regulatory macrophage differentiation or recruitment in non-responders.

Discussion/Conclusion: We show that infliximab induces regulatory macrophages in responders but not in non-responders. This mechanism of action of infliximab may play a role in mucosal healing in patients with IBD.
Enhanced induction of regulatory macrophages upon azathioprine/infliximab combination treatment in vitro

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Introduction: We have previously shown that anti-TNF antibodies induce regulatory macrophages in an Fc region dependent manner in vitro (Gastroenterology 2010). Regulatory macrophages play an important role in wound healing and gut homeostasis and have anti-inflammatory properties. In addition, a recent study has shown that a combination of infliximab and azathioprine therapy is more effective than infliximab or azathioprine monotherapy in inducing and maintaining remission in Crohn’s disease (CD) patients (Colombel et al. New Engl J Med 2010). The aim of this study was to examine the effects of infliximab/azathioprine combination therapy on the induction and function of regulatory macrophages.

Methods: Mixed lymphocyte reactions (MLR) were established using PBMCs from two healthy donors in a 1:1 ratio and treated with either infliximab, azathioprine or both. Macrophage phenotype was evaluated by flow cytometry. Inhibition of T cell proliferation was measured in a secondary MLR containing macrophages and third party lymphocytes. Cytokine secretion was measured using a cytometric bead assay.

Results: Alternatively activated macrophages were induced upon treatment of MLR with anti-TNF antibodies (infliximab or adalimumab). These macrophages showed anti-inflammatory properties in terms of their ability to inhibit proliferation of activated T cells, expression of the regulatory macrophage marker CD206, as well as production of IL-10 in response to LPS. Strikingly, when cells in an MLR were treated with a combination of infliximab and azathioprine, an enhanced induction of regulatory macrophages was observed. Not only was the number of regulatory macrophages increased upon combination treatment, the macrophages also had a more potent phenotype. Compared to regulatory macrophages induced by infliximab monotherapy, regulatory macrophages induced by combination treatment had higher expression of the regulatory macrophage marker CD206 and showed enhanced capacity to inhibit proliferation of activated T cells.

Discussion/Conclusion: We show an enhanced induction of regulatory macrophages upon infliximab/azathioprine combination treatment in vitro. In addition, macrophages induced by combination treatment showed a stronger immunosuppressive phenotype. This mechanism of action may play a role in mucosal healing in patients with IBD and might explain the enhanced efficacy of infliximab/azathioprine combination therapy.
Induction therapy with three doses of infliximab in Polish children with Crohn’s disease

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Background: Infliximab is currently registered to use in Crohn’s disease (CD) in children over 7 years of age. There is so far lack of satisfactory data about infliximab induction therapy in children with CD from Eastern and Medium Europe.

Aim: The aim of this study was to assess clinical efficacy of induction therapy with three doses of infliximab in Polish children with CD.

Patients and methods: 66 CD children aged 14.8; 12.9; 16.3 [median; Q1; Q3] with history of immunomodulation therapy (48 with AZA, 21 with Mtx) were involved to the study. Patients received infliximab (5 mg/kg) in three repeated infusions at 0, 2, 6 weeks. Clinical evaluations (PCDAI, CRP, platelets, BMI) were performed at baseline and week 10. Adverse events monitoring had been conducting. Wilcoxon test was used to compare quantitative variables, p < 0.05 was regarded as significant.

Results: 22 (33%) patients has reached clinical remission (defined as PCDAI score ≤ 10), 26 (39%) clinical response (defined as PCDAI decrease ≥ 15 and PCDAI score ≤ 30); 18 (28%) had no response. The significant decreases (p < 0.05) in: PCDAI score: 52.5; 45.0; 57.5 [median; Q1; Q3] vs. 15.0; 10.0; 30.0, CRP: 1.6; 0.3; 3.5 vs. 0.3; 0.2; 2.1, platelets level 368.0; 287.0; 506.0 vs. 309.0; 263.0; 459.0 and significant increase in BMI: 17.5; 15.4; 19.4 vs. 18.0; 16.7; 20.0 were found when compared data from baseline and week 10. No AE leading to therapy termination was observed.

Conclusions: 1. Induction therapy with infliximab is clinically efficient in 72% of Polish children patients with Crohn’s disease. 2. Induction therapy with infliximab improves nutritional status of these patients.
Seasonal variation by the onset of symptoms and health care seeking behaviour in 282 patients with inflammatory bowel disease (IBD): A single center experience from Turkey

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**Backgrounds and aims:** Environmental factors are believed to trigger the onset of inflammatory bowel disease (IBD) in genetically susceptible individuals. We aimed to evaluate the seasonal variation in the onset of symptoms in patients with IBD. Whether an interaction between seasonality and both age and gender was questioned, too.

**Patients and methods:** Of the 2220 patients with IBD, 282 consecutive patients who have full data were chose from the charts. Demographic features, the month and the age at the onset of presenting symptoms for each patient were analyzed. Cumulative monthly averages analysed by Kruskal Wallis test and Roger’s test. Otherwise, Chi-square, Student’s t-test and Mann-Whitney U analyses were used.

**Results:** Of the 282 patients with IBD, 181 were male (64%). Mean age was $40.1 \pm 14.7$ years (median: 38, range: 14–79 years); $42.8 \pm 14.6$ years (median: 42, range: 17–78) in males vs. $35.4 \pm 13.8$ years (median: 33, range: 14–79 years) in females ($p < 0.0001$). The seasonal pattern showed peak in March with 57% and the lowest point in November with 36% ($p < 0.05$). The seasonal pattern was not influenced by both genders and by age groups in patients with IBD or UC or CD ($p > 0.05$).

**Conclusion:** We investigated the etiologic environment of IBD and found an interaction between the etiopathogenesis of IBD and environmental risk factors or gastrointestinal system infections. This interaction was not influenced by both age and gender.

**Fig. 1:** The IBD Figure shows the distribution of symptomatic onset of disease each month over fourteen years.
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