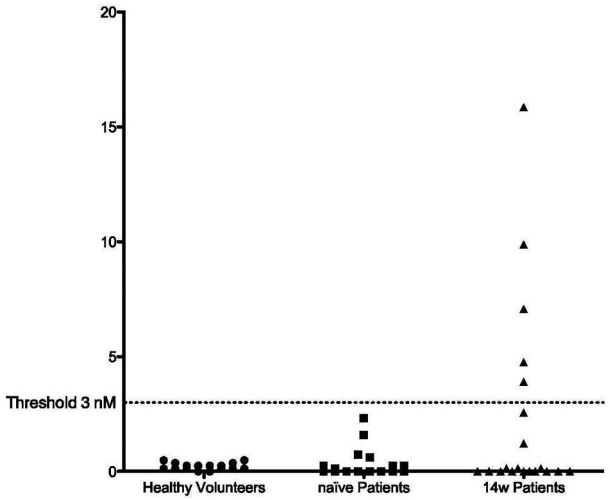

"Figure 1: ATI levels in healthy volunteers, Crohn's disease patients naïve to anti-TNF drugs and after 14 weeks of treatment with IFX"

interaction between IFX and ATI by analysing the changes in the molecular weight of the drug. This characteristic increases the specificity of the method and avoid false-positive Results observed with ELISA based Methods. However, an optimal calibrator to quantify the concentration of ATI is lacking.

Aims: To improve the method for quantifying ATI by the use of an optimal calibrator.

Methods: Human sera from healthy volunteers (n=20) and Crohn's disease (CD) patients (n=19) were analysed. CD patients with active disease (CDAI>150) naïve to anti-TNF treatment were included. Patients received IFX 5 mg/kg at weeks 0, 2, 6 and 14. Biochemical evaluation of ATI was assessed at baseline and week 14. ATI were measured using HMSA. After incubation of human sera with IFX-alexa 488, samples were injected onto Size Exclusion Yarra 3000 HPLC column (Phenomenex, USA) to measure ATI-IFX complexes. The calibration curve in order to quantify ATI in serum was constructed by diluting a pure IgG1 anti-idiotypic anti-IFX antibody generated by HUCAL (Human Combinatorial Antibody Library) technology (Abd Serotec, Germany) in human serum. The purity of the calibrator was corroborated by electrophoresis and subsequent silver staining. The characterization of the molecular weight of the complex between the calibrator and IFX was estimated by SE-HPLC using IFX-alexa 488

Results: We observed a peak for the complex corresponding to 300 kDa as expected for 2 IgG bound. The calibrator showed a high affinity (Kd = 3.9 nM) and a high specificity for IFX. These parameters were assessed by measuring the decrease of fluorescence of 300 kDa peak in presence of unlabelled IFX for the affinity study and of adalimumab or anti-Fc for specificity study. The calibration curve in a range of concentration of 1.3-33.3 nM was linear. The limit of detection was 1.3 nM. At week 14, 26.3% of the patients (n=5) had over 3 nM of ATI concentration (Figure 1).

This concentration could be considered threshold because none of the healthy volunteers and the patients before receiving IFX had over 3 nM of ATI concentration.

Conclusions: A pure IgG1 anti-idiotypic anti-IFX antibody for the calibration curve allows accurately quantifying the concentration of ATI identified by HMSA

P021
Differential plasma microRNA expression profile in ulcerative colitis patients according to their response to corticosteroids

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Background: Corticosteroids (CS) remain the first line treatment for moderate and severe active ulcerative colitis (UC). However up to 40% of patients do not have an adequate response. We use clinical and biological variables to predict a bad response after three days of treatment, but to date we have not been able to predict response before starting therapy. MicroRNA (miR) are small non-coding RNA fragments that modulate gene expression at a post-transcriptional level, playing a critical role in many biological processes. In previous studies we found a differential miR profile in rectal mucosa of patients with UC responders and non-responders to CS. Objective: To compare the miR profile in plasma of patients with active UC responding and non-responding to CS.

Methods: Plasma samples were obtained from UC patients before CS treatment for a moderate-to-severe flare, and also from healthy controls. Patients were grouped according to clinical response (non-responder = moderate or severe activity according to Montreal's classification or need of rescue therapy at day 7; responder = mild activity or remission without rescue therapy at day 7). miR were identified on plasma samples by means of a sequencing method (Illumina kit). After the comparison between groups those miR with a fold change greater than 1.5 and adjusted p-value less than 0.05 were further studied. Potential targets of selected miR were checked in Target Human Scan and miRwalk database, and their impact on biological activity was searched in GeneCodis database.

Results: 10 healthy controls and 20 patients with UC (10 responders and 10 non responders to CS) were included. Comparison between responders and non-responders showed no significant differences. However, we found three miR with differential expression between healthy controls and non-responders (miR-1290, miR-4508, and miR-149-5p). In silico study of these miRNAs showed more than 1000 genes potentially regulated by each, mainly involved in MAPKinasa signaling pathway, regulation of cytoskeletum, calcium signaling pathway, and endocytosis.

Conclusions: The plasma miR profile obteined in the present study is not usefull to identify patients with active UC not responding to CS. However further studies integrating plasma and tissue miR profiles may help us to develop a potential biomarker of response to CS in UC.

P022
Histone deacetylases in inflammatory mucosa distinguish Crohn's disease from ulcerative colitis

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Background: Inflammatory bowel disease (IBD) creates a high burden of both patient disability and health care costs. There is a high unmet need to better understand the pathophysiology of IBD, leading the way to novel therapeutic targets. Histone deacetylation (HDAC) regulates chromatin remodeling and influences inflammatory gene transcription. Sirtuin(SIRT)1, SIRT6 and HDAC9 are members of the HDAC superfamily that exert proinflammatory properties. Interestingly, murine research suggested a critical role for HDAC9 in breaching immune homeostasis in experimental colitis. Considering the advent of a new generation of HDAC inhibitors we aimed to explore the role of HDACs in IBD.
Methods: We collected colon resection tissue from Crohn's disease (CD) patients and ulcerative colitis (UC) patients operated on for therapy refractory disease. From each patient both macroscopically inflamed and non-inflamed areas were collected, and for CD patients stenotic lesions were collected as well. For RNA isolation the lamina propria was separated from the muscularis externa, and a micro array was performed for HDAC and SIRT mRNA expression.
Results: Baseline characteristics were comparable for 15 CD (47% male) and 9 UC (66% male) patients with mean age at operation of 34 +/- 10 years in CD and 37 +/- 10 years in UC pts. Of the CD patients, 53% had ileal and 47% ileocolonic disease, of the UC patients had 44% left sided colitis and 56% pancolitis. Serum CRP levels immediately before surgery averaged 18 mg/L in both cohorts. Expression of HDAC9 was at average 3.81 fold higher in the inflamed mucosa in CD patients compared to the inflamed mucosa of UC patients (p=0.002), in the uninflamed mucosa the expression of HDAC did not differ significantly. Moreover, in the inflamed mucosa of CD patients SIRT6 was significantly upregulated in comparison to UC patients (p=0.003), whereas in the inflamed muscularis mucosa the expression of SIRT1 was higher in CD than in UC patients (p=0.04). Intriguingly, the increased levels of HDAC9, SIRT1 and SIRT6 were only present in inflamed tissue and were found to be the same compared to UC in stenotic or non-inflamed lesions.
Conclusions: Our findings show an increased expression of HDAC9 and SIRT6 in the mucosa of inflamed CD colon compared to inflamed UC colon. Therefore, histone deacetylases expression has the potential to serve as an additional marker to distinguish CD from UC in tissue biopsies. Further research is necessary to investigate the functional properties of histone deacetylation in CD pathogenesis, which could grant opportunities for therapeutical action.

P023
Dipotassium glycyrrhizate normalises the mucosal healing gene expression altered by inflammation in a murine model of colitis

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Background: Dipotassium glycyrrhizate (DPG) is a compound derived from glycyrrhizin, a glycoconjugated triterpene produced by the licorice plant, Glycyrrhiza glabra, whose anti-inflammatory properties are well-known. DPG reduce inflammation though various mechanisms, including the inhibition of the alarmin high mobility group box (HMGB)1 and the enzyme 11beta-hydroxysteroid dehydrogenase 2 (11betaHSD2). We previously demonstrated that DPG significantly reduces the DSS-induced colitis in mice, that showed a surprising recovery of body weight and large intestine length as well as an increase in histological score, the latter indicating the occurrence of a mucosal healing (MH).
The aim of the present study was to deeply investigate the effect of DPG on the expression of genes involved in the mucosal healing (MH) pathway during inflammation
Methods: C57BL/6 mice were divided into 3 experimental groups (5 mice for each group): DSS (3%)-treated mice, DSS (3%)+DPG (8mg/Kg)-treated mice and control mice. After 7 days, mice were sacrificed and the colon removed. Tissue samples were analysed by a PCR array (QIAGEN) able to evaluate 84 key genes central to the wound healing response. To identify the most altered genes, a threshold of 3.5 times was chosen. Selected genes were divided into functional groups. The expression level of the most altered genes inside each group was validated by RT-PCR
Results: DSS treatment significantly up-regulated 19 MH genes, as showed by comparing DSS-treated vs control mice. These genes were significantly down-regulated to control values by DPG treatment, as showed by comparing DSS+DPG mice vs DSS mice.
Altered genes were classified into 6 different functional groups: cytokines (IL-10, IL-1beta, IL-6), chemokines (CCL12, CCL7, CXCL1, CXCL3, CXCL5), extracellular matrix (ECM) components/collagen proteins (Col3a1, Vtn), growth factors (Csf3, Fgf2, Fgf7), remodelling enzymes (Mmp9, Timp1, Plat, Plaur, Serpine1), others (Ptgs2). Expression analysis of most altered genes within each functional group was validated by RT-PCR ($p<0.001$:IL-1beta, IL-6; $p<0.01$: CXCL3, CXCL5, Col3A1, Vtn, Fgf7; $p<0.05$: Mmp9, Serpine1).
Conclusions: We show for the first time that the use of DPG in mice with a DSS-induced colitis strongly improves the MH by modulating the expression levels of genes involved in wound healing response. Due to the total lack of side effects, we believe that DPG could represent a very innovative and useful tool for the management of human intestinal inflammation

P024
Role of Interleukin 27 (IL-27) in the Colonic Mucosa of Patients with Inflammatory Bowel Disease.

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Background: The pathogenesis of inflammatory bowel disease (IBD) is not completely understood. The chronic relapsing inflammation is thought to be the result from a pro-inflammatory microenvironment and an aberrant immune response. The interleukin 27 (IL-27) is an immune-regulatory cytokine, has both anti- and pro-inflammatory properties. As an anti-inflammatory, IL-27 seems to induce a general negative feedback program that limits T and NK-T cell activity and also mediates inflammation during chronic disease. Nevertheless, no previous studies have explored their expression in patients with IBD.
Methods: This is an observational and cross sectional study, we included a total of 30 active UC (aUC), 22 inactive UC (iUC), 20