median age 25.5 years (16.7–69); 45% male) patients across three Belgian IBD centres (University Hospitals Brussels, Ghent and Leuven). New diagnosis was defined as within 3 months after diagnosis, naïve for biologicals and immunosuppressives, and no previous IBD-related surgery. A panel of 91 inflammatory proteins (OLINK) was quantified in the serum sample taken at diagnosis, and of age- and gender-matched healthy controls (n = 80, ± 3.6 years). Wilcoxon rank-sum and t-tests were used as appropriate, and multiple testing correction applied (Benjamini-Hochberg method, R 3.4.2). An adjusted p-value of <0.05 was considered significant.

**Results:** Comparison of protein levels in CD patients with matched controls identified 44 significantly different proteins, with OSM (fold change (FC) = 4.0, p = 1.7E-12) and IL-6 (FC = 3.7, p = 3.9E-12) as the most dysregulated proteins. When comparing UC with controls, 39 significantly different proteins were found, 29 of which were also different in CD, incl. II-6 and OSM (ranked 9th and 10th). The most differentially expressed protein in UC was CXCL1 (FC = 1.7, p = 4.5E-07). We then stratified CD and UC into quartiles based on the age at diagnosis (≥21.5, 21.5–25.5, 25.5–33.5 and >33.5 years for CD; ≥20.6, 20.6–26.0, 26.0–33.7 and >33.7 years for UC), and compared each subgroup with its matched control group. A comparable number of differentially expressed proteins was observed for quartiles 1 to 3 in CD (n = 25–30 proteins), of which 12 overlapped, incl. OSM and II-6. Only one protein, FGF-19, was dysregulated in the oldest CD group. For UC patients, quartiles 1 and 2 showed comparable results (13 and 8 different proteins, overlap of 7), while no differences were observed for the two oldest UC groups.

**Conclusions:** We identified panels of inflammatory markers defining newly diagnosed CD and UC, with some common for both (OSM, II-6), while others appear to be specific for either CD or UC. We found a decreased inflammatory burden with increasing age, providing further evidence for the less severe clinical symptoms in late-onset compared with early-onset IBD.

**P118**

**Identification of colitis-associated bacteria in intestine of inflammatory bowel disease patients**

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**Background:** Dysbiosis of intestinal flora in patients of inflammatory bowel disease (IBD) including ulcerative colitis (UC) and Crohn’s disease (CD) is closely related to intestinal inflammation. To clarify relationship between bacteria and colitis, it is necessary to use the stools of patients to reproduce colitis in animals. In this study, we clarified the characteristics of IBD intestinal flora by metagenomic analysis and identified bacteria causing intestinal inflammation in a mouse model.

**Methods:** Whole genome shotgun sequencing was performed on the stool DNA of 16 UC patients, 8 CD patients, and 13 healthy donors (HD). The composition of bacterial flora was compared by linear discriminant analysis (LDA). Intestinal flora of II10-deficient mice, a model mouse of spontaneous colitis, was sterilised using antibiotics, discriminant analysis (LDA). Intestinal flora of II10-deficient mice, a model mouse of spontaneous colitis, was sterilised using antibiotics, and of age- and gender-matched healthy controls (n = 80, ± 3.6 years). Wilcoxon rank-sum and t-tests were used as appropriate, and multiple testing correction applied (Benjamini-Hochberg method, R 3.4.2). An adjusted p-value of <0.05 was considered significant.

**Results:** Compared with the bacterial flora of the HD patients, there were 43 different bacterial taxonomies in the UC patients and 56 differences in the CD patients (p < 0.05). In particular, Enterococcus faecium in UC and E. coli in CD had the highest LDA scores. In addition, weight gain was less in the UC group than in the HD group, but there was no significant difference between the CD group and the HD group. The pathology score was higher in the UC group than in the HD group, and the expression levels of mif, II1b, and II17 increased in the UC group. In the bacterial flora of the UC group after transplantation, bacteria of the Enterococcus genus were significantly more abundant than those of the HD group (p < 0.05). Mice administered E. faecium showed less weight gain, higher pathology score, and higher expression levels of mif, II1b, II12b, and II17 than those administered the HD feces.

**Conclusions:** The intestinal bacterial flora of UC patients induces colitis and E. faecium might be one of the bacteria causing intestinal inflammation.

**P119**

**The immunohistochemical assessment of CD30+ lymphocytes in the intestinal mucosa facilitates diagnosis of paediatric ulcerative colitis**

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**Background:** Diagnosis of paediatric inflammatory bowel diseases (IBD) remains challenging. We aimed at evaluating the value of immunohistochemical assessment of CD30+ lymphocytes in the intestinal mucosa in differential diagnosis between paediatric Crohn’s disease (CD) and ulcerative colitis (UC) and its utility as a predictor of future differentiation in patients with IBD unclassified (IBDU).

**Methods:** 74 treatment naive paediatric patients with IBD (33 CD, 30 UC, and 11 IBDU) were enrolled into the study. Biopsy samples from six different regions (terminal ileum, caecum, ascending colon, transverse colon, descending colon, and rectum) were immunohistochemically stained with anti-CD30 antibody and number of positive cells per one high power field was quantified.

**Results:** Significant differences between CD and UC were found when compared with all counts of CD30+ cells in median numbers, mean values, and maximal numbers and also for separate counts in terminal ileum, transverse colon, descending colon and rectum. The most profound difference between CD and UC was shown for total median values of CD30+ cells and for the values in rectal localisation. The difference was independent on the intensity of inflammation. A cut-off value of 2.5 CD30+ cells with sensitivity 83% and specificity 90% was found for the rectum. There was no difference between patients with CD and IBDU, but a marked difference between UC and IBDU patients was revealed.

**Conclusions:** Histopathological assessment of biopsy with rectal CD30+ count is reliable and simple method that could help in differential diagnosis among IBD subtypes in children with IBD.

**P120**

**Vitamin D deficiency in Crohn’s disease**

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**Background:** There is increasing evidence that vitamin D (VD) deficiency may play a role in inflammatory bowel disease (IBD). Studies have shown that VD levels are decreased in IBD patients and that supplementation may improve clinical outcomes in Crohn’s disease (CD). There is no consensus on the optimal level of VD needed for optimal health in IBD patients.

**Methods:** We assessed the levels of 25(OH)D in a cohort of IBD patients (n = 101, CD = 65, UC = 36) and healthy controls (n = 50). The study was approved by the institutional review board of the Motol University Hospital. The patients were divided into two groups: naive and previously treated. Statistical analyses were performed using SPSS (version 20).

**Results:** The levels of 25(OH)D were significantly lower in IBD patients compared to healthy controls (p < 0.05). The levels were also lower in previously treated patients compared to naive patients (p < 0.05). A significant correlation was found between the levels of 25(OH)D and the disease activity index (p < 0.05).

**Conclusions:** Our findings suggest that vitamin D deficiency is common in IBD patients and that supplementation may be beneficial in reducing disease activity. Further studies are needed to determine the optimal level of VD needed for optimal health in IBD patients.
Background: Vitamin D deficiency is common among patients with Crohn’s disease (CD) and has been a proposed risk factor for the development of the flare of CD. It remains unclear, however, if this association is a result of the inflammatory process, or a cause. Furthermore, most studies have relied on radioimmunoassays to measure 25-hydroxyvitamin D (25(OH)D), which may be less accurate than the accepted gold standard of liquid chromatography tandem mass spectrometry (LC/MS/MS). Circulating 25(OH)D is metabolised to the metabolically active 1,25(OH)₂D. The alternative pathway involves the production of the inactive 24,25(OH)₂D via 24-hydroxylase prior to elimination. The ratio of 25(OH)D:24,25(OH)₂D may be a more accurate measure of vitamin D status than 25(OH)D alone. We aimed to characterise vitamin D metabolism in patients with active and inactive CD using LC/MS/MS.

Methods: We report the baseline cross-sectional results of a prospective cohort study. Patients were included if they had active CD defined as ulceration at endoscopy; or a Crohn’s disease Activity Index (CDAI) > 220 and a CRP > 10 mg/l or faecal calprotectin > 250 mg/kg. Remission was defined as a CDAI < 150 and normal inflammatory markers. Patients were excluded if they received corticosteroids or vitamin D supplementation in the preceding 4 weeks. Serum was tested for 25(OH)D, epi-25(OH)D, 1,25(OH)₂D and 24,25(OH)₂D using an LC/MS/MS assay. Validated questionnaires were used to estimate vitamin D exposure from diet and sunlight. Spearman’s correlation coefficient was used to test correlations and unpaired t-tests to test differences between active and remission CD groups.

Results: Forty-seven consecutive patients with CD (20 active and 27 remission) were recruited; 55% were male. Median age was 37 years (range 23 to 76 yr.). Fewer patients in the active group were on immunomodulators (30% vs. 61% p = 0.03) or TNF inhibitors (25% vs. 89% p < 0.001). There was no difference in serum 25(OH)D, epi-25(OH)D or 1,25(OH)₂D between the groups. Serum 24,25(OH)₂D levels were significantly lower in the active group (mean 1.3 vs. 2.5 ng/ml p < 0.001) and thus the 25(OH)D:24,25(OH)₂D ratio was higher (49.4 vs. 26.1 p < 0.001). There was an inverse correlation between CDAI and 24,25(OH)₂D levels (r² = 0.33; p = 0.01). Dietary vitamin D intake and sunlight exposure were not different between the groups.

Conclusions: In the setting of active inflammation, levels of 1,25(OH)₂D are maintained by shifting the metabolism of 25(OH)D to 1,25(OH)₂D rather than 24,25(OH)₂D, suggesting a reduction in 24-hydroxylase activity to maintain the active metabolite. The ratio of 25(OH)D:24,25(OH)₂D is increased in active disease and may be a more sensitive marker of vitamin D status in patients with CD.

P122
Vitamin D activates human intestinal fibroblasts


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Background: Vitamin D signals through the vitamin D receptor (VDR) which is a member of the nuclear receptor family of transcription factors. VDR activation has been linked to cell proliferation, cell survival and cell differentiation in multiple cell types. However, the influence of VDR on the function of the human colonic myofibroblast and its role in the pathogenesis of IBD is still unclear. We aimed to evaluate whether activation of VDR can influence the behaviour of human colonic myofibroblasts and determine whether this effect is mediated via the canonical vitamin D receptor pathway.

Methods: The human colonic myofibroblasts were treated with an agonist of VDR (1α,25(OH)₂D₃) for 24 hours in the presence or absence of a VDR antagonist (1α,25(OH)₂D₃-OOH). The experiments were conducted under basal conditions and after stimulation with a pro-inflammatory cytokine (interleukin-1β (IL-1β)). The effects of the VDR agonist on cell proliferation, cell survival and cell differentiation were determined using a cell proliferation assay, an apoptosis assay and an ALP assay, respectively. The effects of the VDR agonist on cell migration were determined using a wound healing assay.

Results: Treatment with the VDR agonist reduced cell proliferation, increased cell survival and increased cell differentiation. These effects were mediated via the canonical vitamin D receptor pathway. Treatment with the VDR agonist also reduced cell migration.

Conclusions: Our results suggest that activation of VDR can influence the function of human colonic myofibroblasts and that this effect is mediated via the canonical vitamin D receptor pathway.