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All Abstracts

Belgian Association for the Study of the Liver (BASL)

A01

Light-to-moderate alcohol intake increases the risk of hepatocellular carcinoma in patients with HCV-related compensated cirrhosis: a prospective study

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Introduction: Whether light-to-moderate alcohol intake increases the risk of complications in patients with HCV-related cirrhosis remains unclear.

Aim: To determine the impact of light-to-moderate alcohol intake and viral eradication on the risk of hepatocellular carcinoma (HCC), decompensation of cirrhosis and death.

Methods: Data on alcohol intake and viral eradication were prospectively collected in 192 patients with compensated HCV-related cirrhosis. Cumulative incidence functions were used to describe the probability of HCC, decompensation of cirrhosis and death.

Results: 74 patients consumed alcohol (median alcohol intake: 15 g/day) and 50 reached viral eradication. During a median follow-up of 47 months, 29 patients developed HCC, 48 experienced at least one decompensation event, and 30 died. Causes of death were liver-related in 23 patients and non-liver related in 7 patients. 19 patients underwent a liver transplantation. The 5-year cumulative incidence rate of HCC was 12.9% (95% CI: 5.7-20.1) in abstainers vs. 28.0% (95% CI: 13.2-42.8) in consumers (p=0.044), and 2.7% (95% CI: 0-8.0) vs. 23.9% (95% CI: 14.4-33.4) in patients with and without viral eradication (p=0.01), respectively. The lowest

risk of HCC was observed for patients without alcohol intake and with viral eradication (0%) followed by patients with alcohol intake and viral eradication (9.1% [95% CI: 0-26.9]), patients without alcohol intake and no viral eradication (17.8% [95% CI: 7.8-27.8]), and patients with alcohol intake and no viral eradication (31.4% [95% CI: 14.5-48.3]) (p=0.024). In multivariate analysis, alcohol consumption was associated with the risk of HCC (hazard ratio: 2.82, 95% CI: 1.25-6.39, p=0.013). Light-to-moderate alcohol intake did not influence the risk of decompensation or death.
Conclusions: Light-to-moderate alcohol intake increases the risk of HCC in patients with HCV-related cirrhosis. There is an increase in the risk of HCC according to alcohol intake and the lack of viral eradication. Accordingly, patients with HCV-related cirrhosis should be strongly advised against any alcohol intake. Patient care should include measures to ensure abstinence.

A02

BROWN ADIPOSE TISSUE STIMULATION AS A NEW APPROACH TO FIGHT OBESITY AND METABOLIC SYNDROME

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Introduction: Obesity, insulin resistance, low grade inflammation and fatty liver are part of the metabolic syndrome. Foz/foz mice, characterized by spontaneous mutation of Alms1, are a model of metabolic syndrome with the particularity of developing non-alcoholic steatohepatitis.
Aim: The aim of this study was to decipher the mechanisms responsible for their metabolic phenotype.
Methods: Male foz/foz and wild-type (WT) littermates were fed a high fat diet (HFD) for 4 weeks. We performed a pairfeeding experiment in which foz/foz mice received the same amount of HFD consumed by WT the day before. Activity level, oxygen consumption and thermogenic adaptation (18FDG PET-scan) were evaluated. Intermittent cold exposure (ICE) (4°C, 2h/day, 5 days/week) and brown adipose tissue (BAT) transplantation (WT BAT to foz/foz mice) were performed to stimulate BAT.
Results: Unlike WT mice, 4 weeks of HFD induced obesity, insulin resistance, steatosis and liver and adipose inflammation in foz/foz mice. Although being hyperphagic compared to their WT littermates (18 vs 14 kcal/day, $p<0.001$), caloric restriction to foz/foz mice failed to restore their metabolic disturbances, notably in terms of adiposity, adipose inflammation, and glucose intolerance. Physical activity and basal metabolism were unaffected in foz/foz mice. By contrast, their thermogenic response to HFD or a cold exposure was severely impaired (44% reduction in 18FDG BAT uptake compared to WT upon cold exposure) due to 2.5-fold lower mitochondrial density in brown adipocytes and lower sympathetic tone (1.5-fold less noradrenaline, $p<0.05$; 2-fold less β3 adrenergic receptor expression, $p<0.05$). ICE and transplantation with WT BAT significantly increased cold-induced expression of thermogenic genes PGC1α, DIO2 and UCP1. Notably, ICE induced restoration of BAT function, decreased body weight gain (11g vs 16g, $p<0.001$) and improved glucose tolerance ($p<0.001$) compared to control foz/foz mice.

Conclusions: Overall, these results suggest that impaired BAT activity drives HFD-induced obesity and metabolic syndrome in foz/foz mice. Induction of endogenous BAT function by ICE or functional BAT transplantation had a significant positive impact on obesity and glucose tolerance, supporting that these strategies could become new effective therapeutic treatment against obesity and its comorbidities.

A03

Kinetics of pulmonary angiogenesis in mouse common bile duct ligation-induced liver fibrosis

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Introduction: Hepatopulmonary syndrome (HPS) is a severe pulmonary complication of liver disease for which no medical treatment is available up till now. In rats, common bile duct ligation (CBDL) has been documented as a model for human HPS, which is characterized by pathological pulmonary angiogenesis. Studies in genetically modified mice could offer opportunities for further research, however, in this species the development of pulmonary angiogenesis in biliary cirrhosis has not been outlined yet.

Aim: We aimed to elucidate the temporal changes in proangiogenic signature of hepatic and pulmonary vasculature after CBDL in mice and in addition identify potential proangiogenic factors contributing to the pathogenesis of HPS.

Methods: Male Swiss mice underwent CBDL or sham surgery and were sacrificed at a weekly basis for 6 consecutive weeks. Pulmonary inflammation was studied by cytology on broncho-alveolar lavage fluid, myeloperoxidase assay and luminex bead based assay. Liver and lungs were collected for protein analysis and histology to assess liver fibrosis and hepatic and pulmonary angiogenesis. Scanning electron microscopy was performed on vascular corrosion casts to visualize pulmonary vasculature during cirrhosis ex vivo.

Results: CBDL progressively induced liver fibrosis from week 1 (F0-1) to 6 (F4). This was accompanied by a gradual increase in hepatic immunopositivity for Endoglin and von Willebrand Factor, two markers of endothelial cell activation ($P<0.0001$). Hepatic levels of vascular endothelial growth factor (VEGF), VEGF receptor 1 and 2 were significantly increased at week 6, whereas placental growth factor (PlGF), which is exclusively involved in pathological angiogenesis, was already upregulated at week 2 ($P<0.0001$). In the pulmonary compartment, CBDL resulted in neutrophil infiltration and increased pro-inflammatory mediators from week 2 to 6 (all $P<0.001$). Pulmonary immunoreactivity for Endoglin and von Willebrand Factor progressively increased from week 4 to 6, while PlGF was already increased from week 2 onwards (all $P<0.0001$). Scanning electron microscopy revealed regions of abnormal vascular architecture, mainly located at the pleural side, decreased intercapillary distance ($P<0.001$) and increased capillary density ($P<0.05$) in lungs of cirrhotic mice compared to sham mice.

Conclusions: CBDL in mice is associated with pathological pulmonary angiogenesis and may represent a model for human HPS. In addition, we point to PlGF as an early indicator of pathological hepatic and pulmonary angiogenesis.

A04

Comparison of the Milan criteria, the alfa-fetoprotein model and the Asan criteria to select hepatocellular carcinoma patients for liver transplantation: a multicentric retrospective study

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Introduction: On behalf of The Belgium Liver and Intestinal Advisory Committee (Be-LIAC) Hepatocellular carcinoma (HCC) is a major indication for liver transplantation (LTx). Selecting the right patients for this potentially curative treatment is of utmost importance as postLTx HCC recurrence dramatically reduces survival. Applying the Milan criteria (1 lesion <5 cm or up to 3 lesions <3 cm) results in a 5-year post-transplant survival of >70%. More recently, extension of these criteria resulting in similar outcomes has been proposed using, among others, the alfa-foetoprotein (aFP) model or the Asan criteria. The aFP model gives points based on tumor number (0 or 2 points for 1-3 or >3 nodules respectively), largest diameter in centimeter (0, 1 or 4 points for <3, 3-6 or >6 cm respectively) and aFP level (0, 2 or 3 points for < 100, 100 – 1000 or > 1000 ng/ml respectively) with a total of > 2 points indicating high risk. The Asan critera are similar to Milan but with different cut-offs (largest lesion ≤5 cm and number of lesions ≤6).

Aim: To compare the performance of the well-established Milan criteria with the aFP model and the Asan criteria in predicting HCC recurrence after LTx.

Methods: We retrospectively reviewed clinical data and radiology reports of 275 patients transplanted for HCC in 4 different Belgian LTx centers. Patients who died in the first three months postTx were excluded unless they had HCC recurrence. Patients were mostly male (80%) with underlying alcoholic liver disease (38%) or chronic hepatitis C infection (32%). Median number of lesions at listing was 1 (range 0 - 10) with the median size of the largest lesion being 29.4 mm (0 - 120). Median aFP level at time of listing was 189 µg/l (1 – 18125). During a follow-up of 50.8 months (range 3 – 157) 40 patients (14.5%) had HCC recurrence and 79 patients (29%) died.

Results: At time of listing 25%, 15% and 11% of the 275 patients were outside the Milan criteria, the aFP model and the Asan criteria, respectively. All three models could significantly predict postTx recurrence (log rank, $p < 0.001$). 5 year recurrence rate were similar for patients inside each model: 10 (+-2.5) %, 12 (+- 2.5) % and 12 (+- 2.5) % for patients inside the Milan criteria, the aFP model and the Asan criteria, respectively. However, increasing recurrence rates for patients outside each model were noted comparing Milan criteria (33 +-7 %), aFP model (39 +- 9%) and Asan criteria (44 +- 11%). Patients outside Milan but still inside aFP model, had an acceptable 5 year recurrence rate of 17 (+-6) % which was significantly better than patients outside both models (log rank, $p = 0.001$). Patients outside Milan but still inside Asan criteria had 24 (+-8)% recurrence risk, which did not differ from patients outside both criteria ($p = 0.09$). Of note, 7% of this multicentric cohort had complete remission of HCC at listing and in 11.5% of cases, no malignancy was detected in the explant specimen.

Conclusions: The Asan criteria are less restrictive compared to the aFP model which is on its turn less restrictive than the Milan criteria. Extension of the Milan criteria is feasible without compromising recurrence rates. In our analysis, patients outside Milan criteria but inside the aFP model still have an acceptable rate of HCC recurrence.

A05

The bile acid tauroursodeoxycholic acid cooperates with N-acetylcysteine in the treatment of experimental acetaminophen-induced hepatotoxicity

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Introduction: Acetaminophen overdose (APAP) in mice causes endoplasmic reticulum stress, which activates the unfolded protein response, and excessive hepatocyte death. Tauroursodeoxycholic acid (TUDCA), a hydrophilic bile acid known to have anti-apoptotic and endoplasmic reticulum stress reducing capacities, is being tested in clinical trials for primary biliary cirrhosis and is FDA approved.

Aim: We aimed at investigating the therapeutic potential of TUDCA in experimental acute liver injury induced by APAP.

Methods: C75Bl/6 mice were fasted for 20 hours prior to receiving 300mg/kg APAP or PBS intraperitoneally. The mice were treated 2 hours post APAP injection with N-acetyl cysteine (NAC), TUDCA, the combination or saline control (n=6/group) and were sacrificed 24 hours later. Liver damage was evaluated by measuring serum transaminases (AST/ALT), liver histology by H&E and TUNEL stainings and induction of pro-inflammatory cytokines by qPCR. Activation of the unfolded protein response was evaluated by qPCR and western blotting.

Results: APAP overdose resulted in increased serum liver enzymes ($p<0.001$) and excessive hepatocyte cell death, evaluated by the level of TUNEL positive cells ($p<0.01$) and necrotic foci on immunohistochemical stained sections ($p<0.001$). APAP induced the pro-inflammatory markers TNF-α and CCL2 ($p<0.001$) and markers of the unfolded protein response Bip, CHOP, GADD34, XBP1s and ATF4. NAC significantly reduced serum transaminases, hepatocyte cell death and UPR markers Bip, CHOP and ATF4 on western blot. However pro-inflammatory cytokines were not significantly reduced following N-acetyl cysteine. TUDCA treatment alone had no therapeutic effect on APAP induced liver damage. However, the combination of NAC and TUDCA significantly improved serum transaminase levels, histopathological liver damage, reduced TNF-α and CCL2 expression and protein levels of ATF4 and CHOP.

Conclusions: These data indicate that a combination strategy of NAC and TUDCA outdoes the standard of care NAC in APAP-induced acute liver injury in mice and might represent an attractive therapeutic opportunity for APAP-intoxicated patients.

A06

Reproducibility of real-time shear wave elastography in the evaluation of liver stiffness and the effect of patient position on liver and spleen stiffness measurements

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Introduction: Real-time shear wave elastography (RT-SWE) is increasingly used to assess liver stiffness, but some practical aspects still need standardization. Information on training and

reproducibility in measurements performed by novice operators is scarce. Also, in some studies, patient position during measurements can vary, particularly for the evaluation of the spleen.
Aim: To evaluate the reproducibility of RT-SWE, performed by an experienced and a novice operator, to assess liver elasticity in healthy volunteers and to evaluate the effect of patient position on liver and spleen stiffness measurements.
Methods: RT-SWE was performed using Aixplorer® (SuperSonic Imagine S.A., Aix-en-Provence, France). The first part of the study included 30 patients examined by two operators, an experienced operator (operator 1) and a novice (operator 2) who received 1.5 hours of theoretical training and 1 hour of practical training. Measurements were performed on the right lobe of the liver through intercostal spaces. Each operator performed 10 consecutive measurements in each volunteer. Intraobserver and interobserver agreement were assessed by intraclass correlation coefficient (ICC), the former between the first 5 and second 5 liver stiffness measurements of each operator, the latter between all 10 measurements of operator 1 and of operator 2. The second part of the study, performed by the experienced operator, included 23 patients with SWE measurements in different patient positions: left lateral decubitus and supine position for liver stiffness and supine position and right lateral decubitus for spleen stiffness.
Results: Intraobserver agreement showed ICC values of 0.93 (95% CI 0.85-0.97) for operator 1 and 0.88 (95% CI 0.74-0.94) for operator 2. Interobserver agreement was 0.89 (95% CI 0.76-0.95). Mean elasticity of the liver was 7.95±2.1kPa and 6.30±1.03kPa in left lateral decubitus and supine position, respectively (p=0.003). Mean elasticity of the spleen was 16.42±2.81kPa and 19.69±3.10kPa in right lateral decubitus and supine position, respectively (p=0.001).
Conclusions: RT-SWE is a reliable and reproducible technique for noninvasive assessment of liver stiffness for both experienced and novice operators. Elasticity measurements of liver and spleen are significantly altered upon repositioning of the patient. Tissue stiffness is higher in left lateral decubitus for the liver and lower in right lateral decubitus for the spleen, compared to supine position. To our knowledge, this is the first study assessing this difference. We would therefore recommend clear description of patient position during elasticity measurements in future studies.

A07

AGEING ENHANCES FIBROTIC RESPONSE IN MICE THROUGH HAMPERING EXTRACELLULAR MATRIX REMODELING

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Introduction: In chronic hepatitis C, liver fibrosis develops faster and is more severe in patients infected at an older age. Ageing is also a risk factor for fibrosis progression in non-alcoholic steatohepatitis.
Aim: Our aim was to evaluate whether ageing modulates the profibrotic response and fibrosis development in a mouse model.
Methods: A fibrotic regimen (CCl4 IP, 3x/week for 4 weeks) was applied to young (aged 7 weeks) and old (aged 15 months) BALB/c or C57Bl/6J mice. Mice were sacrificed 48 hours after the last CCl4 injection unless otherwise specified (96h). Histological and molecular markers of fibrosis, matrix remodeling and inflammation were analyzed.

Results: In response to CCl4, young and old BALB/c mice developed similar significant liver fibrosis with similar collagen deposition evaluated by sirius red morphometry and hydroxyproline quantification, similar increase in activated hepatic stellate cells evaluated by αSMA immunohistochemistry and equal collagen I and TGFβ1 mRNA expression. By contrast, CCl4 caused an enhanced collagen deposition in old C57Bl/6J mice compared to young ones (7.60+/-0.63% versus 2.66+/-0.19% Sirius red stained liver area, respectively, p = 0.0069). The expression of genes related to pro-fibrogenic mechanisms as collagen I and αSMA or to matrix remodeling (Mmp2, Mmp9, TIMP-1, TIMP-2) was equally induced in the two age-groups but not that of Mmp13, the matrix metalloproteinase chiefly involved in fibrosis resolution. Indeed, while CCl4 induced MMP13 by 13 times (p<0.0001) in young mice, MMP13 was barely induced by a factor 2 in old mice (NS). Similarly, CCl4 caused a 2.5-fold induction of CXCL9, a MMP13 inducer, in young (p=0.01) but not in old (p= NS) livers supporting a more dynamic matrix remodeling in young mice compared to old ones. The acceleration in fibrosis resolution in young mice was further confirmed by analysis of residual fibrosis 96hours after CCl4 recovery. At this time point and compared to mice sacrificed 48hours after the last CCl4 injection, liver fibrosis was significantly reduced in young mice with residual collagen deposition nearly returned to control liver levels. By contrast, there was no significant attenuation of liver fibrosis between the 48- and the 96-hour time points in old mice. Kupffer cells play a key role in matrix remodeling and fibrosis resolution. No difference was seen in transcript levels of F4/80 or markers of M1/M2 macrophage polarization between CCl4-treated young or old livers. In young mice, F4/80 positive cells agglomerated in pericentral area and fibrotic bands at peak fibrosis (48h) and homogenously redistributed through the entire lobule upon fibrosis resolution (96h). By contrast, in old mice, enlarged Kupffer cells persisted in the central area and around the fibrotic septa at 96h post last CCl4 doses.
Conclusions: Opposite observations in BalBc and C57 mice support that age-related aggravation of liver fibrosis is determined by the immunological background. Our data support that aggravated fibrosis in older mice is chiefly related to impaired matrix remodeling, linked to altered immune or inflammatory processes.

A08

Sofosbuvir in combination with Simeprevir +/- ribavirin in genotype 4 hepatitis C patients with advanced fibrosis or cirrhosis: real-life experience from Belgium

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Introduction: All-oral, interferon-free regimens that combine direct-acting antiviral drugs have significantly advanced the treatment of hepatitis C (HCV), especially for genotype 1(G1) patients. However, efficacy and safety data of interferon-free regimens in HCV genotype 4 (G4) patients are scarce. In Belgium, Sofosbuvir (SOF) and Simeprevir (SMV) treatment is available since January 2015 for G1 and G4 patients with advanced fibrosis (F3-F4 METAVIR) for 12 weeks.
Aim: The aim of the study was to evaluate the safety and efficacy of the treatment.
Methods: Analysis of HCV G4 patients receiving SOF and SMV treatment in Belgium.
Results: 75 G4 patients were enrolled in this data collection including 31 (41.3%) patients with severe fibrosis F3 and 44 (58.7%) cirrhotic patients defined by histology or non-invasive tests of liver fibrosis. The study population comprised 57.3% male, 77% treatment experienced patients. Median age was 59 [30-81] years and 7 patients were HCV/HIV co-infected. 27(36%) patients received the treatment associated with ribavirin, 11/31 (35.5%) of patients with advanced fibrosis and 16/44 (36.4%) of cirrhotic patients. In cirrhotic patients, median MELD and Child-Pugh score were 9 [6-19] and 5 [5-9], 45.2% had platelet below 100.000/mm³ and 27% had albumin below 35 g/L. In the overall population, undetectable HCV RNA at W4, and at the end of treatment were 30% (18/60) and 86.1% (56/65), respectively while sustained virological response (SVR)12 was 93.6% (44/47). SVR12 in patients treated with and without RBV were 94.4% (17/18) and (93.1%) (27/29), respectively. SVR12 in patients with advanced fibrosis (F3) was100% (21/21) while SVR12 in cirrhotic patients was 88.46% (23/26). Relapses (3 patients) were only observed in cirrhotic patients with advanced disease. All SVR12 data will be available at the time of presentation. No serious adverse event related to the treatment was observed.
Conclusions: Sofosbuvir in combination with Simeprevir +/- ribavirin in G4 HCV patients with advanced fibrosis achieved high SVR12 rates and was well tolerated.

A09

Novel low-cost and efficient method for the isolation of mouse liver sinusoidal cells.

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Introduction: Liver disease encompasses more than just the damage of one, or activation of another cell type to cause an imbalance of liver homeostasis. Upon hepatocyte injury, Kupffer cells (KCs), hepatic stellate cells (HSCs) and sinusoidal endothelial cells (LSECs) try to collectively cope with the altered situation. Complex culture systems can mimic this in vitro and several groups developed methods for the isolation of sinusoidal cells from mice using specific antibodies and fluorescence activated cell sorting (FACS). The disadvantage of these techniques are that they are time consuming and expensive.
Aim: We aimed to develop an isolation procedure of liver sinusoidal cells based on their scavenging features.

Methods: Before liver cell isolation, male Balb/c mice were i.v. injected with secondary antibodies, coupled to alexa 647 (APC) fluorochromes. Livers were enzymatically digested to single cell suspensions and from the non-parenchymal fraction, KCs and LSECs were isolated using a FACS Aria II, on the basis of their UV negativity and APC positivity. HSCs were sorted on the basis of the retinyl ester autofluorescence at 328 nm (UV+). The method was also used with mice that underwent a bile duct ligation (10 days) or were treated with carbon tetrachloride (4 weeks).
Results: After injecting mice with fluorescent antibodies, LSECs (28,7%) and KCs (12,7%) populations could clearly be distinguished on APC-UV FACS-plots. These populations were isolated and their purity was verified using mRNA levels and by immunohistochemical analysis of liver cell specific markers of the sorted cells. Ideal dose and injection time point were determined at 10 µg of fluorochrome coupled antibodies 2 hours before isolation. Although slightly less pure, such isolation can also be used for fibrotic livers. In addition, we observed that 15,1% ± 2,7% of the LSECs are positive for UV autofluorescence, normally used to detect HSCs by FACS. Excluding these UV+ APC+ cells from the total UV fraction, allowed us to obtain ultra-pure HSCs.
Conclusions: We established an easy, rapid and cost efficient method to isolate highly pure KCs, LSECs and HSCs from mouse livers. The procedure, including perfusion, takes only 90 minutes to obtain the different cell types. On average, the costs are at least 50 euros less per mouse when compared to conventional primary antibody-based methods. This method will make in vitro studies of sinusoidal cells more accessible to the entire research community.

A10

Analysis of T and myeloid cells in patients with NASH and diabetes mellitus

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Introduction: The immune system potentially plays a pivotal role in the onset of Non-Alcoholic Steatohepatitis (NASH) and of the associated metabolic disturbances, including diabetes mellitus (DM).
Aim: To study the immune cells in peripheral blood and differentially expressed genes in the liver of preselected patients according to presence or absence of NASH and DM.
Methods: We enrolled 32 patients who underwent liver biopsy because of suspected NASH. The patients were divided in 4 predefined groups according to liver biopsy and glucose parameters: 1) control (NO NASH/NO DM), 2) NASH/NO DM, 3) NASH/DM, 4) NO NASH/DM. A multicolour flow cytometry analysis was performed in order to investigate T, natural killer T cells (NKT), natural killer (NK) cells, monocytes and dendritic cells. Gene expression in the liver was investigated by microarray analysis in patients with similar clinical characteristics.
Results: Significant differences between groups of patients were found among T lymphocyte populations but not in subsets of myeloid cells. Proportions of CD8+ cells with enhanced production of pro-inflammatory cytokines (TNF and INFγ) and cytotoxic molecules (granzyme

A and B, perforin) were increased in patients with NASH and/or DM. The central memory CD8+ cells (CCR7+CD45RA-) were significantly increased in NASH/DM compared to NASH/NO DM, whereas the effector (CCR7CD45RA) and effector memory (CCR7-CD45RA-) CD8+ cells were more potent to produce cytotoxic molecules in all groups of patients with NASH and DM. Gene set enrichment analysis evenso revealed dysregulation of genes associated with activation of CD8+ cells in the liver in NASH/DM compared to NASH/NO DM. We found a trend to increased proportion of NKT cells in patients with NASH. CD4+ T regulatory (Treg) and Th22 cells were increased in NASH/DM compared to NASH/NO DM patients, while Tregs where decreased in NASH patients without DM.
Conclusions: T-cell, bot not myeloid cell, alterations are associated with the presence of NASH regardless of the presence of diabetes. Both flow cytometry and gene expression demonstrate that increased effector functions of cytotoxic CD8+ T cells and CD4+ Th22 and Treg cells are found when NASH is associated with DM, suggesting that these cells are specifically involved in the crosstalk between liver inflammation and metabolic dysfunctions and contribute both to hepatotoxic and hepatoprotective mechanisms.

A11

In Silico Characterization of Liver progenitor cells

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Introduction: Liver progenitor cells (LPCs) have been extensively investigated because of their capacity to differentiate into hepatocytes or cholangiocytes during sever acute and chonic liver injury. Currently, there is much debate whether these cells significantly contribute to liver regeneration, partly due to the lack of an exact identity. Different 'specific' LPC markers (Foxl1, LGR5, HNF1b, MIC1C3, EpCAM) have been used to isolate and characterize LPCs from healthy, damaged and regenerating livers. Whether these studies are actually working with the same cells has never been established.
Aim: Our aim was to obtain a unique LPC gene signature by comparing the expression profiles of recently published array data on LPCs using Foxl1, LGR5, MIC1C3 and HNF1b as markers.
Methods: We compared LPC gene expression data which was published within the last 5 years (Foxl1, Shin et al., GSE28892; LGR5, Huch et al., GSE32210; MIC1C3, Dorrell et al., GSE29121; HNF1b, Rodrigo-Torres et al.). First, our focus was based on gene expression in LPCs that were at least 2 fold increased, using MIC1C3 and HNF1b markers as representative LPC markers in healthy livers. Second, we looked at genes that were 2 times or more upregulated during activation using DDC (MIC1C3, HNF1b), CDE (HNF1b) or CCl4 (LGR5) diet.
Results: More than 600 genes were highly expressed in LPCs and are associated with different gene ontologies, like system development, cell differentiation, tissue development and organ development. Interestingly, KEGG pathways show high significance in Hippo signalling pathway, PI3K-Akt signalling pathway and ECM-receptor interaction. Many of these genes are therefore possible markers and need to be further investigated. Only 3 genes were upregulated during LPC activation in all experimental settings and all isolation methods, indicating that these could be robust markers of LPC activation.
Conclusions: Our analysis shows major difference between all expression profiles of the differently identified LPCs. While there is an overlap in gene expression between these

"different" LPCs, only 3 genes seem to differentially expressed in all LPCs upon activation and not in surrounding liver cells. Progress on the validation of these 3 genes as key markers of LPC activation using additional injury settings and LPC isolations will be presented.

A13

EXPRESSION OF PRO-INFLAMMATORY AND HEPATOPROTECTIVE FACTORS IN ALCOHOLIC LIVER DISEASE IN HUMANS AND THE IMPACT OF SHORT TERM ABSTINENCE

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Introduction: Animal models imperfectly mimic the spectrum of alcoholic liver disease (ALD) seen in humans. Some studies have investigated late stages and severe forms of human ALD, but little is known about the pathophysiological mechanisms occurring in the human liver.

Aim: Here, we investigated inflammatory mechanisms in alcohol dependent patients undergoing a standardized inpatient alcohol withdrawal program.

Methods: Patients with suspicion of significant ALD (ALT, AST increase, fibroscan >7,8 kPa) were randomly assigned to undergo liver biopsy either in the active drinking phase or after 2 weeks of abstinence. Sixty-eight patients (41 active, 27 abstinent) were included in the study. Liver tissue (n=6) from size-reduced liver grafts was used as normal controls. Expression and cellular localization of various factors was assessed by Western-blotting, qPCR and immunohistochemistry and immunofluorescence.

Results: In the active drinking phase, a strong activation of the Kupffer cell (KC) compartment was found with KCs forming clusters adjacent to ballooned, steatotic hepatocytes. KC showed a pro-inflammatory M1 phenotype with activation of NFkB and increased expression of TNFα, IL-1β and iNOS. After 2 weeks of abstinence, the staining pattern of KC returned to normal and NFkB, IL-1β and iNOS levels but not TNFα decreased to almost control levels. In addition, abstinence induced a partial shift to a M2 phenotype with increased production of the anti-inflammatory cytokine IL-10. Interestingly, we did not find activation of TLR4 since TLR4, CD14, and LBP levels remained at control values in active drinkers. By contrast, we found a strong and persistent upregulation of the intracellular TLR3 and TLR7 which correlated with high production of interferon beta and gamma principally located to hepatocytes and bile ducts. Moreover, the hepatoprotective factors IL-6, IL-22, MCP-1 and Stat3 DNA-binding were strongly down-regulated in active drinkers and did not recover after short term abstinence. Hepatocyte Ki67 proliferation index was low in active drinkers and increased modestly but significantly after 2 weeks of abstinence.

Conclusions: A strong pro-inflammatory, KC-dependent response is observed which rapidly reversed upon abstinence. By contrast, down-regulation of hepatoprotective factors is more long lasting and might significantly impair liver repair mechanisms in sustained drinkers.

A14

Liver stiffness measured by real-time shear wave elastography is a good predictor of liver function

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Introduction: Real-time shear wave elastography (RT-SWE; Aixplorer®, SuperSonic Imagine S.A., Aix-en-Provence, France) is a fairly recent tool to assess liver fibrosis by measuring liver stiffness (LS). Recent studies have shown a correlation of liver stiffness measured by RT-SWE with the presence of portal hypertension, overall prognosis and MELD score. Hepatobiliary scintigraphy using mebrofenin (Mebro) has shown to be a good measurement of liver function and is often used to assess liver function before liver resection. Recent studies have shown that a tracer clearance of <5%/min is associated with high post-operative morbidity and mortality.

Aim: The aim of the present study was to evaluate a correlation between LS measured by RT-SWE and tracer clearance (TrCl) by the liver during mebrofenin scintigraphy. We also aimed at assessing the diagnostic performance of LS to predict liver function.

Methods: Retrospective analysis of patients undergoing liver resection that received both LS measurements by RT-SWE and liver function testing by 99m-Technetium mebrofenin scintigraphy.

Results: 60 patients were included, of which 43 had cirrhosis. Median time between both tests was 4 days (IQR: 1-15). LS showed a strong inverse correlation with liver function (rs =-0.858, p<0.0001). When dividing the Mebro results into 4 groups according to liver function [severe (TrCl<4%/min), mild (TrCl 4-7%/min), moderate (TrCl 7-10%/min) and normal (TrCl>10%/min)], the mean elasticity values were 34.9±8.0, 23.0±7.9, 18.2±4.9 and 6.8±2.1 kPa, respectively. There was an overall significant difference between the different groups. AUROC for TrCl<4%, for TrCl<5% and for TrCl>10% were 0.973 (95%CI 0.939-1.000), 0.988 (95%CI 0.967-1.000) and 0.993 (95%CI 0.976-1.000), respectively. LS below a cut-off value of 12.0 kPa to predict the presence of normal TrCl had a sensitivity of 100% and a specificity of 97.8%. LS above a cut-off value of 23.7 kPa to predict the presence of a TrCl<5%/min had a sensitivity of 93.5% and a specificity of 93.1%. LS above a cut-off value of 24.1 kPa to predict the presence of a TrCl<4%/min had a sensitivity of 93.1% and a specificity of 93.5%.

Conclusions: This is the first study to demonstrate a strong correlation between RT-SWE measurement of LS and liver function determined by Mebrofenin scan. LS can be used in this setting to predict liver function with high diagnostic accuracy.

A15

MULTICENTER BELGIAN EXPERIENCE OF SOFOSBUVIR MEDICAL NEED PROGRAM IN PRE- AND POST LIVER TRANSPLANTATION PATIENTS: SAFETY AND EFFICACY RESULTS.

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Introduction: Severe hepatitis C (HCV) recurrence after liver transplantation (LT), and HCV in cirrhotic patients listed for LT have a negative impact on patient survival and Interferon (IFN) based treatment are clearly suboptimal. Sofosbuvir (SOF), Daclatasvir (DCV) and Simeprevir (SMV) have been recently approved in Europe but there are limited data on the use of these drugs in the treatment of these very difficult-to- treat patients
Aim: The aim of this study was to evaluate the safety and efficacy of the treatment.
Methods: Multicenter Belgian retrospective analysis of patients with either severe HCV recurrence after LT or listed for LT receiving SOF with SMV, DCV, PegIFN+ribavirin (RBV) or RBV in medical need program in Belgium.
Results: 35 patients were enrolled in this data collection, 14 cirrhotic patients listed for LT, and 21 LT recipients with severe recurrence. The majority of patients were male (77.1%) and median age was 55[50-67] years. Genotype distribution was: genotype 1(n=28), 2(n=1), 3(n=4), 4(n=1), 5(n=1). Among pre-LT patients, 10/14 (71.4%) had decompensed cirrhosis and 4/14 (28.6%) had compensed cirrhosis with hepatocellular carcinoma. Median MELD score and Child-Pugh scores were 14 [6-40] and 8 [5-14], respectively. Median treatment duration was 154 [1-199] days. Two patients stopped prematurely the treatment, 1 patient because of pancytopenia and liver decompensation and 1 patient died but the death was not related to the treatment. Viral load was not detected at the end of treatment (EOT) in 84.6% (11/13) while SVR12 was 72.7% (8/11). Among post-LT patients, 8/21 (38%) had severe fibrosis (F3) and 13/21 (62%) had cirrhosis. HCV viral load was not detected at EOT in 100 % (21/21) while SVR12 was 94.1% (16/17) because 1 patient died before SVR12. Severe adverse events (SAE) were reported in 10 patients, 8 were not related to the treatment, 1 patient developed pancytopenia after 1 day of treatment and 1 patient had epileptic attack after treatment modification due to possible drugs interactions.
Conclusions: This preliminary experience in pre and post-LT patients shows that SOF in combination with DCV, SMV, RBV or PegIFN is safe and virological response seems to be promising.

A16

Clinical features and outcomes according to the etiology of cirrhosis: a 20-year follow-up study in a monocentric cohort

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Introduction: Cirrhosis is a heterogeneous clinical condition that includes patients at wide-ranging stages of severity. From that perspective, the role of the underlying liver disease on patient prognosis remains unclear. Studies focusing on the epidemiology of cirrhosis and comparing diseases outcomes according to the causes of cirrhosis remain crucial for improving patients' care.

Aim: To determine the relative impact of alcoholic liver disease (ALD), chronic hepatitis C virus (HCV) infection and non-alcoholic fatty liver disease (NAFLD) on the prognosis of patients with cirrhosis.
Methods: Data related to the occurrence of hepatocellular carcinoma (HCC) and deaths were collected during a 20-year period in 838 patients with cirrhosis related to ALD, chronic HCV infection or NAFLD. Cumulative incidence functions were used to describe the probability of HCC and death.
Results: There were 564 (67%) patients with ALD-related cirrhosis, 184 (22%) with HCV-related cirrhosis, and 90 (11%) with NAFLD-related cirrhosis. At the diagnosis of cirrhosis, ALD patients were younger than HCV or NAFLD patients (54 vs. 65 vs. 63 years, respectively; $p<0.001$) and had worse liver function (% of patients with Child-Pugh stages B or C: 47% vs. 13% vs. 19%, respectively; $p<0.001$). Overall, 133 patients developed HCC and 414 died. The 20-year cumulative incidence rates of HCC were lower in ALD patients than in HCV or NAFLD patients (14.1% vs. 42.6% vs. 39.2%, respectively; $p<0.001$). The 20-year cumulative incidence rates of mortality were similar in ALD patients than in HCV or NAFLD patients (75.3% vs. 68.3% vs. 69.3%, respectively; $p=0.5$). In multivariate analyses, ALD was associated with a reduced risk of HCC (HR: 0.37; 95% CI, 0.25-0.55; $p<0.001$). Older age and ALD were associated with a higher risk of mortality (HR for ALD, 1.61; 95% CI, 1.29-2.00; $p<0.001$). ALD patients died less frequently from HCC and more frequently from decompensation than HCV or NAFLD patients.
Conclusions: Patients with ALD-related cirrhosis had a lower incidence of HCC, but died more frequently from decompensation of cirrhosis than patients with chronic HCV infection or NAFLD-related cirrhosis. Cirrhosis related to ALD should be considered as a condition associated with a worse outcome.

A17
Increasing bacterial resistance in spontaneous bacterial peritonitis: a Belgian single center experience.
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Introduction: Spontaneous bacterial peritonitis (SBP) is a frequent complication of liver cirrhosis. It occurs in 10-30% of hospitalized patients with cirrhosis and ascites. Mortality rates due to SBP are high and range between 30 and 90% within the first year after diagnosis. The most prevalent bacteria responsible for SBP are Escherichia coli, Klebsiella pneumoniae and other enterobacteriae. The antibiotic regimen of choice for empiric treatment of community acquired SBP in our center has been amoxicillin/clavulanic acid for more than a decade. For the last years, an increase of antibiotic resistance has been observed, possibly due to increased use of secondary prophylaxis of SBP with Norfloxacin, as proposed by current guidelines.
Aim: The aim of the present study is to investigate the evolution of antibiotic resistance patterns in a Belgian tertiary liver unit in patients with SBP.
Methods: A retrospective study was performed in the department of Hepatology of Ghent University Hospital. All patients admitted to our department with an SBP between 2005 and 2013 were included. From the medical files we retrieved classic demographic data, ascites fluid

culture, resistance patterns of these bacteria and the antibiotic regimen used for treatment of SBP. Data were analysed using chi-square test.
Results: A total of 114 patients were included. The population was divided into two groups: the first group with the diagnosis of an SBP between 2005 and 2009 and the second group with an SBP between 2010 and 2013. Overall, a significant increase in resistant patterns was observed between both groups (p=0.001): e.g. for E. Coli, resistance to any antibiotic increased significantly in group 1 vs group 2 (55.6% vs 68.4%, p=0.039). Significantly more bacteria are resistant to amoxicillin/clavulanic acid than to ceftriaxone (5.3 vs 2.6%; p=0.012). Significantly more bacteria are resistant to amoxicillin/clavulanic acid in group 2 vs group 1 (17% vs 0%; p=0.036). We observed no increase in quinolone resistance in this cohort (21.8% vs 25.8% in group 1 vs group 2; p=0.729).
Conclusions: An increase in bacterial resistance has been found in ascitic cultures in patients with SBP between 2010 and 2013 compared to the period between 2005 and 2009. Regarding the increased resistance to amoxicillin/clavulanic acid, our current first line empiric treatment for community acquired SBP, a third generation cephalosporin might prove the best first line treatment for SBP.

A18

Impaired time-dependent adaptation to methoxamine in steatotic rat livers is not dependent on NO or COX-mediated mechanisms

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Introduction: Early stages of NAFLD, without inflammation or fibrosis, show major intrahepatic vascular alterations and increased portal pressure. We previously demonstrated the presence of endothelial dysfunction, a significant hypersensitivity to the α1-adrenoceptor agonist methoxamine and an attenuated and delayed time-dependent adaptation of intrahepatic vascular resistance after methoxamine administration.
Aim: To study aimed to reveal the underlying mechanisms behind this time-dependent effect upon methoxamine administration.
Methods: Male Wistar rats were fed a methione-choline deficient diet to induce steatosis or control diet for 4 weeks (n=6-7/group). Intrahepatic vascular resistance was studied as a function of time by in situ isolated liver perfusion with a given flow (30 ml/min) and a fixed dose methoxamine (10-4 M). The effects of Nω-Nitro-L-arginine (LNNA), an endothelial nitric oxide synthase (eNOS) inhibitor (3*10-4 M), or indomethacine, a cyclo-oxygenase (COX)-inhibitor (2*10-5 M) were investigated. Subsequently, experiments were performed with equimolar high potassium (K+ 50 mM) to look for receptor-independent vasoconstriction.
Results: Pre-incubation with indomethacine increased the effect of methoxamine at all time-points in control rats, but not significantly, whilst no increase was observed in steatotic rats. Intrahepatic resistance increased substantially in all rats after pre-incubation with LNNA ($p<0.001$ at all time-points). Although time-dependent differences between controls and steatotic rats became statistically insignificant under LNNA, intrinsic time-dependent responses (i.e. attenuated adaption in steatosis) seemed unaltered. Finally, high potassium was able to increase

resistance similarly to methoxamine. Both control and steatotic rats demonstrated an adaptive response to high potassium and differences disappeared.
Conclusions: Our results show that differences in time-dependent adaptation to methoxamine between control and steatotic rats cannot be explained by differences in NO-mediated nor COX-mediated mechanisms. Controls seem to have a COX-mediated vasodilating counter mechanism to methoxamine, which is absent in steatotic rats. The contractile capability, shown by high potassium, was not altered in steatotic rats, nor was adaptation to high potassium. We therefore hypothesize that the observed results are receptor-mediated.

A19

Host-microbiome interactions in primary sclerosing cholangitis

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Introduction: Primary sclerosing cholangitis (PSC) is a cholestatic liver disease, frequently associated with inflammatory bowel disease (IBD). The pathogenesis of PSC remains greatly unknown but 16 risk loci have been associated with PSC, and some of these overlap with known risk loci for IBD.
Aim: As we recently showed that dysbiosis is a feature of patients with PSC, we assessed the influence of genetic risk factors for PSC or IBD on the intestinal microbiota.
Methods: Blood and faecal samples from 52 PSC patients were collected, 41 patients had concomitant IBD (17 Crohn's disease (CD) and 24 ulcerative colitis (UC)). A genetic risk score (GRS) was calculated for each patient, taking into account the risk allele frequency and odds ratio of each single nucleotide polymorfism (SNP). The GRS were calculated using the 14 risk SNPs for PSC, 197 risk SNPs for CD and 191 risk SNPs for UC as generated using Immunochip. Second, a GRS only containing those SNPs implicated in microbiota-interaction was calculated for CD and UC. 16S rDNA paired-end sequencing targeting the V4 hypervariable region was performed using Illumina MiSeq sequencer. Sequencing depth was downsized to 10000 reads/sample. The Ribosomal Database Project classifier was used for taxonomic assignment. Statistical analyses were performed with R, using parametric and non-parametric tests, with multiple testing correction (FDR). Correlation between genera abundances and GRS was performed with Spearman correlation.
Results: Although microbial richness did not differ between patients with a high (n=26) or low (n=26) PSC GRS, and no differences were observed in the abundance of specific phyla, genera, or operational taxonomic units (OTU), the overall microbiota composition was significantly different between the 2 groups (Adonis test on Bray-Curtis dissimilarity, p-value 0.002). Interesting to note, the abundances of 9 genera were significantly different between the 2 groups before multiple testing correction, including Prevotella, Dorea, Methanosfera, and Paraprevotella. No significant association was observed between CD or UC GRS quartiles and microbiota composition, richness, or taxa abundances in this cohort.
Conclusions: The genetic risk for PSC seems to influence the overall gut microbiota composition in patients with PSC, however no specific significant differences in microbial

diversity or differences of OTU, genus or phylum abundances were observed between patients with high and low GRS for PSC. The cumulative effect of multiple small differences at genus level may explain the overall significant difference in community composition. Larger sample size may be needed to evidence significant taxon abundance associations to GRS. Contrarily to PSC GRS, the CD or UC GRS do not seem to influence intestinal microbiota in this population.

A21

Long term in vitro and in vivo replication of feces-derived genotype 3 hepatitis E virus without potent intracellular innate immune responses

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Introduction: Chronic Hepatitis E virus (HEV) genotype 3 (gt3) infections are increasingly diagnosed in immunosuppressed patients in western countries. The immunopathogenesis of HEV is largely unknown. High intrahepatic interferon stimulated gene (ISG) responses are seen during chronic hepatitis C, but not hepatitis B infections, both in humans and in human liver-chimeric mice

Aim: Here we establish the in vitro and in vivo infectivity of different HEV RNA containing human clinical samples and characterize the intrahepatic innate immune response during ongoing HEV gt3 replication.

Methods: Human liver-chimeric uPA+/+Nod-SCID-IL2Rγ-/- mice and human lung adenocarcinoma A549 cells were challenged with HEV gt3 obtained from human plasma, feces or liver-biopsy from 2 different chronic HEV patients. Detection and quantification of HEV RNA was performed in mouse feces, serum, bile, liver and A549 culture supernatant using RT-qPCR and immunofluorescence. Human gene expression of chimeric mouse livers was analyzed using the nanostring nCounter® human immunology panel. Three non-infected non-chimeric mice were used as controls to test cross reactivity. Data were normalized using several human specific housekeeping genes.

Results: Infection of A549 cells showed increasing HEV RNA titers in culture supernatant within 14 days when exposed to HEV derived from feces and liver but not from plasma. Chimeric mice inoculated with feces derived HEV (n=14) contained high HEV RNA levels in liver from week 2-14 post inoculation. Feces of these mice was positive for HEV RNA at least once during follow-up, while HEV viremia was inconsistently detectable. HEV derived from a cryopreserved liver biopsy similarly resulted in moderate to high HEV RNA levels in mouse feces, bile and liver (n=2). In contrast, anti-HEV IgG/IgM negative, HEV RNA positive plasma was not infectious in any of the inoculated chimeric animals (n=4). Mouse livers were examined for 578 immune-associated genes at week 2, 6 or 14 post inoculation. No transcripts from a total of 203 genes (incl. IFNa, IFNb and TNF) were detected. Compared to chimeric livers without detectable intrahepatic HEV RNA, none of the 21 ISG, 11 PRR and 26 cytokine/chemokine genes showed a more than 2-fold regulation in the livers of HEV gt3 infected mice.

Conclusions: In conclusion, infectivity of feces derived human HEV is higher compared to plasma-derived HEV both in vitro and in vivo. HEV gt3 infection does not induce potent intrahepatic ISG, PRR or cytokine and chemokine responses in human liver-chimeric mice.

A23

Early decline of liver stiffness measured by real-time shear wave elastography upon antiviral treatment of chronic hepatitis C.

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Introduction: Real-time shear wave elastography (RT-SWE; Aixplorer®, SuperSonic Imagine S.A., Aix-en-Provence, France) is a fairly recent tool to assess liver fibrosis by measuring liver stiffness (LS). In viral hepatitis, regression of liver fibrosis has been demonstrated both by histology as by a decrease in LS measured by noninvasive elastography in case of treatment success. To date, little is known about the on-treatment effect of antiviral treatment on LS.

Aim: To study the immediate effect of treatment with direct-acting antiviral agents on RT-SWE measurements of LS in patients with chronic hepatitis C (HCV).

Methods: We evaluated liver biochemistry and LS by RT-SWE in HCV patients undergoing antiviral treatment before the start of treatment (BT), at the end of treatment (EoT) and 12 weeks after treatment (SVR12). All patients had fibrosis stage 3 or 4 (METAVIR), determined by histology and/or a combination of noninvasive methods. Results are presented as mean±SD.

Results: These are the preliminary results of 43 patients that reached EoT, of which 24 patients already reached SVR12. All patients were HCV RNA negative at EoT. LS decreased significantly from 16.1±7.8 kPa BT to 13.0±6.2 kPa EoT ($p<0.0001$). AST and ALT significantly decreased from 79±49 to 31±17 IU/mL ($p<0.0001$) and from 97±57 to 35±24 IU/mL ($p<0.0001$) from BT to EoT, respectively. Of the 24 that reached SVR12, 22 had still undetectable HCV RNA. In these 22, LS further significantly decreased from 12.3±5.9 kPa EoT to 11.3±5.6 kPa at SVR12 ($p<0.001$). AST and ALT also further decreased from 29±18 to 24±7 IU/mL ($p=0.12$) and from 31±20 to 25±7 IU/mL ($p=0.09$) from EoT to SVR12, respectively, but this difference did not reach statistical significance. Platelet count did not change significantly from BT to EoT (175±72 to 178±70 10E9/L, $p=0.66$), nor from EoT to SVR12 (173±84 to 183±89 10E9/L, $p=0.26$). In relapser 1, LS did not decrease from BT to EoT (18.2 to 18.9 kPa), in relapser 2 there initially was a decrease of LS from BT to EoT (28.0 to 16.3 kPa), with an increase from EoT to SVR12 (18.6 kPa).

Conclusions: These preliminary results of an ongoing study show a significant decrease of liver stiffness, measured by RT-SWE, early in the course of successful antiviral treatment in patients with chronic HCV. This decrease in LS parallels the decrease in transaminases, suggesting that this early decrease of LS is the consequence of a decrease in inflammation, rather than a regression of liver fibrosis. This important observation should be taken into account when interpreting the results of noninvasive follow-up of the evolution of fibrosis after antiviral therapy.

A24

Impact of nutritional status on cirrhotic inpatients' prognosis: a retrospective monocentric study.

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Introduction: Malnutrition is frequent in cirrhotic patients and is associated with a poor outcome. Role of malnutrition on the prognosis of hospitalized cirrhotic patients is incompletely studied.
Aim: To determine the prevalence of malnutrition, functional scores and their impact on prognosis of hospitalized cirrhotic patients.
Methods: This retrospective study included all consecutive patients with cirrhosis hospitalized in the gastroenterology unit at Saint-Luc university hospital from April 2014 to September 2014. Nutritional status was evaluated according to a score used in our centre including weight loss speed, reduction of intakes and body mass index (BMI). We also investigated the role of Charlson comorbidity index, Braden scale and Onodera's Prognostic Nutritional Index (PNI) depending on both albumin and lymphocyte values. Cirrhosis-related complications or death occurrence were analysed in a one-year follow-up. Patients with unavailable nutritional data or with absence of follow-up were excluded.
Results: On 113 patients with cirrhosis, 18 were excluded, and the remaining 95 patients were assessed for nutritional status and outcomes. There were 71 men (74.8%). Mean age was 60 years (range 20-84 years). Aetiology of cirrhosis was alcoholic in 58.9%, hepatitis B or C virus in 29.5%, NAFLD in 5.3% and other causes in 6.3% of patients. Hepatocellular carcinoma (HCC) was present in 46.3% of patients. Mean BMI was 27.1 kg/m² ± 6.7. Diabetes mellitus was present in 36.8% of patients. Mean MELD was 13.4 ± 6.6 points. 40% of patients had a Child-Pugh A score, 41% a Child-Pugh B score and 19% a Child-Pugh C score. Malnutrition affected 45.3% of patients: 29% with Child-Pugh A, 48.8% with Child-Pugh B and 72.2% with Child-Pugh C. Mean Braden scale was 21.54 ± 2.25; 46.3% of patients had a Braden scale < 23. During the study period, 58.9% of patients developed cirrhosis-related complications (60.7% in the malnutrition group vs. 39.3%, p<0.001, OR 5.06, IC95 1.90-14.58) and 33.7% of patients died (68.75% vs. 31.25%, p=0.002, OR 4.33, IC95 1.62-12.28). In univariate analysis, sodium <135 mmol/L (p<0.001), MELD >15 (p<0.001), PNI <40 (p=0.003), Braden scale <23 (p=0.047) and malnutrition (p<0.001) were significantly associated with mortality. Adjusting for age, sodium, MELD, Charlson index, HCC, platelets, diabetes, PNI and Braden scale, malnutrition was significantly associated with higher mortality and morbidity rates with an OR of 3.56 (IC95 1.55-8.16) and 2.09 (IC95 1.16-3.77) respectively. Braden scale was significantly associated with higher mortality (p=0.027, OR 1.25, IC95 1.03-1.52) whereas PNI was associated with higher morbidity (p=0.001, OR 0.94, IC95 0.90-0.98).
Conclusions: Malnutrition is highly prevalent in hospitalized cirrhotic patients. Malnutrition, low PNI and low Braden scale are associated with poor outcomes in cirrhosis.

A25

Hepatocellular autophagy deficiency in mice modulates the unfolded protein response in a pathway selective manner

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Introduction: Autophagy is an important cellular process involved in the breakdown of cytoplasmic content via a lysosomal pathway in order to maintain cellular homeostasis. Autophagy is constitutionally active, but can be upregulated in case of cellular stress. The unfolded protein response (UPR) is another key homeostatic mechanism activated upon accumulated misfolded proteins in the endoplasmic reticulum. Both autophagy and the UPR are implicated in the pathophysiology of NAFLD. Although the UPR is known to promote autophagic flux, the reciprocal effects are less well known.
Aim: We studied the effects of hepatocellular autophagy-deficiency on the three different UPR-pathways.
Methods: The essential autophagy gene Atg7 was cell-specifically deleted via the Cre-LoxP technology and use of an albumin-promoter to create hepatocyte-specific autophagy-deficient mice. These mice were compared to their wild type littermates at an age of 9-10 weeks. Key mediators and chaperones of the distinct UPR pathways (i.e. IRE1, ATF6, PERK) were studied with rt-PCR and western blotting, as well as the executioner caspase-3/7 activity.
Results: Hepatocellular autophagy-deficient mice did not develop fasting-induced steatosis and exhibit severe parenchymal injury, as previously described. The hepatic expression of IRE1a, its endoribonuclease activity and its downstream effectors were not significantly altered. Although the expression of ATF6 itself was unchanged ($p=0.22$), its active cleaved fragment was robustly decreased in autophagy-deficient mice. The transcription and protein levels of ATF6-regulated chaperones were concordantly reduced ($p<0.05$). In contrast, the PERK pathway was significantly activated in the autophagy-deficient mice ($p<0.05$). CHOP, a central effector of UPR-mediated apoptosis downstream of PERK, was increased in these mice ($p<0.001$) as was confirmed by the increased executioner caspase-3/7 activity ($p<0.01$).
Conclusions: These results demonstrate that autophagy has a reciprocal and pathway-selective effect on UPR, with a diminished cytoprotective ATF6 pathway and an enhanced pro-apoptotic PERK pathway upon autophagy-deficiency. These effects might partially explain the absence of fasting-induced steatosis and the observed significant hepatic lesions.

A26

Sofosbuvir/Velpatasvir Fixed Dose Combination for 12 Weeks Compared to Sofosbuvir with Ribavirin for 24 Weeks in Genotype 3 HCV Infected Patients: The Randomized Controlled Phase 3 ASTRAL-3 Study

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Introduction: Velpatasvir (VEL) is a pangenotypic HCV NS5A inhibitor. In Phase 2 studies, the combination of sofosbuvir (SOF) and VEL for 12 weeks demonstrated high efficacy in patients with genotype 3 HCV.

Aim: This Phase 3 study compared treatment with a fixed dose combination (FDC) of SOF/VEL for 12 weeks to SOF+RBV for 24 weeks, in patients with genotype 3 HCV.

Methods: Patients at 75 sites in North America, Europe, Australia and New Zealand were randomized 1:1 to received SOF/VEL (400 mg /100 mg daily) FDC for 12 weeks or SOF (400mg daily) with RBV (1000-1200mg daily) for 24 weeks. HCV RNA was measured with the CAP/CTM HCV 2.0 assay with LLOQ =15 IU/mL The primary endpoint was sustained virologic response 12 weeks after treatment (SVR12) with a pre-specified non-inferiority margin of 10%.

Results: Of 552 patients randomized and treated, 62% were male, 89% were white, 39% had IL28B CC genotype, 26% had prior treatment failure, and 30% had cirrhosis. Eight patients discontinued treatment due to adverse events, all of which were in the SOF +RBV treatment group. The most common AEs (>10% in either treatment group) were headache (31%), fatigue (24%), nausea (15%) and insomnia (11%) in the SOF/GS-5816 group; headache (28%), fatigue (34%), insomnia (24%), nausea (19%), irritability (11%) and cough (11%) in the SOF+RBV group. Five patients in the SOF/VEL treatment group and 8 patients in the SOF+RBV treatment group experienced SAEs. One SAE, acute generalized exanthematous pustulosis, in a SOF+RBV treated patient was assessed as related to study drugs by the investigator. Hemoglobin decline and total bilirubin increases were more commonly observed in the group treated with SOF +RBV consistent with RBV–induced hemolysis. No other significant lab abnormalities were observed. HCV RNA declined rapidly in both treatment groups with 92% and 88% of patients achieving HCV RNA < LLOQ after 4 weeks of treatment in the SOF/VEL and SOF+RBV treatment groups, respectively. The SVR12 rate in subjects receiving SOF/VEL for 12 weeks was 95% (264/277) and was statistically superior to the 80% (221/275) SVR12 rate in subjects treated with SOF+RBV for 24 weeks ($p<0.001$).

Conclusions: The once daily, all-oral, single tablet regimen of SOF/VEL was well tolerated in treatment-naïve and treatment-experienced genotype 3 HCV-infected patients with and without cirrhosis. There were no discontinuations due to adverse events and a lower incidence of fatigue, insomnia and irritability in patients treated with SOF/VEL for 12 weeks compared to patients treated with SOF+RBV for 24 weeks.

A27

Cathepsin S as a marker of inactivated hepatic stellate cells in the human liver

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Introduction: Hepatic stellate cell (HSC) activation is a key event during liver fibrogenesis. While this process was initially considered to be unidirectional, recent cell fate mapping studies reveal that HSC inactivation is an additional cellular mechanism underlying fibrosis regression in mouse models of toxin-induced liver fibrosis. However, there is no evidence to support that HSC inactivation underlies fibrosis regression in human patients.

Aim: Because fibrosis progression is associated with advancing age, we hypothesize that a liver of advanced age has been exposed to more cycles of injury and repair, therefore potentially harboring primed HSCs with an inactivated phenotype (iaHSCs).

Methods: Freshly isolated, non-cultured human primary HSCs from young and old donors were compared by gene expression profiling using Affymetrix HG-U219 genechips. The expression of CTSS and different liver cell markers on young and old liver was analyzed by immunohistochemistry.

Results: We find that ~1700 genes are differentially expressed between HSCs from old (≥51 years; n=4) and young patients (≤12 years; n=3) (P<0,01; Fold change ≥2). The list of genes with higher expression in old HSCs pertains to response to wounding, response to stress and regulation of cell activation and includes Cathepsin S (CTSS), a lysosomal cysteine proteinase previously identified as a signature gene of iaHSCs. Immunohistochemical double stainings in human livers reveal that CTSS is almost exclusively expressed in vimentin (VIM) positive HSCs and excludes its expression in portal fibroblasts. Quantitatively, CTSS expression was more abundant in the livers of old patients compared to that of young patients. This differential expression is not linked to a difference in activation status, as CTSS expression is not detected in activated myofibroblasts in patients with drug-induced, alcohol-induced and HBV-induced liver cirrhosis.

Conclusions: Our study for the first time identifies CTSS as a potential marker of triggered but non-activated HSCs in the human liver. The differential expression of this gene in HSCs harbors potential as a tool to assess the history of hepatic injury, for example in donor livers.

A28

Turquoise-III: 12-Week Ribavirin-Free Regimen Of Ombitasvir/Paritaprevir/R And Dasabuvir For Patients With HCV Genotype 1B And Compensated Cirrhosis

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Introduction: Hepatitis C virus (HCV) infected patients have historically been more difficult to cure when they have cirrhosis. Treatment with the 3 direct-acting antiviral (3D) regimen of

ombitasvir, paritaprevir boosted with ritonavir, and dasabuvir without ribavirin (RBV) for 12 weeks has demonstrated 12-week sustained virologic response (SVR12) rates of 100% in HCV genotype (GT) 1b patients without cirrhosis, and 99% in GT1b patients with compensated cirrhosis when co-administered with RBV for 12 weeks. We report the safety and efficacy of the 3D regimen without RBV in patients with HCV GT1b infection and compensated cirrhosis.
Aim: -
Methods: Patients enrolled in this phase 3b, multicenter, open-label study received 12 weeks of 3D without RBV. Both treatment-naïve and peginterferon/RBV treatment-experienced patients with compensated cirrhosis with no history of decompensation were enrolled with the following criteria: hemoglobin ≥10 g/dL, albumin ≥2.8 g/dL, platelet count ≥25 x 109/L, and creatinine clearance ≥30 ml/min. Efficacy was assessed by the percentage of patients achieving SVR (HCV RNA below the level of quantitation [LLOQ; <25 IU/mL]) at post-treatment week 12 (SVR12). Efficacy and safety were assessed in all patients receiving study drug.
Results: Sixty GT1b-infected patients with compensated cirrhosis received 3D. The study population comprised 33 (55%) treatment-experienced, 50 (83%) with IL28B non-CC genotype, (13) 22% with platelet count <90 x 109/L, and (10) 17% with albumin <3.5 g/dL. Serum HCV RNA decline was rapid with 37/60 (62%) patients. 60/60 (100%) patients achieved SVR12.
Conclusions: Results indicate the 3D regimen without RBV for 12 weeks is well tolerated and highly efficacious in HCV GT1b-infected patients with compensated cirrhosis.

A29

HEALTH-RELATED QUALITY OF LIFE IMPROVES FURTHER AFTER LIVER TRANSPLANTATION AND IS INFLUENCED BY LENGTH OF STAY.

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Introduction: Liver transplantation (LT) is the only curative treatment for end-stage liver disease (ESLD) with excellent long-term outcomes. However, an important outcome parameter is health-related quality of life (QoL). Improvement of QoL has been described after liver transplantation.
Aim: We wanted to investigate the evolution in QoL in a period of two years after LT and identify factors influencing this evolution.
Methods: We performed a cross-sectional study in a liver transplant unit. Self-report questionnaires (SF36, ICQ and NEO-FFI) were conducted in 177 patients with ESLD: 60 patients pre-transplantation, 60 post-transplantation and control group of 57 patients without perspective of transplantation. Data were analyzed using the Mann-Whitney U test, Spearman's rank correlation coefficient and correlation matrices.
Results: The post-LT patient group consists of 63,0% male individuals and 20,0% had no complications. Mental QoL shows a 19,0% elevation in two years post-LT whereas physical QoL knows a 54,8% elevation in two years post-LT, of which 46,5% the first six months. We observed a significant increase in physical QoL >3 months compared to ≤3 months after LT (p=0,027), as well as a significant increase in disease benefits (p=0,018). In a period of two years post-LT a significant correlation (p<0,001) was found in QoL (physical and mental) compared with length of stay in intensive care (rs=-0,359 and rs=-0,324), length of stay in total (rs=-0,327

and rs=-0,347), helplessness (rs=-,0771 and rs=-0,797), acceptance (rs=0,679 and rs=0,710) and disease benefits (rs=0,514 and rs=0,436). Length of stay on intensive care >6 days post-LT shows a significantly worse physical QoL up to two years post-LT (p=0,023).
Conclusions: These data confirm an increase of QoL during two years post-LT. A better acceptance of illness, a reduction in helplessness and more benefits of the illness after transplantation result in a better QoL. We assume that these findings indicate that patients' illness cognitions are of great influence on QoL. The more time spent in the hospital after LT, the worse QoL gets. A longer stay in intensive care implies longer immobilization, which negatively influences physical QoL. Patients who spent more than six days on intensive care post-LT deserve a more intense physical rehabilitation. These aspects could give new directions in the approach of liver patients after transplantation.

A30

A Phase 3 Double-Blind Placebo-Controlled Evaluation of Sofosbuvir/Velpatasvir Fixed Dose Combination for 12 Weeks in Naïve and Experienced Genotype 1, 2, 4, 5, 6 HCV Infected Patients with and without cirrhosis: Results of the ASTRAL-1 Study

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Introduction: Velpatasvir (VEL, GS-5816) is a pangenotypic HCV NS5A inhibitor. In Phase 2 studies, the combination of sofosbuvir (SOF) and VEL for 12 weeks resulted in high SVR12 in patients with genotype 1-6 HCV infection.
Aim: This Phase 3 study evaluated treatment with a fixed dose combination of SOF/VEL for 12 weeks in patients with genotype 1, 2, 4, 5, or 6 HCV infection (ClinicalTrials.gov Identifier: NCT02201940).
Methods: Patients with genotype 1, 2, 4, or 6 chronic HCV infection were randomized 5:1 to received SOF/VEL (400 mg /100 mg daily) or placebo for 12 weeks. Patients with genotype 5 infection were enrolled to the SOF/VEL treatment group and patients with genotype 3 were

evaluated in a separate study. The primary efficacy analysis was an evaluation of the superiority of SVR12 for the SOF/VEL-treated patients to a pre-specified SVR12 goal of 85%. Secondary endpoints included safety/tolerability, resistance, and additional efficacy outcomes.
Results: 740 patients were enrolled at 81 sites in North America, Europe and Hong Kong: 60% male, 79% white, 30% IL28B CC genotype, 32% treatment-experienced (TE), and 19% compensated cirrhosis. Of the 624 patients treated with SOF/VEL, the genotype distribution was 53% GT1, 17% GT 2, 19% GT 4, 6% GT 5 and 7 % GT 6. Overall SVR12 for SOF/VEL-treated patients was 99.0% (95% confidence interval 97.9% to 99.6%) and the study met its primary efficacy endpoint ($p< 0.001$). SVR12 rates by HCV genotype are presented in the table. Two of 328 patients (0.6%) with genotype 1 infection, including 1 of 73 with cirrhosis, had virologic relapse: 1 genotype 1a treatment-naïve non-cirrhotic and 1 genotype 1b treatment-experienced with cirrhosis. No patients with genotype 2, 4, 5, or 6, including 48 with cirrhosis, had virologic failure. Four patients did not achieve SVR12 for non-virologic reasons (eg. lost to follow-up). Overall, the type, frequency and severity of AEs and laboratory abnormalities were similar in the SOF/VEL-treated patients compared with the 116 placebo-treated patients. One patient discontinued treatment due to adverse events. One SOF/VEL-treated patient died 8 days after completion of treatment of unknown causes. Fifteen (2.4%) SOF/VEL-treated patients and no placebo-treated patients experienced SAEs; none was assessed as related to study drug.
Conclusions: Treatment with the once daily, all-oral, single tablet regimen of SOF/VEL for 12 weeks is well tolerated and results in high SVR12 rates in treatment-naïve and treatment-experienced genotype 1,2,4,5,6 HCV-infected patients with and without cirrhosis.

A31

Self-expanding metal stents in patients with cirrhosis and severe or refractory esophageal variceal bleeding: a systematic review and meta-analysis

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Introduction: Despite recent advances in patient care, the prognosis of patients with cirrhosis and acute variceal bleeding is very poor when the standard-of-care fails to control bleeding, and new treatment modalities are needed. Self-expanding metal stents (SEMS) may be as efficacious and a safer option than balloon tamponade in this setting.
Aim: To synthesize the available evidence on the efficacy of SEMS in patients with cirrhosis and severe or refractory esophageal variceal bleeding.
Methods: Meta-analysis of trials evaluating SEMS in patients with cirrhosis and severe or refractory esophageal variceal bleeding.
Results: Thirteen studies were included. A total of 146 patients with severe or refractory bleeding from esophageal varices were treated with SEMS. More than half of the patients had alcoholic cirrhosis. Sixty percents belong to Child-Pugh stage C. Placement of SEMS was successful in 95% of the cases. The pooled estimate rates were 0.40 (95% CI=0.31-0.49) for death, 0.41 (95% CI=0.29-0.53) for liver-related death, and 0.36 (95% CI=0.26-0.47) for death at day 30, with low heterogeneity between studies. The pooled estimate rates were 0.12 (95% CI=0.07-0.21) for mortality related to variceal bleeding, and 0.18 (95% CI=0.11-0.29) for failure to control bleeding with SEMS, with no or low heterogeneity between studies. The pooled estimate rate were 0.16 (95% CI=0.04-0.48) for rebleeding after stent removal and 0.28 (95%

CI=0.17-0.43) for stent migration, with high heterogeneity. A significant proportion of patients had access to liver transplantation or to transjugular intrahepatic portosystemic shunt (TIPSS) (pooled estimate rate 0.10 [95% CI=0.04-0.21] and 0.26 [95% CI=0.18-0.36], respectively, with no heterogeneity between studies).
Conclusions: Fewer than 40% of the patients treated with SEMS died at one month. SEMS can be used as a bridge to TIPSS or to liver transplantation in a significant proportion of patients. Additional studies are required to identify potential risk factors leading to a poor prognosis in patients with AVB in whom the use of SEMS could be considered.

A32

Next-generation proteasome inhibitor oprozomib synergizes with modulators of the unfolded protein response to suppress hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) responds poorly to conventional systemic therapies. The first-in-class proteasome inhibitor bortezomib has been approved in clinical use for hematologic malignancies and has shown modest activity in a variety of solid tumors, including HCC. However, a considerable proportion of patients fail to respond and experience significant adverse events. Recently, the next-generation orally bioavailable irreversible proteasome inhibitor oprozomib was developed.
Aim: In this study, we aim to assess the therapeutic efficacy of oprozomib in HCC and its effects on the unfolded protein response (UPR), an intracellular signaling cascade that is activated through the IRE1, ATF6 and PERK pathways by the accumulation of unfolded proteins in the endoplasmic reticulum of HCC cells.
Methods: The effects of oprozomib and the role of the UPR were evaluated in HCC cell lines and in diethylnitrosamine-induced and xenograft mouse models for HCC. Next to the expression analysis of UPR-induced targets, MTT, TUNEL immunofluorescence, caspase-3/7 activity and bromodeoxyuridine incorporation assays were performed. In addition, the UPR-regulated protein half-life was determined.
Results: Oprozomib dose-dependently reduced the viability and proliferation of human HCC cells. Unexpectedly, oprozomib-treated cells displayed reduced cytoprotective ATF6-mediated signaling, whereas PERK and IRE1 pathway activation was not observed. However, oprozomib increased pro-apoptotic UPR-mediated protein levels by prolonging their half-life, implying that the proteasome acts as a rapid negative UPR regulator. Supplementary boosting of UPR activity synergistically improved the sensitivity of HCC cells to oprozomib via the PERK pathway. Oral oprozomib monotherapy displayed significant antitumor effects in the orthotopic and xenograft models for HCC, and importantly, the combination of oprozomib with different clinically applicable UPR activators, including the HIV protease inhibitor nelfinavir at a human-relevant dose, enhanced the antitumor efficacy in vivo by stimulating UPR-induced apoptosis without cumulative toxicity.

Conclusions: Next-generation proteasome inhibition by oprozomib results in dysregulated UPR activation in HCC. This finding can be exploited to enhance the antitumor efficacy by combining oprozomib with clinically applicable UPR modulators.

A33

Characteristics of patients with hepatitis B virus and hepatitis C virus dual infection in Belgium: comparison with monoinfected patients and impact of hepatitis C virus replication

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Introduction: There are sparse epidemiological data on patients with hepatitis B (HBV) and hepatitis C (HCV) dual infection in Europe. Updated data focusing on patients with HBV/HCV dual infection are mandatory for assessing changes in epidemiology and to improve patient care.

Aim: To assess epidemiological, clinical, biochemical, virological and histological characteristics of patients with HBV/HCV dual infection and to compare them with those of patients with HBV or HCV monoinfection, and to assess characteristics of patients with HBV/HCV dual infection according to the presence of detectable HCV RNA.

Methods: Data of 23 patients with HBV/HCV dual infection were extracted from a Belgian database of HBs carriers and compared to those of 92 age and sex-matched patients with HBV or HCV monoinfection.

Results: Patients with HBV/HCV dual infection were more often immigrant from Africa or Asia than patients with HCV or HBV monoinfection (52% vs. 20% vs. 24%, p=0.02). IV drug use was the route of transmission in 42% of patients with HBV/HCV dual infection, which was similar to HCV monoinfected patients (51%) but less frequent than HBV monoinfected patients (0%) (p<0.001). The severity of the liver disease was similar among patients with HBV/HCV dual infection and among those with HBV and HCV monoinfection (prevalence of extensive fibrosis or cirrhosis: 19% vs. 29% vs. 14%, respectively, p=0.4), even when fibrosis stage was reported to the duration of infection. Dual infected patients were less likely to receive antiviral therapy than patients with chronic hepatitis B (50% vs. 91%, p=0.002). By contrast, treatment was as frequently considered in patients with HBV/HCV dual infection and in those with HCV monoinfection (50% vs. 62%, p=0.4). In dual infected patients, those with detectable HCV RNA were more frequently male (100% vs. 46%, p=0.02) and had less frequently detectable HBV

DNA although the difference was not significant (50% vs. 71%, p = 0.4). The percentage of patient with extensive fibrosis or cirrhosis was similar in patients with and without detectable HCV RNA (18% vs. 20%, p = 0.9).
Conclusions: Compared to patients with HCV or HBV monoinfection, patients with HBV/HCV dual infection were more often immigrant from Africa or Asia. The severity of the liver disease was similar in patients with HBV/HCV dual infection and in patients with HBV or HCV monoinfection. Among patients with dual HBV/HCV dual infection, detectable HCV RNA was not associated with more extensive fibrosis or cirrhosis.

A34

Female Genital Mutilations have no impact on the incidence of viral hepatitis B and C in West-African women

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Introduction: Viral hepatitis B and C are common in West Africa: the markers of a past exposure to hepatitis B reach 58-98% of the population and a positive HCV serology reach more than 3%. The exact mode of transmission of these viruses remains uncertain in those countries, the horizontal transmission in childhood is widely suspected. In this area of the world, the practice of Female Genital Mutilation (FGM) is also common (for up to 97%), precisely during childhood.
Aim: We studied the prevalence of serological markers of HBV and HCV among pregnant West African women, who have arrived in Brussels, and analysed the role of FGM as a risk factor of infection.
Methods: All pregnant women seen at our clinic, and coming from Mauritania, Sierra Leone, Nigeria, Guinea, Senegal, Ghana, Burkina-Faso and Mali were consecutively included, between January 2013 till December 2014. The presence of FGM was recorded as well as the presence of antibodies (Ab) HBs, HBc, and HCV and the HBs antigen (Ag). The prevalences of serological tests were compared between women who had undergone FGM or not.
Results: There were 206 women (mean age + SD : 28.4 years + 5.6) coming from the previous mentioned countries were studied, among which 154 (74.8%) from Guinea and 18 (8.7%) from Senegal. There were 165 women (80.1%) who had undergone FGM and 26 (12.6%) not (for 15 patients, the information was lacking). Four women had HCV-Ab (1.9%), 124 (67.8%) had HBc-Ab, 10 (4.9%) had HBs-Ag. There were no differences in HCV-Ab frequency among FGM women versus non-FGM (3/165 vs. 1/26, p = 0.50). There were no differences in the HBc-Ab frequency (99/146 in FGM vs. 16/26 in non-MGF, p = 0.53). The HBs-Ag was present in 6/164 in FMG-women vs. 3/26 in non-FMG, p = 0.79).
Conclusions: A past exposure to HBV is common in West African women but a positive HCV serology remains rare. FGM was not identified as a risk factor of HBV and HCV infection in this small series of patients.

A35

Improvement of sleep parameters with the use of rifaximin in Hepatic Encephalopathy and advanced cirrhosis

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Introduction: Hepatic Encephalopathy (HE) is a common complication of advanced cirrhosis and its clinical presentation varies from minimal vigilance disorders (minimal HE) to overt neurological signs (overt HE). Sleep disorders are frequently associated with HE and seriously alter the quality of life of these patients. Rifaximin is currently accepted as the standard of care of overt HE with recurrent episodes.

Aim: The aim of this study was to assess the effect of rifaximin on sleep disorders in patients with overt, refractory HE.

Methods: This study was a pilot, crossover and self-controlled study involving all consecutive patients suffering from cirrhosis and refractory HE admitted in our Liver Unit. All patients systematically underwent a 24-h polysomnography (PSG), followed by 7-days of actigraphy and sleep log. They fulfilled also sleep (Pittsburgh Sleep Quality Index, PSQI) and anxiety-depression (Hopital Anxiety Depression scale, HAD A or D) questionnaires. They received one month of rifaximin and the same assessments were then repeated under this treatment.

Results: Ten patients were included. Six were males, and mean age was 57 +/- 12 years. Child-Pugh scores range from B7 to C15 and MELD scores from 6 to 21. According to West Haven Criteria, mean HE score was 2.5 +/- 0.5. Before rifaximin, PSG revealed a long sleep time distributed on 24 h (698 +/- 240 min). Sleep quality was highly deteriorated with few Stage 3 sleep (6% (0-39)) and REM sleep (6% (3-23)). Actimetry confirmed prolonged sleep time/24h (556 min (183-808)). Questionnaires showed an impaired sleep quality (mean score of 11 +/- 4 on PSQI), and symptoms of anxiety and depression (HAD A 10 +/- 5, HAD D 12 +/- 6). After rifaximin treatment, HE score decreased to 1.7 +/- 0.5. PSG showed an improvement in sleep quality: Stage 3 sleep was increased to 32% (6-56), p=0.04, and REM sleep to 11% (0-27), p=0.04. Total sleep time/24h decreased to 290 (53-701) min, p=0.03 on actigraphy. No changes were observed on questionnaires scores.

Conclusions: In this small pilot study, we showed that the use of rifaximin in overt and refractory HE was associated with an improvement of HE, a decreased sleep time/24h and a significant improvement in sleep quality.

A36

VERSANT HCV GENOTYPE 2.0 LINE PROBE ASSAY (LIPA) MISCLASSIFIES THE CIRCULATING HCV RECOMBINANT RF1_2K/1B IN GENOTYPE 2 PATIENTS

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Introduction: The accuracy of the Versant HCV Genotype 2.0 Line Probe Assay (LiPA) to identify the subtype diversity within genotype 2 has been reported to be suboptimal.

Aim: The aim of the present study was to characterize the HCV subtype diversity of patient samples previously labeled as HCV genotype 2 with the Versant HCV Genotype 2.0 LiPA.
Methods: 89 consecutive HCV-RNA stored samples with genotype 2 status as determined with the Versant HCV Genotype 2.0 Assay (LiPA) were re-genotyped with the HCV NS5B sequencing reference method (Murphy et al., 2007). Concordance for genotype group and subtype between both typing methods was calculated. The results of genotypes and subtypes obtained by NS5B sequencing were considered the reference genotypes.
Results: The HCV polymerase region (NS5B) was successfully sequenced in 86/89 (97%) genotype 2 samples. The two genotyping methods resulted in concordant genotype and subtype in 10/86 samples (12 %). 55/86 (64%) samples were incompletely genotyped (exact genotype result with an unidentified subtype or with an absence of discrimination between two subtypes). Both methods failed to assign a subtype in 8/86 (9 %). 13/86 (15%) were misclassified (wrong genotype or correct genotype associated with a wrong subtype). The circulating HCV recombinant form RF1_2k/1b was detected in 11 of these samples (11/86 (13%)). 9/11 (82%) of these had been labeled as "2a or 2c" with the LiPA 2.0 method.
Conclusions: Direct sequencing of the HCV NS5B polymerase region revealed the presence of the recombinant form RF1_2k/1b in 13% of samples, the majority of which had been labeled as genotype "2a or c" with Versant HCV Genotype -LiPA 2.O. The optimal antiviral treatment regimen for RF1_2k/1b HCV patients needs to be evaluated further.

A37

Treatment and HBeAg-status Differentiate Clinical Outcomes Following ALT Flares – Analysis of Tenofovir Disoproxil Fumarate (TDF) Plus Peginterferon (PEG) Combination Study for Chronic Hepatitis B (CHB)

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Introduction: Factors that drive clinical outcomes following ALT flares in CHB patients are poorly characterized.
Aim: Here, we evaluated clinical outcomes following ALT flares in patients enrolled in a 4-arm interventional CHB study.
Methods: 740 CHB patients without advanced liver disease received either TDF+PEG x48 weeks (Arm A); TDF+PEG x16 weeks followed by TDF x32 weeks (Arm B); continuous TDF (Arm C); or PEG x48 weeks (Arm D). ALT flares, defined as ALT > 2x baseline and > 5x ULN, occurring within the first 24 weeks of treatment were evaluated in context of subsequent clinical outcomes through Week 48.
Results: Overall, 29/172, 25/170, 3/174 and 25/163 patients, respectively, from Arms A-D met criteria for ALT flare; of those, 18, 16, 2, and 14 patients were baseline HBeAg-positive. A greater proportion of patients in Arms A, B and D who experienced ALT flares achieved subsequent HBeAg loss, HBsAg decline equal to or higher than 1log10 IU/ml, or HBsAg loss compared to those who did not experience an ALT flare. HBeAg loss was achieved in 38.9% in Arm A, 18.8% in Arm B and 21.4% in Arm D in patients with an ALT flare, versus 10.4%, 11.4% and 7.7% respectively in patients without an ALT flare. HBsAg decline ≥ 1log10 IU/ml was achieved in 34.5% in Arm A, 28.0% in Arm B and 24% in Arm D of patients with an ALT flare, compared to 11.9%, 4.8% and 11.6% in Arms A, B and D respectively in patients without an ALT flare. HBsAg loss was demonstrated in 24.1% in Arm A, 4.0% in Arm B and 12.0% in Arm D in patients with an ALT flare, compared to 1.7%, 0.7% and 0.4% in Arms A, B and D respectively in patients without an ALT flare. Comparing two combination therapies, Arm A achieved higher rates of HBsAg loss (P=0.016) following flares than Arm B. This is partly driven by HBeAg-negative patients in Arm A who attained disproportionately increasing rates of HBsAg loss (18.2%) and HBsAg decline ≥ 1log10 IU/ml (45.5%) which was not observed in Arm B.
Conclusions: Treatment-associated ALT flares, especially with TDF+PEG combination therapy, are associated with clinical endpoints related to HBV immune control. ALT flares were higher in the TDF+PEG x48w group and associated with the highest HBsAg loss.

A38
The Isolation of Hepatic Progenitor Cells from Human Liver Tissue
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Introduction: Hepatic progenitor cells (HPCs) are small cells with a relative large oval nucleus and a scanty cytoplasm. They are situated in the canals of Hering, the terminal branches of the biliary tree localised in the portal tracts. Phenotypically, HPCs express both markers of (immature) hepatocytes (e.g. α-fetoprotein) and markers of cholangiocytes (e.g. cytokeratin K7 and K19). The mechanisms facilitating proliferation and differentiation of human HPCs are beginning to be unravelled, however, many questions remain. Therefore, it is important to have a good isolation method to obtain a purified HPC fraction which can be used to further characterise human HPCs and their activation/differentiation mechanisms. Until present no such isolation method has been described for human HPCs.

Aim: In order to study human HPCs and their differentiation mechanisms we aim to optimise a HPC isolation method based on the epithelial marker TROP-2 (a.k.a. TACSTD2,) which is known for its capacity to isolate murine HPC fractions.
Methods: The expression of TROP-2 in human HPCs was first determined via immunohistochemical stainings in different stages of (non)alcoholic steatohepatitis (N)ASH, known to have strong HPC activation. Subsequently, human livers with (N)ASH were dissociated and the cell suspension was analysed and separated via Fluorescent Activated Cell sorting (FACS) using a fluorophore-conjugated TROP-2 antibody to obtain the TROP-2 positive fraction enriched with HPCs. The obtained enriched fractions were compared with a whole liver extract and evaluated on both protein level (immunohistochemical staining) and RNA level (high-throughput sequencing).
Results: Based on immunohistochemical stainings, TROP-2 was expressed in HPCs, cholangiocytes and to a lesser extent in intermediate hepatocytes, indicating that TROP-2 can be used to obtain HPC enriched fractions from human livers. Isolation via FACS resulted in clear signal shifts and 90% TROP-2-positive cell isolate. RNA sequence analysis showed an upregulation of HPC markers and a downregulation of hepatocyte markers in the TROP-2 positive fractions.
Conclusions: This study shows the possible use of TROP-2 as a HPC marker to isolate HPC enriched fractions from human liver tissue via a cell sparing method. These fractions can be further used to characterize human HPCs and to understand the molecular mechanisms behind their activation and differentiation. This will be a step forward in human HPC research with the ultimate goal of using HPCs for the treatment of liver diseases.

A39

Monocyte chemoattractant protein-1, a new inflammatory marker of non-alcoholic fatty liver disease?

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Introduction: The growing epidemic of obesity has led to a simultaneously increased prevalence of non-alcoholic fatty liver disease (NAFLD). Emerging data indicate that an impaired release of adipose tissue-derived adipokines could play a pivotal role in the development of NAFLD.
Aim: We aimed to evaluate whether circulating levels of recently described adipokines associate with the histopathological disease severity and thus may contribute to the progression of pure fatty liver into an inflammatory and insulin resistant NASH status
Methods: Serum levels of omentin, chemerin, monocyte chemoattractant protein-1 (MCP-1) and Secreted frizzled-related protein (Sfrp) 4 were measured using enzyme-linked immunosorbent assay (ELISA) in 81 obese patients with biopsy-proven NAFLD and 18 control subjects.

Histopathological grading of NAFLD was scored using the NAFLD activity score (NAS), as verified by Brunt et al.
Results: Serum levels of omentin, MCP-1 and Sfrp 4 did not differ between patients and controls, whereas chemerin serum concentrations were significantly higher in patients with biopsy-proven NAFLD than in controls (P=0.020). When NAFLD patients were subdivided into groups with pure fatty liver, borderline NASH and NASH, insulin resistance and transaminase levels but no adipokine levels were significantly higher in NASH groups and were associated with NAS (r=0.311, P=0.003; r=0.432, P=<0.001 and r=0.480, P=<0.001 for HOMA-IR, AST and GGT, respectively). Only serum MCP-1 levels were significantly associated with the degree of lobular inflammation (r=0.222; P=0.038), independent of insulin resistance.
Conclusions: Our results confirm the relation between insulin resistance and NAFLD severity and additionally suggest that MCP-1 levels, a known inflammatory adipokine, may be related to the degree of lobular inflammation in NAFLD patients.

A40
Experience with Direct Acting Agents for the Treatment of Hepatitis C in Flanders.
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Introduction: The development of direct acting antivirals has revolutionized HCV care. We present the experience in KU Leuven affiliated hospitals with the latest generation of direct acting antivirals without concomitant use of interferon.
Aim: Goal of the study is to investigate the safety and efficacy of the newest generation of direct acting antivirals in the general population in Flanders.
Methods: A retro/prospective cohort study conducted between August 2015 and November 2015 in 6 Belgian centers. Patients infected with HCV, who were treated with the new regimes of DAA following the recent EASL guidelines between March 2013 and November 2015 were included. In case antiviral treatment was started data were collected in a central database, with main focus on treatment outcome and side effects.
Results: In total, 134 patients (age 58 ± 12 years) were treated. HCV genotype 1b was most prevalent, and was present in 74 patients (58%). Genotype 1a was present in 20 patients (16%), genotype 3 in 17 (13%), genotype 4 in 12 (9%), genotype 5a in 2 (2%) and 2 patients with genotype 1 could not be subtyped. 46% of the patients were treatment naïve, 39% were treated once before. 60% of the patients had compensated cirrhosis (F4), 19% had end-stage fibrosis (F3) and 21% were treated without reimbursement criteria in a study setting (F2 or less). Most patients had comorbidities, with an average rate of 1.55 ± 1.6. These were highest in the simeprevir/sofosbuvir group. 59 patients (44%) were treated with daclatasvir/sofosbuvir ± ribavirin, 48 (36%) with simeprevir/sofosbuvir ± ribavirin, and 27 (20%) with ombitasvir/ paritaprevir, ritonavir – dasabuvir ± ribavirin. Today, treatment is completed in 60% of them and in 43% we have SVR (3m). From this group, almost everyone obtained SVR (96.5%).

Subanalysis showed an SVR-rate of 100% (10/10) in the daclatasvir/sofosbuvir ± ribavirin group, 92.6% (25/27) in the simeprevir/sofosbuvir ± ribavirin group, and 100% (20/20) in the ombitasvir/ paritaprevir, ritonavir – dasabuvir ± ribavirin group. Side effects were present in 49%, most frequently fatigue and skin eruptions. In only 10%, this required a change or interruption in ribavirin dosage. Final data will be presented at the meeting.
Conclusions: Real-life experience with DAAs in Belgium shows comparable excellent results in achieving SVR for the treatment of HCV. Side effects were seen especially related to ribavirin, but there was no need to interrupt the DAA.

A41

Cirrhosis and pregnancy. A single center experience

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Introduction: Chronic Liver Failure (CLF) is rarely encountered in women in childbearing years, as cirrhosis related hormonal disregulations predispose to infertility. Nevertheless, pregnancy in cirrhotic patients is frequently observed in African countries, due to the high prevalence of B and C viral hepatitis in developing countries and the lack of national screening programs and supplies for large scale treatment. Currently, limited data are available regarding prevalence and causes of maternal mortality, which is often the result of variceal bleeding (1). Guidelines about the ideal management to prevent peri-partum complications, including prophylaxis for variceal rupture, are not clear.
Aim: We report maternal and fetal outcomes in cirrhotic pregnancies admitted to an African referral institution. Consecutive pregnant patients in follow up at Panzi Hospital during the first trimester of 2015 were included.
Methods: CLF diagnosis, severity and etiology were assessed by clinical (ascites, encephalopathy, gastric or esophageal varices), laboratory (thrombocytopenia, hypoalbuminaemia, coagulopathy, abnormal liver test, HBV/HCV serology), and echographic (free abdominal fluid, dysmorphic and nodular liver, splenomegaly) parameters. 852 pregnant patients were included.
Results: 4/852 (0,5%) patients had liver cirrhosis. Mean age was 30 (+ 8.1). For all patients, CLF was related to HBV chronic hepatitis. Mean bilirubin was 23.3 mg/dL (+ 15.9). 2/4 patients had esophageal varices. One was complicated by esophageal varices rupture (28th week) and was successfully treated by endoscopic banding. Two pregnancies reached the 3rd trimester of gestation (35th and 37th week) and two pregnancies the beginning of the 2nd (14th and 16th week). 4/4 maternal deaths occurred; 2/4 patients died before the delivery (14th and 16th week). Hepatic encephalopathy was the cause of death for 3/4 patients. 1 patient died following vaginal hemorrhage complicating spontaneous abortion. No patient died because of variceal bleeding. 2/4 delivered (vaginal delivery, 35th and 37th week) and died for hepatic encephalopathy respectively 3 and 35 days post-partum. 3/4 fetus died: 2/4 in utero (14th and 16th week) and 1/4 was stillborn.
Conclusions: This study confirms that maternal and fetal mortality are high in cirrhotic pregnancies. Variceal bleeding can occur and can be successfully treated endoscopically by banding, but it is not the only determinant for the high maternal mortality. Efforts should be done

in order to clarify guidelines and to optimize both maternal and fetal management to improve the outcomes.

A42

Influence of inclusion of the liver on the outcome after intestinal transplantation

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Introduction: Intestinal transplantation (ITx) is a procedure that is indicated when life-threatening complications occur following chronic total parenteral nutrition in intestinal failure patients. Several complications (TPN related irreversible liver disease or diffuse splanchnic thrombosis) require simultaneous transplantation of the liver (and sometimes additional viscera). ITx has long been considered as the most difficult solid organ to transplant due to a high risk of rejection and sepsis. On one hand, the liver may facilitate engraftment and protect against rejection (via regulatory and deletional mechanisms). But on the other hand, addition of the liver complicates the surgical procedure and exposes recipients to more risks. Inclusion of the liver in ITx candidates with milder forms of liver disease (to protect the intestinal graft) is controversial.
Aim: To investigate the effect of the inclusion of a liver graft on outcome after ITx.
Methods: We performed a retrospective analysis on our database of ITx recipients, containing recipients of either liver free grafts (LFG) or liver containing grafts (LCG). The data were analyzed on the basis of clinical outcomes, rejection, and survival. We only included patients who received a cadaveric graft.
Results: From 2000 onwards, 15 patients with irreversible intestinal failure received an ITx at our center. Of these, 9 received a LCG and 6 a LFG. 3 were pediatric patients and 12 adults. All received their grafts from brain dead donors that had a negative cross-match. HLA matching was at random. 12 patients (80%) are alive and 11 of these are TPN-independent at home. 1 patient lost her intestinal graft after a biopsy. There were 3 patient deaths: 2 in LCG recipients (1 to invasive aspergillosis and 1 to NSAID related ulcerations) and 1 in LFG recipient (invasive aspergillosis). The 5 year patient survival for LCG and LFG was 88.8% and 83.3% respectively. 5 year graft survival was 88.8% in LCG and 62.3% in LFG. 33% of LCG patients experienced acute rejection against 50% of LFG patients. Of the latter, 2 patients experienced 2 acute rejections each. No chronic rejection was observed. All rejections could be treated by additional immunosuppression. No grafts were lost due to rejection or infection. No donor specific antibodies were detected in any patients.
Conclusions: Addition of the liver to an intestinal graft did not lead to poorer results. On the contrary, there was a trend for less rejection and better graft survival when the liver was co-transplanted. Both liver containing and liver-free ITx represent lifesaving procedures with excellent outcomes.

A43

The leucocyte profile is similar in hepatic venous blood and central venous blood with increased leucocytes, neutrophils and monocytes in both compartments in non-alcoholic steatohepatitis patients with high histological inflammatory activity.

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Introduction: The exact pathophysiological mechanism of non-alcoholic steatohepatitis (NASH) is not yet fully unravelled. Recently, a role of regulatory T-cells (Treg) has been suggested. Previous studies have shown that cytokines might differ when measured in the hepatic venous system compared to the systemic circulation, but studies comparing leucocytes between these compartments are lacking.
Aim: Our first aim was to study the difference in white blood cells [leucocytes (Leu) neutrophils (Neu), lymfocytes (Lym), monocytes (Mono), eosinophils (Eos), basophils (Baso), B-cells, T-cells, helper T cells (Th), cytotoxic T cells (Tc) and Treg] in hepatic venous (HV) and in central venous blood (CV). The second aim was to evaluate the correlation of these cells in both blood compartments.
Methods: HV and CV blood was sampled through transjugular catheterization in suspected NASH patients. Liver biopsy was evaluated using the SAF score (FLIP algorithm). Leucocyte formula was determined through myeloperoxidase staining (Advia 2120i Siemens). Flow cytometry (FC) was performed to further differentiate lymfocytes with Tregs being defined as CD4+CD25+CD127-.
Results: These are the preliminary results of 8 patients who had leucocyte formula testing, of which 6 also had FC testing. Patients were divided histologically into low activity (LAct: SAF = 1, N=3) and high activity (HAct, SAF ≥2, N=5) groups. There was a significant difference ($p<0.05$) between the LAct and the HAct group for CV Leu (4.8±0.9 vs 7.0±2.7), CV Neu (3.0±0.8 vs 3.9±1.0), CV Mono (0.25±0.05 vs 0.36±0.11), HV Leu (4.8±1.0 vs 6.9±2.5), HV Neu (2.9±0.9 vs 3.9±1.0) and HV Mono (0.24±0.03 vs 0.38±0.13), respectively. There was no significant difference between the LAct and the HAct group for Lymf, Eos, Baso, B-cells, T-cells, Th, Tc, Treg in HV and CV blood ($p>0.1$). The power to exclude a significant difference was not yet reached with this patient population. Overall, there was a significant correlation ($p<0.05$) between HV and CV blood for Leu ($rs=0.958$), Neu ($rs=0.976$), Lym ($rs=0.992$), Mono ($rs=0.970$), Eos ($rs=0.976$), Baso ($rs=0.803$), B-cells ($rs=0.886$), T-cells ($rs=0.829$), Th ($rs=0.943$) and Tc ($rs=0.943$). Correlation almost reached statistical significance for Treg ($r=0.785$, $p=0.064$).
Conclusions: Leu, Neu, Lym, Mono, Eos, Baso, B-, T-, Th, Tc and Treg cells showed a very strong correlation between hepatic venous and central venous blood. This suggests that easily obtained systemic blood can be used as a representation of hepatic venous blood when evaluating these white blood cells. This study also showed a significant increase in CV and HV Leu, Neu and Mono in the NASH group with high histological inflammatory activity compared to the group with low activity.

GIREM

B01

Is there evidence for functional 5-hydroxytryptamine 4 (5-HT4) receptors in the equine jejunum?

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Introduction: Colic is still the most important cause of death in horses. Surgical interventions that are needed to resolve problems causing equine colic can be accompanied by important postoperative complications such as ileus, especially in case of surgical intervention at the level of the small intestine. Postoperative ileus has a high fatality rate in horses, explaining the research efforts that are performed to identify potential pharmacological agents to mitigate this problem. In man, selective 5-HT4 receptor agonists such as prucalopride are used as prokinetics, since activation of 5-HT4 receptors on human intestinal cholinergic neurons facilitates acetylcholine release. 5-HT4 receptors, linked to adenylyl cyclase, act via generation of cAMP. None of the 4 in vitro studies on the effect of 5-HT in equine intestine provided evidence for neuronal 5-HT4 receptors, but none used the protocol as described in human studies [1-4].
Aim: Our aim was to investigate whether functional 5-HT4 receptors are present in the equine small intestine.
Methods: In vitro organ bath set up with longitudinal and circular smooth muscle strips of equine mid-jejunum. Electrical field stimulation (EFS) was applied with trains of 10 s at 4 Hz, 0.5 ms and 4 to 20V; the interval between trains was 5 min.
Results: were similar in both muscle layers. In the presence of 0.3 mM NG-Nitro-L-arginine methyl ester and 0.3 μM apamin, excluding effects of the inhibitory transmitters NO and ATP, EFS induced voltage-dependent on-contractions; these were neurogenic as they were abolished by 3 μM tetrodotoxin. At a voltage inducing 50 % of the maximal amplitude, the submaximal EFS-induced contractions were cholinergic as atropine (1 μM) abolished them. Prucalopride (0.3 and 3 μM) did not increase the amplitude of these submaximal EFS-induced contractions. Even in the presence of the nonselective phosphodiesterase inhibitor IBMX, previously shown to enhance the effect of neuronal 5-HT4 receptors by inhibiting breakdown of their 2nd messenger cAMP [5], prucalopride (3 μM) had no influence. Also 5-HT (10 μM), a full agonist at 5-HT4 receptors, tested in the presence of methysergide and granisetron to exclude interaction with other 5-HT receptor subtypes, did not enhance EFS-induced submaximal contractions.
Conclusions: There is no evidence for the presence of 5-HT4 receptors on the cholinergic neurons of the equine small intestine. These results question the application of 5-HT4 prokinetic drugs in horses References: [1]. Weiss et al. 2002, [2]. Nieto et al. 2000, [3]. Delesalle et al. 2006, [4]. Prause et al. 2010, [5]. Priem et al. 2012.

B02

The signaling pathway of 5-HT4 receptors in cholinergic neurons of human colon is controlled by phosphodiesterases

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Introduction: In the canine, porcine and human gastrointestinal tract, activation of 5-HT4 receptors on cholinergic neurons innervating smooth muscle cells facilitates the ongoing acetylcholine release, leading to a sustained increase of smooth muscle contractions. The intraneuronal signaling pathway of these adenylyl cyclase linked 5-HT4 receptors is controlled by phosphodiesterase (PDE) 4 in porcine stomach and colon.

Aim: This study aimed to investigate whether PDEs also control the signaling pathway of 5-HT4 receptors in cholinergic neurons of human colon circular smooth muscle.
Methods: With the approval of the Ethics Committee of Ghent University Hospital, circular smooth muscle strips from human colon (mucosa removed) were loaded with [3H]-choline chloride. Electrical field stimulation (EFS)-induced tritium outflow, as a measure for acetylcholine release, was determined. Two stimulation trains (2 min, 4 Hz, 1 ms, 15 V) were applied at 60 min interval (S1 and S2). Prucalopride (selective 5-HT4 receptor agonist), 3-isobutyl-1-methylxanthine (IBMX; non-selective PDE inhibitor) and rolipram (selective PDE 4 inhibitor) were added before S2. The ratio of acetylcholine release by S2 compared to that by S1 was calculated; results are expressed as mean S2/S1 ratio for n = 7 to 11 strips. Results were compared by ANOVA followed by Bonferroni corrected t-test.
Results: Prucalopride (0.3 µM) significantly enhanced EFS-induced acetylcholine release (S2/S1 from 0.76 in control strips to 0.95; t-test: $p < 0.05$). A tenfold lower concentration of prucalopride (0.03 µM) still significantly enhanced EFS-evoked acetylcholine release (S2/S1 from 0.60 to 0.88; $p < 0.001$). Although not reaching significance, 10 µM IBMX tended to increase the facilitating effect of 0.03 µM prucalopride (S2/S1 0.98); this concentration of IBMX per se did not significantly influence EFS-induced acetylcholine release. The facilitating effect of 0.01 µM prucalopride on acetylcholine release was mild (S2/S1 from 0.74 to 0.85; non-significant). However, in the presence of 10 µM IBMX this effect was significantly enhanced (S2/S1 1.07; $p < 0.05$ versus prucalopride alone; $p < 0.001$ versus controls). 1 µM rolipram per se did not affect the EFS-induced acetylcholine release, but significantly enhanced the facilitating effect of 0.01 µM prucalopride: 0.72 for controls; 0.72 for 1 µM rolipram; 0.78 for 0.01 µM prucalopride and 0.94 for 0.01 µM prucalopride in the presence of 1 µM rolipram ($p < 0.05$ versus prucalopride alone; $p < 0.001$ versus controls).
Conclusions: Also in human colon, the 5-HT4 receptor pathway in cholinergic neurons is controlled by PDEs with a clear-cut role for PDE 4. Further investigation with selective PDE 1, 2 and 3 inhibitors (vinpocetine, EHNA and cilostamide) is currently in progress to exclude the involvement of other PDE subtypes.

B03

Dynamics of postnatal ICC proliferation: investigation in KitCreERT2: R26-confetti mouse gut

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Introduction: Interstitial cells of Cajal (ICC) are mesenchymal cells present in the gastrointestinal tract (GI) musculature which contribute to the regulation of GI motility. The tyrosine kinase receptor Kit is the reference ICC marker and is required for ICC embryonic development and maintenance after birth. Despite several studies, postnatal proliferation of ICC remains poorly documented. Here, we used the transgenic KitCreERT2 (Klein et al., 2013) and R26-Confetti (Snippert et al., 2010) mouse model to unravel the clonal expansion of individual ICC tagged by stochastic induction of the expression of one of the four genetically encoded fluorescent proteins CFP, GFP, YFP and RFP.
Aim: To unravel the dynamic of ICC proliferation along the postnatal and adult mouse gut.
Methods: Neonates (P0) KitCreERT2: R26-confetti were induced with tamoxifen 1x5 mg via the lactating mother. Antrum, small intestine and colon of induced neonates and adults were harvested after 1 month, 3 months and 6 months respectively, fixed in PAF 4% overnight then

cleared using the CUBIC clarification protocol (Susaki et al., 2014). Flat mounts were imaged in spectral (lambda) mode on a Zeiss LSM780 confocal microscope equipped with a C-Achroplan 32X/0.85 W objective and a multiphoton laser (Ex. 920nm). After linear unmixing, images were analysed using Imaris for 3D reconstruction and with CognitionMaster and ImageJ for cell clustering and quantification.
Results: ICCs tagged with CFP, GFP, YFP and RFP, respectively, were observed in the muscularis propria of antrum, small intestine and colon, in all known locations for ICC, although more frequently around the myenteric plexus (ICC-MP). Quantification of ICC-MP in 1 month, 3 months and 6 months gut after neonatal induction revealed small clusters of 2-3 cells only, rarely 4 cells, in antrum and colon. In small intestine, only clusters of 2 cells were seldom detected at each time points. In adult induced mice also, only clusters of 2 cells were seldom observed.
Conclusions: The ICC turnover appears to be surprisingly low in postnatal and adult mouse gut as only clusters of 2-4 cells were mainly found at all ages studied. ICC seems to proliferate (slightly) more in antrum and colon compared to small intestine. These preliminary results suggest that ICC merely proliferate after birth, and mainly during a brief period. Further experiments with earlier induction, at embryonic stages, are now underway to complete the study.

B06

Farnesoid X receptor activation attenuates intestinal ischemia reperfusion injury

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Introduction: Intestinal ischemia, both occlusive (e.g. bowel infarction, strangulation) as non-occlusive (e.g. severe hypotension, shock), is a frequent and clinically devastating condition characterized by high morbidity and mortality. Reperfusion exacerbates the deleterious effect of ischemia, enhancing oxidative stress, activating innate immunity, inflammation and cell death. Compared to other organs, intestinal ischemia reperfusion injury (IRI) is uniquely aggressive, as damage to the mucosal barrier instigates bacterial translocation, sepsis, multiple organ failure and eventually death. Farnesoid X receptor (FXR), member of the nuclear receptor family, is abundantly expressed in the ileum, regulating intestinal innate immunity and homeostasis. In murine models of inflammatory bowel disease and liver disease, treatment with an FXR-agonist decreased pro-inflammatory cytokine expression (NF-κB pathway) and improved epithelial permeability. We hypothesized that activation of FXR would have the same effect in an acute model of intestinal injury.
Aim: For the first time, we investigated whether FXR activation -by obeticholic acid (OCA)- could overcome loss of gut barrier function, suppress inflammation and prevent death in a rodent model of intestinal IRI.

Methods: In a rat model of intestinal IRI (laparotomy + temporary mesenteric artery clamping), 3 conditions were tested (n=16/group): laparotomy only (sham group); ischemia 60min+ reperfusion 60min + vehicle pretreatment (IR group); ischemia 60min + reperfusion 60min + OCA pretreatment (IR+OCA group). Vehicle or OCA (INT-747, 2*30mg/kg) was administered by gavage 24h and 4h prior to IRI. The following end-points were analyzed: 7-day survival; biomarkers of enterocyte viability (L-lactate, I-FABP); histology (morphologic injury to villi/crypts and villus length); intestinal permeability (Ussing chamber); endotoxin translocation (Lipopolysaccharide assay); inflammatory cytokines (IL-6, IL-1-β, TNFα, IFN-γ IL-10, IL-13); apoptosis (cleaved caspase-3); and autophagy (LC3, p62).
Results: IRI was associated with high mortality (90%); loss of intestinal integrity (structurally and functionally); increased endotoxin translocation and pro-inflammatory cytokine production; and inhibition of autophagy. Conversely, OCA-pretreatment improved 7-day survival up to 50% which was associated with prevention of epithelial injury, preserved intestinal architecture and permeability. Additionally, FXR-agonism led to decreased pro-inflammatory cytokine release and alleviated autophagy inhibition.
Conclusions: Pretreatment with OCA, an FXR-agonist, improves survival in a rodent model of intestinal IRI, preserves the gut barrier function and suppresses inflammation. These results turn FXR into a promising target for various conditions associated with intestinal ischemia.

B07

Interaction between enteric glia and myeloid cells as critical players in intestinal immune homeostasis.

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Introduction: In the gastrointestinal tract balance between immune activation and tolerance is essential to maintain intestinal homoeostasis. Recently we have demonstrated that activation of the enteric nervous system (ENS) has potent anti-inflammatory effect on macrophages (MFs) and dendritic cells (DCs) via the release of acetylcholine.
Aim: In the current study, we aim to investigate if also enteric glial cells (EGCs), the mayor cellular constituent of the ENS, may have modulatory effects directly on intestinal immune cells and play a role in preserving intestinal immune homeostasis.
Methods: Immune labeling with specific antibodies was performed to study the interaction between EGCs and intestinal immune cells within the muscularis externa and in the lamina propria. To study the possible anti-inflammatory effect of EGC-released factors, EGCs were isolated from the muscularis externa of wild-type mice and cultured with bone marrow derived MFs or DCs. After co-culture with EGCs or with the glia-derived neurotrophic factor (GDNF), the phenotype of MFs and DCs was analyzed by gene expression. In some experiments, to analyze the participation of enteric glia in vivo during intestinal inflammation, EGCs were FACS sorted from control or mice exposed to 5 days of dextran sodium sulfate (DSS) and gene expression was analyzed.
Results: Anatomical analysis with confocal microscopy revealed that EGCs are in close contact with intestinal immune cells such as MFs and DCs both in the muscularis externa and in the lamina propria. Interestingly, glial-secreted molecules were able to decrease expression of pro-inflammatory cytokine IL-12 in both MFs and DCs after LPS stimulation, while anti-

inflammatory cytokine IL-10 was increased. In addition, typical M2 anti-inflammatory markers such as MRC-1, Lyve-1 and Stab-1 were increased in MFs stimulated with EGC supernatant or GDNF. In vivo after DSS treatment, EGCs express high level of CX3CR1L, a chemokine that typically attracts CX3CR1+ monocytes and genes involved in the synthesis of the tolerogenic molecule retinoic acid (i.e. RALDH3).
Conclusions: In the current study we provide anatomical, in vitro and in vivo evidences suggesting that EGCs exert immunomodulatory effects on intestinal antigen presenting cells. We are currently testing the hypothesis that during inflammation the enteric glia would be able to attract and influence monocytes inducing a tolerogenic phenotype in these cells. Taken together, our data indicate that interaction between enteric glia and the intestinal immune system might be crucial to maintain intestinal immune homeostasis and prevent intestinal immune-mediated diseases such as IBD.

B08

Electrical stimulation of the abdominal vagus nerve is as effective as cervical nerve stimulation in reducing postoperative ileus

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Introduction: Electrical stimulation of the cervical vagus nerve (VNS) prevents postoperative ileus (POI) in mice. This approach is however not attractive to treat POI in human since it would require an additional surgical intervention in the neck. As the vagus nerve is easily accessible at its entrance of the abdominal cavity, we explored electrical stimulation of the abdominal vagus nerve as an alternative approach to treat POI.
Aim: Exploration of electrical stimulation of the abdominal vagus nerve as an alternative approach to treat POI
Methods: The anti-inflammatory effect of abdominal VNS (5 min, 10 Hz, 1 ms, 1mA) was first studied in a murine model of lipopolysaccharide (LPS)-induced sepsis. Five minutes after VNS (anterior (A), posterior (P) or A+P vagal branch), LPS (10 mg/kg) was injected i.p. The mice were sacrificed after 2 hours to collect blood for quantification of serum TNF-α levels. In a second set of experiments, the effect of abdominal VNS was studied on intestinal manipulation-induced POI. The degree of POI was quantified 24 hours postoperatively by assessment of intestinal transit of fluorescent dextran expressed as geometric center (GC). The anti-inflammatory effect was measured by the influx of myeloperoxidase (MPO) positive cells. For both sets of experiments, cervical VNS was also performed as a positive control.
Results: A, P and A+P abdominal VNS (366 ±33 pg/ml, 422 ±88 pg/ml and 407±138 pg/ml respectively, $p<0.05$, n=6-8 mice/group) significantly reduced serum TNFa levels compared to sham stimulation (822±105 pg/ml, n=9). Of note, this effect was comparable to that of cervical VNS (sham: 1812±406 vs cervical VNS: 321±172 pg/ml; $p<0.05$, n=4-6 mice/group). Next, we showed that VNS of the anterior branch significantly improved intestinal transit compared to sham stimulation (GC: 7.8±0.6 vs 5.0±0.2 respectively $p<0.01$, n=12 mice/group), an effect that was comparable to that of cervical VNS (GC: sham: 5.1±0.4 vs cervical VNS: 7.2±0.6; $p<0.05$,

n=8 mice/group). Finally, both cervical (61 ±11 vs sham: 96±11 MPO+ cells/field; $p<0.05$) and abdominal VNS (34±7 vs sham: 71±11 MPO+ cells/field; $p=0.016$) reduced inflammation in response to intestinal manipulation.
Conclusions: Similar to cervical VNS, abdominal VNS significantly reduced serum TNF-α levels during sepsis and improved intestinal transit in our model of POI. These data indicate that peroperative abdominal VNS is an effective alternative for cervical VNS as treatment of POI in human.

B10

Role of nutrient sensing mechanisms in the effects of Roux-en-Y gastric bypass surgery on energy-and glucose homeostasis

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Introduction: Alterations in gut hormone release after Roux-en-Y gastric bypass surgery (RYGB) are hypothesized to contribute to the metabolic improvements after surgery. Taste receptors coupled to the gustatory G-protein, gustducin, on endocrine cells sense the nutrients in the gut and influence gut hormone secretion. During RYGB, nutrients are rerouted to more distal regions of the gut.
Aim: This study aimed to investigate how the new digestive route after RYGB surgery changes the nutrient sensing mechanisms in all regions of the gut which may contribute to the improvements in glucose –and energy homeostasis.
Methods: Wild type (WT) and a-gustducin-/- (a-gust -/-) mice were put on a high-fat high-sucrose diet (HFHSD) for 12 weeks before undergoing sham- or RYGB surgery. Mice were sacrificed 6 weeks after surgery, 15 min after a nutridrink® gavage.
Results: RYGB surgery induced a genotype-dependent decrease in body weight, which was more pronounced in WT mice. This decrease in body weight was accompanied by a decrease in food intake in WT but not a-gust-/- mice and correlated with an increase in plasma ghrelin levels in a-gust-/- but not in WT mice. RYGB induced a genotype-independent increase in plasma GLP-1 which was accompanied by an increased jejunal L-cell count in WT but not a-gust-/- mice. Furthermore RYGB induced a genotype-dependent increase in plasma PYY levels which was more pronounced in a-gust-/- mice. These changes were not accompanied by differences in mRNA levels of the sweet taste/umami receptor subunit TAS1R3 in the jejunum of both genotypes. Nevertheless, RYGB surgery increased mRNA levels of the di-/tripeptidesensor LPAR5 in a-gust-/- mice. RYGB surgery improved glucose tolerance in WT mice. a-gust-/- mice were protected against the diabetogenic effect of the HFHSD, since 12 weeks HFHSD did not induce glucose intolerance in sham operated a-gust-/- mice. RYGB surgery induced changes in bacterial fermentation in the caecum of a-gust-/- mice, which showed increased caecal butyrate and propionate levels compared to WT mice. This resulted in a decrease in colonic free fatty acid receptor 2 (FFAR2) mRNA levels in a-gust-/- mice, while RYGB surgery reduced FFAR3 mRNA levels in both genotypes. RYGB improved colonic permeability independent of the genotype.

Conclusions: Impairment of the gustatory signaling pathway during RYGB surgery affects the expression of taste receptors in the gut.

B11

Analysis of enteric neuronal circuits in different regions of the colon using live calcium imaging

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Introduction: Different regions of the gut have different functions, and therefore, exhibit specific motility patterns. It is well established that the enteric nervous system (ENS) is responsible for the control of gastrointestinal motility. However, currently little is known about how the enteric circuitry is organized to generate these various different gut motility patterns.

Aim: (i) To explore whether live imaging of enteric neurons can uncover differences in ENS circuitry in the proximal and distal colon; (ii) To investigate when different ENS circuits in the large intestine become established during development.

Methods: Ca2+ imaging was performed on myenteric plexus preparations of the proximal and distal colon of Wnt1-Cre; Rosa26-GCaMP3 adult and postnatal day (P)7 mice. Neurons were stimulated by the application of electrical trains using a focal electrode placed on interganglionic fiber tracts. Hexamethonium (an antagonist of nicotinic receptors) was applied to inhibit cholinergic neurotransmission. To investigate differences in the proportion of excitatory and inhibitory enteric neurons, immunohistochemistry was performed against the pan-neuronal marker, HuCD, ChAT (a marker of excitatory neurons) and nNOS (a marker of inhibitory neurons).

Results: Using a low magnification (5X) lens, we were able to record the intracellular Ca2+ changes from a large population of neurons in many ganglia simultaneously (up to 600 µm x 600 µm field of view). In adult tissue, hexamethonium reduced the electrically-evoked intracellular Ca2+ transients in the majority of neurons in comparison to time controls, both in the proximal and distal colon. In some neurons, hexamethonium completely blocked the Ca2+ transient, and this occurred in a larger number of cells in the distal compared to the proximal colon (34.4 ± 11% vs 15.4 ± 6%, $p < 0.05$, two-way ANOVA). Although there appears to be a greater component of cholinergic transmission in the distal colon, the proportion of ChAT-immunoreactive neurons was not different in the proximal vs distal colon, instead, the proportion of nNOS+ neurons was higher in the distal colon. In the postnatal (P7) colon, cells and ganglia were more densely packed, and a larger proportion of cells responded to electrical stimulation compared to adult tissue. Hexamethonium also reduced Ca2+ responses at P7 and this reduction was more pronounced in the distal colon than the proximal colon.

Conclusions: Our findings indicate that there are very subtle differences in the neuronal circuitry of different regions of the colon, as there appears to be greater sensitivity to the inhibition of cholinergic signalling in the distal colon, especially at P7. How this changes during development and how this can impact on differences in colonic motility is subject of our future experiments.

B12

Heteromeric interaction between the human Mas-related G protein-coupled receptors E and F revealed by bioluminescence and FRET-analysis.

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Introduction: G protein-coupled receptors (GPCR's) are leading targets in drug discovery because of their extensive regulatory functions and profound molecular diversity. One decade ago, a new family of GPCR's, referred to as Mas-related G protein-coupled receptors (MRGPR), was discovered and since then shown to be involved in nociceptive sensory perception and mast cell physiology. MRGPR are thus promising targets in drug discovery and potential actors in neuro-immune communication. In the gastrointestinal tract, harboring its own complex innervation and at the same time comprising the largest immune system in our body, MRGPR show marked expression plasticity during inflammatory conditions. In line with their hypothesized role in neuro-immune interactions, these receptors have mainly been located in enteric neurons and mucosal mast cells. Moreover, three MRGPR members, namely MRGPRD, MRGPRE and MRGPRF, are co-expressed in a significant proportion of enteric neurons.
Aim: Given the known concept of GPCR heteromerization, and its effects on receptor functioning, we have set out to investigate heteromerization between the human orthologues of MRGPRD, MRGPRE and MRGPRF.
Methods: A novel luciferase complementation-based platform was used to efficiently screen for interacting MRGPR pairs. Cells were transiently co-transfected with two plasmid constructs, each encoding a MRGPR member coupled to one half of a bioluminescent enzyme. Interaction between receptors of interest was identified through complementation-induced bioluminescence. Further validation of interacting partners was performed with Förster Resonance Energy Transfer (FRET). Acceptor photobleaching was used to determine FRET efficiencies in cells co-expressing eCFP-coupled and eYFP-coupled receptors. For MRGPRD plasmids, receptor functionality was tested by verifying the known MRGPRD-mediated inhibition of KCNQ2-potassium currents in whole-cell patch clamp experiments.
Results: Transient transfections with fluorescently tagged vectors constructs confirmed membrane expression of the plasmid protein product. Moreover, ligand-induced activation of tagged MRGPRD resulted in reversible inhibition of KCNQ2-mediated potassium currents, indicating functionality of C-terminally tagged MRGPR. Interaction screening with luciferase complementation provided clear evidence for heteromeric interactions between MRGPRE and MRGPRF. FRET analysis on transiently co-transfected cells confirmed the MRGPRE-MRGPRF interaction.
Conclusions: This study provides novel insights on the interactions between human MRGPR. The observed MRGPRE-MRGPRF interaction contributes to the further elucidation of the function of these orphan receptors, and suggests the relevance of heteromeric interactions in their receptor biology. Furthermore, we provide a proof-of-concept for the applicability of a new complementation-based screening assay as a high-throughput screening tool for protein-protein interactions.

B13

The effect of anti-interleukin-6 antibodies on gastrointestinal motility, colonic inflammation and mucosal barrier function during in a mouse model of caecal ligation and puncture induced septic ileus.

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Introduction: Sepsis remains a leading cause of mortality in Intensive Care Units. Ileus, the impaired propulsive motility of the gastrointestinal (GI) tract, and mucosal barrier dysfunction will maintain sepsis by the occurrence of bacterial translocation. Previously, we demonstrated that IL6 levels are significantly increased systemically as well as in the colonic wall in our polymicrobial sepsis model (Nullens et al, Shock 2015).

Aim: We therefore aimed to study the effect of therapeutic or curative administration of anti-IL6 antibodies on overall GI motility, colonic permeability and translocation of intestinal bacteria in blood and mesenteric lymph nodes (MLN) in a septic mouse model.

Methods: Sepsis was induced in OF-1 mice by cecal ligation and puncture (CLP). Sham-operated animals served as controls. Mice received either 1 mg/kg of anti-IL6 or an IgG isotype control (IgG) i.p. together (exp1) with or 24h following CLP (exp2). The presence of ileus was confirmed by studying the geometric center (GC) of beads 48h after CLP or sham. In the permeability study, mice were anesthetized 48h following CLP and colonic permeability was studied with the Evans blue (EB) method. RT-PCR was performed on colons to determine mRNA levels of cytokines and tight junction proteins. Serum samples were analyzed for the proinflammatory cytokine IL-6. Blood and homogenized MLN were plated onto agar plates and cultured for 24h to quantify bacterial translocation.

Results: In the preventive exp1, CLP-induced sepsis significantly prolonged GI transit time which was prevented by anti-IL6 treatment (GC: sham+IgG 5.4±0.4, CLP+IgG 2.6±0.2*, CLP+anti-IL6 4.3±0.5). The serum levels of IL-6 rose significantly after CLP and anti-IL6 significantly reduced these (sham+IgG 4.6±2.5, CLP+IgG 276.6±71.6*, CLP+anti-IL6 84.5±18.3 pg/mL). CLP-sepsis increased colonic permeability and anti-IL6 was able to prevent the impaired barrier function (EB: sham+IgG 22.6±1.7, CLP+IgG 52.9±10.4*, CLP+anti-IL6 27.8±5.5 µg/100 mg colon). Sepsis significantly increased the mRNA expression of claudin-1 and anti-IL6 treatment prevented this (relative mRNA-expression with the sham+IgG group as calibrator: sham+IgG 1.44±0.46, CLP+IgG 3.04±0.86*, CLP+anti-IL6 2.21 ± 0.37). Anti-IL6 however was not able to reduce the number of positive cultures from the blood (CLP+IgG 77.8%, CLP+anti-IL6 55.6% positive, p=NS) or MLN (CLP+IgG 100%, CLP+anti-IL6 100% positive). In the curative exp2, anti-IL6 numerically ameliorated the impaired GI transit (GC: sham+IgG 4.6±0.5, CLP+IgG 2.76±0.5, CLP+anti-IL6 3.6±0.7) and significantly decreased serum IL6 levels (sham+IgG 23.5±4.5, CLP+IgG 855.2±462.4*, CLP+anti-IL6 54.7±5.5 pg/mL). The permeability results however were less pronounced when compared to exp1.

Conclusions: CLP resulted in septic ileus with an increased colonic permeability and increased risk of bacterial translocation. Anti-IL6 was able to ameliorate GI motility, suppress inflammation and normalize the permeability of the colonic wall, with the preventive administration being more efficacious than the curative one.

B14

The serine protease inhibitors FUT-175 and SPIx reduce visceral hypersensitivity in a post-inflammatory rat model for irritable bowel syndrome.

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Introduction: Visceral hypersensitivity is an important factor contributing to abdominal pain in irritable bowel syndrome (IBS). Serine proteases have been suggested to play a key role in the pathogenesis of visceral hypersensitivity, but their exact role is currently insufficiently elaborated.

Aim: The aim of this study was to investigate the effect of two serine protease inhibitors in a post-inflammatory rat model for visceral hypersensitivity (PI-IBS): FUT-175 (nafamostat mesylate, a marketed broad-spectrum serine protease inhibitor) and SPIx (a tryptase/matriptase inhibitor, recently developed at our university).

Methods: At day 0, colitis was induced in male Sprague-Dawley rats by the intrarectal administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS); controls received 0.9% NaCl. At day 3, a colonoscopy was performed to confirm the presence of colitis and then repeated every 4 days, from day 10 onwards, to follow up mucosal healing. Three days after complete resolution of colitis, visceral sensitivity was determined using visceromotor responses (VMRs) to colorectal distension (10-60mmHg, 20s, 4min interval); expressed as total area under the curve (AUC; µV/20s). The serine protease inhibitor FUT-175 (0.1-1mg/kg; exp 1) or SPIx (0.01-0.1-1mg/kg; exp 2) or vehicle was injected intraperitoneally (ip), 30 min prior to the VMR experiment. Finally, the inflammatory parameters (colonoscopy, macroscopy, microscopy and myeloperoxidase activity) were scored to verify the post-inflammatory status at the time of the VMR.

Results: At day 3, TNBS rats displayed a mild colitis, which was completely resolved at day 10-18. The post-inflammatory status of the TNBS group at the day of the VMR was confirmed for all animals in both experimental setups. All vehicle-treated post-colitis rats showed significantly higher VMRs compared to controls, indicating visceral hypersensitivity (Exp 1. total AUC: 2465±181 vs 1036±116µV; n=7; p<0,001; Exp 2. total AUC 2539±220 vs 1088±229µV; n=7-9; p<0,01). FUT-175 significantly reduced VMRs in a dose of 0.1 mg/kg (total AUC: 1413±139 vs 2465±181; n=5-7; p<0,001) and in a dose of 1 mg/kg (total AUC: 1731±325 vs 2465±181; n=6-7; p<0,05). SPIx also significantly decreased visceral hypersensitivity with a clear dose-dependent effect: 0.01 mg/kg SPIx had no significant effect (total AUC 1756±317 vs 2539±220 µV; n= 9; ns), whereas 0.1 mg/kg significantly reduced the VMRs (total AUC 1429±228 vs 2539±220 µV; n= 7-9; p<0,01) and 1 mg/kg SPIx completely reversed colitis-induced visceral hypersensitivity (total AUC 991±203 vs 2539±220 µV; n=7-9; p<0,001). The effective doses of FUT-175 and SPIx had no effect on visceral sensitivity in control animals.

Conclusions: Our results indicate that serine proteases could be a potential new target in the search for novel treatments for abdominal pain in post-inflammatory IBS patients. However, complete reversal of the hypersensitivity was only accomplished with the newly developed tryptase/matriptase inhibitor SPIx, highlighting the importance of the inhibitor specificity.

Colonic high-resolution manometry (HRM) evaluation of responses to a meal and to bisacodyl in adults with laxative-refractory slow-transit idiopathic constipation reveals differences between painful and non-painful constipation groups.

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Introduction: In clinical practice colonic manometry is recommended to exclude colonic inertia (no response to meal and to drug stimulation, i.e. bisacodyl) in patients with slow transit constipation not responsive to laxatives (Bharucha 2013).

Aim: As to date, this has not been assessed by colonic HRM in adults, our aim was to evaluate the colonic motor response to a meal and to bisacodyl in patients with slow-transit constipation not responsive to laxatives.

Methods: Consecutive patients with slow-transit and Rome III constipation resistant to laxatives were enrolled. After an overnight fast and tap water enema preparation, during colonoscopy under conscious sedation, an HRM catheter (40 sensors, 2.5 cm spaced) and an infusion tube were advanced as far as possible (caecum) and clipped to the mucosa. Colonic pressures were recorded for three hours before and two after a standardized meal, and for one hour after intra-colonic administration of bisacodyl (10 mg). Number of pan-colonic pressurizations (defined by Corsetti 2015) and of low-amplitude (LAPSs, defined by Dinning 2014) and high-amplitude propagating sequences (HAPSs, defined by De Schryver 2002) were evaluated. A normal response to bisacodyl was identified by the occurrence of at least one HAPS. Data (mean±SD) were compared with those obtained in 10 healthy subjects (HS) (30±11 years, 5 females).

Results: A total of 17 refractory slow-transit constipation patients (43±13 years, 15 females) were studied; 9 of these also referred the presence of pain or discomfort. The total number of pan-colonic pressurizations was significantly lower in patients as a group and in patients without pain as compared to HS (respectively, 32±35 and 9±19 vs 89±40, all $p<0.001$), while it did not differ in patients with pain (53±35). The total number of LAPSs did not differ in patients as a group (25±29) and in patients with pain (40±30), but was significantly lower in patients without pain as compared to HS (6±10 vs 52±39, $p=0.009$). Pan-colonic pressurizations significantly increased after a meal in HS and in patients with ($p<0.02$) but not in those without pain ($p=0.49$). Retrograde LAPSs increased significantly after the meal in HS ($p=0.01$) but not in patients, regardless of the presence of pain (all $p>0.30$). The response to bisacodyl was normal in 8/9 (88%) patients with pain and in 2/8 (25%) of those without pain ($p=0.01$, Fisher's test). Abnormal responses to bisacodyl included absence of contractions, repetitive pan- or distal-colonic pressurizations or atypical HAPSs.

Conclusions: Approximately 50% of patients with laxative-refractory slow-transit constipation also present with abdominal pain. Compared to slow transit patients without pain, these patients have a partially preserved response to meal (increased pan-colonic pressurizations but not LAPSs) and a higher prevalence of a normal response to bisacodyl.

B16

Staphylococcal enterotoxin B triggers a bystander immune response to food antigens leading to visceral hypersensitivity

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Introduction: Bacterial gastroenteritis is a well characterized risk factor to develop irritable bowel syndrome. The mechanism underlying the development of post-infectious irritable bowel syndrome and visceral hypersensitivity (VHS) remains however largely unknown. Previously, we showed that a gastrointestinal infection with Citrobacter rodentium triggers an aberrant immune response to an innocent bystander antigen (ovalbumin, OVA) resulting in mast cell activation and subsequent VHS upon re-exposure to OVA.
Aim: We hypothesize that not only an active infection but also bacterial products, such as superantigens, are able to trigger such an aberrant immune response leading to activation of mast cells and VHS upon re-exposure to the respective antigen. Irritable bowel syndrome features such as VHS and colonic permeability were studied upon re-exposure to OVA in Balb/c mice.
Methods: Three groups of Balb/c mice were studied (n=8-10/group). Group 1 and 2 received Staphylococcal enterotoxin B (SEB) in the absence (SEB/PBS) or presence (SEB/OVA) of OVA, respectively, during three consecutive days. Group 3 only received OVA dissolved in PBS (PBS/OVA). 5 weeks later, all groups received OVA orally every other day. After the 8th OVA re-exposure, group 2 was randomized to one week treatment with either doxantrazole (mast cell stabilizer) or vehicle. Visceral pain was assessed by recording of the visceromotor response to colorectal distension using abdominal muscle electromyography before OVA re-exposure and after 4, 8 and 12 OVA challenges. VHS was considered when the area under the curve of the responses (normalized to maximum pain at baseline) was >4.56 (=95th percentile). Thereafter, mice were sacrificed and the colonic permeability was studied in Ussing chambers. Ear swelling was assessed after injection of OVA or saline in the ear to exclude the development of allergy.
Results: Re-exposure to OVA (at t=5 weeks) did not affect the visceromotor response to colorectal distention in mice that received SEB/PBS or PBS/OVA. In contrast, all mice that received OVA and SEB developed VHS upon re-exposed to OVA. Of note, VHS was reversed in 75% of the doxantrazole compared to 0% in the vehicle treated mice. Moreover, OVA re-exposure increased colonic permeability in SEB/OVA mice compared to SEB/PBS and PBS/OVA mice, an effect that was reversed by doxantrazole. OVA injection did not cause ear swelling in any group (data not shown).
Conclusions: Similar to a bacterial infection, SEB induces an aberrant immune response to innocent bystander antigens, leading to mast cell-mediated VHS and increased mucosal permeability upon re-exposure to the respective antigens. Based on this data, we propose that superantigens, either from microbiota in the nasal cavity or the intestine, may be involved in the pathogenesis of irritable bowel syndrome. Future studies evaluating the presence of these superantigens in patients are therefore warranted.

B17

Neuronal sensitization of TRPV1 by histamine mediated by histamine 1 receptor in an unique cohort of PI-IBS patients.

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Introduction: Three to 36% of individuals develop post-infectious irritable bowel syndrome (PI-IBS) following an episode of bacterial gastroenteritis. Although microscopic inflammation has been proposed as potential pathophysiological mechanism underlying PI-IBS, we recently failed to confirm this hypothesis. In contrast, we identified sensitization of the nociceptor TRPV1 by histamine to be involved in IBS. To what extent this mechanism is also involved in PI-IBS remains however unclear. In December 2010 two Belgian villages suffered from an outbreak of infectious gastroenteritis due to contaminated tapwater. 20% of infected individuals developed PI-IBS.
Aim: In the present study, we invited PI-IBS patients of this cohort to further evaluate the mechanisms underlying PI-IBS.
Methods: Rectal biopsies were taken from 8 PI-IBS patients (1M, med age: 53 yrs IQR [35-54]) and 7 healthy volunteers (HV) (5M, med age: 45 yrs IQR [25-50]). The sensitivity of rectal submucosal neurons to the TRPV1 agonist capsaicin (0.1, 1 and 10nM) was studied using live Ca2+ imaging. In addition, supernatant was collected of rectal biopsies cultured overnight. The effect of this supernatant was evaluated 1. on the response of murine dorsal root ganglion (DRG) neurons to capsaicin using Ca2+ imaging, and 2. on the firing and mechano-sensitization of single serosal afferents in mice by the use of electrophysiological recordings. Inflammatory parameters in rectal biopsies were assessed by RT-qPCR, cytometric bead array, ELISA and immunohistochemistry.
Results: The TRPV1 agonist capsaicin evoked significantly higher Ca2+ peak amplitudes and activated more submucosal neurons in rectal biopsies of PI-IBS patients compared to HV. Moreover, supernatants of PI-IBS, but not of HV, significantly increased the Ca2+ response to capsaicin on murine DRG neurons, an effect mimicked by histamine and blocked by the histamine 1 receptor (H1R) antagonist pyrilamine. In addition, application of PI-IBS supernatant significantly increased the neuronal firing rate of serosal colonic afferents of mice compared to HV supernatant. Furthermore the response to von Frey hair probing was increased after incubation with PI-IBS, but not HV supernatants. Rectal biopsies of PI-IBS patients did not show upregulation of pro-inflammatory cytokines and did not reveal an increase in B-cells, eosinophils, CD3+ T cells, plasma cells, mast cells or their mediators histamine and tryptase compared to HV.
Conclusions: We found sensitization of neuronal TRPV1, mediated by H1R activation, in rectal biopsies of PI-IBS patients. The supernatant of PI-IBS activates and sensitizes colonic murine afferents and sensitizes TRPV1 on DRGs via H1R. Our data indicate H1R-mediated sensitization as an underlying mechanism of PI-IBS and suggest that H1R antagonism may be effective as treatment for PI-IBS.

B18
Effect of aleurone supplementation on postprandial glucose and insulin response in horses
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Introduction: Aleurone, which resides in the outermost layer of the endosperm of the weat kernel, is thought to be responsible for the beneficial health effects of whole wheat products [1]. Aleurone contains several different bioactive phytochemicals such as antioxidants (ea. Ferulic acid), osmolytes (ea. Betaïne), vitamins (ea. Thiamin), essential aminoacids (ea.lysine) and minerals [2]. To date, aleurone-related research has been performed either in vitro or in rodent models focusing on certain pathological conditions such as obesity. However, there is an increasing interest to evaluate the effect of aleurone in healthy subjects, both human and animals. Sports horses are often fed high energy concentrate diets containing high soluble carbohydrate concentrations. It is known that these diets are improper for horses sensitive to pronounced changes in blood glucose levels, because of their reduced insulin sensitivity. Also in humans the negative effects of increased insulin resistance are well known.
Aim: To monitor postprandial glucose and insulin response in trotter horses at rest, fed concentrate feed with and without aleurone supplementation.
Methods: An aleurone dosing trial was done in 8 healthy trotter horses not trained for competition, testing 4 aleurone doses (50, 100, 200, 400g/day) in crossover following a latin square model. Two batches of concentrate feed were manufactured: one pelletized blanco batch in which aleurone was replaced by wheat bran and a pelletized batch containing 20% aleurone. Both batches were mixed to achieve the proper aleurone dose. Each dose was fed during 7 consecutive days, followed by 1 week wash out during which only blanco concentrate feed was fed. The concentrate meal was fed twice a day (8am-aleurone enriched, and 8pm). Horses were housed in individual boxes (14 m2) on wood shavings and had free access to tab water and hay. Horses were customized to the blanco concentrate feed 2 weeks prior to the start of the study. Postprandial glycemic and insulin responses were monitored on day 7 of each week, up to 4h after feeding started for insulin and up to 8h for glucose. The data was organized as a replicated Latin Square design for statistical analysis, with aleurone dose and time after feeding and their interaction as fixed effects. Statistical analysis included aleurone dose regression and curve analysis (time of peak value, peak value, and area under the curve).
Results: The interaction between aleurone dose and time after feeding was significant ($p<0.05$) for both, glucose and insulin. Feeding aleurone delayed time of peak circulating glucose and insulin linearly as aleurone dose in the diet increased ($p<0.05$). Glucose peak value and area under the curve were not affected by aleurone but insulin peak concentration in blood and area under the curve reduced linearly with increasing doses of aleurone in the diet ($p<0.05$).
Conclusions: Aleurone supplementation has a beneficial effect on glucose and insulin response to concentrate meal uptake in horses. More research is needed to evaluate whether these results can be extrapolated to humans. [1]. Lillioja et al., 2013 ; [2] Buri et al., 2004

B19

Improvement of oxazolone-induced colitis by vagus nerve stimulation

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Introduction: The cholinergic anti-inflammatory pathway (CAIP), acting through the vagus nerve, represents a novel mechanism that modulates the immune system, in particular by dampening the activation of macrophages. In the gut, pharmacological activation of the CAIP and vagotomy (VGX) have indeed been shown to respectively improve and increase DSS-induced acute inflammation, a macrophage-dependent model of colitis. To what extent

manipulation of the CAIP also affects colitis mediated by activation of the adaptive immune system remains to be studied.
Aim: We investigated the effect VGX and vagus nerve stimulation (VNS) on Th2-mediated oxazolone-induced colitis.
Methods: Oxazolone colitis was induced in Balbc mice by cutaneous application of 3% oxazolone followed by intrarectal administration of 1% oxazolone 7 days later. In a first series of experiments, mice underwent VGX+pyloroplasty or sham surgery+pyloroplasty 14 days prior to sensitization. In a second series, mice received VNS (5min, 5Hz) or sham stimulation prior to rectal application of oxazolone. Change in body weight, temperature and survival were assessed daily. Colonic inflammation was determined by gene expression of cytokines and by histology (H&E staining). Systemic inflammation was monitored by assessment of cytokine levels in serum. Data are expressed as median plus 25%-75% interquartile ranges. Mann-Whitney U test was used for statistical analysis. Survival rate was assessed using Mantel-Cox test.
Results: In VGX mice (n=6) no clinical differences (weight loss, body temperature) were observed compared to sham mice (n=8). Moreover, comparable levels of Th2 cytokines (IL4, IL5, IL13) were detected in colon samples of VGX and sham-treated mice. Conversely, VNS-treated mice showed a significant improvement in survival (VNS 50% (n=15) vs sham 21% (n=14), p=0.01) and body temperature [VNS 33°C (30-34) vs sham 26.2°C (25-28); p=0.01] 6h post VNS compared to sham-treated mice. IL6 mRNA [VNS 0.09 fold increase (0.01-0.4) vs sham 0.5 fold increase(0.3-0.6); p=0.03] in the colon and IL6 serum level [VNS 0.2 ng/ml (0.06-5.6) vs sham 8.9 ng/ml (1.1-11.7); p=0.02], IL10 [VNS 0.03 ng/ml (0.01-0.1) vs sham 0.8 ng/ml (0.3-3.9); p=0.01] and KC [VNS 0.2 ng/ml (0.2-1.2) vs sham 3.7 ng/ml (0.2-6.7); p=0.01] were significantly reduced in VNS-treated mice (n=15) compared to sham (n=14).
Conclusions: Although VGX did not affect the degree of oxazolone-induced colitis, VNS induced a significant increase in survival rate and reduction in both colonic and systemic cytokines. To further evaluate whether VNS acts through the modulation of splenic and/or colonic immune responses, experiments on mice that underwent splenic denervation are ongoing. Taken together, our data indicate that VNS is also beneficial in Th2-mediated colitis, and suggest that VNS needs to be further explored as novel approach to treat IBD.

B20
Assessment of gastric motility on Functional Dyspepsia (FD) and Joint Hypermobility Syndrome (JHS)
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Introduction: FD is defined as the presence of early satiation, postprandial fullness or epigastric pain/burning, in the absence of underlying organic or metabolic disease. Recent reports have highlighted the co-existence of FD symptoms and JHS, a connective tissue disorder with multi-systemic involvement and a high prevalence of dysautonomia.
Aim: Our aim is to study the impact of the presence of JHS on gastric motor function, nutrient tolerance, dyspeptic symptoms and pre- and post-prandial autonomic tone in FD.
Methods: The intragastric pressure (IGP) was assessed by means of high resolution manometry in healthy volunteers (HVs) and FD patients before, during and after the intragastric infusion (60

ml/min) of a nutrient drink (ND, 1.5 kcal/ml) until maximal satiation. During ND infusion, the stomach relaxes, resulting in a drop in proximal stomach IGP from baseline, followed by a gradual recovery. The presence of JHS was evaluated by a 2-phase interview and clinical examination evaluating major and minor Brighton classification criteria. Sympathetic (LF) and parasympathetic or vagal (HF) modulations were assessed by analysis of Heart Rate Variability (HRV) derived from simultaneous ECG monitoring and computed for 7 minutes before, during, and 14 minutes after the meal.
Results: 15 FD patients (73% females, 42±4 years, BMI 23.2±1.4 kg/m2) and 10 HVs (50% females, 22±1 years, BMI 23.1±0.7 kg/m2) participated. During the meal, the IGP drop and nutrient tolerance were impaired in FD compared to HVs (AUC HVs:-23.4±4.2mmHg and FD:-10.9±3.0mmHg; p=0.02; ingested calories HVs: 1035±143.4 Kcal vs. FD:714±118.6 Kcal, p=0.09). HRV STD was numerically decreased in FD compared to HVs before (p=0.03), during (p=0.06) and after the meal (p=0.06) (Fig1). HF was numerically lower in FD patients before (p=0.3) and during the meal (p=0.4) compared to HVs. After the meal, the HF dropped significantly in HVs (0.03). Based on Brighton criteria, 67% of the FD patients and none of the HVs had JHS. FD patients with JHS reported higher prevalence of early satiation (70% vs. 40%), bloating (90% vs. 60%) and epigastric pain (50% vs. 20%) compared to patients without JHS ($p<0.05$). No difference in IGP drop was observed between JHS and No-JHS patients ($p>0.05$) (Fig 2). JHS patients tended to tolerate higher ND volumes compared to no-JHS (JHS: 801±164 Kcal and no-JHS: 540±124 Kcal, p=0.3). At the current sample size there were no significant differences in autonomic tone between JHS and no-JHS patients.
Conclusions: FD patients have a suppressed IGP drop and lower nutrient tolerance, associated with lower vagal tone. JHS co-exists in a large subgroup, which is characterized by increased symptom severity, but no significantly different IGP, nutrient tolerance or autonomic function compared to the group without JHS. Further evaluation in a larger patient population is warranted.

Case reports

C01
Intraperitoneal cystic lesion discovered incidentally in a woman with anal pain
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Introduction: -
Aim: -
Methods: -
Results: A 59-year-old woman presented with anal pain. Her past medical history is notable for arterial hypertension, hysterectomy, bilateral oviariectomy and thyroglossal duct cyst resection. Upon admission, physical examination was normal. Due to anal pain, abdominopelvic computed tomography was performed and revealed a large, 8cm in diameter, well-circumscribed multiloculated cystic lesion which seems to be centered on the pancreatic head with no evidence of bile and pancreatic ducts dilation. This incidental finding was confirmed by magnetic

resonance imaging (MRI) with cholangiopancreatography. On MRI, the lesion with substantial fluid component, hemorrhagic spots, solid septa and parietal nodules appears to arise from the duodenum and displaces the pancreas with mass effect on the duodenum and on the common bile duct (CBD) but without biliary dilation upstream. To even better characterize the lesion, endoscopy ultrasound (EUS) was performed and confirmed the multiloculated cystic lesion arising from the submucosa of the duodenum with CBD and porto-mesenteric confluence compression without vessels invasion. Biochemical analysis of the fluid obtained by EUS-fine needle aspiration (FNA) demonstrated low carcinoembryonic antigen (1,7ng/ml) and lipase (31 U/L) levels while the cytopathological analysis showed small number of spindle cells. However, pathological material was insufficient to perform immunostaining on the cell block. The patient was scheduled for surgery considering the large size of the cystic lesion to prevent development of mass-related complications (cholangitis or gastric outlet syndrome by CBD/duodenum compression). Moreover, a definitive diagnosis had not yet been established. Gastrointestinal stromal tumor (GIST), which is the most frequent mesenchymal tumor and has malignant potential, was the most likely pre-operative diagnosis. Complete dissection of the paraduodenal lesion was done without break and anal fistula was treated in the same time. Hematoxylin and eosin stained sections demonstrated mesenchymal tumor with spindle cells. Immunohistochemistry revealed positive diffuse staining for S100 protein, while CD117, CD34, smooth-muscle actin and desmin immunostainings were negative. These findings support the definitive diagnosis of intraperitoneal cystic schwannoma. The patient had an unremarkable recovery postoperatively and was discharged home well on the fourth day after surgery. Spindle cells are characteristic of mesenchymal tumors. The most common occurring in the gastrointestinal tract are GIST followed by smooth muscle tumors while schwannomas are very rare and benign. These represent approximately 3% of all gastrointestinal mesenchymal tumors. Most series report a female preponderance. Immunohistochemistry is required for definitive diagnosis. In conclusion, while the discovery of intra-abdominal lesions is often made in asymptomatic patients during radiological examination performed for another reason, EUS and MRI may help to characterize the lesion and to define its precise localization and extension. These informations are of critical importance in the preoperative assessment. However, the definitive diagnosis often requires complete pathological analysis including immunohistochemistry which is not always feasible on a FNA sample.
Conclusions: -

C02

An unusual and underdiagnosed cause of chronic diarrhea

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Introduction: -
Aim: -
Methods: -
Results: Bile acid malabsorption assessed by SeHCAT test: an under-investigated differential diagnosis in patients presenting with chronic diarrhea K. De Groote[1,2], S. Decock[1], P. Van Hootegem[1], J. Arts[1], A. Holvoet[1] In this case report we present a 59-year-old female patient who

consults with watery diarrhea since two years. She has a medical history of psoriatric arthritis, diabetes mellitus type 2, cholecystectomy and lower back surgery. Extensive investigation (including gastro- and colonoscopy, enteroscopy with jejunal biopsies, CT enterography, stool sample including parasites, breath testing for bacterial overgrowth, 24 hour stool collection and chromogranin testing) was negative. Therapy with loperamide, creon, lactose free diet and sandostatin gave no resolution of the diarrhea. Because of persisting diarrhea patient consulted for a second opinion. A Selenium homocholic acid taurine (SeHCAT) test was performed and revealed bile acid malabsorption with a retention rate of 4% after 7 days. Therapy with Colestyramine was started and symptoms of diarrhea clearly improved. The cause of chronic diarrhea in adults is often difficult to ascertain and patients may undergo several investigations without a definitive cause being identified. A common but frequently under-investigated cause of chronic diarrhea is bile acid malabsorption (BAM). There is evidence that more than 25% patients with chronic watery diarrhea or IBS-D have bile acid malabsorption.(Slattery S et al. Aliment Pharmacol Ther. 2015). Diagnosis of bile acid malabsorption is easily and reliably made by the SeHCAT test. In contrast to most countries in Europe a SeHCat test is almost not performed in our country and not reimbursed. In the UK 59% of all Nuclear Medicine Departments are performing this test, with an increase in the last years. This nuclear medicine test involves two scans a week apart and so measures multiple cycles of bile acid excretion and reabsorption. Retention of SeHCAT at 7 days is normally above 15%; values less than 15%, 10% and 5% predict respectively mild, moderate and severe abnormal retention and an increasing likelihood of response to bile acid sequestrants. This case report illustrates the delay in diagnosis and treatment of bile acid malabsorption, which is an under-recognized cause of chronic diarrhoea, and the important role of SeHCat test in confirming the diagnosis. [1] AZ Sint Lucasziekenhuis Brugge [2] Universitair Ziekenhuis Leuven
Conclusions: -

C03
Esophago-jejunal anastomosis defect: Beyond a simple leak!
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Introduction: -
Aim: -
Methods: -
Results: We present the case of a 70 year old man who was diagnosed fortuitously with sub-cardial 3.5 cm GIST. A total gastrectomy with esophago-jejunal Roux-en-Y anastomosis was perfomed. After 2 days, he presented diffuse abdominal pain and fever. Antibiotherapy was started. An upper GI radiological study revealed an important leak at the anastomosis and the patient was referred for endoscopic treatment. Upper GI endoscopy showed an important quantity of pus as well as a complete dehiscence of the anastomosis communicating with a multiloculated collection drained by one of the surgical drains. Using a double-channel therapeutic endoscope, we grabbed the jejunal disconnected segment with a rat tooth forceps and catheterized it deeply with the guidewire. A 20 cm/20-25 mm self- expandable metal partially covered stent was inserted deeply, partially deployed and pulled gently back to move the jejunal

limb close to the esophagus, to cover the fistula and restore the digestive continuity. A second small fistula was identified in the jejunal limb, 5 cm below the distal end of the stent, and confirmed to be drained by a second surgical drain. A naso-jejunal tube was left in place for enteral nutrition and antibiotics were continued. An abdominal CT performed 10 days later showed an additional peri-hepatic collection which was drained percutaneously. Further imaging disclosed persistence of an anastomotic leak, despite complete resolution of collections. Follow-up endoscopy allowed stent removal and showed a residual leak of 8 mm diameter in the left lateral portion of the anastomosis in a circumferential area of neo-epithelialization. The second small distal fistula had closed spontaneously. Three fistula plugs were inserted through the anastomotic fistula by rendez-vous technique to seal the residual leakage and a new partially covered metal stent of 20 cm-20/25 mm was inserted. Four days later, upper GI radiological swallow followed by an abdominal CT showed resolution of the leak and oral intake was authorized. All drains were removed and antibiotics stopped. After one month, the stent was removed. The anastomosis was covered by a neo-epithelium and no remaining fistula was identified. During follow-up, the patient developed dysphagia due to the presence of two strictures that needed several endoscopic dilations, one in the middle third of the esophagus, which was easily calibrated, and one at the anastomosis, needing further dilation sessions. The patient has lost 40 kilograms since surgery but his weight nowadays stabilized, with a body mass index of 24. Complete dehiscence of a surgical anastomosis is a rare complication, but may be succesfully managed by endoscopy in certain cases for which alternative major reparative surgery would be particularly mutilating. Attached file: Video of Endoscopy + fluoroscopy.
Conclusions: -

C04

A colonic mass

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Introduction: -
Aim: -
Methods: -
Results: A 46-year-old man, living in Congo, presented with right upper quadrant abdominal pain for three months, one episode of hematochezia, weight loss of 10 kg and intermittent moderate fever. The patient, on holiday in Belgium, was a pastor in Kinshasa and mentioned occasional work on construction sites. His past medical history was notable for malaria. Upon admission, physical examination revealed mild to moderate right upper quadrant abdominal tenderness. Laboratory test results included the following: hemoglobin 10.3 g/dl, C-reactive protein (CRP) 120 mg/L (N<10mg/L), alkaline phosphatase 617 IU/L (N:53-68 IU/L), gamma-glutamyl transferase 440 IU/L (N:8-61 IU/L). Abdominal computed tomography showed hepatosplenomegaly, parietal thickening stenosis of the cecum with perilesional infiltration and mesenteric lymphadenopathies up to 23 mm in diameter. Coloscopy revealed a large cecal lesion with many burgeoning ulcers and 2 large hemicircumferential ulcers in the ascending and transverse colon. The mucosa in the other parts of the colon was normal. Histological examination of hematoxylin and eosin stained colonic biopsies revealed severely disrupted glandular architecture of the colonic mucosa and lymphoplasmacytic infiltrate associated with

numerous neutrophils. Periodic acid-Schiff staining showed yeast-like microorganisms highly suggestive for Histoplasma capsulatum. Colonic biopsies cultures confirmed the diagnosis. The patient was subsequently diagnosed to be human immunodeficiency virus (HIV) positive with a CD4 count of 52/mm3 (N:388-1851/mm3). The patient was treated for disseminated histoplasmosis with liposomal amphotericin B (3 mg/kg/day) replaced by oral itraconazole after 5 days (200 mg three times a day for 3 days, then twice a day for 1 year) due to development of acute renal failure. Treatment against HIV was also initiated. Resolution of symptoms, normalization of CRP and improvement of liver function tests were observed after 12 days of treatment. The patient is still doing well at 9 months follow-up. Histoplasmosis is an opportunistic invasive fungal infection that occurs almost exclusively in immunodeficient patients, especially those with AIDS. It presents as pulmonary lesion or as disseminated. Clinical suspicion, especially in endemic areas and in immunocompromised patients, is essential to make the correct diagnosis for appropriate therapeutic management. Although the clinical, biological, endoscopic and radiological presentation was in favor of an inflammatory/infectious disease, the final diagnosis of disseminated histoplasmosis with prominent colonic involvement and absence of respiratory symptoms was nevertheless surprising and uncommon.
Conclusions: -

C05

Enuresis nocturna : a malignant symptom ?

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Introduction: -
Aim: -
Methods: -
Results: J.L. is a 5-year old boy, born from non-consanguineous Belgian parents. His previous medical history was uneventful as well as the familial history. During workup for secondary enuresis nocturna, abdominal ultrasonography revealed a cirrhotic aspect of the liver and splenomegaly. Liver MRI confirmed these findings and additionally showed minimal 3 lesions suspect for hepatocellular carcinoma. Serum alpha-fetoprotein was 200 µg/L. Chest CT showed a nodular lesion in the inferior right lobe of the lung suspect for metastasis. To obtain a definitive diagnosis, the third liver segment was resected. The nodular lesions were confirmed to be hepatocellular carcinoma and the remainder of the liver tissue to be completely cirrhotic (inactive state). An extensive metabolic workup was performed. However, no underlying cause could be identified. Liver function tests were normal. Serum IgG was normal and liver-specific autoantibodies were negative. HBV and HCV serology was negative. Cholesterol and triglycerides were normal. Lysosomal acid lipase (LAL) deficiency was excluded. Sweat test was normal. Furthermore, there was no evidence for alpha-1 antitrypsin deficiency, biliary atresia, hereditary tyrosinemia or a glycogen storage disease. Mitochondrial respiratory chain disorders could not be identified. Genetic analysis for type 2 progressive familial intrahepatic cholestasis is pending. Reevaluation of the lung nodule after 1 month did not show any progression.

Considering the importance of a complete resection and the cirrhotic state of the liver, an orthotopic living related liver transplantation (with the mother as donor) was performed. Anatomopathological examination of the explant liver showed multifocal hepatocarcinogenesis. There were multiple high-grade dysplastic nodules with well-differentiated hepatocellular carcinoma in some nodules without evidence of lymphovascular invasion. The underlying cirrhosis was inactive. Electron microscopic examination did not show glycogenosis, tyrosinemia or mitochondrial defects. After 3 weeks, a revision of the biliary anastomosis was necessary because of a bile leak. To date, besides this complication, the patient did well. Maintenance immunosuppression consisted of tacrolimus for 6 weeks, which was subsequently switched to sirolimus. Chest and abdominal imaging 4 months after transplantation did not show any sign of relapse. In conclusion, this case describes the rare presentation of a cryptogenic cirrhosis and multifocal hepatocellular carcinoma in a 5-year old boy. According to adult transplantation criteria, this patient would not have been eligible for liver transplantation. However, as several studies have reported pediatric patients, successfully treated by transplantation, that were well outside of University of California, San Francisco and Milan criteria and since the pathogenesis of hepatocellular carcinoma in children often differs from that in adults, the establishment of pediatric transplantation criteria for HCC is warranted.
Conclusions: -

C06

Eosinophilic cholangiopathy, a diagnosis dilemma. A case series

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Introduction: -
Aim: -
Methods: -
Results: A 29-year-old Mediterranean woman presented with a few weeks history of pruritus, jaundice and cholestasis. Medical history was significant for childhood asthma. Clinical examination revealed epigastric pain and jaundiced. Laboratory tests showed elevated liver function tests (LFTs): AST: 150 IU/L, ALT: 240 IU/L, ALP: 288 IU/L, gGT: 287 IU/L and bilirubin: 3.12 mg/dl. MRCP demonstrated intra-hepatic bile ducts (IHBD) dilation predominant in the right segments without visible tumor. CEA and CA 19-9 were respectively 2.3ng/ml and 75.3 IU/l. IgG and IgG4 levels were normal. Viral and autoimmune serologies were also negative. ERCP showed a hilar stricture, followed by cholangioscopy revealing nodular endoluminal tissue within the hilum which was brushed and biopsied. Three biliary plastic stents were inserted. Histopathology suggested cholangiocarcinoma. The patient underwent right portal embolization and enlarged right hepatectomy. Pathologic specimen revealed thickened bile ducts and mixed inflammatory infiltrate rich in eosinophils, which evoked eosinophilic cholangitis. Parasite research revealed anti-fasciola hepatica antibodies, therefore eosinophilic cholangitis could be secondary to a parasitic infection. The second case is about a healthy 27 year old Burundian man whose clinical complaints were pale stools for several months and more recently a notion of jaundice. A recent blood test showed moderate cytolysis and cholestasis. Auto-

immune and viral serologies, IgG/IgG4 levels, as well as tumor markers were normal. CT revealed IHBD dilatation predominantly in the left side. ERCP showed a complex hilar stricture which was biopsied. Histopathology didn't revealed malignancy. MRCP showed hilar stricture with upstream left IHBD dilation without visible mass. Repeat ERCP combined with cholangioscopy demonstrated inflammatory changes in hilar structure. Biopsies confirmed eosinophilic cholangitis. A retrospective review of the first biopsy showed an important eosinophilic infiltrate. Anti-strongyloïd antibodies were positive. The last case is a 74 year old man with a history of prostate adenocarcinoma recently treated with Bicalutamide and Triptoreline who developed progressive jaundice with pruritus. He noted an 11 kg weight loss over the last 3 months. Bilirubin was 24.2mg/dl, ALP and transaminases were 2 and 1.5 fold the normal range respectively. CA 19-9 was 68 U/ml. IgG4 level was normal. MRCP revealed proximal CBD thickening with IHBD dilatation. ERCP showed a long CBD stricture with upstream dilatation which was brushed and biopsied. After biliary sphincterotomy, two plastic stents were inserted. Biopsies suggested eosinophilic cholangitis. Bicalutamide adverse effect was suspected and was discontinued. Steroids were started. After 3 months, the patient became asymptomatic. Cholangiogram control didn't show any stricture. These three cases of secondary eosinophilic cholangitis due to drugs and parasites were characterized by the absence of raised peripheral eosinophil count. Eosinophilic cholangitis is a rare entity which can be mistaken for cholangiocarcinoma, leading sometimes to subsequent unnecessary surgery. In undetermined bile duct stricture, cholangioscopy might help in making the diagnosis. Although renowned to have a specificity of 100% for the diagnosis of cholangiocarcinoma, bile duct cytologic brush sample can have false positive results.
Conclusions: -

C07
Omental infiltration and abdominal pain
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Introduction: -
Aim: -
Methods: -
Results: Introduction Erdheim-Chester disease (ECD) is a non-Langerhans cell histiocytosis usually involving the long bones. Extra-skeletal infiltration may be encountered in up to 50% of cases. To our knowledge, only a few cases have been reported with an intra-abdominal localisation. Case report In this case, we describe an involvement of the retroperitoneum and the omentum in a 44 year-old-man suffering from fluctuant diplopy, weight loss and aspecific chronic abdominal pain. A biologic inflammatory syndrom of unknown aetiology was found. An abdominal CT-scan was performed and showed a diffuse infiltrate of the omentum and the peri-renal region. Results The biopsy of the omentum revealed a fibrous and nodular tissue filled by large sheets of foamy histiocytes. Those were PAS and Ziehl-negative and strongly expressed CD68 but not protein S100 and CD1a. V600E mutation of BRAF was detected. Supplementary X-ray investigations showed metaphyso-diaphyseal osteoslerotic lesions of femur, tibia and humerus with endosteal and periosteal reaction. A thickening of the pituitary gland was found at

RMI. FDG-PET scan did not highlight any pathological metabolic activity. The patient was treated by interferon alpha with a dose of 135 microgram per week with a good tolerance. During the follow-up of one year, the patient became asymptomatic. A partial regression of the adenohypophyseal mass was also observed but status quo of other lesions. Discussion The differential diagnosis of lympho-histiocytic (CD68+) infiltration of the omentum includes infectious diseases (Mycobacterium, Histoplasma, Whipple disease, malakoplakia), inflammatory pseudotumor, lymphomas (in particular histiocytic variant of anaplastic large cell lymphoma) and non-Langerhans cell histiocytoses (Rosaï-Dorfman disease and ECD). Conclusion ECD can be part of the differential diagnosis of chronic inflammatory disease of unknown aetiology with intra-abdominal diffuse infiltrate. In the presence of CD68+/S100-/CD1a- foamy histiocytes, the pathologist should be aware of this entity. While the presence of V600E BRAF mutation represents a good help, clinico-radiological and pathological confrontation is mandatory for the final diagnosis.
Conclusions: -

C08

Culture-negative hemodynamic shock with high conjugated bilirubin and renal failure: an unusual suspect.

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Introduction: -
Aim: -
Methods: -
Results: A 57 year-old male patient was transferred to our intensive care unit with a combination of acute renal failure, thrombocytopenia, elevated bilirubin and hypotension. Six days prior to admission the patient had developed fever and myalgia of the upper legs. Before transfer to our ICU, noradrenaline, dobutamine, hydrocortisone, vancomycine and ciprofloxacin had been started. Ceftriaxone in high dose was added at admission in the Antwerp University Hospital. The patient had not traveled abroad recently, nor was there a family member with similar symptoms. Lumbar punction, urine culture, repetitive blood cultures and thoracoabdominal CT were normal. Transesophageal ultrasound showed a normal contractility without evidence for endocarditis. Hepatitis A, B and C serology, galactomannan antigen and malaria thick smear were negative. Consecutive chest X-rays showed development of acute respiratory distress syndrome. Bilirubin (predominantly conjugated) further increased to a level of 20 mg/dL with normal transaminase values and normal INR. Though, under the initiated treatment, the patient gradually improved with ameliorating renal function and decreasing inflammatory parameters. After retaking a detailed (medical) history, one unusual event surfaced, being the removal of a dead rabbit out of an outside water reservoir. Two days after admission, Leptospirosis IgM came back as positive. As Ceftriaxone is the recommended treatment for Leptospirosis infection, it was continued with lowering of the dose to 2g daily. The condition of the patient further improved with complete clinical recovery and normalization of renal function and bilirubin level. Leptospirosis is a freshwater-borne zoonotic spirochete capable of infecting a variety of

mammalian hosts and is shedded intermittently in the urine of carriers where it will remain viable for days to months in soil or water. Whilst being endemic in some countries, it is an infrequent, though not rare disease in our country, with recently several infections after the sporting event 'titan run' in Nijlen where participants had to cross infected water. Leptospirosis infection can range from a subclinical or mild infection to a very severe and potentially fatal one. Fast diagnosis should be based on detailed history and clinical suspicion, because the current testing modalities can have a long turnaround time and are not always clinically useful in an acutely ill patient. With the correct antimicrobial therapy and proper supportive care, most leptospirosis patients recover completely.
Conclusions: -

C09
Early recurrent spontaneous hepatic hemorrhage after laparoscopic left lateral hepatectomy. A case report of a 45 year old woman taking ginseng, green tea, venlafaxine and non-steroïdal anti- inflammatory drugs.
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Introduction: -
Aim: -
Methods: -
Results: INTRODUCTION. Herbal supplements are over the counter accessible in Belgium. Some of these are known for their potentially adverse effects. Interaction of venlafaxine with non-steroïdal anti-inflammatory drugs may cause an increased risk of bleeding. CASE: We report a case of a 45 year old woman with recurrent hepatic hemorrhage during intake of ginseng, green tea, venlafaxine and non-steroïdal anti-inflammatory drug. After presenting with a spontaneaous subcapsular haematoma, patient was admitted for an elective laparoscopic left lateral hepatectomy for suspected adenoma as underlying cause for the hematoma. During the early postoperative period (second postoperative day), patient developed an new hepatic hemorrhage, right sided. Beside the intake of green tea, ginseng, venlafaxine and non-steroïdal anti inflammatory drugs, there was no other cause for bleeding diathesis. Venlafaxine and herbal medications were stopped, the patient is bleeding free since more than 6 months. CONCLUSION Herbal supplements and the combination of non steroidal anti inflammatory drugs with venlafaxine drugs are known to increase risk of bleeding (via the platelet aggregation inhibition pathway), as demonstrated in this case. This matter cannot be emphasized enough, as every practitioner should be aware of these potentially life-threatening adverse events or interactions.
Conclusions: -

Belgian Society for Gastrointestinal Endoscopy (BSGIE)

G01
A PROSPECTIVE MULTICENTER BELGIAN REGISTRY OF RADIOFREQUENCY ABLATION FOR BARRETT'S ESOPHAGUS
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Introduction: Radiofrequency ablation (RFA), combined with endoscopic resection (ER) of visible lesions, can be used as a primary treatment for low grade dysplasia (LGD), high grade dysplasia (HGD) and early adenocarcinoma (EAC) in Barrett's esophagus (BE). In prospective multicenter controlled trials, high rates of complete remission of dysplasia (CR-D) and intestinal metaplasia (CR-IM) has been reported.
Aim: The aim of this study is to monitor outcome and efficacy of RFA in a setting of absence of reimbursement in a multicenter national prospective registry.
Methods: Between February 2008 and August 2015, data from 7 centers performing RFA was collected in the Belgian RFA registry. All procedures were monitored for indication, treatment before RFA, short/long term complications and prospective long-term pathological outcome. Primary endpoints was combined CR-D/CR-IM. Secondary endpoints were safety and need for escape treatments.
Results: 538 RFA procedures were registered in 279 different patients (mean age 65; 84.5% men). In 60.2% a previous EMR/ESD was performed. Baseline worst histology prior to RFA (including ER) was: 2% IM (5), 8% LGD (22), 52% HGD (146), 37% EAC (102), 1% unknown (4). At the time of analysis 44 patients were still under treatment and 16 patients discontinued treatment. In an intention to treat analysis, 193/235 patients (82%) achieved CR-IM/CR-D after a median of 2 RFA sessions. 14 % of these (27) needed rescue treatment to achieve CR-IM/CR-D: 7% APC (14), 5% EMR (10), 1% ESD (2), 1% ESD+APC (1). Treatment failure was not disease related in 18 patients, giving a per protocol CR-IM/CR-D in 89%. 131/193 (68%) patients remained in CR-IM/CR-D after a median follow-up of 795 days. Recurrent disease was limited to IM in 37 patients (19%). Recurrent neoplasia occurred in 24 patients (12.5%) : 7 LGD , 17 HGIN/Ca. There was no higher risk for recurrence between EAC or HGD pre RFA (p= 0.5). Mean time to recurrence was 510, 464 and 372 days for IM, LGD and HGD/EAC respectively. In total 3 (1.5%) patients were referred to surgery after CR-IM/CR-D. 15 were treated endoscopically and 3 with LGD were followed. Immediate complications occurred in 4.5% of the procedures in 21 different patients (7.5%): 17 small mucosal lacerations (9 after sizing), 7 bleedings, 1 pneumonia, no perforations. Late adverse events occurred in 8.2% of the procedures in 42 different patients (15 %): 19 stenosis(mean 4 dilatations), 7 bleedings, 2 poor healing, 9 prolonged hospitalization for general symptoms, 4 general symptoms without prolonged hospitalization, 1 pneumonia.
Conclusions: Our data confirms that combined ER-RFA is an efficient and safe treatment for Barrett's associated neoplasia. In the absence of reimbursement, more escape treatments were performed in comparison to published controlled trials. Outside clinical trials, meticulous follow-up appears to be even more important to detect and treat early recurrence. We suggest a systematic registration of RFA practice to monitor long term outcome and efficacy.

G02
Quality in standard colonoscopy: a prospective randomized single-center study

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Introduction: Critical analysis of quality in colonoscopy remains crucial in evaluating performance status of Endoscopy units. However, multiple variables including patient characteristics, medical and logistic parameters can have their influence on analysis.

Aim: We prospectively monitored recommended quality parameters during standard colonoscopy performed by medical staff in a large tertiary hospital.

Methods: Data from 2136 colonoscopies, prospectively collected between December 2010 and June 2014 , were analyzed. Exclusion criteria were history of inflammatory bowel disease (IBD), genetic predisposition, major colon resection and inadequate bowel preparation (5.6%). All colonoscopies were performed by graduated staff members, consultant or resident gastroenterologists. Patients were randomly assigned to 3 endoscopy systems in combination with 4 modalities (normal white light, high-definition white light, virtual chromoendoscopy and high-definition virtual chromoendoscopy) as part of another study.

Results: The overall adenoma detection ratio (ADR) was 34.9 +/- 1.3%. The average retraction time was 10m44s +/- 35s. For 1 endoscopy system a significant longer total procedure time was documented (22m59s vs. 19m37s vs. 19m47s) while retraction time was significantly different between all 3 systems (9m58s vs. 10m22s vs. 11m53s). However, no significant differences in ADR were noted between the 3 systems (34.3 +/- 0.8% vs. 32.4 +/- 1.6% vs. 37.1 +/- 2.7%), between normal and high-definition (33.2% vs. 34.4%) or between white light and virtual chromoendoscopy (34.4% vs. 35.5%). While there was a significant correlation ($R^2 = 0.998$) between inspection time and ADR, no significant difference in ADR was detected between staff members, consultant and resident gastroenterologists (29.8% vs. 30.2% vs. 33.4%). Between systems, no differences in polyp shape (flat vs. sessile/pedunculated) or type of adenomas was observed. High-definition colonoscopy resulted in a significantly higher percentage of serrated adenomas (5.5% vs. 2.6%) and carcinomas (2.7% vs. 0.5%), while no difference was observed between white light and virtual chromoendoscopy. Smoking was associated with a significant higher ADR (36.8% vs. 30.8%). Surprisingly, intake of aspirin was also associated with a significant higher ADR (42.3% vs. 31.0%) but after correction this observation appears merely age related (68 +/- 9 years vs. 58 +/- 12 years).

Conclusions: Recording quality parameters in standard colonoscopy is recommended to monitor service performance for adherence to international guidelines, as well as for providing personal feedback. Our data suggests a better detection of serrated adenomas and carcinomas in high-definition colonoscopy but we did not found an additional value for the use of virtual chromoendoscopy for adenoma detection.

G03

Safety and feasibility of an endoluminal-suturing device (Endomina®) as an aid for endoscopic gastric reduction

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Introduction: Endoscopic suturing with full thickness gastric sutures is still challenging due to the complexity of suturing maneuvers. We currently report first human experience using a suturing station assembled into the stomach.
Aim: This pilot trial explored the safety and the technical feasibility of a novel triangulation platform "ENDOMINA®(EndoToolsTherapeutics, Belgium)" to perform full thickness endoscopic suturing and anterio-posterior apposition into the stomach. ClinicalTrials.gov NCT02534662
Methods: This single use triangulation platform, assembled on the endoscope into the stomach, adds a 5Fr bendable channel and a 5Fr non-bendable channel to an endoscope. ENDOMINA® is used in combination with a Transmural Antero-Posterior Endoscopic Stitcher (TAPES) in the bendable channel with a preloaded tag and tightening system. The procedure included performing 2 large plicatures with serosa to serosa apposition tightened to each other after mucosal ablation and resulting in antero-posterior apposition. This was repeated 4 to 6 times in every case.
Results: Twelve obese (BMI 34.6 +/-2.1kg/m2) patients (8 women, mean age: 36 +/- 10 years) were included between May 2015 and July 2015. One patient was excluded because of gastric ulcers. The median (range) duration of the procedure was: 2h06 (1h15 – 3h20) in this first human experience. No severe adverse event was observed. Mean weight loss and % excess weight loss were 5.8kg (SD 2.7) and 21% (SD9) at 1 month (n=11);10.6kg (SD 4.6) and 40% (SD 22) at 3 months (n=7); 10.6kg (SD 7.2) and 40% (SD 31) at 5 months (n=9).
Conclusions: This single use triangulation platform and suturing systemappears to be safe and effective at short term in creating gastric restriction and inducing weight loss. More patients and follow-up are mandatory.

G04
Treatment of Zenker's diverticulum through a flexible endoscope with a transparent hood attached to the tip and a monopolar forceps: results of a consecutive series of 76 patients
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Introduction: Zenker's diverticulum was commonly treated by means of external transcervical diverticulectomy, myotomy or diverticulopexy, or by means of an endoscopic myotomy through a rigid endoscope. Gastroenterologists first described flexible endoscopic therapy for Zenker's diverticulum in 1995.
Aim: In our single-center study we report the safety, feasibility and results of endoscopic myotomy through a flexible endoscope, performed at a secondary referral centre.
Methods: The median age of the patients was 75.4 years (range 47 - 97 years). In this series were 45 men and 31 women. The initial diagnosis was based on clinical, endoscopic, and radiological findings. Dysphagia was the main indication for treatment of the Zenker's diverticulum. Afterwards, the patients were systematically followed and treated until they were completely symptom-free. Procedure:Prophylactic antibiotics were not administered, only conscious sedation was used. Fluoroscopy was not used. A nasogastric tube was introduced over the guide wire into the stomach. The presence of the nasogastric tube improves the endoscopic view of the Zenker bridge and protects the contralateral side of the esophagus from thermal damage. A transparent hood, 14.7 mm in diameter was attached to the tip of the gastroscope for adequate visualization of the Zenker bridge and to protect the surrounding tissue during

cauterization. A monopolar coagulation forceps with alligator spoons was used to graspe the Zenker bridge in the midline and to pull it into the transparent hood. Then it was cut using a blended current (cutting 120 watt maximum/coagulation 60 watt maximum) .This was repeated until a smooth passage to the esophagus was achieved. Patients with residual dysphagia were retreated again using the same endoscopic technique.
Results: Sixty(60) patients (78.9 %) were and remained symptom -free after one single session of endoscopic therapy. In 15 patients (19.8 %) a second session was needed and in only one patient (1.3%) a third session. Adverse events were limited to transient cervical emphysema in a single patient. Two patients developed cervical emphysema following the procedure. Contrast radiography using water-soluble contrast medium, however, did not show extraluminal extravasation. These patients were given antibiotics and were able to resume oral feeding after 3 days without any problem and there were no long-term sequelae. Apart from these cases of cervical emphysema, no other complications occurred. No hemorrhage, mediastinal emphysema, or mediastinitis was encountered.
Conclusions: This endoscopic technique is an efficient, safe and minimally invasive method for the treatment of Zenker's diverticulum. General anesthesia is not necessary and oral feeding can be resumed the next day. In view of the excellent results and minimal complications, it can be considered a safe and effective treatment of Zenker's diverticulum. The intention-to-treat success rate has been 100 %: in 78.9% after one single session, in 19.7% after a second and in only 1.3% after a third session of endoscopic therapy. The complication rate is very low (2.6%) and very mild.

G05

Performance of the Glasgow Blatchford score in upper gastrointestinal bleeding: for which patients, for what outcome and with which threshold?

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Introduction: Acute upper gastrointestinal bleeding (UGIB) is the most common medical emergency managed by gastroenterologists. It accounts for 5% of presentations to the emergency department (ED) and 2% to 3% of hospital admissions. Overall mortality remains around 8%. Several practice guidelines and risk scores, combining clinical and endoscopic parameters, have been developed to predict the outcomes of these patients. Their aim is to assist physicians in the early stages of decision making. In 2000, Blatchford developed a clinical score that predicts the need for treatment in acute UGIB. The score ranges from 0 to 23 and the risk of requiring treatment increases with increasing score. Patients presenting to the ED with UGIB and a GBS score of 0 have been shown to be safe for discharge and can be managed in an outpatient setting. This is supported by Belgian guidelines published in 2011 but has not been studied for use in any Belgian medical facility yet.
Aim: To validate the Glasgow Blatchford score, to assess its accuracy compared with previous studies and to determine the optimal cut-off value for the safe discharge of low-risk patients with acute upper gastrointestinal bleeding presenting at a Belgian tertiary care medical center.
Methods: Retrospective study of all patients presenting to the emergency department with upper gastrointestinal bleeding over 3 years. We defined UGIB as hematemesis, melena, and/or rectal bleeding, the latest only if it was accompanied by anemia (haemoglobin < 10 g/dl) and/or syncope. Primary outcome was the need for clinical intervention: blood transfusion, endoscopic,

radiological or surgical intervention. We assessed the accuracy of the Glasgow Blatchford scoring system by plotting its receiver-operating characteristic (ROC) curve.
Results: We included 257 patients in final analysis. A total of 50.2% patients received one or more transfusion with packed red blood cells, fresh-frozen plasma and/or platelet concentrates. Sixty three (24.5%) patients underwent endoscopic hemostasis. Six patients (2.3%) underwent emergency surgery and 7 (2.7%) received interventional radiology. Mean GBS was 9.4 (±4.1). No patient who needed clinical intervention (blood transfusion, endoscopic, surgical, or radiologic management) was not identified by the GBS. The area under ROC curve reaches 92.3%. Thirty nine (15.2%) patients had a GBS ≤ 4. None of them needed intervention.
Conclusions: We validated the Glasgow Blatchford score in the setting of a Belgian university hospital. Given its high sensitivity, it is probably the safest scoring system to predict the need for treatment of patients presenting to the emergency department with symptoms of acute upper gastrointestinal bleeding. To reduce unnecessary admissions, a cut-off value of ≤ 4 can be used safely and effectively in our institution as it predicts the need for clinical intervention with 100% sensitivity.

G07

ERCP in patients with Billroth II gastrectomy : Comparison of conventional duodenoscope and single-balloon enteroscope

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Introduction: Billroth II partial gastrectomy precludes conventional endoscopic retrograde cholangiopancreatography (ERCP) because of altered anatomy. It renders ERCP more difficult because of the intubation of the afferent limb and the orientation of the intact papilla.
Aim: Comparison of ERCP procedures performed with the conventional duodenoscope and the single-balloon enteroscope (SBE) in Billroth II patients in 2 university endoscopy units. Patients and methods: 34 Billroth II patients underwent 64 ERCP procedures between 2006 and 2014. Technical aspects, therapeutic success and complications were recorded.
Methods: Male / female ratio was 20/14 (59%/41%) with a mean age of 72±1 (48-91) years. The initial choice of endoscope type was at the endoscopist's discretion.
Results: 29 (45%) ERCPs were started using a duodenoscope of whom 25 (86%) were successful and 3 were completed using SBE. 21 (33%) ERCPs were started using SBE of whom 19 (91%) were successful and 2 were completed using a pediatric colonoscope. 5 (8%) ERCPs were started using a pediatric colonoscope of whom 4 were completed with a duodenoscope and 2 with the SBE. In total 9 (14%) procedures needed a change of endoscope type in order to complete the procedure. Overall therapeutic success rate using a duodenoscope was 87% vs 81% using SBE (P>0.05; Chis-square), whereas success rate using a pediatric colonoscope was only 43% (P<0.05; Chi-square). Complication rate using a duodenoscope was 7% (pancreatitis; embolism) vs 14% (pancreatitis; biliary leak) using SBE (P>0.05; Chi-square), without mortality. The use of a duodenoscope allowed complete sphincterotomy and both plastic and metallic stent placement, whereas the use of SBE often needed to combine sphincterotomy with additional sphincteroplasty (8-15 mm) and only 7 Fr plastic stent placement was possible due to the 2.8 mm working channel diameter. However, SBE allowed easy access to the papilla in the afferent limb and sphincteroplasty often allowed direct cholangioscopy using SBE. Indications

were bile duct stones (55%), chronic pancreatitis (26%), cholangitis (16%), livertransplantation (3%).
Conclusions: Therapeutic ERCP success rate is high in patients with Billroth II gastrectomy using either a conventional duodenoscope or the SBE, with an acceptable and comparable complication rate. The choice of endoscope may depend on the endoscopist's experience, postoperative anatomy (gastrojejunostomy and length of afferent limb) and therapeutic indication (metallic stent placement and direct cholangioscopy).

G08

Prospective record of 7 consecutives colonoscopic perforations: a monocentric colonoscopy registry.

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Introduction: Colonoscopic perforation is defined as a traumatic defect or a leakage in the wall intestine. Colonoscopic perforations (CP) has been reported in 0.003% to 0.8% of diagnostic colonoscopies and flexible sigmoidoscopies but may be seen in up to 5% of therapeutic colonoscopies. Risk factors widely recognized are therapeutic colonoscopies, age over 75 years, multiple comorbidities, history of diverticular disease, previous intra abdominal surgery and females. Although CP is a rare complication, it is associated with a high morbidity and mortality rate.
Aim: The aim of this study was to characterize perforations that occurred over 22 months period of prospective registry of colonoscopy in our center.
Methods: We reviewed all patients undergoing colonoscopy between 6 january 2014 and 15 november 2015 at CHU Charleroi, Belgium, for which a systematic prospective registry was hold on. Colonoscopic perforation is defined as a traumatic defect in the wall intestine visualized during procedure or extravisceral free air on imaging.
Results: 7736 colonoscopies were performed over 22 months. We reported 7 perforations which corresponds to an incidence of 0.09% (5 women, 2 men). The median (min-max) age was 75 (60-82) years. Three perforations were identified during the examination (43%), 1 (14%) within the first 24 hours and 3 (43%) were delayed (more than 24 hours). In the 3 immediate perforations, 2 were treated using an over the scope clip and one by surgery. Perforations occur in therapeutic colonoscopies in 57% (4 cases) and in diagnostic colonoscopies in 43% (3 cases). Of the 7 perforations, 3 occured in the sigmoid (43%), 3 in the descending colon (43%) and 1 in the ascending colon (14%). Two of perforations (29%) were treated by endoscopic closure, two by conservative treatment (29%) and three by surgery (42%). Endoscopic closure consisted by launching an over the scope clip using the "twin grasper" with a successful closure. In the group treated surgically, all the patients required primary repair. The median (range) duration of hospitalization was 21 (10-35) days. No death was recorded at 28 days after perforation.
Conclusions: The setup of a systematic registry of the colonoscopies performed in our institution allows to assess our complications rate and especially CP incidence of 0.09% which is comparable to published series. No mortality was associated to CP in our series.

G09

Cecal intubation failure during colonoscopy: a prospective monocentric analysis of 7736 consecutive low endoscopic procedures

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Introduction: Colonoscopy is actually considered as gold standard for colon investigation but also has some limitations. Cecal intubation rate is considered as one of important quality criteria to measure good clinical practice.

Aim: The aim of our study was to analyze institutional cecal intubation rate performance and etiology of failure.

Methods: Between 6 January 2014 and 15 November 2015, we prospectively record informations relating to: prior colonoscopy criteria (indication), the review (quality of preparation by Boston score, cecal intubation and reason of potential failure, number of polyps identified and resected) and complications (immediate and long term).

Results: Between 6 January 2014 and 15 November 2015, 7736 low endoscopic procedures were realized in our institution, including 6725 total colonoscopies intent examinations. Global cecal intubation rate is 95.2% (6404/6725). The most frequent reason of failure was insufficient preparation and occurs in 79.5% (255/321) of cases. 82.7% of patients were prepared in hospital. Median age (min-max) of bad preparation population was 66 (18-94) years. Male to female ratio was 137/118 patients. Diagnostic colonoscopies represent 92.5% (236 cases) but constipation represent only 10% (24/255) of main indication of colonoscopies. Impassable neoplasic stenosis occurs in 9.3% (30/321) of failure (4 rectal, 17 sigmoïd, 4 left colon, 2 transverse, 3 right colon). Irreductible loop occurs in 7.8% (25/321) of failure procedure (progression to sigmoid in 7 patients, to left colon in 5 patients, to transverse in 3 patients and in right colon without cecal access in 10 patients). Impassable non neoplasic stenosis occurs in 2.2% (7/321) of cases and early discontinuation for anesthesia-related adverse event occurs in 1.2% (4/321) patients.

Conclusions: Systematic prospective analyze of quality indicator as cecal intubation rate can help to improve institutional good medical practice of colonoscopies. This analyze show that institutional cecal intubation rate is within recommendation and insufficient intra-hospital preparation is the main reason of failure.

G10

Endoscopic management of intragastric migrated bands or rings for restrictive surgery: a single center experience.

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Introduction: Intraluminal migration or dysfunctions of bands and rings may be observed in the long-term follow-up of laparoscopic adjustable gastric banding (Lap-Band) and vertical banded gastroplasty (VBG). They typically require revisional surgical procedures, which are burdened with high morbidity rates.

Aim: Assess feasibility and safety of endoscopic treatment of dysfunctional or migrated bands or rings in a secondary care center.

Methods: Retrospective review of patients who underwent endoscopic removal of intraluminal migrated bands or rings from October 2011 to November 2015 in CHU Vésale & Charleroi. Endoscopic procedures were performed in 1 or 2 sessions following the policy described by Blero and al (GIE (2010), 71: 468 – 474). The ring or band was removed after its prior section with a wire cutting-system, which consisted of a guide wire threaded on a mechanical lithotripter (G-Flex; GF500). According to the level of band erosions/migration a self-expandable plastic stent (SEPS) was placed across the ring or band and both SEPS and band/ring were removed 4 weeks after the first endoscopic procedure.
Results: We included 9 patients, with a mean age of 51.4 years old, 3 males and 6 females – 5 with eroded Lap-Bands and 4 with eroded Silastic rings. Median delay to band/ring removal after surgery was 105months (range 46 – 273 months). All bands or rings were successfully removed in 1 (n=7) or 2 (n=2) sessions. Two patients (1 Lap Band and 1 VBG) required placement of a SEPS to induce intraluminal migration prior to extraction. We observed 2 immediate complications. We documented 1 pneumoperitoneum, without perforation, 24 hours after the procedure, which was treated by a percutaneous drain exsufflation. The second immediate complication was persistent dysphagia in another patient, which presented outlet stoma dysfunction due to severe cicatricial stenosis and deformation. Patient was successfully treated by a stenting of the stenosis (Endoflex; Fully covered self expandable metallic stent, 80mm 20/24) during 19 weeks. No delayed complications were observed within a mean follow up of 21 months. Four patients benefited from a gastric bypass (GBP), 3 after the extraction of the band or ring and 1 before. Mean delay between endoscopic treatment and GBP procedure was 12.3months (n=3). Mean follow-up after GBP was 11,3 months, with no complication observed.
Conclusions: Endoscopic treatment of intraluminal migrated ring or bands as described by Blero and al. seems feasible and safe in secondary care center.

G12

Long-term results of endoscopic treatment for Zenker's diverticulum: a retrospective analysis and a new symptom evaluation score.

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Introduction: Flexible endoscopic myotomy is a feasible and efficacious treatment for symptomatic Zenker's diverticulum (ZD). For the evaluation of symptoms pre- and post-treatment, studies often use only a dysphagia score. Besides dysphagia, patients also suffer from regurgitation, coughing, aspiration related pneumonia and weight loss.
Aim: To evaluate the long-term efficacy of endoscopic myotomy in ZD and to define an adapted symptom score and giving additional symptoms an appropriate weight in the score.
Methods: Single center, retrospective analysis of patients that underwent endoscopic myotomy for ZD during the last 5 years. Review of patient files and patient questioning for adverse events and immediate and long-term efficacy with regard to ZD related symptoms. The proposed score includes dysphagia (for solids, semisolids or liquids), regurgitation, respiratory symptoms (cough, aspiration and pneumonia due to aspiration) and weight loss, and also takes into account the frequency of the dysphagia and the regurgitation (table 1).

_____ Table 1. Zenker diverticulum scoring system (15)

_____ Dysphagia (0-6) 0 No dysphagia 1 Dysphagia for solids 2 Dysphagia for semisolids 3 Dysphagia for liquids x2 if symptoms at every meal

_____ Respiratory symptoms (0-3) 0 No respiratory symptoms 1 Chronic cough 2 Aspiration / choking 3 Pneumonia due to aspiration

_____ Regurgitation (0-3) 0 No regurgitation 1 Incidental regurgitation 2 Daily regurgitation 3 Regurgitation at every meal

_____ Weight loss (0-3) 0 No weight loss 1 Weight loss < 5 kg 2 Weight loss 5-10 kg 3 Weight loss > 10 kg

Results: Eighteen consecutive patients with ZD were selected in the data collection. Four patients died during follow-up, 1 patient was excluded due to epiglottal cancer (5 years post myotomy). In 13 patients mean follow-up time was 28 months (range 2-52 months). At follow-up, 10 out of 13 patients had complete resolution of symptoms; in 3 partial resolution was achieved. Two of them consider a new myotomy. In 1 patient transient fever and retrosternal pain was noticed post procedure, with negative work-up for mediastinitis. The mean score for ZD related symptoms dropped from 4.46 pre-treatment to 0.38 post treatment (0-15). Subscores dropped from 2.08 to 0.15 for dysphagia (0-6), from 1.23 to 0.15 for regurgitation (0-3), from 0.92 to 0.08 for respiratory symptoms (0-3) and from 0.23 to 0 for weight loss (0-3).
Conclusions: Taken into account that we only surveyed a small test group, our analysis confirms that endoscopic treatment of ZD is effective and safe. A new scoring system that includes dysphagia, regurgitation, respiratory symptoms and weight loss, can provide a more accurate analysis of symptoms in patients with ZD. However, further research, including prospective studies, is needed to confirm these findings and evaluate the proposed score.

G13

Helicobacter pylori infection in patients with and without human immuno-deficiency virus infection: Are endoscopic findings similar?

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Introduction: Gastroesophageal disease is common in obese individuals and may also occur frequently in people infected with human immunodeficiency virus (HIV).
Aim: To evaluate the frequency of peptic esophagitis and gastroduodenal ulcers among Helicobacter pylori-infected patients with and without HIV infection.
Methods: We prospectively collected data on consecutive HIV-infected patients who underwent their first upper gastrointestinal endoscopy with gastric biopsy between 2008 and 2013 and a control group comprised of non-HIV-infected obese patients (HIV - ob.) who were candidates for bariatric surgery during the same period. Data for those with Helicobacter pylori infection (HP+) on gastric biopsy were extracted and data for HP+/HIV+ versus HP+/HIV- ob. patients were compared.

Results: Data from 233 HP+/HIV+ patients and 458 HP+/HIV- ob. patients were analyzed. Compared to HP+/HIV- ob. patients, HP+/HIV+ patients were younger, more often male, and were more frequently chronically co-infected with hepatitis B or C ($p < 0.05$). Endoscopic diagnoses that were more common among HP+/HIV+ patients compared to HP+/HIV-ob. patients included esophageal candidiasis, esophageal or gastric varices, and Kaposi's sarcoma ($p<0.05$). The prevalence of duodenal or gastric ulcer was similar in both groups ($p=0.3$). The subset of HP+/HIV+ patients on antiretroviral therapy with CD4 lymphocyte count > 200 cells/μL had an increased frequency of peptic esophagitis compared HP+/HIV- ob. patients ($p=0.04$).
Conclusions: Helicobacter pylori-HIV co-infected patients on antiretroviral therapy with improved immune function had an increased prevalence of peptic esophagitis but identical prevalence of gastric or duodenal ulcer compared to Helicobacter pylori-positive/HIV-negative obese patients.

G14
Yield of upper gastrointestinal endoscopy in the era of modern antiretroviral therapy
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Introduction: Gastrointestinal disorders are common in human immunodeficiency virus-positive patients.
Aim: Gastrointestinal disorders are common in human immunodeficiency virus-positive patients. The aim of this study was to determine whether lymphocyte T CD4 cell counts were correlated with indications for endoscopy in these patients and with endoscopic diagnosis.
Methods: We prospectively collected data from consecutive human immunodeficiency virus-positive patients undergoing upper gastrointestinal endoscopy between 2007 and 2013, and included 265 patients who had been receiving antiretroviral therapy for at least 6 months. Parameters studied were demographics, immune parameters, co-morbidities, co-medications, indications for endoscopy, and endoscopic, pathologic, and microbiologic findings.
Results: The most frequent indications for upper gastrointestinal endoscopy were gastroesophageal reflux, epigastric pain and other. Peptic esophagitis, esophageal candidiasis, and normal endoscopy were the most common diagnoses. H. pylori infection and neoplasia were found in 26.4% and 1.8%, respectively. Patients with CD4+ counts >200 cells/μl had significantly lower rates of antibiotics use, fewer comorbidities, and were less likely to have acquired immuno-deficiency syndrome than patients with lower counts. They were also more likely to have normal UGI endoscopy and had a higher frequency of H. pylori infection. Acquired immuno-deficiency syndrome status and the presence of co-morbidities were independent predictors of endoscopic abnormalities.
Conclusions: Upper gastrointestinal endoscopy remains a key diagnostic procedure for human immunodeficiency virus-positive patients with upper gastrointestinal symptoms. Acquired immuno-deficiency syndrome and co-morbidities are risk factors for the presence of mucosal lesions among human immunodeficiency virus-positive patients on antiretroviral therapy.

IBD Research Group (BIRD)

I01

A variable number of tandem repeat polymorphism in the promotor region of the neonatal Fc receptor affects anti-TNF serum levels in IBD

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Introduction: Ample evidence exists that anti-TNF trough levels (TL) during induction determine short and long-term outcome in IBD. Sex, CRP, albumin and BMI are known to influence these concentrations. The neonatal Fc (FcRn) or Brambell receptor is responsible for extending serum half-life of albumin and IgGs and harbours a variable number of tandem repeats (VNTR) polymorphism in the promotor region of the FcRn gene.[1]

Aim: We hypothesized that anti-TNF TL are affected by this VNTR polymorphism.

Methods: We analysed infliximab (IFX) TL in 368 IBD patients (215 CD, 153 UC), all IFX naïve, who received IFX 5mg/kg at week 0, 2 & 6. A second independent cohort of 139 IBD patients (100 CD, 39 UC), adalimumab (ADM) naïve and treated with ADM 160/80/40mg at week 0, 2 & 4, was analysed for ADM TL. All patients were genotyped for the VNTR polymorphism with PCR as previously described.[1] TL were measured using an in-house-developed ELISA. The median TL during anti-TNF induction (from week 2 & 6 for IFX, from week 2 & 4 for ADM) was calculated for every patient. Genotype, patient- and disease-related factors were analysed in multiple linear regression with median TL as dependent variable.

Results: Distribution of VNTR genotypes was similar as previously described.[1] Only genotypes with a frequency > 5% (VNTR2/3 (n=83 or 16,4%) and VNTR3/3 (n=414 or 81.6%)) were further considered for analysis. Multiple regression in the IFX group withheld several factors highly associated with IFX TL (UC diagnosis, male sex, prior different anti-TNF use, BMI, albumin, CRP and later on development of ATI, all $p<0.01$) but not the VNTR genotype. When excluding patients who later on developed antibodies to IFX (ATI) (n=53), predictors remained unchanged but now also included the VNTR2/3 genotype ($p=0.03$), resulting in a predicted 16% lower median TL as opposed to VNTR3/3 genotype. Prior anti-TNF use other than IFX predicted a 30% lower median TL than anti-TNF naïve patients ($p=0.001$). In the ADM group, the VNTR2/3 genotype predicted a 23% lower median ADM TL ($p=0.007$). The combined presence of VNTR2/3, male sex and prior IFX predicted a 40% lower median ADM TL as opposed to those without these risk factors ($p=0.045$).

Conclusions: The VNTR2/3 genotype in the FcRn gene is associated with lower IFX but also lower ADM induction TL. Developing anti-drug antibodies for both IFX or ADM later on and the prior use of another anti-TNF agent were also associated with lower induction TL. We could furthermore confirm previously identified risk factors for lower induction TL. We therefore propose that patients, in whom these risk factors are present, might benefit from a higher anti-TNF induction dose to ensure optimal disease outcome.

[1]. Sachs et al. A VNTR polymorphism influences the transcriptional activity of the neonatal Fc receptor a-chain promoter. Immunology 2006; 119: 83-89.

I02

Infliximab trough level measured during treatment induction may be predictive of the loss of response to Infliximab during treatment maintenance in Inflammatory Bowel Disease patients: A longitudinal observational retrospective study

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Introduction: Infliximab (IFX), is indicated for the treatment of inflammatory bowel disease (IBD) (ulcerative colitis (UC) or Crohn disease (CD)). Nevertheless, loss of response (LOR) to IFX is reported in up to 20-30% over 12 months of treatment. Importantly, pro-active monitoring of IFX pharmacokinetics may help prevent LOR during treatment maintenance.

Aim: Our objective is to analyze in a cohort of 263 patients the pharmacokinetics early on in order to predict LOR during maintenance treatment.

Methods: 263 IBD patients (188 CD and 75 UC) have been treated with IFX on follow-up (median + range), (EC approved). 2331 samples were prospectively collected and measured retrospectively by ELISA in parallel with clinical data. Statistical analysis used Mann- Whitney test after determination of non-normality of compared distributions thanks to $\chi 2$ goodness-of-fit test.

Results: During the maintenance, the median IFX Trough level (TL) was statistically higher in patients on combo-therapy (IFX combined with immunomodulator, n=107) compared to patients on mono-therapy (n=81) (1.32 µg/ml [0-12.09µg/ml] VS 2.14 µg/ml [0-11.98µg/ml], $p<0.000001$). Median IFX TL was higher under combo when patients were sequentially treated first with combo- then mono-therapy (4.17 µg/ml [0.12-11.98µg/ml] VS 3.22 µg/ml [0.08-11.98 µg/ml], $p < 0.01$, n=55). On the contrary, there was no statistical difference between mono- and combo-therapy when patients were sequentially treated first with mono- then combo-therapy (2.02 [0.11-12.09µg/ml] µg/ml VS 2.49µg/ml [0.11-12.09µg/ml], $p<0.17$, n=49). During maintenance, 19% of the patients (n= 49) experienced LOR, defined as secondary non responders (NRII), requiring treatment optimization by either shortening of the dosing interval and/or by increasing the dose. 57% of these patients (n= 28), defined as secondary non responders end (NRIIend), did not respond to any optimization strategy and were switched to another treatment, while 43% of patients (n= 21), defined as secondary responders to optimization (RIIopt), responded to optimization. Looking at the TLs during induction (week 2 and 6), median IFX TL at induction was not statistically different in NRII (n=49) compared to patients with sustained response (n=170) (6.66 µg/ml [0.23-19.93µg/ml] VS 11.91 µg/ml [0.11-19.93 µg/ml], $p <0.08$). However, median IFX TL at induction was statistically lower in NRIIend (n=23) compared with the patients with sustained response (1.69 [0.30-19.93µg/ml] VS 11.91 µg/ml [0.11- 19.93µg/ml], $p <0.05$).

Conclusions: This study suggests that patients who do not respond to any optimization strategy (NRIIend) seem to have lower IFX TLs at induction. IFX TLs measured at induction may predict clinical response to IFX during maintenance.

I03

Functional translation of IBD-associated genetic variation in patient-derived intestinal epithelial cells

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Introduction: In contrast to the general acceptance that IBD represents a spectrum of disease phenotypes with distinct behavioral features, overall management of IBD is quite homogenous. With the imminent introduction of new therapeutic classes for IBD patients, adequate characterization and improved stratification of patients based on the underlying disease mechanisms will be essential. Large-scale studies have identified bacterial recognition & handling, autophagy and ER stress signaling as interlinked inflammatory signaling pathways driving IBD pathogenesis. Genetic variation in these pathways may affect normal (immune) function of the intestinal epithelium, responsible for conserving host-microbial interactions and tissue homeostasis.
Aim: We studied if genetic variation in IBD-associated pathways translates into distinct epithelial cell functional activity. These functional read-outs could in turn be used for an improved stratification of IBD patients.
Methods: Endoscopically-derived mucosal biopsies were obtained from the colon of 35 IBD patients, all genotyped by immunochip. Intestinal epithelial crypts were isolated and plated in collagen-coated wells, resulting in a monolayer of intestinal epithelial cells (IECs). We recently developed and validated a short-term culture system for deriving IECs from IBD patients. These cells were assessed for correlation of autophagic activity and/or ER stress level with 8 IBD-associated SNPs linked to ER stress (XBP1, ORMDL3) and autophagy (ATG16L1, IRGM, ULK1, MTMR-3, LRRK2). ER stress level and autophagic activity were evaluated by ELISA-based quantification of BiP/GRP78 (with(out) thapsigargin) and p62/SQSTM1 (after addition of chloroquine).
Results: All patients were selected based on the distribution of risk alleles (Q1 or Q4): patients in Q1 (3≤ autophagy- or ≤1 ER stress-related risk alleles) were considered to have a low genetic risk for IBD-associated pathway perturbation while patients in Q4 (≥5 autophagy- or ≥2 ER stress-related risk alleles) were considered to be at high genetic risk. Presence of a high autophagic genetic risk score showed a trend of increased ER stress (low 1.8 to high 2.7 ng/ml BiP) and decreased autophagic activity (low 1.4 to high 1.2 ng/ml p62) compared to low autophagic genetic risk patients. Epithelial cell cultures of high ER stress-related genetic risk patients showed a trend of increased ER stress (low 1.9 to high 2.5 ng/ml BiP) and increased autophagic activity (low 1.2 to high 1.5 ng/ml p62).
Conclusions: Genetic variation in IBD-associated pathways (ER stress, autophagy) tends to affect the level of ER stress and the autophagic activity measured in patient-derived epithelial cell cultures and supports the potential of translating molecular clues into personalized management of IBD.

I04

Tauroursodeoxycholic acid prevents Crohn's disease-like ileitis and restores gene expression involved in ileal bile acid reuptake

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Introduction: One of the hallmarks of ileal Crohn's disease (CD) is bile acid malabsorption, mainly resulting from defects in ileal bile acid reuptake. Bile acids are important modulators of the intestinal inflammatory response.

Aim: We determined whether tauroursodeoxycholic acid (TUDCA), a bile acid which prevents murine colitis, also improves ileitis. Secondly, because the integrity of the bile acid enterohepatic circulation is preserved by bile acid transport systems, whose expression is tightly regulated by nuclear receptors, we investigated their expression in ileal inflammation.
Methods: At 4 weeks of age, TNFDeltaARE/WT mice and wild-type littermates were treated daily with TUDCA or placebo until the age of 15 weeks. Ileal histopathology and inflammatory markers were measured and gene expression levels of ileal bile acid transporters and nuclear receptors were determined. In addition, gene expression data were compared with a cohort of ileal biopsies of CD patients with active ileitis versus healthy controls.
Results: Daily treatment with TUDCA reduced the level of ileal inflammation in TNFDeltaARE/WT mice, as indicated by improved body weight gain (P=0.042), reduced histological inflammatory cell infiltration (P=0.01) and decreased ileal IL-6 expression (P=0.042). The ileal bile acid uptake transporter ASBT was downregulated in inflamed ileum of both TNFDeltaARE/WT mice (17% of control values, P=0.001) and CD patients (32% of control values, P=0.004). Expression levels of the basolateral efflux transporter complex OSTa/OSTb were significantly reduced in TNFDeltaARE/WT mice, but not in CD. As compared to placebo-treated TNFDeltaARE/WT mice, expression levels of ASBT and OSTa/OSTb were higher in ileal tissue of TUDCA-treated TNFDeltaARE/WT mice (P=0.001, P=0.006 and P=0.08, respectively). In wild-type mice, however, TUDCA had no effect on these transporters. Additionally, ileitis in TNFDeltaARE/WT mice was associated with a significant downregulation of the nuclear receptors FXR, PXR and VDR (41%, 48% and 34% of controls, respectively). A similar decrease was observed for PXR (48% of controls, P=0.024) and VDR (49% of controls, P=0.054), but not FXR, in ileal biopsies of CD patients. Chronic administration of TUDCA to TNFDeltaARE/WT mice prevented the dysregulation of these regulatory receptors.
Conclusions: TUDCA alleviates murine ileitis and partially restores the expression of bile acid transporters and regulatory receptors. Therefore, TUDCA may represent a potential drug for the treatment of CD ileitis.

I05

Revised roles of matrix metalloproteinase/MMP-9 in inflammatory bowel diseases/IBD: from target to biomarker

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Introduction: MMP-9 is elevated in blood and intestinal tissue of IBD patients and is suggested by knock-out (KO) mouse and inhibitor studies as a key causal factor. MMP-9 antagonists are presently evaluated in clinical trials for IBD.
Aim: Our aim was to re-investigate the role of MMP-9 in acute and chronic intestinal inflammation.
Methods: MMP-9 KO mice were backcrossed for 13 generations into C57BL/6J mice and bred under identical environmental conditions for more than 15 years in our animal facility. In 8-10 weeks old MMP-9 KO mice and their WT littermates, acute colitis was induced by oral administration of 3% dextran sodium sulphate (DSS) for 7 days followed by 2 days of regular water. Chronic colitis was induced by 3 cycles of 1 week 1.75–2.0% DSS each followed by a recovery phase of 2 weeks. Intestinal inflammation and fibrosis were assessed by macroscopic parameters, histopathology analysis and tissue collagen levels. In colonic tissue, MMP-9 levels were determined by gelatin zymography analysis and gene expression differences were assessed with RNA sequencing (Illumina HiSeq2500, fold change [FC]>2, 10% false discovery rate [FDR]). Pharmacological inhibition of MMP-9 was tested by administration of two peptide inhibitors (CPU1 and CPU2) to acute DSS-treated C57BL/6J mice via daily intraperitoneal injections (250 µg) and via implanted osmotic pumps (30 mg/kg/day). The inhibitory effect was evaluated by clinical, histopathological and qRT-PCR analyses.
Results: In contrast to previously reported phenotypes, clinical and histopathological parameters were not attenuated in MMP-9 KO mice after acute DSS administration compared with WT littermates. Zymography analysis confirmed absence of MMP-9 in our KO mice and showed increased MMP-9 levels after DSS in WT mice only. Similar expression of host genes in WT and MMP-9 KO control mice was observed, with exception of Mmp9 (FC=3.8), Rims4 (FC=-6.0) and Slpi (FC=2.5). After induction of colitis, 11 genes involved in antimicrobial response were differentially expressed between WT and MMP-9 KO mice. Development of fibrosis was not altered in chronic DSS-treated MMP-9 KO mice, although less goblet cell loss was observed compared with WT mice. Pharmacological inhibition of MMP-9 with CPU1 and CPU2 did not improve parameters of intestinal inflammation. On the contrary, increased Mmp3, Mmp8 and Mmp9 expression was observed in DSS-treated mice that received CPU2 compared with saline.
Conclusions: Against prevailing evidences, we demonstrate that MMP-9 deletion or inhibition does not lead to clinical and histopathological attenuation of DSS-induced colitis. Whereas MMP-9 remains an excellent inflammatory marker in IBD, ongoing clinical trials with MMP-9 inhibition as a therapeutic option for IBD need to be carefully followed.

I06

CCR2-monocytes are essential for the resolution of inflammation and tissue repair in colitis.

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Introduction: Monocyte recruitment in the gut wall via C-C chemokine receptor 2 (CCR2) is a major hallmark in the pathogenesis of inflammatory bowel disease (IBD). Classically, monocytes are considered as the main mediators of tissue damage during colitis. However, monocyte-derived macrophages may be essential for the resolution of inflammation.
Aim: In the current study, we aim to identify the function of monocytes recruited during colitis and to define whether monocytes play a role in the induction of inflammation and/or are crucial for tissue repair.

Methods: To study the contribution of monocytes, acute colitis was induced in wild type (WT, C57BL/6) and CCR2-/- mice by 2,5% dextran sodium sulfate (DSS) in drinking water for 5 days. Disease progression was assessed via a standardized disease activity index (DAI) including body weight loss, stool consistency and blood in the feces. To study the role of monocytes during chronic colitis, mice were subjected to 3 cycles of 2% DSS. Monocyte and neutrophil recruitment and macrophage differentiation in the colon were assessed by flow cytometry and cell sorting. Histological evaluation of colonic tissues was performed.
Results: During acute DSS colitis (day 5), CCR2-/- mice displayed a reduced DAI and body weight loss as compared to WT mice indicating that inhibiting monocyte recruitment reduces tissue inflammation. On the contrary, during the recovery phase (day 10 to 14), CCR2-/- mice showed increased DAI and body weight loss compared to WT (24.5% vs 8.6%). Interestingly, the induction of chronic colitis confirmed that CCR2-monocytes are crucial for the resolution of inflammation. During the 1st cycle of DSS, body weight loss in WT mice (6.8%) was 10-fold higher than in CCR2-/- animals (0.67%). Interestingly from day 10 onward, CCR2-/- mice continued to lose weight, showing altered stool consistency and blood in the feces. During the 2nd and 3rd subsequent DSS cycles, CCR2-/- mice failed to recover body weight and presented increased disease severity compared to WT. This phenotype in CCR2-/- mice correlated with increased colonic tissue alterations such as epithelial erosion, cell infiltration and fibrosis. Flow cytometry analysis showed increased accumulation of ROS-producing neutrophils in CCR2-/- mice (WT:9.5x10^5; CCR2-/-:37.6x10^5; $p<0.05$), while immune infiltrate in WT mice mainly consisted of differentiated MHCII+ macrophages expressing typical M2 markers such as Arg-1, IL-10, CD163, MRC1, Lyve1 and Stab1.
Conclusions: Our data demonstrate a dual role of monocytes during colitis, inducing the inflammatory response during the first phase, and playing a crucial role in the resolution of inflammation and tissue repair at a later stage. Further understanding of the mechanisms leading to immune-regulation and mucosal repair is vitally important to improve treatment for patients suffering from IBD.

I07

Correlation of small intestinal permeability, faecal calprotectin and barrier genes in multiple-affected families with inflammatory bowel disease

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Introduction: Impaired intestinal permeability (IP) is a crucial factor in the pathogenesis of inflammatory bowel disease (IBD). However, its causal role and association with genetic and environmental determinants remain unclear.
Aim: We performed an in-depth characterisation of small IP in affected and unaffected individuals from multiplex IBD families (=at least 3 affected first-degree relatives), and correlated this with faecal calprotectin and SNPs in barrier genes.
Methods: Eleven multiplex IBD families were studied, including 26 affected IBD patients (21 Crohn's disease, 5 ulcerative colitis), and 39 unaffected family members (33 first-degree relatives, 6 spouses). Fifteen unrelated healthy individuals served as controls. Demographic (sex, age, BMI, smoking) and clinical (age at diagnosis, disease location and behaviour, surgery,

medication use) characteristics were collected. Small IP was measured using the 2-hour lactulose-mannitol urinary excretion test. Acute and chronic stress levels were determined by cortisol in saliva and the Perceived Stress Questionnaire, respectively. Faecal calprotectin was measured as marker for intestinal inflammation. The contribution of genetic risk factors to IP was assessed using a polygenic risk score, including SNPs (located in 128 barrier genes) that were nominally ($p<0.05$) associated with IBD within the families.
Results: Quartile analysis of the lactulose-mannitol ratio (LMR) showed a higher proportion of IBD patients (40%) in Q4, compared to unaffected first-degree relatives (19%), spouses (17%) and healthy controls (13%) ($p=0.05$). We found high intraclass correlation for the LMR in affected members of our families (0.64), while this was much lower in unaffected family members (0.10), suggesting that IP is more disease-dependent than family-dependent. In addition, age was significantly positively correlated to IP in our families ($r=0.34$, $p=0.01$). Neither stress nor calprotectin levels were correlated with the LMR, although the median calprotectin concentration was significantly higher in affected versus unaffected family members (117 [interquartile range IQR 39-338] vs. 41 [IQR 30-91] µg/g, $p=0.002$). Fifty SNPs were associated with IBD in our families, comprising 9 barrier genes (MUC1, MUC21, MUC22, MAGI1, MAGI3, LAMB2, CDH1, F11R, CXADR). The polygenic risk score, however, did not influence the LMR.
Conclusions: We observed higher small IP in a subset of IBD patients compared to unaffected first-degree relatives, spouses and healthy controls. Interestingly, calprotectin was not associated with small IP, suggesting that barrier defects also occur independent of inflammation. Our study furthermore indicates that genetic factors are not the primary determinants for barrier dysfunction in IBD. Other serologic and environmental risk factors, including the microbiota, will be added in future analyses.

I08

Effects of introduction of an IBD nurse position on the quality of delivered care

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Introduction: Inflammatory Bowel Diseases (IBD) are chronic gastrointestinal conditions that require long-term outpatient follow-up ideally by a dedicated, multidisciplinary team. More and more, a key role has been proposed for the IBD nurse. IBD nurses serve as the first point of contact for patients and provide a frequent and easy access to education, advice and support aimed at improving in outcomes.
Aim: We investigated the effect of the introduction of an IBD nursing role on the delivered quality of care within an IBD referral center.
Methods: In our tertiary referral IBD center an IBD nurse position was instituted in Sep 2014. All contacts (phone, e-mail or personal contact) and outcomes were prospectively recorded over a 12-month period using a logbook kept by the nurse. Interventions were categorized as prescriptions and attestations, flare management, starting new therapy, follow-up on therapy, disease information and support, questions about side effects, psychosocial support, and planning of follow-up.
Results: Between Sep 2014 and Aug 2015, 1312 patient contacts were recorded (42% male, median age 38 years, 72% Crohn's disease). Most of those were phone calls (48%) and personal contact (46%) and a minority e-mails (6%). The contacts increased with time: Q1 (Sep-Nov 2014): 144, Q2: 322, Q3: 477 and Q4: 370. More than half of the patients who contacted the IBD

nurse (58%) were taking biologicals. The vast majority of contacts (94%, 1229/1312) could be handled independently by the nurse whereas medical staff back up was needed for 6%. 10% (137/1312) of the contacts were for patients with recently diagnosed disease (<1 year). Most of the contacts of the IBD nurse were assigned to the start of new therapy (12%) and the follow up of these medications (22%), to the planning of follow-up (22%) and to providing disease information (16%). In addition 9% of the patients contacted the IBD nurse for flare management, 8% for prescriptions and attestations, 7% for psychosocial support and 3% for questions about side effects. In the study period, 30 emergency room visits could be avoided by convertion into outpatient clinic visits through the intervention of the IBD nurse and 133 unscheduled outpatient appointments could be avoided through counseling. The IBD nurse provided faster access to procedures and other departments related to IBD for 136 patients.
Conclusions: The role of IBD nurses as first point of contact and counseling is evident from a high volume of nurse-patient interactions that increased three fold within the first 6 months. Avoidance of emergency room and unscheduled clinic visits, and improved access to procedures were associated with these contacts.

I09
Variability in Vedolizumab Exposure between Patients with Inflammatory Bowel Disease
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Introduction: Vedolizumab (VDZ), a humanized monoclonal antibody that specifically targets the α4β7 integrin, has been approved for the treatment of patients with moderate to severe Crohn's disease (CD) and ulcerative colitis (UC), following successful completion of the GEMINI phase 3 programs. Nothing is known about the relation between drug exposure and efficacy in real-life practice.
Aim: The aims are to (i) develop assays for quantifying VDZ and anti-drug antibodies (ADAbs), (ii) describe and explain the pharmacokinetic (PK) variability between patients and (iii) correlate PK with outcome.
Methods: Enzyme-linked immunosorbent assays for measuring serum VDZ concentrations and ADAbs were developed in house. A total of 37 VDZ naïve patients (24 CD, 13 UC) were sampled at trough during induction treatment (300 mg IV administered at weeks 0, 2 and 6). All patients but one had prior anti-tumor necrosis factor-alpha exposure. VDZ PK during induction was described based on trough concentrations and baseline patient factors influencing variability were tested (sex, (lean) body mass, disease type (CD or UC), C-reactive protein (CRP), albumin and lymphocyte count). Biological response, based on CRP, was correlated to the VDZ serum concentrations.
Results: A substantial interindividual variability in VDZ serum concentrations was observed at week 2 (median 27.5 µg/mL, IQR 15.6 µg/mL) and week 6 (median 24.9 µg/mL, IQR 18.8 µg/mL). Two patients with CD had undetectable VDZ trough concentrations at week 6, of which one had detectable ADAbs. No significant difference in week 2 and 6 trough concentrations was seen between CD or UC. Patients with a baseline CRP above 10 mg/L had a significantly lower VDZ trough concentration at week 2 (p=.03). A baseline serum albumin below 40 g/L was associated with significantly lower VDZ trough concentration at week 6 (p=.04). Women had significantly higher VDZ trough concentration at week 2 (p=.0005). A significant negative

correlation between (lean) body mass and the VDZ trough concentration at week 2 was found (p=.01 for body mass and p=.001 for lean body mass). Patients who had a decrease in CRP (n=23, excluding ADAb+ patient) between week 0 and week 6 had significantly higher VDZ trough concentrations at week 6 (median 31.7 µg/mL, IQR 20.8 µg/mL), compared to patients who had an increase in CRP (n=13) (median 16.4 µg/mL, IQR 10.8 µg/mL) (p=.046).
Conclusions: This first real-life experience with VDZ shows substantial PK variability between patients. High CRP and low albumin at baseline, both indicators of disease severity, were shown to predict lower VDZ trough concentrations during induction. Higher (lean) body mass is shown to be associated with lower VDZ trough concentrations at week 2. Early biological response, judged by a decrease in CRP by week 6, is associated with significantly higher VDZ exposure at week 6, already suggesting an early exposure-response correlation.

I10

Mucosal healing and dysplasia surveillance in a large referral center cohort of patients with Crohn's disease and ulcerative colitis treated with vedolizumab.

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Introduction: Vedolizumab has been approved for the treatment of Crohn's disease (CD) and ulcerative colitis (UC) based on controlled trials extending to one year. Long term data on mucosal healing and on dysplasia surveillance in CD and UC are lacking.
Aim: To document the incidence of endoscopic disease activity and of colonic dysplasia in CD and UC patients treated with vedolizumab maintenance therapy.
Methods: All patients recruited at a tertiary referral center in the open label extension phase of the Gemini studies (GEMINI LTS) with at least one year of continued vedolizumab treatment, 300 mg on a 4 weekly dosing scheme, were analyzed through retrospective electronic chart review. Endoscopic healing was defined as a Mayo score of 0 or 1 for UC. For CD, marked improvement in endoscopic lesions with persistent ulceration was defined as partial healing, and the absence of ulcers as complete healing. Regular surveillance endoscopy was prospectively scheduled for all UC and CD patients with longstanding colitis. Targeted biopsies were obtained at advanced imaging with Methylene-blue or NBI (narrow band imaging) performed by expert IBD endoscopists. All biopsies were read by the same expert inflammatory bowel disease pathologist.
Results: Of 118 initially recruited patients in the GEMINI LTS study, a total of 68 patients (29 CD/39 UC) received vedolizumab beyond one year. Median treatment duration was 38 (range 13-73) months. Endoscopy data were available for 23 CD and 34 UC patients. At the last colonoscopy, 70% of UC patients maintained mucosal healing. In CD, 44 % had complete and 38% had partial healing. In a total of 32 (CD) and 50 (UC) colonoscopies throughout the study and performed after a median of 2.7 years, complete healing in CD was observed in 44%, partial healing in 47%, no healing in 9% and in UC Mayo score 0 was observed in 54%, Mayo score 1 in 20%, Mayo score 2 in 16% and Mayo score 3 in 10%. Low grade dysplasia (LGD) was detected in 10 % of patients. No high grade dysplasia (HGD) or colorectal cancer was found in biopsies or resected lesions, although HGD was found in the colectomy specimen of a refractory patient with LGD in biopsies.

Conclusions: Long-term endoscopic healing is observed in a selected group of CD and UC patients treated with vedolizumab. While even larger cohorts are needed, no new colonic dysplasia signal was found in this study.

I11

The need for surgery in stricturing ileal CD is linked to clinical and imaging factors but independent of NOD2 genotype.

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Introduction: The hallmark of Crohn's disease (CD) is transmural inflammation of the bowel wall leading to stricturing and penetrating complications in a majority of patients. Stricturing disease phenotype has been associated to NOD2 genotype. Overall, factors increasing the need for and influencing timing of surgery in patients with stricturing CD are understudied. Those predictors could prioritize surgery and avoid unnecessary expensive biologic therapies.

Aim: The aim of the study is to define whether a specific profile of stricturing CD could be identified which is associated with ultimate need for surgery.

Methods: All computed tomography (CT) or magnetic resonance (MR) scan performed at our institution (tertiary referral center) for CD between 2005 and 2015 were reviewed. The electronic charts of all patients with ileal stricturing CD were retrospectively reviewed for smoking, Montreal classification, first stricture presentation, penetrating complications, CD therapy, C-reactive protein, previous surgery, imaging features of stricturing CD, endoscopic dilation, clinical symptoms and hospitalization. The NOD2 variants were genotyped.

Results: A total of 1803 CT or MR scans were performed in a total of 957 CD patients. A total of 244 patients were diagnosed with an ileal stricturing disease. Nine patients were excluded for missing data. Overall, 161 patients (68.5%) needed surgery for stricturing CD. In 99 patients (61.5%) this was the first surgery. Univariate analysis identified several significant factors, associated with the need for surgery: prestenotic dilation of the small bowel on imaging, stenosis >5 cm, hospitalization for CD complications (especially obstruction), ileal B3 phenotype (especially abscess) in addition to stricturing CD, symptomatic strictures and impossibility for endoscopic dilation (all $p < 0.05$). In multivariate regression analysis, stenosis >5cm (p=0.0007, OR 3.31 [1.65-6.62]), prestenotic dilation (p= 0.006, OR 2.74 [1.33-5.62]), symptomatic stricturing CD (p=0.03, OR 3.18 [1.12-9.09] and hospitalization for stricturing CD (p= 0.04, OR 2.04 [1.05-3.99]) significantly impacted on the need for surgery. The interval between diagnosis of stricturing CD on imaging and surgery was only influenced by the length of stenosis with a greater delay for longer stenosis (p=0.048, OR 1.825 [1.01-3.32]). Surgery was independent of NOD2 genotype. Risk stratification according to the number of significant factors resulted in an increasing risk for surgery of 23%, 51%; 65%, 77%, 91% and 100% for patients with respectively 0, 1, 2, 3, 4 and 5 risk factors.

Conclusions: Abdominal surgery in stricturing ileal CD is mainly linked to clinical and imaging factors. A clinically valid risk stratification model can be built to aid physicians in deciding on surgery in ileal stricturing CD and may avoid unnecessary delay of surgery.

I12

Changes of practice in ileoanal pouch surgery in a single referal center

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Introduction: Ileal pouch-anal anastomosis (IPAA) has become the standard of care for the 20-25% of patients with ulcerative colitis (UC) who will undergo surgery during their disease course despite protracted medical therapy. This study aims to assess changes in surgical techniques and outcome in a single center over a 25 year period.

Aim: To evaluate 25 years of experience with IPAA in a single referal center

Methods: Demographic, perioperative, and follow-up data of patients with a restorative proctocolectomy for UC from 1990 to 2015 in UZ Leuven were analyzed from a database to audit our 25-years experience with this technique. Primary outcomes are leakage and ultimate pouch failure. The year of pouch construction was dichotomized according to three periods (Group A:1990 – 1999; Group B:2000 – 2009; Group C: 2010 – 2015).

Results: Between 1990 and 2015, 337 patients with a median age of 40.3 years (interquartile range: 32-49 years) (males=58.4%) underwent an IPAA for UC. 125 patients had surgery before 2000, 125 patients were operated during the second time period, and 87 patients were operated during the last period. Patients were younger at the time of surgery in group A (36.5 years versus 41.1 years versus 43.6 years ($p<0.001$)), duration disease was shorter in group A (6.6 years versus 8.7 years versus 11.2 years ($p<0.001$)). The overall rate of defunctioning ileostomies evolved from 94.0% in the early period to 13.9% in the late period ($p<0.001$). There was an important evolution in surgical management over the studied period. We described an important shift from restorative proctocolectomy with derivative ileostomy to colectomy and completion proctectomy without ileostomy (2.6% in group A versus 29.4% in group B versus 62.4% in group C) ($p<0.001$). The three-staged procedure was performed in 20.5% of the cases in group A, while only 8.4% of the patients underwent this sequence in group B and 4.9% in group C. These technical evolutions did influence, however not significantly, the leakage rate, which decreased from 13.6% to 5.9% ($p=0.25$), with a more pronounced decrease in clinically significant leakages. The overall pouch failure rate was 5.9% with an important decrease in the latest period (7.7% in the early period versus 0.9% in the late period), but this difference was not statistically significant ($p=0.13$).

Conclusions: Different important aspects of IPAA surgery changed over time and seem to reduce early postoperative morbidity. Septic complications decrease over time and suggest an ongoing learning curve.

I13

Analytical and clinical validation of a rapid point-of-care assay for infliximab quantification in patients with ulcerative colitis

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Introduction: Therapeutic drug monitoring of infliximab (IFX) has been widely accepted as a tool to optimize IFX outcomes, but the available (commercial) assays to monitor IFX concentrations lack the speed to implement treatment algorithms immediately. In addition, most assays require sample analysis in batch to reduce cost per sample.
Aim: To develop and validate a rapid, point-of-care assay for quantitative determination of IFX and assess thresholds associated with short term mucosal healing (STMH) in patients with ulcerative colitis.
Methods: Samples were prospectively collected from 29 anti-TNF naïve patients with ulcerative colitis starting IFX induction therapy, included between June 2010 and February 2012. All patients had a Mayo endoscopic sub-score ≥ 2 at baseline. STMH was evaluated at week 10-14 and defined as a Mayo endoscopic sub-score ≤ 1. IFX trough concentrations were determined during induction (week 2 and 6) and during maintenance (week 14 onwards) of IFX treatment with a novel lateral-flow based assay (LFA). The LFA was developed using the highly specific monoclonal anti-IFX antibody – clone mAb-IFX6B7 – for the detection of IFX bound to capture antibody. Conjugated gold nanoparticles were used for signal enhancement generating a colorimetric signal, which was read with a portable LFA reader. The LFA was benchmarked with the RIDASCREEN® IFX Monitoring ELISA.
Results: The LFA revealed linear dose-response curves in the 1 to 20 ng/mL range. To allow appropriate quantification of IFX concentrations within this range, a 1:2000 and 1:500 dilution was applied for induction samples and maintenance samples, respectively. The LFA showed an excellent agreement with ELISA for quantification of IFX as observed from Pearson and intraclass correlation coefficients of 0.95 and 0.95 during induction and 0.92 and 0.87 during maintenance therapy, respectively. In total, 45% of patients achieved STMH. Post-induction IFX trough concentrations (week 14) were higher in patients with STMH and receiver operating characteristic analysis withheld an IFX trough concentration ≥2.1 µg/mL (AUC: 0.819, p=0.008) in LFA as a factor associated with STMH with 100% sensitivity and 50% specificity. After one year follow-up, 92% of patients with STMH were still receiving IFX therapy versus 38% of patients without STMH.
Conclusions: The LFA is sensitive, selective and highly specific for IFX and identified an IFX threshold of 2.1 µg/mL at week 14 to be associated with STMH in patients with ulcerative colitis. With a turn-around time of 20 minutes, individual sample analysis and user-friendliness, the LFA outplays ELISA as a rapid, accurate tool to monitor IFX concentrations facilitating clinical decision making.

I14

Validation of the Simplified Geboes Score for ulcerative colitis

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Introduction: A Simplified Geboes Score (SGS) that includes the presence of basal plasmacytosis, eosinophils and neutrophils in lamina propria (LP), neutrophils in epithelium and epithelial injury has been recently proposed for histological assessment of ulcerative colitis (UC). The aim of this study was to validate the SGS prospectively.

Aim: The aim of this study was to validate the SGS prospectively.
Methods: UC patients treated at our tertiary referral centre were prospectively included from January 2011 to March 2014. Patients were followed up until a) Endoscopic re-assessment due to clinical symptoms, change of medication or screening for dysplasia or b) End of the follow-up period in October 2015. Endoscopic activity was defined as Mayo Endoscopic Subscore ≥1. Histological activity was assessed with the Original Geboes Score (OGS) and the SGS by 2 trained readers (readers A and B) and an expert gastrointestinal pathologist (reader C) blinded to clinical data.
Results: Ninety-two UC patients were included (72% males, mean age 47 years). Forty-six (50%) presented with Mayo 0, 18 (20%) with Mayo 1, 11 (12%) with Mayo 2, and 17 (18%) with Mayo 3. Overall, 260 slides were scored. Histological activity, defined as a presence of neutrophils in the biopsy (SGS≥2B.1), was present in 52/92 patients (57%): 39/46 (85%) of patients with Mayo ≥1 and 13/46 (28%) of patients with Mayo 0 at inclusion (p<0.001). Basal plasmacytosis and eosinophils in LP were significantly associated with histological activity (p<0.001). At the end of follow up, 67/92 (73%) patients underwent endoscopic control and 38 of them (57%) showed endoscopic activity: persistence of active endoscopic disease in 26/67 (39%) and relapse of previously endoscopically inactive UC in 12/67 (18%). Basal plasmacytosis (OR 4.7 [IC95% 1.6-14.2], p=0.006), eosinophils in LP (OR 4.3 [IC95% 1.4-13.7, p=0.01), neutrophils in LP (OR 7.1 [2.4-21.3], p<0.001), neutrophils in epithelium (OR 7.4 (IC95% 2.5-21.8, p<0.001), and epithelial injury (OR 6.8 (IC95% 2.3-20.3, p=0.001) at the baseline endoscopy were identified as predictors of endoscopic activity in univariate analysis, and neutrophils in epithelium (OR 7.4 [IC95% 2.5-21.8], p<0.001) as an independent predictor of endoscopic activity in multivariate analysis. Only neutrophils in LP (OR 7 [IC95% 1.3-38], p=0.02) and epithelium (OR 12 [IC95% 1.2-124], p=0.04) were predictors of relapse in patients with Mayo 0. Inter-observer agreement was better for the SGS than for the OGS (kappa 0.7 vs 0.6 for readers A-C and kappa 0.7 vs 0.5 for readers B-C).
Conclusions: The SGS is a useful and valid histological score for the assessment of UC. All its components (basal plasmacytosis, eosinophils and neutrophils) have been identified as predictors of endoscopic activity . Only neutrophils have been associated with relapse in patients with Mayo 0. The inter-observer agreement is better with the SGS that with the OGS.

I15

Hospital resources and co-morbidities associated with Crohn's disease and ulcerative colitis in Belgium

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Introduction: Crohn’s disease (CD) and ulcerative colitis (UC) are chronic diseases that in many cases are difficult to control leading to treatment escalations, hospitalizations and in some cases surgery. However, little is known regarding the hospital resources used by severe CD/UC patients.
Aim: This study aimed at estimating the cost and the length of stay (LOS) associated with hospitalizations due to CD/UC (i.e. with CD/UC as main cause of hospitalization) in Belgium. It also describes, within the hospitalized CD/UC population, the most common other causes of hospitalization and the percentage of patients undergoing gastrointestinal surgery. All objectives were addressed using retrospective data.

Methods: This study was conducted using the longitudinal IMS RWD Hospital Data -Belgium database (full year 2014), including data (diagnoses, procedures, costs, basic demographics) on 25% of all acute hospital beds in Belgium. Eligible population was selected based on the ICD-9-CM diagnosis codes (primary and secondary) for CD (555) and UC (556). Cost of hospitalizations due to CD/UC was calculated taking into account hospitalizations with CD or UC recorded as primary diagnosis. Other causes of hospitalization were identified by capturing all the primary diagnoses other than CD/UC documented in the eligible population during the study period.
Results: Crohn's disease A total of 3,988 distinct CD patients were retrieved in the database, adding up to 6,964 hospitalizations. Among these hospitalizations, 3,741 were directly attributable to the CD diagnosis: 767 in full-hospitalization setting (corresponding to 655 distinct patients; average cost: €5,609; average LOS: 7.5 days) and 2,974 in day clinic setting (1,697 patients; average cost: €416). Other common reasons for hospitalization in CD patients included mainly complications of CD: "Intestinal obstruction without hernia" (ICD-9-CM code '560'; 3.7% of patients), "Gastritis and duodenitis" ('535'; 1.4%), "Non-infectious gastroenteritis and colitis" ('558'; 1.3%) and "Anal fissure and fistula" ('565'; 1.1%). Gastrointestinal surgery was performed in 60.8% of the hospitalized CD patients (with 56.4% undergoing excision, incision or anamostosis of intestine). Ulcerative colitis A total of 2,029 distinct UC patients were retrieved, corresponding to 2,904 hospitalizations and 1,717 with a primary diagnosis of UC: 337 in full-hospitalization setting (corresponding to 310 distinct patients; average cost: €6,214; average LOS: 9.8 days) and 1,390 in day clinic setting (947 patients; average cost: €421). Other common reasons for hospitalization in UC patients included "Benign neoplasm of digestive system" (ICD-9-CM code '211'; 3.8% of patients), "Diverticula of intestine" ('562'; 1.6%) and "Other disorders of intestine" ('569'; 1.3%). About 75% of the UC patients underwent gastrointestinal surgery (and 70.1% excision, incision or anamostosis of intestine).
Conclusions: Real-life data show that hospitalized CD/UC patients can be costly and that the vast majority of them will undergo surgery over the course of the year.

I16

Predictive factors of stricture's evolution in Crohn's disease

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Introduction: Stricturing Crohn's disease affects around 30% of patients over ten years after the diagnosis. This behaviour is often refractory to medical treatment and is the leading cause to surgical resection.
Aim: Our goals were to assess strictures in Crohn's disease and study the different characteristics of these lesions at their diagnosis which could predict the disease's evolution.
Methods: We performed a retrospective monocenter study. Patients with CD type B2 were selected from our computerised register. For each patient were searched different factors (clinical, biological, imaging and endoscopic) to correlate to stricture's outcome. Outcomes studied were endoscopic dilation and surgery. Time to outcome was assessed by Kaplan Meier curve and risk factors were studied by Cox model. Factors studied were patient's clinical characteristics (gender, BMI, Tobacco, age at CD's diagnosis, age at stricture's diagnosis, maximal treatment before surgery), biological factors (CRP, Albumin, Fecal Calprotectin, ASCA), imaging factors (contrast-enhancement, bowel wall thickening, comb sign, ulceration,

mesenteric infiltration, complications like abscess and fistula), endoscopic factors (stricture's location, mucosal inflammation, length). An inflammatory stricture was defined by ulceration at endoscopy or MRI and/or CT scanner factors like mesenteric infiltration, comb sign or stratified contrast-enhancement.
Results: Among the 170 patients with pure stricturing disease (B2), the descriptive analysis shows that strictures were mainly ileal (67.7%), colic (16.5%) or anal (5.3%), last 10,5% were mixte, they were predominantly inflammatory (74.7% vs. 12.3% fibrotic vs 13% undefined), affecting more frequently women (62.9%) with mean BMI of 22.4 ± 3.8 kg/m², arising a median of 11 years (IQR=3 - 19 years) after CD's diagnosis. Median time to surgery or dilation was 31.1 months (IQR = 3.3 - 110 months). In univariate analysis, predictors of this outcome were female gender (HR = 1.74, IC95% = 1.16 - 2.60, p=0.007), existence of mesenteric infiltration on MRI or CT scanner (HR = 1.96, IC95% = 1.01 - 3.81, P= 0.047) and low albumin (HR = 0.95, IC95% = 0.92 - 0.99, p = 0.018). No variable was selected in multivariate analysis. Median time to surgery only (endoscopic dilatation excluded) was 53 month (IQR = 7.8 - 197 months). Predictive factors of this outcome were female gender (HR = 1.64, IC95% = 1.06 – 2.54, p=0.026), existence of mesenteric infiltration on MRI or CT scanner (HR = 2.40, IC95% = 1.2 - 4.80, p= 0.013), low albumin (HR = 0.94, IC95% = 0.90 - 0.98, p = 0.002), and active smoking compared to previous smoking (HR=2.7, IC95% = 1.1 - 6.3, p= 0.041). No variable was selected in multivariate analysis.
Conclusions: Half of the patients with CD's stricture were operated within 4 years and half. Main risk factors are female gender, active smoking, presence of mesenteric infiltration on MRI or CTscanner, and low albumin.

I17
Generation and characterization of a unique panel of anti-adalimumab specific antibodies and their application in therapeutic drug monitoring assays
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Introduction: A number of assays are currently available to support therapeutic drug monitoring of adalimumab. However, a complete characterization of the assays and comparison of different assays has not been performed.
Aim: To generate and characterize a panel of specific monoclonal antibodies towards adalimumab (MA-ADM) for use in novel assays to determine adalimumab concentrations; to assess the impact of tumor necrosis factor (TNF) and (non-)neutralizing antibodies on adalimumab detection and to compare the performance of assays.
Methods: A panel of MA-ADM was generated, characterized and applied in a TNF-coated (A) and a MA-coated ELISA (B). Serum samples of adalimumab-treated Crohn's disease patients were used for an external validation using the assay of Sanquin (C) and the apDia kit (D).
Results: Ten specific MAs recognizing adalimumab were generated of which four revealed a neutralizing potency of >78%. MA-ADM40D8 was selected as detecting antibody to determine

adalimumab in the TNF-coated ELISA and the MA-ADM28B8/MA-ADM40D8 antibody pair was chosen for use in the MA-coated ELISA. The impact of TNF and antibodies was similar on both ELISAs. All adalimumab assays showed excellent Pearson correlation: r = 0.96 for A versus B, 0.96 for A versus C, 0.94 for A versus D, 0.97 for B versus C, 0.95 for B versus D and 0.94 for C and D.
Conclusions: We generated 10 specific MA-ADM enabling the development of two highly specific adalimumab ELISAs. The ELISAs are in excellent agreement with two available ELISAs allowing harmonization of treatment algorithms in and between different hospitals/infusion centers.

I18

Effects of education and information on vaccination behavior in IBD patients

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Introduction: Despite the existence of (inter)national guidelines, vaccination in patients with inflammatory bowel diseases (IBD) has not yet been optimally integrated.
Aim: We investigated the current vaccination status in our IBD population and evaluated the efficacy of thorough education and information on vaccination rates.
Methods: Between Dec 2014 and Mar 2015, the vaccination status of 505 consecutive IBD patients (53% male, median age 44 years, 72% Crohn's disease, 27% ulcerative colitis) was collected by either a fellow in training or certified gastroenterologist at our outpatient clinic. Thereafter, patients were randomly assigned to group A receiving routine clinical practice, or intervention group B receiving additional thorough education by an IBD nurse, including an information brochure and vaccination card. After 8 months, all patients were contacted again to check for actual vaccination history. We looked for factors associated with compliance to vaccination recommendations.
Results: Analysis of the current vaccination status demonstrated that 31% of the IBD patients were completely vaccinated according to national guidelines, 80% for influenza, 62% for pneumococci, 53% for hepatitis B, and 82% for tetanus. Since 94% of the patients showed previous exposure to VZV, the effect of VZV vaccination was not further explored. Of the 346 patients that were not vaccinated following the recommendations, 140 (40%) were randomized to the intervention group B. After 8 months, vaccination rates were significantly greater in group B vs. group A, namely 36% vs. 10% for influenza (p=0.001), 62% vs. 23% for pneumococci (p<0.001), 27% vs. 5% for hepatitis B (p<0.001), and 33% vs. 2% for tetanus (p<0.001). Overall, 33% of group B vs. 6% of group A followed all vaccination recommendations 8 months after randomization (p<0.001). In univariate analysis, a higher educational level was associated with compliance to pneumococci vaccine recommendations (p=0.01) while the use of immunosuppressive therapy was associated with good compliance to influenza guidelines (p=0.015). Patients seen by certified gastroenterologists showed a better compliance to both influenza and tetanus vaccination guidelines (p=0.011 and p=0.027, respectively). In multivariate analysis, thorough education and information was the only consistent factor associated with a better compliance to vaccination guidelines for influenza [3.76 (1.20-11.78), p=0.023] as well as for pneumococcus [5.56 (2.92-10.58), p<0.001], hepatitis B [7.60 (3.12-18.52), p<0.001] and tetanus [23.72 (2.83-198.78), p=0.004]. Compliance to all vaccination recommendations after 8

months was significantly higher in the intervention group B in comparison with group A [7.38 (3.67-14.76), p<0.001].
Conclusions: Approximately one third of the IBD patients were vaccinated according to local guidelines. The introduction of vaccination education, an information brochure and a vaccination card significantly increased compliance to vaccination recommendations from 6% to 33%. Since compliance was far from optimal, there is still need for further education of both patients and health care professionals.

I19

Intestinal organoids derived from patients with inflammatory bowel disease show unaltered transcriptional profiles when compared to healthy controls

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Introduction: Various mechanisms contribute to the pathogenesis of inflammatory bowel diseases (IBD), including microbial dysbiosis and defects in epithelial barrier, Paneth cell or goblet cell function. The epithelium is constantly renewed by intestinal stem cells (ISCs) located at the bottom of the crypts. The ex vivo ISC-containing organoids may be a suitable model to investigate IBD pathogenesis.
Aim: We evaluated if the organoid forming capacity of colonic crypts, as well as organoid transcriptional profiles, from IBD patients differ from that of healthy controls (HC).
Methods: Colonic mucosal biopsies from 10 HC, 11 ulcerative colitis (UC) patients, and 11 Crohn's disease (CD) patients were used to culture organoids as previously described. After 2 passages, organoids were differentiated for 4 days by withdrawal of Wnt3a, nicotinamide and p38-inhibitor from the medium. RNA and histology samples were processed and analyzed using qPCR and immunohistochemistry (IHC) for ISCs and differentiated cell types as well as proliferation and apoptosis (Ki-67 and cleaved caspase 3 staining). We also investigated how inflammation (IL8, TNFa, CXCL3, among others) evolved when organoids were established from macroscopic inflamed areas. We included 3 patients per group (HC, UC, CD) and cultured organoids from crypts derived from both macroscopic inflamed and non-inflamed tissue. RNA was isolated from fresh biopsies and 1 week old organoids derived from the same tissue.
Results: There was no difference in initial organoid forming capacity between crypts of controls and IBD patients (HC 77%, UC 79%, CD 80% P=0.51). Additionally, no changes in expression of the ISC-marker Lgr5 were found. The expression of Hath1, a positive regulator of goblet cell differentiation, was decreased between controls and both UC and CD in differentiated as well as undifferentiated conditions (P=0.0242 and P=0.0240 for UC and CD under differentiation and P=0.0147 and P=0.0120 for UC and CD undifferentiated conditions, respectively). Also, chromogranin A expression in UC patients was decreased under differentiation (P=0.0402), while in differentiated organoids from CD patients a decrease in mucin2 expression was observed (P=0.0482). Moreover, preliminary data showed an unexpected increase in IL8 expression when both macroscopic and non-inflamed tissue was cultured as organoids, while expression of CXCL3 was down-regulated.
Conclusions: Our data shows that intestinal crypts isolated from IBD patients form organoids as efficient as crypts from healthy controls. Gene expression of markers of stemness and differentiation showed only subtle differences, and the biological implications remain to be

clarified via immunohistochemistry. We are validating these results by expanding the cohort and testing more genes.

I20

Variability in golimumab exposure: a "real-life" observational study in active ulcerative colitis

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Introduction: Golimumab is approved recently to treat refractory moderate-to-severe ulcerative colitis (UC). To date it is not clear why a considerable fraction of patients do not respond, or lose initial response, to golimumab therapy.

Aim: Our aim was to investigate whether a low golimumab serum concentration and/or a positive anti-golimumab antibody status reduces the efficacy of this drug in patients with UC.

Methods: Serum samples of 21 patients with moderate-to-severe UC were collected during the first 14 weeks of golimumab therapy. For measurement of golimumab serum concentrations, both a TNF-coated ELISA and a sandwich-type ELISA were developed. Anti-golimumab antibodies were measured using a bridging ELISA and a newly-developed drug-tolerant immunoassay. Clinical response and mucosal healing were assessed 14 weeks after start of treatment.

Results: Out of 21 patients, 10 (48%) reached partial clinical response at week 14. Median [interquartile range] serum golimumab concentration was significantly higher in partial clinical responders than in non-responders: 10.0 [7.8-10.5] µg/ml versus 7.4 [4.8-8.3] µg/ml at week 2 (P=0.035) and 5.1 [4.0-7.9] µg/ml versus 2.1 [1.8-4.2] µg/ml at week 6 (P=0.037). Four out of 21 UC patients developed anti-golimumab antibodies, detectable only using a drug-tolerant immunoassay, and three had a partial clinical response at that time. Clinical non-responders had a significantly more severe colitis, indicated by a higher endoscopic mayo score at baseline compared to partial clinical responders (P=0.048).

Conclusions: Adequate exposure to golimumab drives clinical response. A worse disease at baseline influences clinical response rate negatively.

I21

Evaluation of the efficacy of octreotide LAR in the treatment of Crohn’s disease associated refractory diarrhea

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Introduction: Diarrhea is one of the main characteristics of Crohn’s disease (CD). Its mechanisms are complex and multifactorial. Diarrhea is usually significantly improved with specific CD treatments. Loperamide and Cholestyramine may also be useful. However in some cases, diarrhea becomes refractory because of an incomplete control of the intestinal inflammatory reaction, despite an optimization of CD treatment, or due to persisting functional diarrhea. In these situations, octreotide may be useful, through its effects on gastrointestinal tract secretion, motility and its potential immunomodulatory effect.

Aim: To assess the safety and efficacy of octreotide in CD refractory diarrhea, in addition to CD treatments.
Methods: Three regional centres included fifteen patients aged 18 or older with diarrhea related to CD and refractory to CD treatments, Loperamide and/or Cholestyramine. Two patients were unfortunately lost to follow-up. Persisting diarrhea was defined by at least an average of five smooth or liquid stools per day, despite a normal CRP and an optimized CD treatment. A subcutaneous injection of 100µg of octreotide was performed three times a day during three days (V2, day 0-day 2). When the drug had been well tolerated, an intramuscular injection of 30mg octreotide (Sandostatin LAR 30®) was realized (V3, day 3). An evaluation of the effect of octreotide was done at V4, day 31. The criterion of evaluation was the number of smooth or liquid stools per day between 22 and 28 days after octreotide injection. Secondary endpoints were the number of patients responding to octreotide, the effect on CD activity (Harvey-Bradshaw Index (HBI)), on health related quality of life (IBDQ) and on fecal calprotectin and CRP. A significant response was defined by a decrease of at least 25% of the average number of liquid or smooth stools per day (measured over the last 7 days).
Results: Fifteen patients were included from December 2011 to July 2015. The median age was 49.3 ± 11.6 years. Eleven (73.3%) had previous ileo-colic resection. Eight (53.3%) were treated with anti-TNF, seven (46.7%) with immunosuppressive drugs, four (26.7%) with steroids and two (13.3%) with mesalazine. A significant reduction ($p<0.0001$) of the average number of smooth or liquid stools, from 6.6 ± 1.1 to 4.1 ± 2.0, was observed between V2 and V4. The maximum number of stools also significantly decreased ($p=0.0006$), from 8.5 ± 2.2 to 5.2 ± 2.0. Four (26.7%) patients presented adverse events characterized by abdominal pain or nausea. Nobody presented serious adverse events. Ten (76.9%) patients on thirteen responded to octreotide. We also observed a significant decrease ($p=0.0006$) of the HBI. The improvement of the IBDQ was also significant ($p=0.0012$). No significant change was observed in fecal calprotectin and CRP.
Conclusions: In this uncontrolled open-labelled study, octreotide appeared safe and effective in patients with CD associated refractory diarrhea, in addition to CD specific treatments. It significantly improved the number of liquid or smooth stools, the HBI and the IBDQ.

I22
Vedolizumab exposure in pregnancy: Outcomes from clinical studies in inflammatory bowel disease
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Introduction: Vedolizumab (VDZ) is a gut-selective immunoglobulin G1 monoclonal antibody to α4β7 integrin with demonstrated efficacy and safety in the treatment of Crohn's disease (CD) and ulcerative colitis (UC) in adults. Placental transfer of VDZ is anticipated to be similar to all other immunoglobulin G1 therapeutic antibodies and increases in a linear fashion as pregnancy progresses, with the largest amount transferred during the third trimester.

Aim: There are no controlled studies with VDZ in pregnant women. Here we report the effect of VDZ on pregnancy outcomes for female study participants and partners of male patients exposed in clinical studies.
Methods: Data from the VDZ clinical development programme up to 27 June 2013 were reviewed. According to the study protocols, female participants who became pregnant were to discontinue the study. The outcomes of pregnancies for female participants who became pregnant during the study and male patients with pregnant partners were summarised descriptively.
Results: The number of pregnancies reported were 27 in females (25 in patients with UC or CD, 2 in healthy volunteers) and 20 pregnancies in the partners of male patients in 6 clinical studies (placebo and VDZ were administered in 2 single dose studies and 2 multiple dose 1-year studies; VDZ was also administered in 2 long-term, open-label, multiple dose studies of 78 weeks and 4 years [ongoing]; Table). Of the 24 VDZ-treated females, 11 resulted in live births (2 premature). A congenital anomaly of agenesis of the corpus callosum was reported in the healthy volunteer with an obstetric history of 2 spontaneous abortions and 1 ectopic pregnancy who had received a single dose of VDZ 79 days prior to the estimated date of conception. Among the 16 VDZ-exposed partner pregnancies, there were 9 live births, 2 spontaneous abortions, 2 elective terminations, and 3 undocumented outcomes at the last follow-up.
Conclusions: Although female participants were discontinued if they became pregnant during the study, data from the VDZ clinical development program provide some insight into pregnancy outcomes of VDZ-treated patients. An observational pregnancy registry enrolling patients with UC or CD on VDZ is currently in development to observe and evaluate the long-term safety of VDZ in pregnancy.

I23

Evolution of patient reported outcomes from the CDAI to define remission in the Gemini 2 study.

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Introduction: Clinical trials in Crohn's disease (CD) are evolving towards including patient-reported outcomes (PROs) to evaluate drug efficacy; however, no validated PROs are available.
Aim: In post hoc analyses of data from the placebo (PBO)-controlled GEMINI 2 study, we evaluated the use of the PRO components of the CD Activity Index (CDAI) to define vedolizumab (VDZ)-induced remission.
Methods: Patients (pts) with moderately to severely active CD (CDAI 220-450) who responded to 6 weeks (wks) of VDZ induction therapy were re-randomised to maintenance therapy with PBO or VDZ for 46 wks. PRO components of the CDAI (stool frequency [SF], abdominal pain [AP], and general wellbeing [GWB]) were evaluated alone and in combination (PRO-2 [SF and

AP] and PRO-3 [SF, AP, and GWB]) at wks 0, 6, and 52. Contributions of PROs to baseline and mean change from baseline CDAI total scores were analysed. The sensitivity of PRO-based definitions of remission (PRO-2 [SF ≤3 and AP ≤1]; PRO-2 ≤75; and PRO-3 ≤80) to detect clinical remission (CDAI ≤150), their agreement with CDAI ≤150, and the percentage of GEMINI 2 pts who met PRO-based entry criteria (SF ≥4 and/or AP ≥2) were evaluated.
Results: The SF, AP, and GWB subscores contributed 25%, 21%, and 31%, respectively, to the mean baseline CDAI score. PRO-2 and PRO-3 accounted for, respectively, 53% and 91% of the mean change from baseline CDAI score at wk 6, and 47% and 82% at wk 52. PRO-2 (SF ≤3 and AP ≤1), PRO-2 ≤75, and PRO-3 ≤80 detected clinical remission with a sensitivity of, respectively, 67%, 76%, and 57% at wk 6, and 74%, 95%, and 77% at wk 52. PRO-3 ≤80 had the best agreement with CDAI remission at wk 6 and PRO-2 ≤75 had the best agreement at wk 52. PRO-based entry criteria of SF ≥4 and/or AP ≥2 were met by 86% of GEMINI 2 pts.
Conclusions: The PRO components of the CDAI contributed most to baseline and treatment-induced changes in CDAI score. Different PRO-based definitions of remission (PRO-2 ≤75 or PRO-3 ≤80) had the best agreement with clinical remission at wks 6 and 52. Interpretations of these post hoc analyses are limited, but applying the cut-offs used here to other datasets could clarify the reliability and clinical meaningfulness of PROs as outcome measures.

I24

Genetic risk for Crohn's disease has little impact on intestinal microbiota composition

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Introduction: Crohn's disease (CD) has a multifactorial pathogenesis with input from genetics, immunologic factors, and environmental triggers, including intestinal microbiota. To date, 174 risk loci have been identified for CD, with a remarkable predominance in pathways associated with host-microbiota interactions. Dysbiosis with reduced richness and diversity is a replicated finding in patients with CD. Whether this is related to the genetic imprinting of a patient or linked to inflammation is unknown.
Aim: We investigated if these genetic risk variants associate to intestinal microbiota composition differences.
Methods: Fecal and blood samples were collected from 30 CD patients from whom Immunochip data were available. A genetic risk score (GRS) was calculated for each patient, taking into account the risk allele frequency and odds ratio of each single nucleotide polymorfism (SNP). The GRS associated with CD was calculated with 197 SNPs available on the Immunochip. Autophagy, ER stress and NOD2 associated GRS were also generated. Patients were divided into 4 groups according to quartiles of the general GRS. 16S rDNA paired-end sequencing targeting the V4 hypervariable region was performed using Illumina MiSeq sequencer. Sequencing depth was downsized to 10000 reads/sample. The Ribosomal Database Project classifier was used for taxonomic assignment. Statistical analyses were performed with R package phyloseq, using

parametric and non-parametric tests, with multiple testing correction (FDR). Correlation between genera abundances and genetic risk scores was performed with Spearman correlation.
Results: The microbiota richness (alpha diversity) and overall microbiota composition were not significantly different between patients belonging to Q1 (n=8) or Q4 (n=8) of their GRS. At genus level, no differences were observed. When looking specifically to particular pathways, we observed that microbiota richness (anova p value 0.045) and community composition (Bray-Curtis dissimilarity adonis p value 0.03) differed according to autophagy GRS. However, no significant taxon abundance differences were observed at phylum, genus or operational taxonomic unit (OTU) level. Noteworthy, some differences at genus level (eg. Anaerostipes, Roseburia and Megamonas) were observed between the different groups of autophagy GRS before multiple testing correction.
Conclusions: Host-genetics seem to influence the intestinal microbiota composition through pathways associated with host-microbiome interactions, particularly autophagy. However, this influence is small. Larger sample size may be needed to detect small differences at genus level. Environmental factors seem to have a larger impact on gut microbiota than host-genetics.

Nutrition

N04
The role of FODMAPs in gastric accommodation and upper gastrointestinal motor activity
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Introduction: There is accumulating evidence for the benefit of a diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) for the management of irritable bowel syndrome (IBS) symptoms. Whether FODMAPs alter the upper GI response to nutrients, including gastric accommodation (GA), remains to be assessed.
Aim: The objectives were to assess the role of different FODMAPs in the intragastric pressure (IGP) response to nutrient ingestion (which reflects GA), upper GI motility, meal-induced satiation and symptom generation.
Methods: A high resolution manometry and infusion catheter were positioned in the proximal stomach of healthy volunteers. After a stabilization period and when the subjects were in late phase II of the migrating motor complex (MMC), one of four solutions (fructans (38g/L), fructose (100g/L), FODMAP mix (80g/L) or glucose (100g/L) as control) were intragastrically infused at 60 mL/min, three days to one week apart in a single-blind randomised cross-over order. The FODMAP mix consisted of 20g of fructans, 10g of galacto-oligosaccharides, sorbitol and mannitol and 30g of fructose dissolved in 1L of water. The infusion ended when subjects scored maximum satiation (0-5 scale). IGP was recorded for the duration of the drink infusion and for the following 3 hours. IGP was presented as change from baseline (mean ± SEM). Intensity of epigastric and GI symptoms were rated before the infusion, and then every 15 minutes using a 100 mm VAS. Results were compared using a repeated measures ANOVA.
Results: 20 healthy volunteers (19-32 y, 10 men, 18-44 BMI) were randomized. Two were smokers and none had GI symptoms or history of GI disease. Total ingested volumes at maximal satiation differed significantly between fructose and FODMAP mix ($p = 0.0197$) (fructans 1383±193 mL; fructose 1032±113 mL, FODMAP mix 1302±129 mL, glucose 1125±98 mL). In all subjects and with each infusion, the IGP decreased initially to gradually recover thereafter.

The mean IGP drop during infusion was significantly less for fructans (-2.25±0.15 mmHg), when compared to all other solutions (fructose -3.21±0.26 mmHg, p=0.0005; FODMAP mix -3.78±0.23 mmHg, p<0.0001 and glucose -2.98±0.19 mmHg, p=0.0113). Furthermore mean IGP differed significantly between FODMAP mix and glucose (p=0.0075). After recovery of the IGP drop, although all solutions remained constantly high in their pressure across the three hours post infusion, the fructans maintained a significantly higher intra-gastric pressure (3.7±0.19 mmHg) compared to the fructose, FODMAP mix and glucose (2.7±0.19, 1.5±0.16 and 1.7±0.15 mmHg, respectively) (p<0.0001). In comparison to the glucose, differences in symptoms were reported for wind following the fructans, fructose and FODMAP mix and for cramps following the fructose and FODMAP mix solutions (p<0.05).
Conclusions: This study indicates that fructans induce a significantly lower IGP response in the healthy state, when compared to the other FODMAPs and glucose. Unraveling the sensory, neural and/or hormonal pathways involved in the effect of fructans on gastric physiology require further mechanistic studies. The findings also offer opportunities to identify whether ingestion of fructans contribute to symptoms associated with impaired GA seen in functional GI patients, including IBS.

N05
Oral administration of a Spirulina extract protects old mice against hepatic "inflammaging" "
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Introduction: The process of aging predisposes to hepatic functional and structural impairment leading to inflammation -called inflammageing- and favours non-alcoholic fatty liver disease (NAFLD). Spirulina is a cyanobacterium within the Oscillatoraceae family which is used as a food additive. Previous studies suggest the beneficial effects of Spirulina on immune functions and against NAFLD, inflammatory disorders, hyperglycaemia and hypercholesterolaemia in mouse models of metabolic syndrome.
Aim: The aim of the present study was to test the potential hepatoprotective effects of Spirulina extract supplementation in aged mice and to determine whether these effects can be related to a modulation of the gut microbiota.
Methods: Male C57Bl6J mice of 3- and 24-months old were fed a control diet supplemented with or without 5% Spirulina extract (Biores, Belgium) during 8 weeks.
Results: Aged mice exhibited inflammation and oxidative stress in the liver tissue (higher expression of TNF-α, IL-6, IL-1beta, MCP-1, CD68, COX-2, CD11c, TLR4, NADPHoxidase) as compared to mice of 3 months. The supplementation with Spirulina extract reduced those hepatic inflammatory and oxidative markers in 24-months mice. Interestingly, the expression of transcription factor involved in immune system regulation (FoxP3 in T Regulatory cells) and the expression of antimicrobial peptide (Reg3gamma) were upregulated in the ileum of Spirulina-treated mice. Combination of pyrosequencing and qPCR analyses of the 16S rRNA gene revealed a decrease in total bacteria and -among specific changes of gut microbiota composition- an increase in lachnospiraceae population by Spirulina treatment.
Conclusions: Our study shows for the first time that the oral administration of a cyanobacterium (Spirulina) is able to modulate the gut microbiota, to activate immune system in the gut, thereby

improving hepatic inflammation in aged mice. Those data allow to envisage new therapeutic tools, based on gut microbes-host interactions, in the management of systemic diseases, such as NAFLD.

N10

Evaluation of the flowchart of nutritional management of adult

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Introduction: Dans le cadre d'une prise en charge nutritionnelle (PECN) systématique des patients adultes hospitalisés en médecine, chirurgie, et revalidation au CHR de La Citadelle, le département infirmier (I) a mis en place à l'aide du CLAN un logigramme (L) de PECN au 01/12/2014. La 1ère partie du NRS-2002 a été intégrée dans l'anamnèse infirmière.

Aim: Le but est d'évaluer l'application de ce logigramme pluridisciplinaire après 5 mois de fonctionnement.

Methods: Une étude rétrospective a été conduite d'avril à août 2015. Le travail des diététiciens (D) (12 D interrogés) et des I a été analysé à l'aide de questionnaires spécifiques (47 I interrogés). Ensuite, un audit de dossiers infirmiers (DI) (334 dossiers I audités) a été réalisé ainsi qu'un audit des lettres de sortie des médecins correspondants aux DI.

Results: Cinq mois après la mise en place du L, 2/12 D ne notent pas le score NRS-2002 et/ou le grade de dénutrition. Le L permet une PECN ciblée. Nous voyons que 75% des I interrogés connaissent le NRS-2002 et 25% le connaissent mais pas de nom. 94% des I contactent le D en cas de NRS-2002 1ère partie > 0. L'audit des DI révèle que 21% avaient un NRS-2002 1ère partie complet, 63% étaient incomplets c.à.d. avec au moins 1 des items demandés non complété et 16% n'étaient pas complétés. Pour les 63% de dossiers incomplets, l'item principal manquant était l'IMC. La mise à disposition de roulettes IMC pourrait être une solution. Le poids est trouvé pour 78% des DI via le NRS-2002 1ère partie contre 48 % de poids trouvés ailleurs dans les dossiers. Le NRS-2002 1ère partie est problématique pour les US à Turn over important. Les I demandent des formations. Peu ou pas d'informations nutritionnelles sont retrouvées dans les lettres de sortie des médecins lorsqu'une PECN a été effectuée. Ceci constitue un manque pour la justification des durées de séjour et la qualité de la PECN.

Conclusions: Cinq mois après la mise en place du logigramme, il est bien appliqué par les infirmiers et les diététiciens. Les infirmiers sont sensibilisés à la dénutrition mais désirent mieux comprendre le NRS-2002 pour éviter l'application automatique. Les résultats des questionnaires I et de l'audit des dossiers I ont été remis à chaque Unité de Soins pour analyse. Le logigramme sera expliqué lors de formations continuée des I et aux nouveaux agents. Il est essentiel d'impliquer d'avantage les médecins dans la PECN par l'introduction des items nutritionnels dans les lettres de sortie au risque de perdre la qualité du travail réalisé en amont.

Belgian Group for Digestive Oncology (BGDO)

O01

Tumor Regression Grading according to Mandard (ypT) in Esophageal Carcinomas after Neoadjuvant Treatment followed by Surgery.

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Introduction: Tumor regression grading (TRG) systems aim to categorize the amount of regressive changes on the primary tumor after neoadjuvant treatment in order to demonstrate potential prognostic information based on objectively determinable histopathologic findings. In literature Mandard grade 1 is usually considered as the only prognosticator for good survival, as compared to the other grades.

Aim: To evaluate the prognostic information from prospectively recorded Mandard TRG on overall and disease-free survival in adenocarcinoma (ADC) and squamous cell carcinoma (SCC).

Methods: From our multidisciplinary oncologic consult (MOC) meetings between 2005 and 2014, we retrieved 455 patients who were treated by esophagectomy after neoadjuvant treatment. Ten patients with a histology other than ADC or SCC were excluded, as well as 66 patients who received neoadjuvant chemotherapy alone. Three hundred and seventy nine patients were withheld for this analysis. Mandard TRG was prospectively recorded. Overall survival and disease free survival were calculated by means of Kaplan-Meier curves and compared by log rank tests.

Results: There were 290 (76%) males and 89 (24%) females with a mean age of 62 years (range 34-83). ADC were predominant (n=246 or 65%). Mandard TRG grade 1 (complete regression) was found in 121 (32%); grade 2 (fibrosis with scattered tumor cells) in 124 (33%); grade 3 (fibrosis and tumor cells with preponderance of fibrosis) in 54 (14%); grade 4 (fibrosis and tumor cells with preponderance of tumor cells) in 60 (16%) and grade 5 (tissue of tumor without signs of regression) in 7 (2%) patients. In 13 (3%) patients Mandard TRG was not reported. Both overall survival (OS) and disease-free survival (DFS) showed no significant difference between grade 1 and 2 ($p=0.236$ and 0.151 for OS and DFS respectively). The remaining three grades (3 till 5) were significantly different (all $p<0.05$) from these two grades. The same effect is still present when both ADC and SCC are analyzed separately. Therefore grades 1 and 2 were classified as ‘major response’ (MaR) and grades 3 till 5 as ‘minor response’ (MiR). The prevalence of MaR was significantly lower in the ADC group (60.1%) compared to 72.9% for SCC ($p=0.028$). Local recurrence rates were significantly lower in MaR compared to MiR (11.5% versus 26.0% for ADC; $p= 0.004$ and 8.2% versus 36.4% for SCC; $p<0.0001$). Incorporating the tumor depth (ypT) in the Mandard TRG shows a significant ($p<0.0001$) decrease in OS with increasing ypT-stages.

Conclusions: Mandard TRG is a valuable prognostic tool in assessing pathologic response on the primary tumor after neoadjuvant chemoradiationtherapy. Our findings suggest that not only Mandard TRG grade 1 is carrying a significant better prognosis, but TRG grade 2 shows equal favourable prognosis. Based on our findings in OS, DFS and local recurrence patterns a clear distinction between major (TRG 1 & 2) and minor (TRG 3-5) response is possible. Nevertheless location of residual tumor cells (ypT) also seems to play a crucial role.

O02

Expression profiling of budding cells in colorectal cancer suggests an EMT-like phenotype and molecular subtype switching

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Introduction: Tumour budding is referred to as the presence of single cells or small clusters of up to five tumour cells at the invasive margin of colorectal carcinoma and is considered as an additional prognostic marker besides TNM-staging. It is generally hypothesized, but not yet proven, that the feature of budding is the histologic representation of epithelial-mesenchymal transition (EMT).

Aim: In the present study, we aim to investigate the molecular signature of tumour budding and the corresponding tumour bulk regions.

Methods: Tumour bulk and budding areas were microdissected from eight fresh-frozen (FF) colorectal cancer samples and processed for RNA-sequencing. Since little RNA was obtained from budding cells, a special low-input (~1ng) mRNA library preparation protocol was used. Gene expression profiles of budding as compared to tumour bulk were investigated for established EMT signatures, consensus molecular subtype (CMS), gene set enrichment and pathway analysis.

Results: A total of 296 genes were differentially expressed with a FDR<0.05 and at least a twofold change between tumour bulk and budding regions. Genes that were upregulated in the budding signature were mainly involved in cell migration and survival while downregulated genes were important for cell proliferation. Supervised clustering according to an established EMT gene signature categorized budding cells as EMT-positive, whereas tumour bulk samples were considered EMT-negative. TGFβ1, TGFβ3 and CTNNB1 were potential upstream regulators of the differential expression profile hinting for active signalling through WNT and TGFβ pathway. Furthermore, a shift from the consensus molecular subtype CMS2 (epithelial) to CMS4 (mesenchymal) was observed as tumour cells transit from the tumour bulk to the tumour budding regions.

Conclusions: Tumour budding regions are characterized by a phenotype switch compared to the tumour bulk, involving the acquisition of migratory characteristics and a decrease in cell proliferation. In particular, most tumour budding signatures were EMT-positive with activation of both WNT and TGFβ signalling and a switch from an epithelial subtype (CMS2) in the tumour bulk to a mesenchymal subtype (CMS4) in budding cells.

O03

Metastatic colorectal cancer has heterogeneous immune microenvironment and mutational expression

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Introduction: Understanding the colorectal cancer (CRC) metastasis process is a major clinical challenge for treatment efficacy. CRC cells must successfully negotiate a series of complex steps for progression and establishment in a foreign tissue environment.
Aim: We plan to analyze the immune microenvironment within all resected metastases (Ms) and corresponding primary tumor (PT) to investigate the possible tumor immune heterogeneity and the consequence for personalized-medicine approaches.
Methods: We performed a full whole-slide quantification of immune cell densities (CD3/CD8/CD45RO/CD20/FoxP3) in the core (CT) and invasive margin (IM) on all resected synchronous (Sync) and metachronous (Metac) Ms (n=338) and available corresponding PT (n=69) from a cohort of 114 operated metastatic CRC patients (pts). The mean density value was calculated for each marker in each tumor region (CT/IM) with a dedicated image analysis software on whole-slide images. Additionally, a somatic mutation profiling (Ion Torrent PGM technology) of 50 onco- and tumor suppressor genes from Ms (Sync and Metac) and PTs was performed on a subgroup of 12 pts with the highest tumor immune heterogeneity. Comparisons were made using the t-test and Wilcoxon-Mann-Whitney test
Results: The global immune infiltration was mostly heterogeneous in each tumor region (CT/IM) within and across Ms (Sync/Metac) and PTs from all pts. CD3, CD8 and CD45RO densities were significantly higher in the Ms (CT/IM, Sync/Metac) compared to the PTs ($p<0.05$). Conversely, higher CD20 and FOXP3 densities were observed in PTs (CT; $p<0.05$). For pts with multiple Ms, the global T-cell infiltration was increased in small-sized Ms (CT/IM; $p<0.05$). The analysis of the highest infiltrated metastasis per patient, showed that pts with a large number of Ms had a significantly higher number of infiltrating immune cells compared to pts with few Ms ($p<0.05$). Focusing on the group of pts with the highest tumor immune heterogeneity, a diverse mutational expression (TP53, PIK3CA, KDR, KIT, APC and KRAS) was observed between Sync and Metac Ms and PTs.
Conclusions: A global heterogeneity of the tumor immune environment and mutational expression was observed in metastatic CRC, suggesting a genetic evolution of tumor clones during progression. It could pose major challenge to personalized-medicine and makes tumor-biopsy challenging for biomarker discovery.

O08

Proteins in early CRC stages - from proteomic discovery to immunohistochemistry tissue distribution characterisation

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Introduction: Colorectal cancer (CRC) is one of the most common cancers. Early CRC detection shows great potential due to better prognosis and elimination of precancerous lesions at high CRC transformation risk.
Aim: We performed a retrospective study in order to identify proteins involved in CRC progression and to highlight new potential diagnosis markers leading to development of new therapeutic tools.
Methods: We tested FFPE tissue samples of 20 controls (patients with diverticular disease using paired inflamed and healthy tissues) and 36 CRC (pT1N0M0 and pT2N0M0). After enrichment of epithelial cells by macrodissection, we used FFPE-FASP kit (Expedeon) for sample preparation. The protein digests obtained were analyzed using 2D-nanoAquity UPLC separation online with Q-Tof Synapt HDMSTM G2 using ion mobility as additional separation (Waters). We performed protein identification/quantitation and differential analysis using Progenesis QI for proteomics (Nonlinear Dynamics-Waters). Moreover, we validated some selected potential markers previously associated with cancer by immunohistochemistry (IHC) on an independent cohort of patient samples including precancerous grade (low and high grades adenomas) and cancerous lesions (pTis to pT4 grades).
Results: Using proteomics, Olfactomedin-4 (OLFM4) and Sec24C were found at a significantly higher level in early CRC tissue as compared non inflammatory controls. They were selected for further tissue distribution characterisation in a confirmatory cohort of patients. The OLFM4 and Sec24C stains increased significantly in dysplasia tissues compared to normal tissue and with the highest signal obtained for pT2N0M0 tissues. By contrast the pT3 and pT4 CRC stages provided lower staining with signal distribution qualified as moderate or heterogeneous.
Conclusions: This FFPE retrospective study comparing T1 and T2 CRC highlighted proteins already previously identified as potential cancer biomarkers. These proteins may help to better understand early CRC tumor progression and to discriminate early from late pTNM CRC stages.

O09

PURE LAPAROSCOPIC LIVER RESECTIONS IN THE POSTEROSUPERIOR SEGMENTS IN SEMIPRONE POSITION. SINGLE CENTER EXPERIENCE AND ANALYSIS OF LEARNING CURVE.

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Introduction: Performing pure laparoscopic liver resections in the posterosuperior segments (LRPSS) remains challenging. As reported by several series laparoscopic resections of tumors located in segment IVA, (upper part of) segment IV, segment VII or VIII is as difficult as a major liver resection because exposure is difficult in supine position and the transection line is often curved or angled. Therefore many surgeons are tempted to perform a laparoscopic right hemihepatectomy for tumors located in these segments. However, several papers reported a

prolonged survival in patients with colorectal metastases when parenchymal preserving liver resections were compared to major hepatectomies. Furthermore, right hemihepatectomy has been associated with an increased risk of hepatic insufficiency when compared to peranchymal sparing resections.
Aim: We analyzed our experience with pure laparoscopic resections of tumors located in the posterosuperior segments. All resections were performed with the patient positioned in the semiprone position.
Methods: Retrospective review of prospectively collected database on operative and postoperative characteristics and surgical outcomes of all patients in whom LRPSS was performed by one surgeon between September 2011 and September 2015.
Results: Forty nine patients underwent LRPSS.Median age was 64 years(range 23-82).In total 58 resections were performed in the posterosuperior segments.Seven patients underwent additional resections in other segments.Indication for surgery was mainly colorectal liver metastases (n=31;63.3%).There were 4 postoperative complications (Clavien Dindo I or II (1 bile leak)).Ninety day mortality was 0.There were 2(4.1%) conversions.Median operative time was 140min(50-260).Median intraoperative blood loss was 150 mL(0-1500) .A Pringle maneuver was never used.Median hospital stay was 7days (3-14).R0 resection rate was 100%.There was no difference in operative times when comparing the first 25 cases with the last 24 cases (130 minutes(IQR 100-140) - first 25 cases;150 minutes(IQR 125-185) - last 24 cases($t = -1.75$; $p = 0.086$)).There was no difference in blood loss when comparing the first 25 cases with the last 24 cases (150 mL(IQR 100-350) - first 25 cases;165 mL(IQR 75-300) - last 24 cases($r = -0.20$;$p = 0.84$)).
Conclusions: LRPSS in semiprone are safe and feasible.Placing the patient in semiprone improves visualization, mobilization, enables this laparoscopic technique to provide safe and effective parenchyma preserving liver resections for lesions in these difficult segments and avoids a steep learning curve.

O10
Myoferlin: an indispensable component in VEGF-A secretion by pancreas cancer cells
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Introduction: Ferlin family proteins have been reported to participate in plasma membrane fusion, repair, and endocytosis concretely but not exclusively, it has been reported in skeletal muscle development and repair (myoferlin and dysferlin) and presynaptic transmission in the auditory system (otoferlin). While some reports have implicated a member of ferlin family proteins, myoferlin, in cancer; the extent of its expression and contributions to cancer are not well established.
Aim: Myoferlin, a member of the ferlin protein family was recently identified in our laboratory as a new accessible biomarker for human pancreatic ductal adenocarcinoma. In addition to its potential suitability for targeted therapy, we aim to examine the biological role of this protein in the development of pancreatic cancer.

Methods: SiRNA mediated silencing technique was adopted to ensure the depletion of myoferlin to reveal the biological function of myoferlin during tumor progression in vivo and in vitro. Scoring of immunohistochemically stained patients' cohort sections was taken up to investigate correlation between myoferlin expression and micro-vessel density in patients.
Results: SiRNA-mediated myoferlin-silencing significantly reduced the volume of BxPC-3 tumors developed onto the chorioallantoic membrane of fertilized chicken eggs. Intriguingly, aside their reduced volume, myoferlin-silenced tumors appeared whitish and exhibited a significant decrease of blood vessel density as shown by FITC-conjugated Sambuccus nigra agglutinin staining. This observation suggested that, in addition to an inhibition of BxPC-3 cell growth after myoferlin silencing, this protein may exhibit a pro-angiogenic activity. Accordingly, we next showed that myoferlin-silencing significantly inhibited VEGF-A secretion without decreasing VEGF-A gene expression. Immunofluorescence revealed that VEGF-A seemed to accumulate in the cytosol at the vicinity of the plasma membrane concomitantly together with vesicle like structures, seen by the electron microscopy, in myoferlin depleted cells. Furthermore, immunofluorescence techniques showed a colocalization of myoferlin with Sec5/Exoc2, a component essential for exocytosis, raising the hypothesis that myoferlin plays a role in VEGF-A secretion.
Conclusions: Our work highlight a new function of myoferlin in pancreatic cancer progression. We show that myoferlin is essential for pancreatic cancer cell lines proliferation and tumor growth. We also report for the first time myoferlin as a key regulator in VEGF-A secretion by controlling the exocytosis of VEGF-A secretory granules in the tumor stroma.

O11

EXPEL: A Novel Non-Destructive Method for Mining Soluble Tumor Biomarkers

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Introduction: The search for biomarkers able to detect and evaluate disease such as cancer at an early stage, or to predict resistance and response to therapies, has been and remains a major challenge. Despite very important progresses in all fields of omics technologies, the success of discovery of clinically valuable biomarkers is surprisingly disappointing. Difficult mining of secreted proteins in biological fluids poses the first major hurdle, mainly because the concentration of interesting proteins in serum or urine is generally very low. The second key limitation in the field is the inaccessibility of tissue specimens from early lesions. Those are routinely required in their integrity for the complete histological evaluation in the clinical routine, leaving no residual material for research.
Aim: Aim of the study is the discovery of new soluble biomarkers for non invasive diagnosis of colorectal cancer and its liver metastasis.
Methods: Here we present an innovative procedure that we have named EXPEL, which entirely overcomes the mentioned limitations. It makes any tissue, regardless of its size, available for both omics research and histological investigation.

Results: Our original device and approach extracts soluble tumor biomarkers and small metabolites within few minutes and without altering the tissue morphology. For this purpose a small tissue biopsy is incubated in a slightly hypertonic extraction buffer while subjected to alternating pressure. Upon extraction the tissue is fixed in formalin and can be used for histological analysis. The soluble extract is further prepared for proteomic and metabolomic analysis. In a proof of concept study we have extracted and analyzed soluble biomarkers from human colorectal carcinoma liver metastases (N=10) as well as primary colorectal tumors (N=10). Pathology validation demonstrates that EXPEL procedure does not alter tissue morphology or subsequent molecular and clinical tests. The comparison of proteins and metabolites identified in tumor lesions with those found in adjacent normal tissues revealed a promising group of novel and differentially expressed targets. Their potential usefulness as diagnostic or predictive markers is currently being explored.
Conclusions: The Expel method provides clinicians with a new tool enabling them to non-destructively discover new biomarkers and preserve precious tissues (like colon polyps) for pathology evaluation

O12

Quality Control of pancreatic tissue samples in Tumorbank@UZA

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Introduction: Since 2008, snap frozen tumor tissue and FFPE material is being collected for translational and clinical research in Tumorbank@UZA. In this study a total of 87 frozen (-80°C) tissue samples from 52 patients with pancreatic cancer and their corresponding uninvolved tissue samples (if available) were selected for quality control.
Aim: We checked if there was a correlation between the RNA quality based on the RIN value and pre-analytical variables such as cold ischaemia time and/or sample age. We also evaluated if there was a difference in RNA quality between tumour tissue versus reference pancreatic tissue.
Methods: A trained pathologist evaluated the hematoxilin eosin stainings for the presence of tumour and estimation of tumour cell percentage. From the samples that passed this first quality control, DNA and RNA was simultaneously isolated using the Qiagen Allprep Micro kit. Concentration of nucleic acids was measured by spectophotometry and RNA quality, expressed as RNA integrity number (RIN) was measured with the Agilent Bioanalyzer.
Results: From all selected samples, 70% were acceptable for downstream research after evaluation of the HE stainings. Rejection of samples was based on the presence of tumour cells in samples that were annotated as reference material and vice versa (16%) or on difficulties in assessment of the slide due to presence of artefacts (14%). An overall mean RIN value of 4,2 reflects that pancreatic tissue is among the most difficult tissues to isolate RNA due to the abundant presence of nucleases. In this sample set there was no significant correlation between cold ischaemia time or storage time and RNA quality. However, RNA quality was significantly higher for tumour tissue as compared to reference tissue (mean RIN respectively 5,1 and 3,2).
Conclusions: In conclusion, our findings underline the necessity of quality control of banked tissue samples in order to find “fit-for-purpose” applications.

O13

Discovery of Novel Accessible Proteins for Therapeutic Targeting of Hepatocellular Carcinoma

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Introduction: Hepatocellular carcinoma (HCC) is world's sixth most common and third most deadly malignancy. The clinical management of HCC is difficult. Apart from liver transplantation in a minority of operable patients, there is currently no effective treatment to eradicate HCC. On-going clinical trials are exploring predominantly small molecules that have not been specifically tailored for HCC. Such therapies have shown only modest success in other types of tumors. We know today that small molecules (such as tyrosine kinase inhibitors) do not have the ability to selectively accumulate in neoplastic lesions. In contrast to this, monoclonal antibodies (mAb) can achieve good tumor to blood ratios. However, in "naked" format mAb rarely have the necessary toxicity to eradicate the tumor. Antibody-drug conjugates (ADC) are potent derivatives of classical antibodies that are able to deliver cytotoxic payloads to the tumor. Unfortunately, today only one ADC is tested for efficacy in HCC (anti-TROP2 antibody conjugated with irinotecan), suggesting that new ADC-compatible targets for HCC are desperately needed.

Aim: The present study is motivated by this unmet need, aiming at a de novo discovery of accessible tumor biomarkers in HCC. Accessible proteins are membrane-bound and extracellular proteins that are reachable by systemically delivered homing antibodies.

Methods: The isolation of accessible proteins was performed using fresh human HCC tissues as well as matched normal livers from 5 individual patients. Fresh biopsies were first soaked in biotinylation reagent (Sulfo NHS-SS biotin), followed by isolation of target proteins using streptavidin affinity columns. The isolated proteins were further identified and quantified owing to an MS-assisted proteomic approach.

Results: The analysis identified over 1500 potentially accessible proteins, of which at least 200 targets were uniquely expressed in HCC. Bioinformatic evaluation focusing exclusively on proteins with known subcellular localization revealed more than 20 novel therapeutic candidates. Validation studies using immunohistochemistry on larger cohort of patients (N=200) confirmed the overexpression of several selected proteins in HCC.

Conclusions: Current efforts are underway to explore the targeting ability of specific monoclonal antibodies directed against these biomarkers in HCC in vivo models.

O14

The correlation between imaging and resection specimen of colorectal liver metastases.

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Department of Hepatology and Gastroenterology, [3] ASZ, Aalst, Belgium, Department of Radiology, [4] ASZ, Aalst, Belgium, Hepatobiliairy and Abdominal surgery
Introduction: Colorectal cancer (CRC) is the third most common cancer in Belgium (26,846 new cases in 2010). Due to a haematogenous spread, more than 50% of the patients with colorectal cancer develop liver metastases in the course of the disease. Only a small proportion of patients with colorectal liver metastases (CRLM) are eligible for surgery at the moment of diagnosis. Yet surgical resection is the only treatment for CRLM that is associated with a survival rate between 40-50%. The selection for resection relies on imaging techniques (CT/MRI), these demonstrate the exact number, localization and size of metastases. Several studies showed that MRI is more sensitive than CT for lesions <10mm.
Aim: This retrospective study aims to determine the correlation between imaging techniques and the anatomopathology of the resection specimen of CRLM.
Methods: In this study we evaluated fifty patients with colorectal carcinoma operated for liver metastases from September 2010 until June 2015. We compared the largest diameter on imaging with the largest diameter on the anatomo-pathological evaluation. All metastases were evaluated with the last imaging before surgery; in 82% of the cases CT was the last imaging, 18% had MRI.
Results: In total there were 114 lesions evaluated, 97 of them were diagnosed pre-operatively on imaging and 17 lesions were found during surgery. All of 17 were CRLM on final pathology. 88 of the 97 lesions (90,7%) could be confirmed as CRLM, 8 could not be confirmed as CRLM. 1 of 97 (1%) lesions was defined as benign. We presume that 8 of 96 lesions (8.3%) pre-operatively considered as CRLM are lesions with complete response. The time between the imaging and surgery was on average 34 days (range = 1-165 days). 80% of the patients received neoadjuvant chemotherapy. Pre-operative imaging (CT/MRI) showed 97 lesions (mean = 1.9 lesions per patient, range = 0-6), the mean of the largest diameters was 18.9 mm (range = 17.6 - 122.3mm). Eventually anatomical pathology revealed 105 metastases (mean =2.1 lesions per patient, range = 0-7), with a mean diameter of 17.6 mm. There is a significant correlation (R=0.7; p = <0.01) between the diameters on imaging and on anatomopathology. However there is in general an overestimation of 8% on imaging: a remarkable difference is noticed depending on the size of the metastases. Lesions between 9 – 25 mm were better estimated on imaging than lesions larger than 25mm. Lesions below 9mm are underestimated. There is no significant correlation between overestimation and whether the patient had neo-adjuvant chemotherapy, CT or MR.
Conclusions: As expected there is a significant correlation between the diameter of the lesion on imaging and the diameter of the resected specimen. Even though there is an overestimation of the diameter of CRLM on imaging. An intraoperatively systematic ultrasonographic survey of the liver needs to be done, to prevent missing of accessory lesions.

Belgian Pancreatic Club (BPC)

P03
The role of laparoscopic pancreatic surgery
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Introduction: The first laparoscopically performed pancreaticoduodenectomy has been reported by Gagner et al. in 1994. Since then laparoscopic pancreatic surgery has only slowly gained enthousiasm.
Aim: To clarify the role of laparoscopy in pancreatic surgery.
Methods: Reviewing the recent literature and answer the following questions.
Results: • Should Distal Pancreatectomy for benign and premalignant lesion always be performed laparoscopically ? There are recent reviews and meta-analyses showing that laparoscopic distal pancreatectomy for these lesions is superior over open approach in terms of blood loss, time to first oral intake, hospital stay and spleen preservation rate but with longer operation time. • Can Distal Pancreatectomy for adenocarcinoma be performed laparoscopically with the same oncologic results as in open setting ? In a review and meta-analysis by Ricci, the laparoscopic approach could reach similar percentage R0 resection rate, a similar number of resected lymph nodes with shorter hospital stay in pancreatic tail adenocarcinoma up to 3.5 cm. • Is there a 'standard' technique for Laparoscopic Pancreaticoduodenectomy ? A short movie concerning the essential steps in Laparoscopic Pancreaticoduodenectomy will be showed. • Is Laparoscopic Pancreaticoduodenectomy for pancreatic ductal adenocarcinoma as safe as the open approach ? A large series from the Mayo Clinic shows that with laparoscopic pancreaticoduodenectomy they can achieve a similar R0 resection rate and a similar number of resected lymph nodes with lower blood loss and transfusions rate, but at the cost of longer operation time as in open surgery. The laparoscopically treated patients have more often adjuvant chemotherapy which in their hands results in lower local recurrence rate and better progression free survival. • Is Laparoscopic Pancreaticoduodenectomy more costly than open approach ? The costs are not necessarily higher when these procedures are performed in high volume hospitals (> 20 pancreaticoduodenectomies per year). • Defining the learning curve for Laparoscopic Pancreaticoduodenectomy ? The first 10 cases represent the biggest hurdle introducing this procedure. Further reduction in operation time and blood loss after 50 cases. • The role of robot-system in Laparoscopic Pancreaticoduodenectomy has to be clarified.
Conclusions: Concerning the Distal Pancreatectomy: the laparoscopic approach is an established value for benign and premalignant disease. In malignant disease, LDP can achieve results similar to open surgery in tumors up to 3.5 cm. Concerning the Laparoscopic Pancreaticoduodenectomy : Some expert centres can achieve similar results to open technique, costs are not necessarily higher, the learning curve requires 10-50 cases and the added value of robot still has to be proven.

P06

Long-term outcome of endoscopic therapy of chronic pancreatitis in children

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Introduction: Chronic pancreatitis (CP) occurs rarely in children. Previous publications have shown that endoscopic therapy with ductal drainage, with or without extracorporeal shock wave lithotripsy (ESWL), is safe and efficient. However, long-term results are scarce. The aim of this retrospective study was to evaluate the long-term effects of endoscopic therapy on pain relief in children with CP and to compare the results with a group of adult CP patients, undergoing endoscopic therapy during the same period.
Aim: -

Methods: During a period of 21 years (1991-2012), 42 children with CP had endoscopic therapy. A group of 84 adults with CP having undergone endoscopic therapy were matched (1:2) to the paediatric group according to the chronological period that the treatment took place (same month). Clinical status was evaluated after therapy, based on the number of hospital admissions (for pain or subsequent endoscopic procedure) during follow-up (FU). Results were considered excellent if no other admission was required, satisfactory if < 5 admissions or ≥ 5 but with no pain or ongoing endoscopic therapy for minimum 12 months at the end of FU, and poor if surgery was required or ≥5 admissions were noted with ongoing endoscopic therapy at the end of FU.
Results: 42 patients (female: 26(62%)) were included (median age:10(2-17)). 15 were ≤ 7 years old. Twelve children (27%) had hereditary CP associated with cationic trypsinogen mutation. CP was characterized as severe according to the Cambridge classification in 29 (69%) children. Clinical presentation varies between children and adults; RAP was seen in 25 (60%) with a median of 3 (0.6-18) admissions/year in the children's' group versus 30% in the adult group (p=0.005). Pancreas divisum was seen in 6/42 children (15%). Endoscopic therapy consisted of pancreatic sphincterotomy of the major (n=35) and/or the minor papilla (n=12) for all patients. ESWL for pancreatic calcifications was required in 9 (21%). Only 2 patients had pancreatic stent insertion during initial therapy. Mild post-ERCP AP occurred in 8 (19%) and median length of stay was 4 days (2-8). Median FU of 85 months (7-240) was obtained in 37 children. Median frequency of admissions per year (0.66 (0-3.4)) was significantly decreased after therapy (p=0.001). Results were excellent in 5 (14%) and satisfactory in 26 (70%) patients. Finally, poor results were observed in 6 children (16%), who required surgery (n=1) and/or frequent admissions for ongoing endoscopic therapy (n=5). Comparatively, children tended to have better outcomes and response to therapy compared to adults; 84% of patients with excellent and/or satisfactory results in the children's' group compared to 64% in the adults' population (p=0.081)
Conclusions: Therapeutic ERCP in a pediatric population of CP can lead to clinical improvement and can be considered as the initial treatment of choice. Furthermore, it can be applied during childhood, as children seem to respond better than adults to endoscopic therapy.

P07

Ultra-deep targeted resequencing of 38 pancreatic neuroendocrine tumors reveals tumor heterogeneity for actionable mutations

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Introduction: Pancreatic neuroendocrine tumors (PNETs) are rare tumors arising from the endocrine pancreas. Low-coverage exome sequencing of primary PNET tumor samples revealed PI3K-AKT-mTOR pathway genes to be mutated in 16% of all PNETs, in addition to highlighting mutations in other genes, including MEN1 (44% of all patients), DAXX (25%), ATRX (18%). Recently, intra-tumor heterogeneity has been described as a driving factor for tumor progression and therapy resistance in different tumor types. Our pre-clinical research suggests that intra-tumor heterogeneity plays a role in PNET therapy resistance.

Aim: To study tumor heterogeneity and identify subclones within tumors, high coverage next generation sequencing data sets from PNETs are needed and currently lacking.
Methods: Formaline-fixed paraffin-embedded paired tumor-normal tissue from 38 invasive grade 1-2 PNETs was collected at Erasmus MC and University Hospital Antwerp. After DNA extraction and enrichment with an in-house-developed Agilent Haloplex 24-gene-panel, all tumor samples were ultra-deep sequenced on the Illumina HiSeq 1500 platform. Single-nucleotide variants (SNVs) and insertions and deletions (indels) were detected using the Genome Analysis ToolKit using a ploidy setting of 40, allowing detection of alterations present in 5% of tumor cells. Using VariantDB, SNVs and indels, predicted to be damaging by PolyPhen2, SIFT, PROVEAN or MutationTaster, were filtered to have an allelic fraction (AF) >0.025, alternative allele depth >20, mapping quality >50, Fisher-scaled strand bias <20, snpEff annotations ≠ noncoding, only RefSeq stopgain, stoploss, and nonsynonymous SNVs.
Results: Average target base coverage over all samples was 2602-fold. A total of 3572 mutations were identified, with 70,3% of these mutations only present in less than 30% of all reads, pointing to subclonal tumor cell populations containing specific mutations. All genes in the panel, but KRAS, showed mutations in at least one tumor sample. All tumors showed mutations in PTCH2, CYFIP2, MUC17 & MUC 16. The mutation load was highest in MUC16, PTCH2 and TSC2. DAXX and ATRX mutations were seen in 89,5% and 73,7% of all tumors, respectively. MEN1 was found mutated in 94,7% of included tumors. Components of the PI3K-AKT-mTOR pathway, including PIK3C2A, MTOR and PTEN, were mutated in sub-clonal cell populations in more than 80% of all tumors, possibly explaining limited efficacy of mTOR-inhibitor everolimus in PNETs.
Conclusions: This first study using ultra-deep targeted sequencing in PNETs reveals genetic tumor heterogeneity. Known PNET mutations, such as in ATRX/DAXX and MEN1 were seen in higher fractions in our study population than reported in literature. Additionally, actionable mutations in the PI3K-AKT-mTOR pathway were found. Further validation and correlation with clinical data is ongoing.

P08

Focal pancreatic lesions suggestive of neuro-endocrine tumors (NET) in a patient with a previous history of a resected meningioma.

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Introduction: Abstract concerns a case report, cannot be send in this format.
Aim: Abstract concerns a case report, cannot be send in this format.
Methods: Abstract concerns a case report, cannot be send in this format.
Results: Abstract concerns a case report, cannot be send in this format.
Conclusions: Abstract concerns a case report, cannot be send in this format. I send the text of the abstract to myriam delhaey of the belgian pancreatic club

P09

The "PPP syndrome". About a case. F-P Mouthuy (1), A Sibille (1), J-P Dufour (2), P-P Roquet (3), A De Coster (3), P Warzee (1) (1)Gastroenterology Unit (2) Rheumatology Unit (3) Dermatology unit Grand hôpital de Charleroi (GHdC) 3 Gd Rue 6000 Charleroi

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Introduction: -

Aim: -

Methods: -

Results: A 60-year-old patient known for vascular peripheral diseases and chronic pancreatitis was admitted for progressive (10 days) hyperalgic distal polyarthralgias non responding to Colchicine. One month before he experienced at home a slight access of pancreatic pain spontaneously resoluting in 3 days. At physical examination it was noted no temperature, no abdominal pain but very painfull swelling of the wrists, ankles, fingers and toes. Laboratory values were WBC 25000/mm3, CRP 263 mg/L (0-10), LDH 334UI/L (< 245), lipase 20475 UI/L (73-393) and amylase 3410 UI/L (25-125). Abdominal CT revealed moderate peripancreatic fat infiltration, parenchymateus calcifications, a cephalic pseudocyst (30mm) without enlargement of the wirsung and partial portal vein thrombosis. Dermatologic examination was compatible with panniculitis and articular puncture revealed oily brownish liquid containing spumous cells. Bone scintigraphy showed diffuse inflammation of distal long bones and suspected osteonecrosis of fingers, tarsus and carpus confirmed by RMN. Based on progressive temperature and persisting hyperlipasemia endoscopic drainage of pseudocyst by double pigtails was performed followed by dramatic improvement of lipasemia. Septic events were controlled by antibiotics. Fortunately no bone superinfection was noted, but osteoarticular recovery needed long-lasting revalidation and use of biphosphonates. In summary the association of pancreatitis with panniculitis and polyarthritis, known as "PPP syndrome", is a rare but potentially severe complication of pancreatitis. Cutaneous and osteoarticular damages are probably due to longlasting very high levels of activated pancreatic enzymes, such as lipases, into the bloodstream, leading to lipolysis and secondary inflammation in several sites (as subcutaneous adipose tissue, bone marrow, synovium). In case of severe distal polyarthritis a pancreatic cause should be ruled out and treated. Although endoscopic treatment of underlying pancreatic cause of systemic fat necrosis is usually followed by dramatic improvement in serum lipases, osteoarticular rescue may require months of revalidation and be life-threatening in case of bone superinfection.

Conclusions: -

P10

Exciting cause of upper abdominal pain in Ulcerative Colitis

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Introduction: -

Aim: -

Methods: -

Results: A 27-year-old non-smoking man presented with severe upper abdominal pain. He has a history of severe extensive ulcerative colitis (UC) currently treated with azathioprine (150 mg/day) since the initial diagnosis in March 2012 and oral mesalazine (2g/day) for the last 22 months. The patient was in clinical and endoscopic remission before symptom onset. He had no history of alcohol consumption. Upon admission, physical examination was normal except for upper abdominal tenderness. Laboratory test results included the following: hemoglobin 12.1g/dL [N:13.5-17.6g/dL], white blood cell count 3.8x103/mm3 [N:4.2-11.4x103/mm3], platelet count 184x103/mm3 [N:155-346x103/mm3], C-reactive protein 17mg/L [N<10mg/L], total bilirubin 0.34mg/dl [N<1.2mg/dl], lipase 803 IU/L [N<75 IU/L] and lactate dehydrogenase 131 IU/L [N<225 IU/L]. Abdominal computed tomography revealed pancreas enlargement with rim-like enhancement and mild main pancreatic duct (MPD) dilation. Additional laboratory tests including IgG, IgG4, antinuclear antibody, calcium, triglycerides and tumor marker carbohydrate antigen 19-9, were normal or negative. Magnetic resonance imaging (MRI) with cholangiopancreatography showed diffuse pancreas enlargement with delayed enhancement and MPD dilation (3.5mm) with secondary ducts dilation upstream of an isthmic substenosis with no evidence of mass neither of gallstones. These radiological findings were suggestive of autoimmune pancreatitis (AIP). Due to patient young age, no serum IgG4 elevation and association with inflammatory bowel disease (IBD), more specifically with UC, type 2 AIP was suspected. Steroid trial with 0.6mg/kg per day of oral methylprednisolone was initiated and resulted in complete recovery with normalization of lipase within a few days. Response to therapy was evaluated at day 15 by MRI showing improvement of pancreas enlargement with resolution of MPD dilation. These data support the diagnosis of AIP. However, a definitive diagnosis of type 2 AIP would have required histological analysis. The patient will be followed by MRI, laboratory tests and clinically with slow tapering in methylprednisolone dose. Thiopurines, gallstones and alcohol are the most frequent causes of acute pancreatitis (AP) in IBD patients. Thiopurine-induced as well as mesalazine-induced AP, usually uncomplicated and self-limited, typically occurs within a few weeks after initiation of treatment. Therefore, drug-induced AP was less likely in our patient. AIP is a relatively newly recognized disease and is increasingly diagnosed in IBD patients, particularly type 2 AIP in UC patients. Type 1 and type 2 AIP are not only two distinct histological subtypes, but also two distinct clinical entities. Type 1 AIP is acknowledged to be part of IgG4-related disease associated with elevated levels of serum IgG4, while type 2 AIP appears to be a pancreas-specific disorder with no serum IgG4 elevation. Corticosteroids are effective in both types. A correct diagnosis is of critical importance for appropriate therapeutic management. In conclusion, acute pancreatitis must always be thoroughly investigated in IBD patients in order to seek most common etiologies but also less common causes such as AIP which may require specific treatment, like corticosteroids, besides conservative management.
Conclusions: -

Radiology, Pathology and Nuclear Medicine

R02

A clinical history of longstanding Ulcerative Colitis: do not forget the lymph nodes…

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Introduction: Development of colorectal cancer is a long-term complication of colonic inflammatory bowel disease, following a “chronic inflammation-dysplasia-cancer” pathway. Endoscopic surveillance is recommended in those patients but the detection of dysplasia and cancer can be difficult as the lesions can be multifocal and developed on a macroscopically normal mucosa.
Aim: The aim of this abstract is to illustrate the multifocal and potentially aggressive development of such dysplastic lesion.
Methods: We report the endoscopic description and the histological findings of a patient suffering from longstanding ulcerative colitis.
Results: We report the case of a 65 year old woman suffering from ulcerative colitis since 1980. The disease was a pancolitis that required one course of corticosteroids in 1986 followed by a long term treatment with Azathioprine and Mesalazine. This treatment allowed clinical remission until autumn 2012 when the patient developed a new flare. A rectosigmoidoscopy was done and showed an active colitis and, in the sigmoid, a high grade dysplastic lesion surrounded by inflammatory mucosa (DALM). A full colonoscopy was then performed and two lesions were found in the sigmoid at 20 and 30 cm from anal margin, composed of high-grade dysplasia and surrounded by inflammatory and dysplastic mucosa. Five months later the patient underwent a coloproctectomy with total mesorectal excision (TME), lymph nodes dissection, ileo-anal anastomosis and J pouch. The histological analysis showed severe chronic active ulcerative colitis with low grade and high grade dysplasia. Twenty-five lymph nodes were analyzed and surprisingly two were infiltrated by adenocarcinoma. As no macroscopic lesion was found, new histological examination of the rectosigmoid was performed. Finally, two lesions of well differentiated and infiltrating adenocarcinoma were discovered (staging T1N1).
Conclusions: In this case we illustrate the development of dysplasia and colorectal cancer in longstanding ulcerative colitis. We also show the multifocal location of dysplasia and the potential difficulties to find the colorectal neoplastic lesion.

R03

Clinico-pathological characterization of patients with colorectal cancer and restricted immunohistochemistry overexpression of p53.

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Introduction: Restricted overexpression of p53 in immunohistochemistry (p53IHC RO) was previously described as a different pattern from the negative or intense and diffuse p53 IHC expression. p53IHC RO is characterized by a limited number of homogenously scattered strongly positive colorectal cancer (CRC) cells. We previously reported, on a limited and selected patient cohort that p53IHC RO was associated with MSI CRC tumor.

Aim: The aim of this work was to confirm this association on a larger and unselected cohort of operated CRC patients.
Methods: p53 and MMR (MLH1, MSH2, MSH6 and PMS2) proteins IHC expression were performed on CRC tumor of patients who underwent surgery at the Cliniques universitaires St-Luc between 1998 and 2014. p53 IHC results were defined as negative, intense and diffuse or restricted expression. Clinico-pathologic CRC characteristics must be available for this analysis. Between p53 overexpression groups, comparisons of numerical and categorical clinico-pathological data were evaluated respectively by Mann-Whitney and Fisher exact tests. Results were considered as statistically significant for a p-value <0.05.
Results: Among CRC tumors analyzed from 673 patients, p53 IHC expression was categorized as restricted (n=207), intense and diffuse (n= 340) or negative (n=126). As compared with diffuse and negative IHC pattern, p53IHC RO was significantly associated with MSI CRC (33.3 vs 13.3%) older patient age (69 vs 66 y-old), female sex (54.1 vs 44.4%), colon cancer (73.4 vs 57.5%), proximal tumor location (35.2 vs 18.5%) and mucoid adenocarcinoma (19.3 vs 5.8%). p53IHC RO was also associated with better clinico-pathological CRC characteristics: lowest lymph node ratio (0.062 vs 0.121), less venous (16.9 vs 29.2%) and lymphatic permeation (29.5 vs 37.6%) and less advanced stage (stage III/IV) at presentation (38.7 vs 49.1%). Among the patients with MSS CRC (n=542), p53IHC RO were significantly associated to the same clinico-pathological characteristics except for age at surgery, tumor location and venous/lymphatic permeation. Lowest LNR (0.074 vs 0.117) and less metastatic stage at presentation (6.5 vs 13.1%) remains also significantly associated with p53IHC RO MSS CRC.
Conclusions: p53IHC RO is closely related to MSI CRC and its related clinico-pathological features. Nevertheless, when only considering MSS tumors, p53IHC RO remains associated with the MSI-related clinico-pathological characteristics and a less advanced disease stage at diagnosis. Prognostic impact of p53IHC RO must be further characterized especially for MSS CRC.

R04

Characterization of HER2 gene/protein and Ki67 protein expressions in colorectal carcinoma variants with relation to clinicopathological parameters and prognosis

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Introduction: There is extensive ongoing research on alternative therapeutic targets and agents other than chemotherapy for the management of cancer. One of these targets is the human epidermal growth factor receptor 2 (HER2). The advent of HER2 directed therapies has dramatically affected the outcome of patients with HER2 positive breast and gastric or gastroesophageal cancers; however, the results have been discouraging in other HER2 overexpressing cancers. It is currently unclear whether HER2 is a potential therapeutic target in patients with colorectal carcinoma (CRC) or not.
Aim: The data on the frequency and pattern of HER2 expression in CRC and its clinical significance are ambiguous. Little is known about HER2 status in CRC variants and its relation to proliferative activity and clinical outcome. Nevertheless, the exact prognostic value of proliferative and cell cycle-associated markers in CRC remains vague. Such knowledge may be of potential value for therapeutic decision making in CRCs. We, therefore, conducted this study

to determine the frequency and clinical significance of HER2 and Ki67 expressions in CRC variants.
Methods: The HER2 gene/protein status was assessed by fluorescence in situ hybridization and immunohistochemistry in a tissue microarray of 150 CRCs and correlated with the expression of the proliferation marker Ki67, clinicopathological factors, and prognosis.
Results: CRCs were categorized into conventional adenocarcinoma (CA), 47 cases; adenocarcinoma with mucinous component (AMC), 28 cases; mucinous adenocarcinoma (MA), 56 cases; and signet ring cell carcinoma (SRCC), 19 cases. Compared to other variants, CA was significantly associated with favorable clinicopathological features, higher overall survival (OS), and disease-free survival (DFS), while SRCC and MA were significantly associated with ominous clinicopathological features, lower OS, and DFS. Cytoplasmic HER2 overexpression was detected in 14.2% of CRC cases and showed a significant agreement with gene amplification ($P<0.001$). None of these cases showed membranous HER2 staining. High Ki67 expression was detected in 48% of CRC cases. The interrelation between HER2 and Ki67 expression in CRC variants was not statistically significant, and neither had any significant relation to OS or DFS in any of the CRC variants. However, AMC showed significant HER2 and ki67 overexpression than the other variants ($P= 0.015$ and 0.028 respectively). HER2 overexpression was significantly associated with fungating tumors ($P=0.027$), negative lymphovascular emboli ($P=0.001$), negative lymph node metastasis ($P=0.002$), lower stages ($P=0.001$) and absence of associated schistosomiasis ($P=0.042$). On the other hand, high Ki67 expression was significantly associated with absence of peritumoral lymphocytic infiltrate (Crohn's-like response) ($P=0.002$).
Conclusions: We conclude that mucinous histology infers an adverse prognosis in CRC. The upregulation of HER2 and Ki67 appears to play an important role in colorectal oncogenesis. A subset of early stage CRC patients with HER2 overexpression, and possibly of a distinct variant (AMC), may benefit from intracellular HER2-targeted therapies (as lapatinib). The documented high agreement between HER2 cytoplasmic expression and gene amplification implies that the latter is not restricted to membranous expression and justifies the use of IHC as a method of screening for HER2 gene amplification in CRCs.

R06

A VALIDATION OF C-MET TESTING IN GASTRIC AND GE JUNCTION CARCINOMAS

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Introduction: C-Met is an epithelial receptor tyrosine kinase with one known ligand, hepatocyte growth factor (HGF). When inappropriately activated, C-Met contributes to features of malignancy in several cancers a.o. lung, gastroesophageal. C-Met protein overexpression and gene amplification are associated with a worse overall survival in gastric and GE junction adenocarcinomas. Targeted c-Met therapy is actively investigated in clinical trials for these cancer types. Consequently there is a need for validation of C-Met testing strategies such as immunohistochemistry (IHC) and silver in situ hybridisation (SISH).
Aim: We carried out a primary validation study of c-Met IHC and SISH in gastric and junction adenocarcinomas. Our first goal was to check the technical feasibility of the IHC and SISH test methods currently available in our lab. Secondly, we analysed the relationship between IHC membranous versus cytoplasmic staining, and we determined the optimal IHC scoring method.

Thirdly, we investigated the relation between IHC scores and patterns of gene amplification. Fourth, we tested the reproducibility of the scoring systems by determining inter-observer variability between an expert and a non-expert pathologist reader.
Methods: Our study was conducted on 11 formalin-fixed paraffin-embedded tissue samples from endoscopic biopsies and surgical resections. Three of these were tested previously at another institution and were shown to harbour a MET gene amplification. IHC was performed with an anti c-Met rabbit monoclonal primary antibody (clone SP44, Ventana) on the BenchMark ULTRA platform. Stainings were evaluated with the already published histo (H) score, which combines an intensity read (0-3) with a surface calculation (0-100%). H-scores were obtained and compared for membranous and cytoplasmic staining. SISH was by dual color in situ hybridisation with a MET DNP probe and chromosome 7 DIG probe (Ventana). Signals were counted in 25 nuclei and MET was considered amplified when the MET/CEN7 signal ratio was higher than 2.
Results: All IHC slides were of good quality and were interpreted consistently by both observers. There was a strong positive correlation between membranous and cytoplasmic H-scores. The 3 known MET-amplified cases had membranous H-scores of 250 – 280 and 3+ staining intensity was always observed. The 8 non-amplified samples had H-scores ranging from 0 to 130. There was no significant inter-observer variability for the SISH data. For the 3 known amplified cases, we obtained a high amplification ratio (with a range between 6.47 and 7.46). The 8 non-amplified samples had ratios between 0.92 and 1.35.
Conclusions: Based on this small validation study, we conclude that c-Met IHC and SISH testing of gastric and GEJ tumors is technically feasible in our lab. We recommend using the membranous H-score for IHC evaluation. A cut-off of 200 or the presence of 3+ staining intensity strongly suggest that the MET gene is amplified. This amplification can be detected reproducibly by SISH. Moreover, in this series we only found high-level gene amplification. We will submit these tests for BELAC accreditation.

R08

Preoperative staging with Magnetic Resonance Imaging (MRI) and EndoRectal Ultra-sonography (ERUS) for locally advanced rectal cancer (LARC) after chemoradiotherapy (CRT): accuracy with histopathologic findings

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Introduction: Pre-operative CRT followed by total mesorectal excision (TME) is nowadays the standard of care for patient with LARC (cT3-T4N0 or cTxN+). Rectal surgery increases morbidity with late bowel dysfunction. Currently, pathological complete response occurs in +/-

15% after CRT suggesting a non-operative management could be proposed for these patients. The challenge remains to adequately identify the complete responder patients.
Aim: Our aim was to determine whether preoperative staging by MRI and ERUS after CRT could accurately predict a pathological response.
Methods: Between 1998 and 2014, 331 patients with LARC who underwent TME after CRT were identified. Only patients with available and complete results for both MRI and ERUS were used for the analysis. T and N preoperative staging (MRI / ERUS) were compared to pathological staging. Relevant categories of clinical and pathological downstaging (yT0N0: complete; yT1-2N0: present; yT3-T4N0 or yTxN+: absence) were compared.
Results: 154 patients (sex ratio M/F 1.96; mean age 62.0 years-old; upper (15,5%), mid (26.6%) and low rectum (57.7%)) were analyzed. T-stage was accurately estimated by MRI and ERUS in respectively 53.2% and 55.8%. Clinical T overstaging was more frequent (MRI: 35.1%; ERUS: 33.8%) than understaging (MRI: 11.7%; ERUS: 10.4%). Kappa coefficient was of 0.489, corresponding to a moderate agreement between MRI and ERUS staging. N-stage was accurately estimated by MRI and ERUS in respectively 64.3% and 63.6% of the patient. Clinical N overstaging was more frequent with MRI (23.4%) than with ERUS (14.9%) and understaging appeared more prevalent with ERUS (21.4%) than with MRI (12.3%). Kappa coefficient was of 0.326, corresponding to a fair agreement between MRI and ERUS staging. High concordance between clinical (MRI/ERUS) and pathological categories was found in absence of tumor downstaging (yT3-T4N0 or yTxN+). Pathological stage MRI accuracy ERUS accuracy T0 (n=23) 30,4% (n=7) 30,4% (n=7) T1-T2 (n=48) 25,0% (n=12) 16,7% (n=8) T3-T4 (n=83) 75,9% (n=63) 85,5% (n=71) N0 (n=102) 64,7% (n=66) 77,4% (n=79) N+ (n=52) 63,4% (n=33) 36,5% (n=19) ypT0N0 (n=20) 25,0% (n=5) 30,0% (n=6) ypT1-2N0 (n=37) 13,5% (n=5) 35,1% (n=13) ypT3-4N0; ypTxN+ (n=97) 85,5% (n=83) 87,6% (n=85)
Conclusions: While preoperative clinical imaging (MRI/ERUS) after CRT for LARC poorly coincides with pathological tumor downstaging, it often corresponds with an absence of pathological stage regression. Regarding our results, conventional clinical imaging (MRI/ERUS) is unhelpful in defining an optimal clinical response and a possible conservative approach.

R09

Tailored step-up approach results in beneficial long-term disease outcome in the prospective Belgian paediatric Crohn's disease registry (BELCRO)

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Introduction: The prolonged use of biologic agents with or without immunomodulators (IM) remains controversial in the management of paediatric Crohn's disease (CD).

Aim: To determine associations between treatment and outcome variables in CD patients with and without anti-TNF treatment.

Methods: Five-year follow-up (FU) data from the BELCRO, an observational prospective cohort of children (< 18 years) diagnosed with CD in Belgium, were analysed. Disease severity was scored as inactive, mild or moderate-to-severe on a 3-point PCDAI scale and monitored yearly. Treatment and outcomes were recorded from diagnosis until 5 yrs FU. Remission was defined as inactive disease and sustained remission when achieved for ≥ 2 yrs FU. Univariate analyses were performed between patients with or without anti-TNF and Spearman's correlation between treatment and outcomes.

Results: A total of 91 patients (median (IQR) age 12.7 (10.9 – 14.8) yrs, 53% male) were included. Disease location was 12% ileal, 23% colonic (L2), 64% ileocolonic, 76% upper GI and 30% perianal. Disease severity was 25% mild and 75% moderate-to-severe. Anti-TNF was started in 73% after median (IQR) 1.1 (0.6 – 2.2) yrs with duration of 3.9 (2.5 – 4.7) yrs of which 89% combination therapy with duration of 1.3 (0.6 – 2.0) yrs. Older age (13.1 (11.5 – 15.2) vs. 11.8 (8.7 – 13.8) yrs; p< .05) and location L2 (29% vs. 8%; p= .04) were associated with need to start anti-TNF. Despite shorter delay to corticosteroids (CS) (0 (0 – 0.02) vs. 0.02 (0 – 0.06); p= .04), total duration of CS was similar and total duration of IM (2.5 (1.4 – 4.7) vs. 4.7 (3.6 – 5.2); p= .001) was shorter in the anti-TNF group. Time to first (1.1 (0.5 – 1.8) vs. 0.6 (0.3 – 1.1); p= .01) and sustained (2.9 (2.3 – 3.9) vs. 2.3 (2.1 – 2.9); p= .03) remission was longer with anti-TNF use. Mean disease severity (1.7 (1.4- 1.9) vs. 1.4 (1.3- 1.6); p< .01) during 5 yrs FU was higher with anti-TNF but rates of inactive disease (65% vs. 76%; p= .32) after 5 yrs FU were similar with less ongoing CS (41% vs. 72%; p= .008) in the anti-TNF group. Delay to IM treatment was correlated with mean disease severity (r= .26; p= .02) and duration of CS with duration of sustained remission (r= -.24, p= .03), though not significantly after correction for multiple testing. Rates of perianal flares, hospitalizations or surgery were similar and no serious opportunistic infections, cancer or deaths were reported with use of anti-TNF.

Conclusions: Prospective data from the BELCRO demonstrate beneficial long-term outcomes using a step-up approach with anti-TNF in over 2/3 of patients, limiting IM treatment. The gain of top-down and early combination therapy remains to be determined in paediatric CD.

R12

Type of treating physician is associated with long-term disease outcome in the prospective Belgian registry of paediatric Crohn's disease (BELCRO)

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Introduction: Treatment and outcomes in paediatric Crohn's disease (CD) have not been compared between treating physician and centre of care.

Aim: To determine associations between treatment and outcome variables in different levels of care for paediatric CD.

Methods: Data from the BELCRO, an observational prospective cohort of children (< 18 yrs) diagnosed with CD in Belgium, were analysed. Disease severity was scored as inactive, mild and moderate-to-severe using a 3-point scale and monitored yearly. Remission was defined as inactive disease and sustained remission when achieved for ≥ 2 yrs follow-up (FU). Univariate analyses were performed between paediatric or adult and secondary or tertiary centre of care.

Results: A total of 91 children (median (IQR) age 12.7 (10.9 – 14.8) yrs, 53% male) were included. Disease location was 12% ileal, 23% colonic, 64% ileocolonic, 76% upper GI or 66% proximal (L4A) and 30% perianal. Disease severity at diagnosis was 25% mild and 75% moderate-to-severe. Level of care was 70% paediatric and 71% tertiary. Younger age (11.9 (9.8 – 13.4) vs. 15.1 (13.8 – 16.7) yrs; p< 10-7) and location L4A (77% vs. 41%; p= .02) were associated with paediatrics. Young age was associated with lower disease severity (11.4 (8.7 - 13.8) yrs for mild and 13.2 (11.6 – 15.3) yrs for moderate-to-severe; p= .02). Time to biological (1.5 (0.7 – 2.6) vs. 0.6 (0.4 – 1.4); p= .003) and combination (18.5 (8.0 – 32.5) vs. 7.0 (3.0 – 12.0); p= .006) therapy was longer and duration on biologicals (3.6 (2.0 – 4.4) vs. 4.6 (2.6 – 5.0); p= .01) was shorter in paediatrics. Biological (60% vs. 26%; p= .008) and combination (65% vs. 26%: p= .005) therapy were initiated more often after first remission by paediatricians. Rate of sustained remission (95% vs. 67%; p< .001) was higher for paediatric but similar for tertiary care. Time to first (0.7 (0.3 - 1.4) vs. 1.2 (0.7 – 2.5); p= .01) and sustained (2.6 (2.1 – 3.2) vs. 3.1 (2.5 – 3.9); p< .05) remission was shorter and duration of sustained remission (2.8 (1.6 – 4.2) vs. 2.1 (1.3 – 3.5); p= .004) was longer in paediatrics. Mean disease severity during 5 yrs FU (1.5 (1.3 – 1.8) vs. 1.8 (1.6 – 2.0); p= .008) was lower in paediatrics. Rate of inactive disease after 5 yrs of FU (73% vs. 56%; p= .09) was similar with more ongoing immunomodulator treatment (56% vs. 33%; p< .05) in paediatric compared to adult care.

Conclusions: Paediatric care is associated with longer delay to and shorter duration on biological or combination therapy with better disease control, using a step-up approach. However, outcomes after 5 years are similiar with adult care and use of top-down strategies for more severe disease course in older patients.

R13

INCIDENCE AND PHENOTYPE AT DIAGNOSIS IN VERY EARLY COMPARED TO LATER-ONSET PEDIATRIC INFLAMMATORY BOWEL DISEASE: A POPULATION-BASED STUDY (1988-2011)

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Introduction: More and more studies are published on very early-onset (<6 years) inflammatory bowel disease (VEO-IBD), but their phenotype is still poorly known. Age at diagnosis of inflammatory bowel disease in children has taken an important role. Our goals were to asnwer two questions: (1) Is the incidence of VEO-IBD increasing? (2) Is there a different phenotype depending on age at diagnosis?

Aim: We aimed to compare the incidence and phenotype at diagnosis of VEO-IBD and IBD in older children (6-17 years) from a French population-based study over a 24-year period.

Methods: Data were obtained from a cohort of 1412 children (<17 years) with IBD enrolled in a prospective French population-based Registry from 1988 to 2011. Incidence, initial classification, clinical presentation and phenotype at diagnosis were compared according to age at diagnosis (<6 years and 6-16 years).

Results: In total, 1412 children (8% of all IBD) have been recorded including 42 (3%) with VEO-IBD. The incidence remained stable among VEO-IBD children (0.4/10^5 from 1988-1990 to 2009-2011) while it increased from 4.43 per 100.000 in 1988 - 1990 to 9.54 per 100.000 in 2009-2011 (+115%, p <10^-3). The incidence of overall IBD in children increased from 3.0/105 in 1988-1990 to 6.3/105 in 2009-2011 (+110 %; p<10^-3. The initial classification as ulcerative colitis (UC) or IBD unclassified (IBDU) was more common among the VEO-IBD group (40% vs 26%; p=0.05). The diagnosis of IBD is most often done in hospital in <6 years (69% vs 42%). Rectal bleeding and mucous stools are more common in children under 6 years; weight loss and abdominal pain are less frequent than in 6-16 years group. Among the children with CD, isolated colonic disease is more common in <6 years group (39% vs 14%).

Conclusions: In this large population-based study, the incidence of VEO-IBD was low and remained stable from 1988 to 2011. Children diagnosed with VEO-IBD were more often diagnosed in hospital than those diagnosed after the age of 6. CD is most present in two age groups, but UC or IBD-U was more common among the VEO-IBD group. VEO-CD children presented more rectal symptoms, presumably in relation to a high prevalence of isolated colonic CD.

R16

Hirschsprung disease and CAKUT: A coincidence ?

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Introduction: Hirschsprung disease and congenital anomalies of the kidney and urinary tract (CAKUT), two pathological entities with their own, complex, genetic background, can be associated based on the common RET-pathway.
Aim: To explore this association we designed a literature review in combination with a retrospective cohort study of 60 patients with Hirschsprung disease and follow-up at UZ Leuven from 1980 until 2014.
Methods: Ten patients were excluded due to the lack of anatomopathological diagnosis or because they had a known genetic disease/syndrome. Thirty-three patients (66%) underwent an ultrasound.
Results: Within this group, there were 6 patients (18,2%) detected with CAKUT. These results are in line with the known literature.
Conclusions: We can thus conclude that there seems to be an association between Hirschsprung disease and CAKUT. Our cohort study emphasizes the need for more systematic nefrological screening of patients with Hirschsprung disease. We therefore suggest a new diagnostic approach which consists of a screening protocol where all patients with Hirschsprung disease must undergo an ultrasound of the kidneys, a urinanalysis and a blood pressure measurement. Broader studies are still necessary to confirm the diagnostic association between both diseases, to illustrate and explore the genetic association and to determine the influence of certain patient- or disease specific factors in these new guidelines.

R17

Intragastric pressure measurement during nutrient intake: a novel minimally invasive method to measure gastric accommodation in functional dyspepsia

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Introduction: Functional dyspepsia (FD) in pediatrics is defined as the presence of upper abdominal symptoms in the absence of organic or metabolic disease likely to explain the symptoms. Impaired gastric accommodation (GA) is one of the main proposed pathophysiological mechanisms. The gastric barostat is the gold standard to measure GA. However, this procedure is very invasive and it might alter the normal gastric physiology. We proposed the measurement of intragastric pressure (IGP) during nutrient intake as a potential alternative for assessing GA. This technique uses a thin manometry (HRM) catheter that measures the IGP over the entire length of the stomach.
Aim: The aim of this study is to introduce the HRM as new minimally invasive technique to measure GA and nutrient tolerance in children.
Methods: The HRM probe and a second infusion catheter were positioned in the stomach of the subjects. The IGP was measured before and during the intragastric infusion of a nutrient drink (300 Kcal, 60 ml per minute). The patients were asked to score their hunger, satiation and epigastric symptoms at 5-minute intervals. The experiment ended when the subjects scored maximal satiation at 1-minute intervals on a scale graded from 0–5 (1, threshold; 5, maximum satiety).

Results: 13 FD pediatric patients (92% female, 14.8 ± 0.8 years old, BMI: 19.5±0.8) and 12 healthy volunteers (HVs) (100% female, 22.2 ± 0.4 years old, BMI: 21.2 ± 0.3) were recruited. In both groups, intragastric infusion of nutrient drink induced a rapid drop in proximal stomach IGP. The average AUC change from baseline was -44.7±11.0 mmHg in patients and -48.4±24.1 mmHg in HVs. Patients tended to score maximal satiation at lower volumes compared HVs (433.9±64.2 ml and 600.0±67.6mL respectively, p=0.01). All FD patients and HVs tolerated the catheters and could finalize the study.
Conclusions: The IGP measurement during intragastric nutrient drink infusion is a promising method to assess GA accommodation and nutrient tolerance.

R18

Aberrant expression of Calretinin, D2-40 and Mesothelin in mucinous and non-mucinous colorectal carcinomas and relation to clinicopathological features and prognosis

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Introduction: Colorectal carcinoma (CRC) is a heterogeneous disease in terms of morphology, invasive behavior, metastatic capacity, and clinical outcome. Recently, many so-called mesothelial markers, including calretinin, D2-40, WT1, thrombomodulin, mesothelin, and others, have been certified. Many studies had examined the expression of these mesothelial markers in various epithelial tumors with pointing to certain prognostic implications. The expression of these markers in CRC was not extensively studied, and if far of being settled, especially in the histologic variants of CRC; as mucinous adenocarcinoma (MA); that have specific lower survival rates than conventional adenocarcinoma (CA).
Aim: The aim of this study was to assess the immunohistochemical expression of calretinin and other mesothelial markers (D2-40 and mesothelin) in colorectal MA and CA specimens and relation to clinicopathological features and prognosis.
Methods: Files of all resected CRC cases in surgical pathology lab at Gastroenterology Center, Mansoura, Egypt, were revised during the period from 2007 to 2011. MA cases were selected and revised. Cases with incomplete clinical data and those that were composed completely of pools of mucin with very few epithelial cells were excluded. Seventy five cases with MA were fulfilling selection criteria. Another 75 cases of CA were chosen randomly for comparison from the same period. The patients didn't receive any neoadjuvant therapy. All clinicopathological data of these 150 cases were revised with re-examination of all their slides. Three high density manual tissue microarrays were constructed using modified mechanical pencil tips technique and immunohistochemistry for calretinin, D2-40 and mesothelin was done.
Results: Calretinin was positive in 5.3% of CRC, with CA showing statistically significant positivity than MA (P=0.029). D2-40 showed aberrant nuclear staining in 9.3% of CRC; all of CA group with totally negative MA cases (P<0.001). Mesothelin showed luminal cytoplasmic positivity in 4% of CRC, with no statistically significant difference between CA and MA. However, luminal mesothelin expression was significantly associated with cytoplasmic calretinin (P=0.002) and nuclear D2-40 (P<0.001) expressions in CRC. There were no statistically significant relations between any of the clinicopathological or histological parameters and any of the three markers. In a univariate analysis, neither calretinin nor D2-40 expressions showed any significant relations to disease-free (DFS) or overall survival (OS). However, mesothelin luminal expression was significantly associated with worse DFS. Multivariate Cox regression analysis

proved that luminal mesothelin expression was an independent negative prognostic factor in CA (HR: 0.307, 95% CI 0.118 – 0.799, P = 0.016).
Conclusions: Calretinin, D2-40 and mesothelin are frequently expressed in CA than MA. Aberrant expression of these mesothelial markers was not associated with clinicopathological or histological features of CRCs. Only Mesothelin expression appears to be a strong predictor of adverse prognosis in CRC patients.

R19

Role of of B-cell transcription factors Pax-5, Oct-2 and Bob-1 in colorectal adenoma-carcinoma sequence with relation to clinicopathological features and prognosis

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Introduction: Colorectal carcinoma (CRC) is a major cause of morbidity and mortality throughout the world. It is the third most common cancer and the fourth most common cause of death worldwide. Pax5, Oct2 and Bob1 are B-cell transcription factors that designate true B-cell differentiation. The expression of these B-cell transcription factors was described as a frequent event in some epithelial malignant neoplasms, but whether they are expressed in CRC is not known yet.
Aim: The aim of this study was to assess the immunohistochemical expression of Pax5, Oct2 and Bob1 B-cell transcription factors in colorectal adenomas and carcinomas with exploration of their relation to clinicopathological features and prognosis.
Methods: This retrospective study was carried out in surgical pathology lab at Gastroenterology Center, Mansoura, Egypt. 150 cases of CRC were studied. All clinicopathological data of these 150 cases were revised with re-examination of all their slides. Ten normal colonic mucosal tissues and 10 colorectal adenomas were also included in the study. Three high density manual tissue microarrays were constructed using modified mechanical pencil tips technique and immunohistochemistry for Pax5, Oct2 and Bob1 was done. All relations were analyzed using established statistical methodologies.
Results: The CRC cases included 47 cases of ordinary adenocarcinoma (OA), 28 cases of adenocarcinoma with mucinous component <50% of the tumor (OAMC), 56 cases of mucinous adenocarcinoma (MA) (mucinous component > 50% of the tumor) and 19 cases of signet ring cell carcinoma (SRCC). OA was significantly associated with good prognostic factors, while SRCC was the worst. Pax-5, Oct-2 and Bob-1 were totally negative in all normal colorectal mucosal tissues. Only 2 adenomas showed focal nuclear Pax-5 and faint luminal cytoplasmic Bob-1 expressions. Pax-5 was positive in 29 cases (19.3%) of CRC, Oct-2 showed aberrant cytoplasmic staining without any nuclear staining in 21 cases (14.0%) while Bob-1 showed positive staining in 16 cases (10.7%). Pax-5, Oct-2 and Bob-1 expressions were not significantly different between normal colorectal mucosae, adenomas and CRCs (P=0.305, 0.202 and 0.346 respectively). OA showed significantly higher Pax-5 expression (29.8%) than other subtypes (P=0.047), while OAMC showed significantly higher Oct-2 expression (28.6%) than other subtypes (P=0.036). no significant differences in Bob-1 expression were detected between CRC subtypes (P=0.179). Pax-5 expression was not significantly associated with any of the clinicopathological and histological parameters. In contrast, aberrant Oct-2 expression was significantly associated with more depth of invasion (P=0.031) and more microscopic abscess formation (P=0.003), while more peritumoral lymphocytic infiltrate was significantly associated with negative Oct-2 expression (P=0.048). Bob-1 expression was significantly associated with older age (P=0.030), while rectosigmoid location was significantly associated with negative Bob-

1 expression (P=0.046). By multivariate analysis, only Bob-1 expression was proved to be an independent prognostic factor in MA patients (P=0.042).
Conclusions: B-cell transcription factors are expressed in about 15-20% of CRC cases, with no detected role in adenoma-carcinoma sequence. Oct-2 expression is associated with bad prognostic factors, but with no relation to survival. Bob-1 expression was proved to be an independent prognostic factor in MA patients.