

ABSTRACTS

XXVIth Belgian Week of Gastroenterology 2014

ABSTRACTS

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B01 — B18	OG-FWO
P01 — P14	Belgian Pancreatic Club (BPC)
G01 — G13	Belgian Society for Gastrointestinal Endoscopy (BSGIE) and Small Bowel Group
I01 — I31	IBD Research Group (BIRD)
N01 — N05	Research Group of Clinical Nutrition and Metabolism (SBNC)
O01 — O17	Belgian Group for Digestive Oncology (BGDO)
R01 — R17	Pathology Club, Radiology, Nuclear Medicine
C01 — C11	Case Report Session
D01 — D14	Plenary Session
S01 — S10	Seven Societies Postgraduate Course

TIME – DEPENDENT EFFECTS OF HYPOXIA ON LIVER PROGENITOR CELL ACTIVATION IN HEPATOCELLULAR CARCINOMA. E. Bogaerts (1), A. Paridaens (1), Y.P. Vandewynckel (1), A. Geerts (1), F. Heindryckx (2), H. Van Vlierberghe (1). (1) Ghent University, Gent, Belgium ; (2) Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

Introduction : In hepatocellular carcinoma (HCC), hypoxia can be induced by factors like fibrosis, inadequate circulation to fast growing tissue or treatment. Current treatment strategies for HCC often aim to deprive the tumour of its nutrient and oxygen supply, creating hypoxic conditions. However, this could lead to an altered liver progenitor cell (LPC)-niche, creating a more aggressive tumor phenotype.

Aim : Compare the effect of hypoxia at different time-points in hepatocarcinogenesis, to determine the time-dependent effect of hypoxia on LPC activation.

Methods : HCC was induced in mice by diethylnitrosamine (DEN) injections for 22 weeks. To mimic a hypoxic reaction, pan-prolyl-hydroxylase-domain inhibitor dimethyloxaloylglycine was administered from week 1-5, 16-22 or therapeutically from week 22-27. Expression of LPC-markers and metastatic-marker MMP9 was assessed by RT-qPCR. Cytokeratin (CK) 19+ singular cells, cholangioma and (pre)malignant HCC-nodules were detected through Immunohistochemical, Sirius Red and reticulin stainings.

Results : Increased Epcam and CD44 mRNA expression and number of CK19+ singular cells was seen in all DEN induced mice, except for the treatment PBS-control group. Showing loss of LPC characteristics after 27 weeks of hepatocarcinogenesis only without external hypoxic-stimuli.

Interestingly, all LPC markers were upregulated after 22 weeks of DEN and dimethyloxaloylglycine from week 1-5 compared to week 16-22, coinciding with a borderline significantly increased expression of MMP9, this was not detectable after 16 w of DEN and early dimethyloxaloylglycine. Indicating an important delayed effect of early hypoxia.

Conclusions : This shows only late hypoxia-inducing treatment leads to increased LPC activation, possibly detrimental for tumour progression. However, there also is a marked delayed effect of hypoxic conditions during tumour initiation causing increased metastatic potential.

ROLE OF TUMOR NECROSIS FACTOR- α DURING THE DUCTULAR REACTION IN MOUSE MODELS OF LIVER INJURY. J. Best (1), S. Verhulst (2), N. Van Hul (3), W. Syn (4), H. Femke (5), V.V. Hans (5), R. Hendrik (2), L. Isabelle A (3), L.A. Van Grunsven (2), L. Dolle (2). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) Free University (VUB), Brussels, Belgium ; (3) Laboratory of Pediatric Hepatology and Cell Therapy, Institute of Experimental & Clinical Research, Université Catholique de Louvain, Brussels, Belgium, Brussels, Belgium ; (4) Imperial College, London, United Kingdom ; (5) Ghent University, Gent, Belgium.

Introduction : Liver progenitor cells (LPC) are quiescent in healthy liver and are activated during severe liver injury. Many cytokines have been proposed to regulate LPC activation but in vivo studies are lacking.

Aim : Here, we used strategies to compromise TNF- α signalling, and evaluated the impact on ductular reaction (DR)/LPC response.

Methods : We used ethionine-supplemented choline-deficient diet (CDE, hepatocytic injury) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine diet (DDC, cholangiocytic injury) to induce DR in mice, and treated mice with liposome-encapsulated Clodronate to deplete KC, with anti-TNF- α -antibody (Remicade™) to inhibit TNF-signalling or with the anti-inflammatory corticosteroid dexamethasone. We evaluated the consequences of these treatments on phenotype/microenvironment of the DR by IHC techniques (K19, Ki67, laminin, endoglin, α -SMA, F4/80 and Sirius Red stainings). Cytokine mRNA levels (Il-6, TNF α , MCP1, Wnt3a, and Jagged1) were determined by qPCR.

Results : In both models, DR is associated with up-regulation of hepatic TNF- α and Il-6 expression. KC depletion, dexamethasone and anti-TNF α blunted the CDE and DDC-induced TNF- α and Il-6 up-regulation. While KC depletion in CDE did not influence LPC proliferation but attenuated their invasive behaviour, in DDC-treated mice it affects the ductular reaction in their proliferative and expansive capacities. There is a profound inhibition of the K19-expressing cell expansion in all strategies used. A significantly decreased amount of myofibroblasts and ECM deposition in both liver injury settings is observed with the 3 different regimens. Only in biliary regeneration, Jagged1/Notch levels were down-regulated by dexamethasone.

Conclusions : The TNF- α -NF κ B-IL6 pathway is a key regulatory pathway of LPC expansion in mouse models of hepatocytic and cholangiocytic liver injury.

ACTIVATION OF INTERFERON AND NF KAPPA B PATHWAYS IN EARLY HUMAN ALCOHOLIC LIVER DISEASE DEVELOPMENT. P. Starkel (1), N. Lanthier (2), C. De Saeger (3), C. Sempoux (4), I. Leclercq (1), Y. Horsmans (1). (1) Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium ; (2) Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium ; (3) Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium ; (4) Service d'Anatomie Pathologique, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium.

Introduction and aims : Animal data suggest a pathogenic role for TLR4 with subsequent activation of NF κ B in alcohol liver injury. Here, we assessed cytokines, TLR and TLR-dependent pathways expressions in human ALD.

Methods : Liver biopsies from 41 actively drinking ALD patients were compared with normal liver tissue (n = 6) and with liver biopsies from ALD patients after 2 weeks of alcohol abstinence (n = 27). Quantitative PCR and Western blotting were used for mRNA and protein analysis, respectively, immunohistochemistry for cellular localization of inflammatory processes. The study was approved by our local ethics committee.

Results : Compared with normal livers, already early ALD (with minimal fibrosis) showed a significant activation of NF κ B with increased total and phosphorylated p65 protein expression and up-regulation of NF κ B responsive genes mRNA levels TNF α and IL-1 β . P65 immunohistochemistry evidenced positive staining mainly in inflammatory cells in ALD patients. Interestingly, TLR4 mRNA expression remained unchanged whereas CD14 and TNF receptor 1 mRNA were down-regulated in active ALD. Only IL-1 β returned to control levels after abstinence.

STAT3 phosphorylation was also significantly induced in early ALD patients. Intriguingly, STAT3 mRNA expression as well as STAT3 responsive genes SOCS3, IL-6 and MCP-1 were significantly downregulated in ALD patients suggesting an overall inhibition of the STAT3 pathway. STAT3 inhibition did not recover following 2 weeks of alcohol abstinence.

The interferon pathway was strongly activated in early ALD with significant increase in total and phosphorylated interferon regulatory factor-3, up-regulation of interferon-beta and interferon responsive genes ISG6-16 and OAS-1 mRNA levels. Interferon-beta levels significantly correlated with up-regulation of TLR3 and 7 mRNA. Activation of the interferon pathway was not reversed after abstinence.

CD68 mRNA was significantly increased and both CD68 and CD163 immunohistochemistry revealed enlarged, aggregated Kupffer cells and small inflammatory cells in early ALD compared to scattered Kupffer cells in normal liver.

Conclusions : Besides NF κ B, early activation of the interferon pathway could play an important role in human ALD pathogenesis. Inhibition of the STAT3 pathway could contribute to impaired regenerative capacity in ALD.

A LINK BETWEEN AUTOPHAGY AND ER STRESS DURING HEPATIC STELLATE CELL ACTIVATION. L.F. Thoen (1), I. Mannaerts (1), A. Paridaens (2), I. Colle (2), L.A. Van Grunsven (1). (1) Laboratory of Liver Cell Biology, VUB, Brussels, Belgium ; (2) Department of Hepatology and Gastroenterology, Universiteit Gent, Ghent, Belgium.

Introduction : The ability of a cell to sense, respond to and circumvent stress is essential for maintaining homeostasis. The process of protein folding is particularly sensitive to stress. The endoplasmic reticulum (ER) is the site of synthesis, folding and modification of proteins where unfolded proteins can accumulate in stress conditions. This accumulation initiates an adaptive response called unfolded protein response, which consists of three main signaling pathways, *i.e.* IRE1, PERK and ATF6, aiming at restoring organelle function. It has been suggested that ER stress plays a role in the chronicity and liver damage during alcohol abuse, infection with the hepatitis B and C virus, non-alcoholic steatohepatitis and can even promote hepatocarcinogenesis. In addition, ER stress can also induce autophagy. This is a metabolic process by which cells degrade and metabolize own constituents with many connections to human disease and physiology.

Aim : To study the role of ER stress during early HSC activation and investigate a possible link between ER stress and autophagy in primary mouse HSCs.

Methods : Mouse HSCs were isolated from Balb/c mice and were i) plated on plastic culture dishes to induce *in vitro* activation, ii) seeded on substrates with different softness's and iii) cultured as aggregates. In all conditions, the expression of activation and ER stress markers was analyzed by RT-qPCR. Analysis of the autophagic flux in primary mouse HSCs after induction or inhibition of ER stress *in vitro* was performed using a DsRed-GFP-LC3B encoding plasmid. *In vivo* induction of HSC activation was triggered by CCl₄ injection.

Results : The expression of ER stress markers, *i.e.* XBP1spliced, BiP and chop, during *in vitro* HSC activation showed an early peak 10 hours after seeding primary HSCs on plastic culture dishes followed by a decreased expression at 24 h.

At later time points in culture, the expression levels did not differ from the 24 h time point. The peak in XBP1spliced could also been seen in freshly isolated HSCs isolated from mice 10 h after 1 CCl₄ injection. HSCs seeded on a soft substrate (0.48 kPa) showed prevention of the early ER stress peak and expression of activation markers was inhibited compared to HSCs plated on plastic. Similar results were observed when HSC aggregates were analyzed. HSCs treated after 1 day in culture with the ER stress inducer tunicamycin, showed a significantly up regulated autophagic flux. This up regulation could be blocked by pretreating the cells with a specific IRE1 inhibitor.

Conclusions : ER stress is induced during early HSC activation *in vitro* and *in vivo*. Inhibition of ER stress by growing cells on soft substrates or culturing cells in aggregates inhibits HSC activation while induction of ER stress induced autophagic flux in an IRE1 dependent manner in cultured HSCs.

- A05 -

SINUSOIDAL OBSTRUCTION SYNDROME (SOS) : A LIGHT AND ELECTRON MICROSCOPY STUDY IN HUMAN LIVER. C. Vreuls (1), A. Driessen (2), S. Olde Damink (1), G. Koek (1), H. Duimel (1), M. Van Den Broek (1), C. Dejong (1), F. Verheyen (1), F. Braet (3), E. Wisse (1). (1) Maastricht University Medical Centre, Maastricht, Netherlands ; (2) Department of Pathology, Antwerp University Hospital, Antwerpen, Belgium ; (3) Australian Centre for Microscopy & Microanalysis, Sydney, Australia.

Introduction : Oxaliplatin is an important chemotherapeutic agent, used in the treatment of colorectal liver metastases. This treatment can induce sinusoidal obstruction syndrome (SOS) and lead to higher morbidity with prolonged hospital stay. Current knowledge of the pathophysiology of SOS is based on a rat model : it is assumed that the aetiology of SOS is direct toxic damage of oxaliplatin to the sinusoidal endothelial cells (SECs). Comparable data however lack in humans.

Aim : Therefore, the aim of this study was to perform a detailed and comprehensive study of the features of SOS secondary to oxaliplatin use, in human liver at time of surgery, using both light microscopy (LM) and electron microscopy (EM).

Methods : Included were all patients of whom wedge liver biopsies were collected during a partial hepatectomy for colorectal liver metastases, between September 2005 and September 2009. The wedge biopsy were perfusion fixated and processed for LM and EM. The SOS lesions were selected by LM and details were studied in EM.

Results : Material was available in 30 patients, of whom 28 patients received neo-adjuvant oxaliplatin treatment. Eighteen (64%) of the 28 patients showed SOS lesions in both LM and EM. The EM lesions consisted of detachment of the SECs from the space of Disse. In the enlarged space of Disse a variable amount of erythrocytes were located. Patients with SECs detachment had a larger median cumulative amount of oxaliplatin and a shorter interval between the last administration of oxaliplatin and surgery, respectively 752.5 versus 495 mg/ m² and 60.5 and 64 days. In the control group no SOS lesions were observed.

Conclusions : SECs detachment was present in human SOS, accompanied by enlargement of the space of Disse and erythrocytes in this area. These findings, originally described in a rat model, were now for the first time confirmed in human livers under clinical relevant settings.

- A06 -

THE NON-CANONICAL WNT/CALCIUM/NFAT PATHWAY CONTROLS BILIARY DIFFERENTIATION. J.B. Beaudry, S. Cordi, Y. Achouri, F. Lemaigre. Université Catholique De Louvain, Brussels, Belgium.

Introduction : Elucidating the mechanisms of hepatic cell differentiation is critical for understanding how progenitor cells give rise to mature cells during liver regeneration and disease. Also, defective differentiation of embryonic cholangiocyte precursors can lead to severe biliary dysfunction. Therefore, much information has been gained from studies on embryonic development of the liver, during which cells undergo the full differentiation process from precursor to differentiated cell. In embryonic liver, cholangiocyte precursors arise from hepatoblasts and form the ductal plate, a single-layered sleeve of cells located around portal veins. These precursors eventually give rise to functional bile ducts, canals of Hering and periportal hepatocytes. The Wnt/beta-Catenin signalling pathway is thought to regulate cholangiocyte development, yet the molecular mechanisms are unclear.

Methods : Transgenic mice expressing the GFP marker in cholangiocytes were used to isolate and characterize embryonic biliary cells. *Cre-loxP* technology was used to generate mutant mice that lack or overexpress the Wnt mediator beta-Catenin in the whole liver or specifically in cholangiocytes. Cultured hepatoblasts were used to investigate the mechanisms of Wnt-induced biliary differentiation.

Results : We demonstrate that, unexpectedly, Wnt stimulates biliary differentiation in a β Catenin-independent manner. *In vitro*, repression or activation of beta-Catenin do not influence Wnt-induced biliary gene expression. *In vivo*,

suppression of beta-Catenin does not impair cholangiocyte specification, differentiation and maintenance, while its activation in cholangiocyte precursors perturbs biliary differentiation. Instead, we provide evidence that the non-canonical Wnt/Calcium/NFAT pathway controls biliary differentiation. The NFAT pathway is active in normal cholangiocytes and increased in models of ectopic biliary development. Inhibition of NFAT abolishes Wnt-induced biliary differentiation while its activation stimulates cholangiocyte gene expression and represses hepatocyte differentiation in vitro.

Conclusion : These data support an unprecedented role for non-canonical Wnt/Calcium signalling in liver development and suggest that NFAT is a critical mediator of cholangiocyte differentiation.

- A07 -

CORRELATION OF HUMAN LIVER PPAR GENE EXPRESSION WITH HISTOLOGICAL NASH : A LONGITUDINAL STUDY. S. Francque (1), A. Verrijken (1), S. Caron (2), J. Prawitt (2), R. Paumelle (3), B. Derudas (3), M.R. Taskinen (4), W. Van Hul (5), I. Mertens (1), G. Hubens (1), E. Van Marck (1), P. Michielsen (1), L. Van Gaal (1), B. Staels (2). (1) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium ; (2) Institut Pasteur, Lille, France ; (3) Institut Pasteur, Lille, France ; (4) Helsinki University Central Hospital and Biomedicum, Helsinki, Finland ; (5) University of Antwerp, Antwerpen, Belgium.

Introduction : Peroxisome proliferator-activated receptors (PPARs) have been implicated in the pathogenesis of NASH, merely based on animal data. Gene expression data in NASH patients are scarce.

Aim : We aimed at studying liver PPAR α , β/δ and γ expression in a large cohort of obese patients assessed for the presence of NAFLD at baseline and after 1 year follow-up.

Methods : Patients presenting to the obesity clinic underwent a metabolic and hepatic work-up. If NAFLD was suspected, a liver biopsy was performed and scored using the Chalasani definition and the NASH CRN Scoring System. Gene expression was studied by mRNA quantification. Patients were reassessed after 1 year.

Results : 125 patients were consecutively included (mean age 45.0 ± 12.4 y, mean BMI 38.7 ± 6.67 kg/m²). Liver PPAR α expression negatively correlated with the presence of NASH ($p = 0.001$) and with the severity of steatosis ($p = 0.003$), ballooning ($p = 0.001$), the NASH activity score ($p = 0.008$), and fibrosis ($p = 0.003$). PPAR α expression was positively correlated to adiponectin ($R^2 = 0.345$, $p = 0.010$) and inversely correlated to visceral fat ($R^2 = 0.343$, $p < 0.001$), HOMA IR ($R^2 = 0.411$, $p < 0.001$) and CK18 ($R^2 = 0.233$, $p = 0.012$). Liver PPAR β/δ and PPAR γ expression did not correlate with any of the histological features nor with glucose metabolism or serum lipids. At 1 year, correlation of PPAR α expression with liver histology was confirmed. In longitudinal analysis, an increase in PPAR α expression was significantly associated with histological improvement ($p = 0.008$).

Conclusions : Human liver PPAR α gene expression negatively correlates with NASH severity, visceral adiposity and insulin resistance and positively with adiponectin. Histological improvement is associated with an increase in PPAR α expression. These data suggest that PPAR α is a potential therapeutic target in NASH.

- A08 -

ROLE OF ANGIOGENIC FACTORS/CELL ADHESION MARKERS IN SERUM OF PATIENTS WITH HEPATOPULMONARY SYNDROME. S. Raevens (1), S. Coulon (1), C. Van Steenkiste (2), R. Colman (3), H. Van Vlierberghe (1), A. Geerts (1), T. Perkmann (4), T. Horvatits (4), V. Fuhrmann (4), I. Colle (1). (1) Ghent University Hospital, Ghent, Belgium ; (2) Maria Middelaars Ziekenhuis, Ghent, Belgium ; (3) Ghent University, Ghent, Belgium ; (4) Medical University, Vienna, Austria.

Introduction : Hepatopulmonary syndrome (HPS) is a complication seen in 4% to 47% of patients with chronic liver disease and results in a significant increase in morbidity and mortality. HPS is caused by intrapulmonary vascular dilations and direct arterio-venous connections (also known as shunts or collaterals) with a devastating influence on the physiology of the gas exchange. No effective medical therapies are available, only liver transplantation can cure HPS. The mechanisms responsible for these pathologic changes are still poorly understood. Many reports suggest an imbalance between vasodilators and vasoconstrictors. In recent literature, evidence mounts for a process of (neo-) angiogenesis in the pathogenesis of HPS.

Aim : The aim of this study was (1) to identify possible angiogenic factors and cell adhesion molecules in serum of patients with HPS and (2) to study the possibility to predict the presence or absence of HPS by these factors.

Methods : Multiplex assays (Meso Scale Discovery, Gaithersburg, USA) were used to measure the concentration of several angiogenic factors and cell adhesion molecules in the serum of patients with HPS ($n = 30$) and without HPS ($n = 30$). The following factors were measured : cKit, VEGFR2 (Growth Factor Panel II), bFGF, PlGF, sFlt1, Tie2,

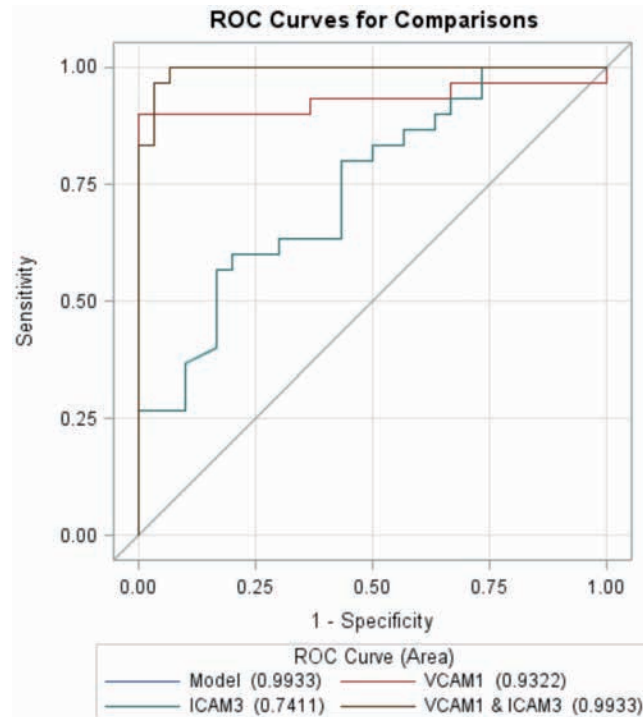


Fig. 1. — ROC Curves of VCAM1 (AUC = 0.932), ICAM3 (AUC = 0.741) and VCAM1 & ICAM3 (AUC = 0.993)

VEGF, VEGFC, VEGFD (Angiogenesis Panel I), e-selectine, p-selectine, ICAM3, Throm, (Vascular Injury Panel I), CRP, SAA, ICAM1 and VCAM1 (Vascular Injury Panel II). The diagnosis of HPS was made on the basis of alveolo-arterial oxygen gradient above 20 and positive contrast echocardiography.

Results : Patients with (n = 30) and without HPS (n = 30) had a similar MELD score (mean MELD score of 12.84 vs. 12.52 ; p = 0.852) and PaCO₂ (34.33 vs. 36.37 ; p = 0.059). PaO₂ values were significantly lower in the HPS group compared to the group without HPS (78.23 vs. 85.67 ; p = 0.009). Logistic regression was performed on all our results and we established a model with each predictor. Based on Area Under the Curve (AUC) data and p-values the best predictors were determined : VCAM1 (AUC = 0.932 ; p < 0.001) and ICAM3 (AUC = 0.741 ; p = 0.003). Combining these 2 factors results in an AUC of 0.993 and after cross-validation the AUC was still 0.988 (Fig. 1). Moreover, when adding age to the logistic regression model as an independent variable, VCAM1 and ICAM3 were still significant predictors for HPS. In the population with HPS, a moderate Spearman correlation between ICAM3 and Child-Pugh Score was observed (r = 0.48 ; p = 0.007).

Conclusions : Our results indicate that VCAM1 and ICAM3 might be promising biomarkers for predicting the presence of HPS. Combining these 2 factors result in an AUC of 0.993. However, the use of these biomarkers should be validated in a larger group of patients.

- A09 -

CD4+RORGAMMAT+ CELLS AND TREGS IN HFD-INDUCED NON ALCOHOLIC STEATOHEPATITIS (NASH) MOUSE MODEL. L. Vonghia (1), N. Ruysers (2), D. Schrijvers (3), B. De Winter (4), P. Pelckmans (1), P. Michielsen (1), L. De Clerck (5), E. Jirillo (6), D. Ebo (5), C. Britts (5), S. Francque (1). (1) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium ; (2) Laboratory of Experimental Medicine and Pediatrics, University of Antwerp, Antwerp, Belgium ; (3) Laboratory of Pharmacology, University of Antwerp, Antwerp, Belgium ; (4) Laboratory of Experimental Medicine and Pediatrics, Division of Gastroenterology, University of Antwerp, Antwerp, Belgium ; (5) Department of Immunology, Allergology and Rheumatology, University of Antwerp, Antwerp, Belgium ; (6) Department of Basic Medical Science, University of Bari, Bari, Italy.

Introduction : The differential role of several immune cells and inflammatory mediators and their cross-talk in different metabolic active organs in the pathogenesis of non-alcoholic steatohepatitis (NASH) is poorly understood.

Aim : To study CD4+RORgammat+ T-helper cells and their counterpart, the CD4+CD25+FOXP3+ T-regulatory cells (Tregs) in the liver, subcutaneous (SAT) and abdominal adipose tissue (AAT) in a high fat diet (HFD) mouse model.

Methods : C57BL6 mice were fed a HFD or a normal diet (ND) for 36 weeks. Liver enzymes, metabolic parameters and liver histology were assessed. The expression of CD4+RORgammat+cells and Tregs in different organs (blood, liver, AAT and SAT) were analysed by flowcytometry. Cytokine and adipokine tissue expression were studied by RT-PCR.

Results : Mice fed a HFD developed NASH (evidenced histologically) and showed a significant higher weight gain (p :0.001), increased liver enzymes (p :0.01) and altered glucose metabolism (p :0.02) compared to ND. CD4+cells with high expression of RORgammat (CD4+RORgammatHi) were significantly increased in liver (p :0.02) and AAT (p :0.01), while an increase of Tregs was observed in SAT of mice fed HFD (p :0.02) compared to ND. IL17a and IL10 gene expression were upregulated in liver, AAT and SAT of HFD fed mice compared to ND. Moreover an increase of leptin gene expression was found in adipose tissue after HFD, most pronounced in SAT.

Conclusions : ROgammaRtHi CD4+cells and Tregs contribute to NASH pathogenesis but mechanisms are different in liver, AAT and SAT, the latter also showing significant alterations. These site-specific differences are associated with increased expression of inflammatory cytokines and differential leptin upregulation.

- A10 -

CONTROLLED ATTENUATION PARAMETER FOR THE EVALUATION OF HEPATIC STEATOSIS IN ALD AND NAFLD. A. Lepida (1), F. Puleo (1), D. Degre (1), L. Verset (2), P. Demetter (2), T. Gustot (1), J. Schreiber (1), M. Adler (1), E. Trepo (1), C. Moreno (3). (1) Hépatogastroentérologie, Hôpital universitaire Erasme ULB, Brussels, Belgium ; (2) Erasme Hospital, Brussels, Belgium ; (3) Hépatogastroentérologie, Cliniques Universitaires St. Luc, Brussels, Belgium.

Background and Aims : During the last years, new non invasive tools have been developed for the evaluation of liver fat content. Our aim was to determine the accuracy of Controlled Attenuation Parameter (CAP), a new method for the evaluation of hepatic steatosis using transient elastography, in an alcoholic (ALD) and non-alcoholic fatty liver disease (NAFLD) population and to identify specific cut-offs which predict the severity of steatosis. We also studied the possible impact of histological inflammation and fibrosis on CAP.

Methods : Ninety-eight consecutive ALD or NAFLD patients candidate for a liver biopsy, were also evaluated for the amount of steatosis with CAP. The percentage of steatosis among total hepatocytes was assessed histologically as follow : S0 : < 5%, S1 : 5-33%, S2 : 34-66%, S3 : 67-100%. The Fatty Liver Index (FLI), a composite serum marker of steatosis was also calculated. Areas under receiver operating characteristics (AUROC) curves were used to evaluate the performance of CAP for diagnosing steatosis compared with histology. Inflammation was evaluated by the NAFLD activity score (NAS) in the NAFLD population and by the presence of histological lesions of alcoholic hepatitis (AH) in the ALD population.

Results : Characteristics of the patients included were : median age 51.5 years, median BMI 27 kg/m², ALD 58%, NAFLD 42%. The prevalence of steatosis in the whole population was : S0 23.5%, S1 40.8%, S2 17.3%, S3 18.4%. The presence of significant steatosis (≥ 5%) on the liver biopsy was significantly associated in univariate analysis with the median CAP values (p = 0.011), FLI (p = 0.035), triglycerides (p = 0.039) and albumine levels (p = 0.049). The median CAP was higher among patients with significant steatosis (299 dB/m vs 235 dB/m ; p = 0.01) and the AUROC for this outcome was 0.78. A cut-off of 266.5 dB/m was 71% sensitive, 78% specific and had positive and negative predictive values of 91.3% and 45.2% respectively. The diagnostic accuracy of FLI for the detection of significant steatosis was lower compared to the CAP (AUROC 0.69). Using CAP vs FLI score, the AUROCs were 0.75 vs 0.64 for the diagnosis of steatosis ≥ S2 and 0.67 vs 0.61 for the diagnosis of steatosis ≥ S3 respectively. There was no influence of inflammation on CAP ; it was not associated with NAS scores ≥ 5 in the NAFLD group (p = 0.56) and also not associated with histological AH in the ALD group (p = 0.60). Finally CAP was not associated with histological fibrosis (p = 0.73).

Conclusions : CAP is an appealing novel non-invasive method to detect and semi-quantify hepatic steatosis with simultaneous assessment of fibrosis using liver stiffness measurements. It has many advantages compared to other modalities and can be used as a screening-friendly method to detect steatosis in the general population.

- A11 -

HEPATOCYTE AUTOPHAGY DEFICIENCY INDUCES LIVER INJURY, INHIBITS STEATOSIS AND IMPROVES SERUM LIPIDS. W.J. Kwanten (1), W. Martinet (2), B.Y. De Winter (3), V. Van Hoof (3), P. Bedossa (4), P.P. Michielsen (5), S.M. Francque (5). (1) Laboratory of Experimental Medicine and Pediatrics, Division of Gastroenterology, University of Antwerp, Antwerp, Belgium ; (2) Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium ; (3) University of Antwerp, Antwerpen, Belgium ; (4) Department of pathology, Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, Clichy, France ; (5) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium.

Introduction & Aim : Autophagy is a cellular degradation pathway delivering cytoplasmic content to lysosomes. Evidence that autophagy interferes with the lipid metabolism and perhaps with pathogenesis of NAFLD increases, though controversy on its function (lipolytic vs. lipogenic) exists. This study investigates autophagy in the hepatocellular lipid metabolism of mice.

Methods : Hepatocyte specific autophagy deficient C57Bl/6J mice (*Atg7^{Flox}*) were created using the Cre-LoxP technology with an albumin-promoter of Cre to delete the autophagy gene *Atg7* specifically in hepatocytes and were compared with their control countermates (*Atg7^{WT}*). *Atg7^{WT}* and *Atg7^{Flox}* were fed control diet (CD) or methionine choline deficient diet (MCDD) (respectively n = 16/16/11/13) for 3 weeks before analysis. Statistics were performed using 2-way-ANOVA followed by 1-way-ANOVA with Bonferroni post-hoc analysis when appropriate.

Results : Liver/body weight ratio and liver enzymes were significantly higher in *Atg7^{Flox}/CD* mice compared to *Atg7^{WT}/CD* mice (Table 1). *Atg7^{WT}/CD* mice showed normal liver histology and fasting-induced steatosis, whereas *Atg7^{Flox} mice* showed hypertrophic hepatocytes, inflammation, (pre-)apoptotic cells and pronounced ductular reaction. The fibrosis area (Sirius-red, morphometry) and alpha-SMA positive stellate cell number (semi-quantitative scores) were significantly increased in *Atg7^{Flox}*. Lipid droplets were virtually absent in *Atg7^{Flox}/CD*. Serum triglycerides were significantly lower, whilst HDL and total cholesterol were higher. *Atg7^{WT}/MCDD* had comparable liver/body weight and increased hepatocyte injury compared to *Atg7^{WT}/CD*. Liver fat content (Oil-red-O, morphometry) was similar to CD fed mice. *Atg7^{Flox}/MCDD* showed the most severe histological lesions and significantly more fibrosis. Although lipid droplets were present in these mice, this was considerably less compared to *Atg7^{WT}/MCDD*.

Table 1. — All data are presented as mean ± SEM. Sample size (n) of the serum analyses are represented between the brackets. Data were analysed using two-way ANOVA

	<i>Atg7^{WT}/CD</i>	<i>Atg7^{Flox}/CD</i>	<i>Atg7^{WT}/MCDD</i>	<i>Atg7^{Flox}/MCDD</i>
Liver/body weight ratio	0.049 ± 0.001	0.242 ± 0.009 ^a	0.054 ± 0.009	0.168 ± 0.006 ^{bc}
Serum analysis				
- ALT (U/l)	20.14 ± 2.62 (15)	737.68 ± 146.21 (10) ^a	161.37 ± 89.44 (9)	299.58 ± 56.51 (10) ^c
- AST (U/l)	175.15 ± 22.42 (15)	1855.06 ± 279.86 (10) ^a	422.88 ± 124.91 (9) ^a	1033.03 ± 17.82 (10) ^c
- gGT (U/l)	5.6 ± 0.16 (15)	14.40 ± 0.70 (10) [§]	5.86 ± 0.42 (9)	12.10 ± 1.30 (10) [§]
- Alk. phosphatase (U/L)	127.59 ± 8.22 (15)	335.69 ± 12.48 (10) ^a	122.92 ± 10.78 (9)	147.82 ± 11.37 (9) ^c
- Total bilirubin (mg/dl)	0.10 ± 0.00 (15)	0.11 ± 0.00 (10)	0.18 ± 0.03 (9) [#]	0.13 ± 0.01(9) [#]
- Glycemia (mg/dl)	106.95 ± 5.70 (15)	87.44 ± 6.44 (10)	55.41 ± 5.63 (9) [#]	53.73 ± 4.23 (10) [#]
- Total cholesterol (mg/dl)	73.51 ± 4.14 (15)	140.84 ± 8.18 (10) ^a	50.00 ± 0.00 (9) ^a	61.17 ± 3.92 (10) ^c
- HDL (mg/dl)	63.48 ± 5.22 (15)	129.32 ± 7.56 (9) ^a	19.20 ± 3.85 (9) ^a	55.60 ± 5.51 (8) ^{bc}
- LDL (mg/dl)	6.60 ± 0.83 (15)	12.81 ± 2.14 (10) ^a	7.17 ± 0.88 (9)	5.56 ± 0.63 (9) ^c
- Triglycerides (mg/dl)	67.90 ± 7.30 (15)	15.10 ± 3.76 (10) ^a	35.83 ± 3.37 (9) ^a	16.41 ± 1.41 (10)
Histology				
- Sirius-red (%Area)	0.102 ± 0.102	1.648 ± 0.414 [§]	1.173 ± 0.268 [#]	3.166 ± 0.381 ^{§#}
- Oil-red-O (%Area)	4.947 ± 0.765	0.000 ± 0.000 [§]	5.352 ± 1.839	1.125 ± 0.692 [§]
- aSMA activity index (/12pnts)	0.44 ± 0.38	3.82 ± 0.55 ^a	0.50 ± 0.44	1.39 ± 0.31 ^c

([§] p < 0.001 for genotype, [#] p < 0.001 for diet) followed by a one-way ANOVA with Bonferroni post-hoc analysis when appropriate (^a p < 0.01 vs *Atg7^{WT}/CD*, ^b p < 0.01 vs *Atg7^{WT}/MCDD*, ^c p < 0.01 vs *Atg7^{Flox}/CD*).

Conclusions : Hepatocyte specific autophagy deficiency leads to severe parenchymal damage, but prevents hepatocellular lipid accumulation and improves serum lipids, both in CD and in MCDD fed mice. Lesions are more pronounced after MCDD. Autophagy therefore seems to be crucial and protective in liver cell homeostasis, although it negatively impacts on lipid metabolism.

SIMEPREVIR EFFICACY/SAFETY IN PREVIOUSLY RELAPSING HCV GT 1-INFECTED PATIENTS : PROMISE PHASE 3 TRIAL. E. Lawitz (1), X. Forns (2), S. Zeuzem (3), E. Gane (4), J.P. Bronowicki (5), P. Andreone (6), A. Horban (7), A. Brown (8), M. Peeters (9), O. Lenz (9), S. Ouwkerk-Mahadevan (10), R. Kalmeijer (11), M. Beumont-Mauviel (10). (1) Texas Liver Institute, University of Texas Health Science Center, San Antonio, United States ; (2) Liver Unit, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain ; (3) J.W. Goethe University Hospital, Frankfurt, Germany ; (4) Auckland Hospital Clinical Studies Unit, Auckland, New Zealand (Aotearoa) ; (5) INSERM U954, Université de Lorraine, Centre Hospitalier Universitaire de Nancy, Vandœuvre-lès-Nancy, France ; (6) Dipartimento di Scienze Mediche e Chirurgiche, University of Bologna, Bologna, Italy ; (7) Medical University of Warsaw, Warsaw, Poland ; (8) Imperial College Healthcare NHS Trust, London, Great Britain (UK) ; (9) Janssen Infectious Diseases BVBA, Beerse, Belgium ; (10) Janssen Research & Development, Beerse, Belgium ; (11) Janssen Global Services, LLC, Raritan, United States.

Introduction : Simeprevir (SMV, TMC435) is a potent, once-daily (QD), oral hepatitis C virus (HCV) NS3/4A protease inhibitor. PROMISE (TMC435-HPC3007 ; NCT01281839) is a Phase III, randomised, double-blind, placebo-controlled trial assessing the efficacy, safety and tolerability of SMV plus peginterferon alpha-2a/ribavirin (PR) versus placebo (PBO)/PR in chronically infected HCV genotype 1 patients who relapsed after previous interferon-based therapy. Safety and sustained virological response at Week 12 (SVR12) results from a primary analysis at Week 60 are presented.

Methods : Patients (N = 393) were randomised (2 : 1) to receive SMV (150 mg QD) plus PR (n = 260) or PBO/PR (n = 133) for 12 weeks, followed by PR alone. Patients were stratified by HCV genotype 1 subtype and *IL28B* genotype. Total treatment duration was 24 or 48 weeks based on response-guided therapy (RGT) criteria (SMV group ; HCV RNA < 25 IU/mL Week 4 and undetectable Week 12) or 48 weeks (PBO group).

Results : SMV/PR was superior to PBO/PR ; SVR12 : 79% vs 37%, respectively (p < 0.001). A significant proportion of patients had bridging fibrosis (METAVIR F3 ; 15%) or cirrhosis (METAVIR F4 ; 15%), 42% had HCV genotype 1a infection, and 24% were *IL28B* CC genotype. Most (93%) SMV-treated patients met RGT criteria and completed treatment at Week 24. Of these patients, 83% achieved SVR12. SMV/PR treatment led to SVR12 rates of 82% in patients with METAVIR score F0-F2 and 74% in those with F3-F4. The corresponding SVR12 rates following treatment with PBO/PR were 41% and 24%. SVR12 rates in patients with HCV genotype 1a/other and 1b were 70% and 86% after SMV/PR treatment, and 28% and 43% after PBO/PR, respectively. Rates of SVR12 in patients with *IL28B* genotypes CC, CT and TT were 89%, 78% and 65% in the SMV/PR group and 53%, 34% and 19% in the PBO/PR group, respectively. Overall, 77% of SMV-treated patients and 3% of PBO-treated patients achieved rapid virological response. Treatment with SMV/PR treatment also led to a lower on-treatment failure rate and a lower relapse rate, compared to PBO/PR (3% vs 27% and 19% vs 48%, respectively). The most common adverse events were fatigue, influenza-like illness, pruritus and headache. Rates of anaemia and neutropenia in the simeprevir group compared to placebo were 17% versus 20% and 18% versus 22%, respectively. Rates of pruritus and rash were comparable between SMV and PBO (27.7% vs 27.8% and 23.1% vs 22.6%, respectively).

Conclusions : SMV 150 mg QD, in combination with PR, was generally safe and well tolerated in patients with prior relapse after previous PR therapy, leading to SVR12 rates of 79% overall, 83% in those meeting RGT criteria and 74% in F3-F4 patients. Most patients (93%) receiving SMV could shorten therapy to 24 weeks.

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SOFOSBUVIR + RIBAVIRIN FOR 12 OR 24 weeks FOR PATIENTS WITH HCV GENOTYPE 2 OR 3 : THE VALENCE TRIAL. S. Zeuzem (1), G. Dusheiko (2), R. Salupere (3), A. Mangia (4), R. Flisiak (5), R. Hyland (6), A. Lleperruma (6), E. Svarovskaia (6), D. Brainard (6), W. Symonds (6), J. Mchutchison (7), O. Weiland (8), H. Reesink (9), P. Ferenci (10), C. Hezode (11), R. Esteban (12), J. Piessevaux (13). (1) J.W. Goethe University Hospital, Frankfurt, Germany ; (2) Royal Free Hospital, London, Great Britain (UK) ; (3) Tartu University Hospital, Tartu, Estonia ; (4) "Casa Sollievo della Sofferenza" Hospital, San Giovanni Rotondo, Italy ; (5) Medical University of Bialystok, Bialystok, Poland ; (6) Gilead Sciences, Foster City, United States ; (7) Gilead Sciences, Foster City, United States ; (8) Karolinska University Hospital, Stockholm, Sweden ; (9) Academic Medical Center, Amsterdam, Netherlands ; (10) Medical University of Vienna, Vienna, Austria ; (11) Hôpital Henri Mondor, Créteil, France ; (12) Hospital Universitario Val d'Hebron, Barcelona, Spain ; (13) Gilead Sciences, City of Brussels, Belgium.

Introduction : In phase 3 trials, 12 weeks of sofosbuvir (SOF) + ribavirin (RBV) has demonstrated high SVR rates in patients with genotype 2 and 3 HCV infection, with higher response rates in patients infected with genotype 2 than in those infected with genotype 3 HCV.

Aim : VALENCE is a Phase 3 study conducted in Europe assessing the safety and efficacy of SOF+RBV administered for 12 or 24 weeks.

Methods : Treatment-naïve or treatment-experienced patients infected with HCV genotype 2 or 3 were randomized 4:1 to receive SOF+RBV for 12 weeks or matching placebo. The study was subsequently amended to extend treatment duration to 24 weeks for patients with genotype 3 HCV irrespective of prior treatment history. The primary end point is SVR12.

Results : 421 patients were randomized : 334 received SOF+RBV, 85 received placebo, and 2 did not initiate treatment. Of those randomized to receive SOF+RBV, 261 (78%) had HCV genotype 3, 195 (58%) were treatment-experienced, 70 (21%) were cirrhotic, and 114 (34%) had the *IL28b* CC genotype. All patients receiving SOF+RBV became and remained HCV RNA negative while on treatment ; relapse has accounted for all virologic failures to date. Of the 73 genotype 2 patients, 68 (93%) achieved SVR4. The genotype 3 patients have not yet reached post-treatment Week 4. Final SVR12 data for all patients will be presented. SOF+RBV for 12 or 24 weeks was generally well tolerated ; 2 (< 1%) patients have discontinued treatment early due to adverse events (malaise and headache in one patient, and suicide attempt in the other). Adverse events and laboratory abnormalities were generally consistent with the safety profile of RBV. The most frequent adverse events in patients who received SOF+RBV were : headache, 28% ; fatigue, 27% ; pruritus, 24% ; asthenia, 22% ; nausea, 17% ; insomnia, 14% ; dyspnoea, 11% ; and dry skin, 11%.

Conclusions : SOF+RBV was well tolerated in a predominantly treatment-experienced patient population treated for 12 (genotype 2) or 24 (genotype 3) weeks. Efficacy in genotype 2 patients is similar to that observed in recent Phase 3 studies. Data on the efficacy of SOF+RBV for 24 weeks in genotype 3 HCV-infected patients is critical to optimize the treatment duration for HCV genotype 3 infections and offer improved SVR rates.

- A14 -

SIMEPREVIR WITH PEGINTERFERON/RIBAVIRIN FOR TREATMENT OF CHRONIC HCV GENOTYPE 4 INFECTION IN TREATMENT-NAÏVE OR -EXPERIENCED PATIENTS : INTERIM RESULTS OF A PHASE III TRIAL. S. Bourgeois (1), C. Hezode (2), P. Marcellin (3), S. Francque (4), D. Samuel (5), F. Zoulim (6), J.D. Grange (7), U. Shukla (8), O. Lenz (8), S. Ouwkerk-Mahadevan (9), M. Peeters (8), W. Jessner (8), C. Moreno (10). (1) ZNA campus Stuivenberg, Antwerpen, Belgium ; (2) Hôpital Henri Mondor, Créteil, France ; (3) Hôpital Beaujon, Clichy, France ; (4) Universitair Ziekenhuis Antwerpen, Antwerpen, Belgium ; (5) Hôpital Paul Brousse, Villejuif, France ; (6) La Croix-Rousse Hospital, Lyon, France ; (7) Hôpital Tenon, Paris, France ; (8) Janssen Infectious Diseases BVBA, Beerse, Belgium ; (9) Janssen Research & Development, Beerse, Belgium ; (10) Erasme Hospital, Brussels, Belgium.

Introduction : Simeprevir (SMV) is a potent, once-daily (QD), HCV NS3/4A protease inhibitor. In genotype 1 (GT1) Phase III studies, SMV 150 mg QD (12 weeks) plus peginterferon/ribavirin (PR) significantly improved sustained virologic response (SVR) rates over PR alone with a shorter total treatment duration of 24 weeks in most patients. We present interim results from a GT4, Phase III, multicentre, uncontrolled, open-label study evaluating SMV/PR in treatment-naïve/-experienced patients (RESTORE ; NCT01567735).

Methods : Treatment-naïve and prior-relapser patients received SMV 150 mg QD with PR (12 weeks), plus PR (12 or 36 weeks) determined by response-guided therapy (RGT). Prior non-responders received SMV 150 mg QD with PR (12 weeks) plus PR (36 weeks). Primary efficacy endpoint : SVR 12 weeks after planned end of treatment (SVR12, EOT).

Results : 107 patients received treatment (male, 78.5% ; median age, 49 years ; Black, 28.0% ; METAVIR F4, 28.8% ; GT4a/4d/4 other, 42.5/23.6/33.9% ; treatment-naïve, n = 35 ; relapsers, n = 22 ; partial responders, n = 10 ; null-responders, n = 40). In 15 (14.0%) patients treatment was still ongoing. 52/61 (85.2%) patients achieved SVR12 (treatment-naïve, 28/32 ; relapsers, 19/21 ; partial responders, 1/3 ; null responders, 4/5). SVR12 was achieved in 66.7% and 83.0% of patients with METAVIR F4 and *IL28B* CT/TT, respectively. 69/104 (66.3%) patients achieved rapid virologic response (RVR) (treatment-naïve, 28/35 ; relapsers, 18/20 ; partial responders, 4/10 ; null responders, 19/39). Of 57 treatment-naïve/relapser patients, 51 (89.5%) had HCV RNA < 25 IU/mL at Week 4 and undetectable at Week 12, thus meeting RGT. Of these, 47 (92.2%) achieved SVR12. 25/84 (29.8%) patients experienced on-treatment failure (treatment-naïve, n = 4 ; relapsers, n = 2 ; partial responders, n = 2 ; null responders, n = 17). 5/84 (6.0%) patients experienced viral relapse (treatment-naïve, n = 2 ; relapsers, n = 1 ; partial responders, n = 1 ; null responders, n = 1). Adverse events (AEs, first 12 weeks) were mainly grade 1/2 ; serious AEs were infrequent (5 patients [4.7%], none were SMV-related) ; no fatal AEs occurred. AEs of interest were pruritus/rash (20.6%/13.1%), anaemia (9.3%), neutropenia (4.7%) and hyperbilirubinaemia (1.9%). No photosensitivity AEs occurred.

Conclusions : SMV 150 mg once daily for 12 weeks with PR was well tolerated and showed promising efficacy in treatment-naïve/-experienced (including prior relapser) HCV GT4 patients, consistent with previous observations in HCV GT1 patients.

Acknowledgements : This study was sponsored by Janssen Research and Development, Beerse, Belgium.

- A15 -

Invited lecture

Liver transplantation for postethylic liver cirrhosis and acute alcoholic hepatitis.

Prof. Philippe Mathurin (CHRU, Lille, France)

CONTROLLED DCD DONATION IS PART OF THE SOLUTION TO LIVER GRAFT SHORTAGE, REGARDLESS OF DONOR AGE. O. Detry (1), N. Meurisse (1), J. Delwaide (1), A. Lamproye (1), B. Bastens (2), C. Brixko (3), V. Putzeys (3), B. Servais (4), M. Meurisse (1), A. Deroover (1), P. Honore (1). (1) CHU Liege, Liège, Belgium ; (2) CHC, Liège, Belgium ; (3) CHR Citadelle, Liège, Belgium ; (4) CHBA, Seraing, Belgium.

Aim : Results of donation after circulatory death (DCD) liver transplantation (LT) are impaired by ischemic bile duct lesions caused by procurement warm ischemia. Donor age is a risk factor in deceased donor LT, and particularly in DCD-LT. At the authors institute, age is not an absolute exclusion criterion to discard DCD liver grafts, controlled DCD donors receive comfort therapy before withdrawal, and cold ischemia is minimized. The aim of the present study was to report on the results of the first 10 years of this experience, and particularly on graft survival and the rate of post-transplant biliary complications, according to DCD donor age.

Methods : The authors retrospectively studied a consecutive series of 70 DCD-LT performed from 2003 to 2012, with at least one year of follow-up. This series was divided according to donor's age, including 32 liver grafts from donors < 55years, 20 between 56 and 69 years, and 18 from older donors > 69 years. The three groups were compared in terms of donor and recipient demographics, procurement and transplantation conditions, peak laboratory values during the first post-transplant 72 hours, and results at one and four years. Median follow-up was 43 months.

Results : Overall graft survival was 98.5%, 91.4% and 69.5% at 1 month, 1 year and 4 years, respectively, without graft loss secondary to ischemic bile duct lesions. Cancer was the primary cause of graft loss and patient death. No difference other than age was noted between the three groups in donor and recipient characteristics, and in procurement conditions. There was no primary non-function but one patient needed re-transplantation for artery thrombosis. Biliary complications occurred similarly in the three groups. Graft and patient survival rates were not different at one and four years between the three groups. During the study period, there was an increasing liver procurement and transplantation activity, and in 2012, 30% of performed LT were DCD-LT, allowing a mean LT waiting time of 66 days.

Conclusions : This study shows comparable results between controlled DCD-LT from younger and older donors. Donor age > 50 years should not be a contraindication to DCD-LT if other donor risk factors (such as warm and cold ischemia time) are minimized. DCD-LT with short cold ischemia may provide a significant source of liver grafts, decreasing waiting time.

PROGRESSION OF LIVER DISEASE IN PIZZ ALPHA-I- ANTITRYPSIN DEFICIENCY PREDOMINATES IN MALE CHILDREN. X. Stephenne (1), S.L. Eta (2), F. Smets (1), E. Sokal (1). (1) Université Catholique de Louvain, City of Brussels, Belgium ; (2) Université Catholique de Louvain, Brussels, Belgium.

Introduction : About 10% of PIZZ infants with Alpha-I-Antitrypsin Deficiency (AATD) develop neonatal cholestasis and of these about 25%-30% develop end-stage liver disease requiring liver transplantation in the first decade of life. AATD present also with incidental finding of elevated liver function tests while few patients may present with cirrhosis at diagnosis. Sex prevalence of liver disease associated to AATD has so far not been reported in paediatric cohort.

Methods : We reviewed retrospectively data on 37 PIZZ AATD (24 males & 13 PIZZ females) referred in our paediatric liver unit. To compare categorical variables, data were analyzed using the Fischer exact.

Results : At the time of AATD diagnosis, 23 of 37 (62%) PIZZ patients had clinical liver disease such as neonatal cholestasis and/or cirrhosis. Neonatal cholestasis was diagnosed at presentation in 18 PIZZ patients (11 males and 7 females) while cirrhosis was diagnosed in 5 male patients and not in females. Thirteen (56%) of 23 liver affected patients developed end-stage liver disease requiring liver transplantation (LT), among which 11 were males, accounting for 46% of male patients (11/24) and 2 were PIZZ females (2/13, 15% of female patients) ($p = 0.0006$ and $p = 0.06$ respectively). Six of the 11 male transplanted patients (55%) had presented initially with neonatal cholestasis, and 5 with compensated cirrhosis. The 2 transplanted girls had presented initially with neonatal cholestasis. Of 24 patients who did not require LT, 10 (42%) presented with neonatal cholestasis (5 males & 5 females) and 14 had no history of liver disease.

Conclusions : In a tertiary pediatric liver unit, cirrhosis at presentation in PIZZ AATD is associated with male gender, and males account for 85% of the transplanted cases. Despite a recruitment bias, our finding suggests a more severe disease evolution in male children.

TRANSCRIPTOMIC AND EPIGENOMIC PROFILING OF DISTINCT HUMAN LIVER CELLS AND CULTURE-ACTIVATED HSCS. A. El Taghdouini (1), A. Sørensen (2), M. Coll (3), I. Mannaerts (4), C. Øie (5), Å. Fretland (6), B. Rosøk (6), M. Najimi (7), E. Sokal (7), A. Luttun (8), B. Smedsrød (5), P. Sancho-Bru (3), P. Collas (2), L. Van Grunsven (9). (1) Laboratory of Liver Cell Biology, VUB, Brussels, Belgium, Brussels, Belgium ; (2) Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway ; (3) IDIBAPS, Barcelona, Spain ; (4) Free University (VUB), Brussels, Belgium ; (5) Dep. of Medical Biology, University of Tromsø, Norway, Tromsø, Norway ; (6) Oslo University Hospital, Rikshospitalet, Oslo, Norway ; (7) Laboratory of Pediatric Hepatology and Cell Therapy, Institute of Experimental & Clinical Research, Université Catholique de Louvain, Brussels, Belgium, Brussels, Belgium ; (8) Dep. of Cardiovascular Sciences, KU Leuven, Leuven, Belgium, Leuven, Belgium ; (9) Laboratory of Liver Cell Biology, VUB, Brussels, Belgium, Brussels, Belgium.

Introduction : Following liver injury, hepatic fibrosis can develop as a reversible wound-healing response. However, if the insult is chronic further aberrant accumulation of extracellular matrix and inflammation can lead to irreversible cirrhosis. The primary source of ECM in the fibrotic liver is the hepatic stellate cell (HSC), a liver cell type residing in adjacency to liver specific endothelial cells (LSECs) and hepatocytes in the space of Disse. Following liver injury HSCs undergo a process of activation, characterized by an increase in ECM production and secretion to encapsulate the injury. **Aim** : By performing gene expression and DNA methylation profiling of freshly isolated, non-cultured liver cell types and culture-activated HSCs, we aim to identify new liver cell type specific genes and evaluate the transcriptomic and epigenomic response of HSCs to culture-induced activation.

Methods : Hepatocytes were isolated by collagenase perfusion of the liver segment. Quiescent HSCs (qHSCs) (UV+CD32-CD45-) and liver-specific endothelial cells (LSECs) (CD32+CD45-) were FACS sorted from non-parenchymal fractions. Homogeneous populations of culture-activated HSCs were obtained after 4 passages of nycodenz-isolated HSCs. Gene expression profiling was performed using Affymetrix HG-U219 genechips. The promoter methylome was examined by MeDIP, coupled to promoter array hybridization.

Results : Our procedure allows the isolation of pure liver cell populations as assessed by RTq-PCR for classical markers. Of the 20216 genes examined, 0.46% (including PDGFRB, PPARG), 0.44% (including LYVE1, STAB2) and 5,1% (including ASGR1, HNF4A) are uniquely expressed by qHSCs, LSECs and hepatocytes, respectively. Uncultured liver cells display ~7000-8000 methylated promoters, with 60-70% similarity between all cell types, while ~500-1000 promoters are uniquely methylated. More than 7% of the genes are upregulated upon culture activation of HSCs and the promoter methylome deviates dramatically from that of their quiescent counterparts.

Conclusions : To our knowledge, our data provide the first comparative gene expression profiles and genome-wide maps of promoter DNA methylation in human purified and uncultured adult liver cell types. They reveal marked similarities in profiles between the different cell types, but also unique expression and epigenetic patterns. The massive changes in HSCs as a response to culture appear to be, at least in part, reminiscent of that elicited in fibrotic liver.

MANAGEMENT OF HEPATITIS C VIRUS (HCV) DISEASE BURDEN WITH THE NEXT GENERATION DIRECT ANTIVIRAL AGENT. P. Starkel (1), W. Laleman (2), C. Moreno (3), D. Vandijck (4), H. Van Vlierberghe (5), S. Hindman (6), H. Razavi (6), P. Van Damme (7). (1) Hépatogastroentérologie, Cliniques Universitaires St. Luc, Brussels, Belgium ; (2) Hepatology, University Hospitals Leuven, Leuven, Belgium ; (3) Hépatogastroentérologie, Hôpital Universitaire Erasme ULB, Brussels, Belgium ; (4) Ghent University, Ghent, Belgium ; (5) Hepato-gastroenterology, Ghent University Hospital, Ghent, Belgium ; (6) Center for Disease Analysis (CDA), Louisville, United States ; (7) University of Antwerp, Antwerpen, Belgium.

Introduction : Novel DAAs are expected to become the primary treatment for HCV in the Belgian system, once on the market (2016 – 2020). These therapies are expected to bring higher sustained viral response (SVR) and higher compliance due to shortened treatment duration and fewer side-effects.

Aim : Our aim was to evaluate different realistic and efficient strategies to control the projected increase in HCV-related disease burden, and examine the effect that delayed and timely access to DAAs has on disease burden.

Methods : Based on literature review, expert opinions, and historical assumptions, HCV-disease progression and mortality in Belgium was modeled to 2030. After validation of the base case with an expert panel, a strategy was developed to decrease HCV related mortality by 50% by 2030, assuming full access and uptake of novel treatment by 2018.

Results : Assuming no change in treatment practices, access to therapy, or therapy efficacy (710 patient treated annually, SVR rates of G1- 60%, G2-65%, G3/4- 40%) the total number of HCV infections is projected to decrease from 56,000 viremic cases in 2013 to 36,500 cases by 2030. Despite a decrease in total cases, the number of cases with cirrhosis is estimated to increase by 45%, decompensated cirrhosis by 50%, HCC by 85%, and liver related deaths by 75% by 2030 as the population ages.

Compared with the base case, a scenario that increases SVR to 85-90% and treats 1,990 F2-F4 cases annually beginning in 2018 is estimated to decrease liver related deaths by 54%, by 2030. The number of cases of decompensated cirrhosis and HCC are expected to decrease by 60%. By 2030, this strategy is projected to decrease the total number of viremic cases to 22,000, a 40% improvement from the base case. To obtain comparable outcomes with F0-F4 cases, 2,890 patients should be treated.

The same strategy was modeled with a two year delay (implementation in 2020). By 2030, the delay resulted in an 8% increase in the total number of HCV cases, and increased HCV-related mortality by 930 deaths (19%), as compared to the 2018 implementation. Delayed implementation also increased the number of cases of HCC and cirrhotic decompensation by 20%.

By accelerating the strategy by two years (implementation in 2016), an additional 7% decrease in the number of HCV cases was achieved by 2030 (1,580 fewer cases than with the original strategy). Additionally, 1,070 HCV related deaths were averted, and 40 cases of HCC were prevented.

Conclusions : HCV morbidity and mortality is forecasted to increase under current treatment practices, but the future availability of higher SVR DAAs provides an opportunity to mitigate the burden of disease. Timely access to these new therapies will significantly improve the outcomes of the HCV population in Belgium.

- A20 -

OBETICHOIC ACID, AN FXR AGONIST, REDUCES BACTERIAL TRANSLOCATION IN EXPERIMENTAL CHOLESTASIS. L. Verbeke (1), R. Farre (1), K. Covens (1), B. Verbinnen (1), T. Vanuytsel (1), I. Vander Elst (1), P. Windmolders (1), J. Verhaegen (1), M. Komuta (1), T. Roskams (1), J. Trebicka (2), F. Nevens (1), W. Laleman (1). (1) KU Leuven, Leuven, Belgium ; (2) University Hospital, Bonn, Germany.

Introduction : Bacterial translocation (BT) drives the pathogenesis and complications of cirrhosis. The farnesoid-X receptor (FXR) is considered a key transcription regulator involved in intestinal barrier function, bile and liver metabolism.

Aim : We studied potential intestinal FXR-dysfunction in a cholestatic animal model and evaluated the impact of obeticholic acid (INT-747), an FXR-agonist, on BT, gut permeability, inflammation and liver damage.

Methods : Rats were gavaged with INT-747 5 mg/kg or vehicle every 2 days during 10 days of bile-duct ligation (BDL ; n = 14+20) and assessed for changes in BT (by culture of ascites and mesenteric lymph nodes (MLN)), permeability (Ussing-chambers), tight-junction protein expression, intestinal immune cell recruitment & cytokine-expression (by FACS + RT-PCR in ileum, MLN and spleen) and liver injury. Healthy rats served as control (n = 17). Auxiliary in-vitro BT-mimicking experiments, using the CaCo2(human enterocyte-like)-cell line were performed in Transwells™.

Results : Vehicle-treated BDL-rats showed reduced ileal FXR pathway expression (P = 0.02 vs. healthy control) which was associated with increased BT, ileal permeability (through increased claudin-2-expression), recruitment of macrophages, natural killer and dendritic cells and interferon- γ (IFN- γ)-expression (P < 0.05 vs. control). Following INT-747-treatment, immune cell recruitment and IFN- γ expression were markedly reduced which was associated with normalized permeability (by up-regulated claudin-1, occludin and zonula-occludens-protein-1) (P < 0.05 vs. BDL+vehicle). Following INT-747-treatment plasma bilirubin decreased from 12.4 ± 0.4 to 8.4 ± 0.7 mg/dl in BDL-rats (P < 0.01). In vitro, IFN- γ induced increased permeability and E. coli translocation which remained unaffected with INT-747-preincubation.

Conclusions : In experimental cholestasis, FXR-agonism restores ileal permeability and decreases BT by attenuating intestinal inflammation, demonstrating a crucial protective role for FXR in the gut-liver axis.

- A21 -

ERCP IN THE MANAGEMENT OF PEDIATRIC BILO-PANCREATIC DISEASE, A SINGLE CENTER EXPERIENCE. I. Scheers, C. De Magnee, H. Piessevaux, P. Deprez, E. Sokal. Clin universitaires St-Luc, UCL, Brussels, Belgium.

Introduction : Endoscopic retrograde cholangiopancreatography (ERCP) is increasingly being used in the evaluation and management of bilo-pancreatic disorders in children.

Aim : The aim of this study was to review the records of pediatric patients who underwent ERCP procedures in the last year and report eventual complications.

Methods : Medical records of the eight patients followed in our tertiary referral center, who underwent ERCP for any indication during the last year were retrospectively analyzed. Indication for ERCP and serious adverse events including bleeding, perforation, pancreatitis or death were reported.

Results : Twelve ERCPs were performed on 8 patients. Patient age was 6.6 ± 4.6 years and the sex ratio was 1M/1F. The indications were : complications of pancreatic trauma (3 cases), choledochal cyst (1), pancreatic tumor (1), Wirsung duct lithiasis related to chronic pancreatitis (1) and biliary derivation (2). A therapeutic intervention was performed in 6 patients (62.5%), representing 10 ERCP procedures, and technical success was achieved in 7/10 ERCPs. Reason for therapeutic failure were biliary duct paucity (1 case) and impossibility to recanalize distal pancreatic duct after traumatic Wirsung disruption (2 cases). Two (2/8) patients presented a moderate adverse event as they developed uncomplicated acute pancreatitis after the intervention.

Conclusions : ERCP indications are rare in the pediatric population. However the technique is as efficacious as in adults with low rates of adverse events in the hands of experienced endoscopists working in high-volume centers.

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PERIPHERAL AND HEPATIC VEIN CYTOKINE LEVELS IN NON ALCOHOLIC FATTY LIVER DISEASE. L. Vonghia (1), T. Magrone (2), A. Verrijken (3), P. Pelckmans (1), P. Michielsen (1), L. Van Gaal (3), E. Jirillo (2), S. Francque (1). (1) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium ; (2) Department of Basic Medical Science, University of Bari, Bari, Italy ; (3) Department of Endocrinology, Metabolism and Clinical Nutrition, University Hospital Antwerp, Antwerpen, Belgium.

Introduction : Haemodynamic impairment, inflammatory mediators and glucose metabolism impairment have been implicated in the pathogenesis of Non Alcoholic Fatty Liver Disease (NAFLD).

Aim : To study the cytokine profile in patients with NAFLD/Non Alcoholic Steatohepatitis (NASH) in peripheral (P) and hepatic vein (HV) blood and to compare with histology, haemodynamic and metabolic parameters.

Methods : 40 NAFLD/NASH obese patients who underwent a transjugular liver biopsy were enrolled. Besides an extended liver and metabolic work-up, IL1B, IL4, IL6, IL10, IL17a, IL21, IL23, TNFalpha and INFgamma were measured in plasma obtained from P and HV blood by means of multiplex immunoassay. The Th1/Th2 (INFgamma/IL4), the M1/M2 [(TNFalpha+IL6+IL23)/IL10] and the IL10/IL17a ratios were calculated.

Results : A decrease of the P-IL10/IL17a-ratio and an increase of the P-M1/M2-ratio ($p < 0.05$) was observed in NASH versus NAFLD patients. A P-M1/M2-ratio increase was detected also in patients with portal hypertension in comparison with patients without it ($p < 0.05$). Moreover diabetic patients showed an increase of the P-Th1/Th2-ratio in comparison with non diabetic ones ($p < 0.05$). The P-M1/M2 ratio positively correlated with steatosis grade ($r : 0.39$, $p : 0.02$) and insulin ($r : 0.47$, $p : 0.003$). The HV-M1/M2 ratio positively correlated with fasting insulin and Hepatic Vein Pressure Gradient ($r : 0.47$, $p : 0.003$). IL6 correlated with the visceral fat amount ($r : 0.36$, $p : 0.02$). The P- and HV-IL10/IL17a ratios negatively correlated with fasting insulin (respectively $r : -0.4$, $p : 0.005$; $r : 0.4$, $p : 0.01$).

Conclusions : A proinflammatory cytokine state is associated with more disturbed metabolic, histological, and hemodynamic features in NAFLD obese patients. An increase of the M1/M2 ratio and a decrease of the IL10/IL17a ratio play a key role in this process.

- A23 -

THE ROLE OF HYPOXIA ON LIVER PROGENITOR CELL ACTIVATION IN HEPATOCELLULAR CARCINOMA. E. Bogaerts (1), A. Comhaire (1), A. Paridaens (1), Y.P. Vandewynckel (1), P. Carmeliet (2), A. Geerts (1), F. Heindryckx (3), H. Van Vlierberghe (1). (1) Ghent University, Ghent, Belgium ; (2) Vesalius Research Center VIB-KULeuven, Leuven, Belgium ; (3) Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

Introduction : Hepatocellular carcinoma (HCC) is the 5th most common cancer worldwide and is often detected in an advanced state. Current treatment options are mostly based on depriving the tumor from its oxygen and nutrient supply by decreasing angiogenesis, thus creating hypoxic conditions. This could lead to an alteration in the liver progenitor cell (LPC)-niche, creating a more aggressive tumor phenotype. Understanding more about the influence of oxygen deprivation on progenitor cells and their differentiation is of vast importance when using and improving the use of hypoxia inducing therapies. In a previous study, we have shown that weekly injections with diethylnitrosamine (DEN) in prolyl hydroxylase domain 2 haplodeficient (PHD2^{+/-}) mice -who have increased HIF stabilization (mimicking hypoxic signaling)- induces a mixed cholangiohepatocellular carcinoma, an LPC-derived tumor, while wild type (WT) mice develop only HCC.

Aim : Assess the effect of increased hypoxia inducible factor (HIF) stabilization on LPC activation and differentiation in a DEN-induced HCC mouse model, by using PHD2^{+/-}-mice.

Methods : HCC was induced in WT and PHD2^{+/-} mice by weekly DEN-injections and euthanized after 20, 25 and 30 weeks. RTqPCR analysis and Immunohistochemical staining (IHC) for LPC markers was performed.

Results : qPCR analysis revealed an increase in cytokeratin (CK) 7, CK19 and CD44 expression after 30w in all DEN-treated groups compared to control mice ($p < 0,05$). In PHD2+/- mice, CK7 and CK19 mRNA concentrations increased drastically compared to WT mice after 20 weeks ($p < 0,01$) but drop back to WT levels after 25 weeks. For CD44, no significant difference between PHD2+/- and WT groups could be marked. However, IHC for CD44 showed increased expression with longer induction and was higher in PHD2+/-

Conclusions : During DEN-induction, LPCs are possibly in a proliferating state, as seen by the increased levels of CK7 and CK19 and CD44 in WT and PHD+/- mice. Increased CK7, CK19 and CD44 expression in early PHD2+/- tumors demonstrates that activation of HIF-pathway probably influences LPC proliferation and differentiation mostly in earlier stages of primary liver tumors, pushing them towards a more stem cell like phenotype. This could contribute to the development of more aggressive tumors with worse prognosis and has repercussion on patients receiving long term anti-angiogenic treatment.

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NEONATAL CHOLESTASIS AND VITAMIN K DEFICIENT BLEEDING DIATHESIS AS A CLUE FOR CYSTIC FIBROSIS. L. Dossche, M. Van Winckel, S. Vande Velde, R. De Bruyne, S. Van Biervliet. Ghent University Hospital, Gent, Belgium.

Introduction : Neonatal cholestasis is caused by a broad spectrum of liver and bile duct diseases. Exclusion of biliary atresia by stool evaluation and liver biopsy is fairly urgent as early surgery reflects in a better outcome. However, a sweat chloride test can avoid unnecessary liver biopsies. We report 3 patients diagnosed in the past 2 years with cystic fibrosis (CF) related neonatal cholestasis and vitamin K deficient coagulation disorder.

Case Reports :

CASE 1 : A premature (gestation 33w) male neonate born, part of a dizygotic twin, presented with necrotising enterocolitis and perforation. Because of surgical complications, long term total parenteral nutrition (TPN) was indicated. At the age of 50 days he developed jaundice, first attributed to the long term TPN. Despite oral feeding, liver transaminases (SGOT 106, SGPT 79, GGT 246 IU/L) and cholestatic icterus (total bilirubin (TB) 9,8, direct bilirubin (DB) 8,4 mg/dl) deteriorated. A stepwise investigation of the cholestasis was performed including a sweat chloride test. This revealed CF which was later genetically confirmed.

CASE 2 : A dysmature (2.37 kg), term male neonate had persistent bleeding after a blood sampling at the age of 2.5 m. A discrete cholestasis (TB 1,86 (nl < 1,2), DB 0,76 mg/dL (nl < 0,2)), elevated liver enzymes (SGOT 71, SGPT 42, GGT 374 U/L) and vitamin K deficient bleeding disorder (PT < 5%, INR > 5) despite oral vitamin K supplements during the first month and a hydrolised MCT containing-formula feeding thereafter, was documented. The sweat test confirmed the diagnosis of CF.

CASE 3 : A 1-month old exclusively breastfed male neonate, was hospitalised for prolonged icterus and acholic stools. He received oral vitamin K supplements. Blood tests revealed cholestasis (TB 9,5, DB 7,77 mg/dl) vitamin K deficient coagulation disorder (PT 64%, INR 1,33) and normal liver enzymes. The liver biopsy showed hepatocellular iron storage. A sweat test confirmed the diagnosis of CF.

Discussion : CF as cause of neonatal cholestasis is rare (0.76%). There is however, a paucity in the literature on this topic. Many features of CF associated neonatal cholestasis such as acholic stools, vitamin K deficiency and hepatomegaly are misleading. If an assumed TPN related cholestasis doesn't respond to the usual therapy (oral feeding, reduced IV fat load,...), differential diagnosis should be expanded. As a sweat chloride test is non invasive and results are known within 24H, there should be no hesitation to perform it early in the diagnostic work-up. A confirmation of CF will avoid an unnecessary liver biopsy. Moreover, a liver biopsy in CF related cholestasis will only reveal non-specific histological findings.

Conclusion : Neonatal cholestasis can be the presenting sign of cystic fibrosis. Therefore it seems important to perform as soon as possible a sweat chloride test to avoid an unnecessary liver biopsy.

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Marc Hautekeete Lecture
Mitochondrial defects affecting gastrointestinal function.
Prof. Rudy Van Coster (UG)

POSTERS

- A26 -

THE TELAPREVIR EARLY ACCESS PROGRAM : EFFICACY RESULTS OF 1587 HCV GT1 PATIENTS WITH F3 OR F4. C. Moreno (1), P. Ferreira (2), M. Colombo (3), P. Urbanek (4), S. Strasser (5), I. Fernández (6), D. Abdurakhmanov (7), A. Streinu-Cercel (8), G. Gaeta (9), A. Verheyen (10), W. Iraqi (11), R. Demasi (12), A. Hill (13), J. Läufer (14), I. Lonjon-Domanec (11), H. Wedemeyer (15). (1) Hôpital Erasme, City of Brussels, Belgium ; (2) Outpatient Clinic to HIV and Viral Hepatitis Division of Infectious Disease, Federal University of São Paulo, Sao Paulo, Brazil ; (3) Department of Medicine, Division of Gastroenterology, Fondazione IRCCS Ca` Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy ; (4) Department of Internal Medicine, First Medical Faculty, Charles University, and Central Military Hospital Prague, Prague, Czech Republic ; (5) Royal Prince Alfred Hospital, University of Sydney, Sydney, Australia ; (6) Hospital Universitario 12 de Octubre, Sección de Aparato Digestivo, Madrid, Spain ; (7) E. M. Tareev Clinic for Nephrology, Internal and Occupational Medicine, Moscow, Russian Federation ; (8) University of Medicine Bucuresti and Institute for Infectious Disease IBI, Bucharest, Romania ; (9) Viral Hepatitis Unit, Department of Infectious Diseases, Second University, Naples, Italy ; (10) Janssen Pharmaceutica, Beerse, Belgium ; (11) Janssen Pharmaceuticals, Paris, France ; (12) Tibotec Inc., Titusville, United States ; (13) MetaVirology Ltd, London, Great Britain (UK) ; (14) Janssen-Cilag AG, Baar, Switzerland ; (15) Medizinische Hochschule Hannover, Hanover, Germany.

Introduction : HEP3002 is an ongoing, open-label, early access program of telaprevir in 16 countries, for patients with genotype 1 hepatitis C with severe fibrosis or compensated cirrhosis.

Methods : Patients were treated with telaprevir, pegylated interferon-alpha and ribavirin (PR) for 12 weeks, followed by PR. Liver biopsy or non-invasive tests showing severe fibrosis (Metavir F3 or Ishak 3-4) or cirrhosis (Metavir F4 or Ishak 5-6) and platelet count > 90,000/mm³ were required at entry. This interim (ITT) analysis included 16 week data from the first 1587 patients.

Results : Mean age was 53 years and mean weight 78kg ; 64% were male and 98% Caucasian, 66% had HCV RNA levels ≥ 800,000 IU/mL, 47%/53% had severe fibrosis/cirrhosis, 22% had genotype 1a, 20% were treatment naïve, 34% prior relapsers, 43% prior non-responders and 3% had prior viral breakthrough. HCV RNA responses (percent undetectable) at weeks 4 and 12 (ITT analysis) are shown below :

Percent HCV RNA not detected / Week	Week 4 (RVR)	Week 4+12 (eRVR)	Week 12
Naïve (n = 321)	65%	60%	85%
Relapser (n = 531)	70%	65%	88%
Partial responder (n = 203)	59%	53%	80%
Null responder (n = 436)	46%	41%	72%
Overall (n = 1587)	60%	55%	82%

Overall 40/1587 patients (2.5%) met the stopping rule for early discontinuation of TVR. In multivariate analysis, four baseline factors were associated with a higher chance of eRVR : baseline viral load < 800,000 IU/mL (OR = 1.47, 95% CI = 1.18-1.85), Genotype 1b (OR = 1.52, (95% CI = 1.16-1.96), AFP < 10 pg/mL (OR = 2.36, 95% CI = 1.82-3.23) and naïve, relapser or prior partial response versus prior null response (OR = 2.0, 95% CI = 1.56-2.5). The rates of eRVR were 20/50 (40%), 77/235 (33%), 262/500 (52%), 349/584 (60%) and 173/200 (79%) for patients with 0, 1, 2, 3 or 4 of these predictive factors, respectively. Follow-up results on SVR24 data will be presented at BGW in February 2014 based on the original posters presented at AASLD on November 2013.

Conclusions : In this telaprevir early access program for patients with severe fibrosis or compensated cirrhosis, 82% of patients had undetectable HCV RNA by week 12 (ITT), while 55% had eRVR. The strongest predictors of eRVR were baseline HCV RNA, sub-genotype, AFP and prior response.

THE TELAPREVIR EARLY ACCESS PROGRAM : RESULTS OF PATIENTS OVER 65 YEARS OLD WITH GT1 HCV WITH F3 / F4. C. Moreno (1), H. Wedemeyer (2), I. Fernández (3), P. Ferreira (4), S. Strasser (5), P. Urbanek (6), D. Abdurakhmanov (7), A. Streinu-Cercel (8), G. Gaeta (9), F. Beeldens (10), W. Iraqi (11), R. Demasi (12), A. Hill (13), J. Läubler (14), I. Lonjon-Domanec (11), M. Colombo (15). (1) Hôpital Erasme, City of Brussels, Belgium ; (2) Medizinische Hochschule Hannover, Hanover, Germany ; (3) Hospital Universitario 12 de Octubre, Sección de Aparato Digestivo, Madrid, Spain ; (4) Outpatient Clinic to HIV and Viral Hepatitis Division of Infectious Disease, Federal University of São Paulo, Sao Paulo, Brazil ; (5) Royal Prince Alfred Hospital, University of Sydney, Sydney, Australia ; (6) Department of Internal Medicine, First Medical Faculty, Charles University, and Central Military Hospital Prague, Prague, Czech Republic ; (7) E. M. Tareev Clinic for Nephrology, Internal and Occupational Medicine, Moscow, Russian Federation ; (8) University of Medicine Bucuresti and Institute for Infectious Disease IBI, Bucharest, Romania ; (9) Viral Hepatitis Unit, Department of Infectious Diseases, Second University, Naples, Italy ; (10) Janssen Pharmaceutica, Beerse, Belgium ; (11) Janssen Pharmaceuticals, Paris, France ; (12) Tibotec Inc., Titusville, United States ; (13) MetaVirology Ltd, London, Great Britain (UK) ; (14) Janssen-Cilag AG, Baar, Switzerland ; (15) Department of Medicine, Division of Gastroenterology, Fondazione IRCCS Ca` Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy.

Introduction : HEP3002 is an ongoing, open-label, early access program (EAP) of telaprevir in 16 countries, for patients with genotype 1 hepatitis C with severe fibrosis or compensated cirrhosis.

Methods : Patients were treated with telaprevir, pegylated interferon-alpha and ribavirin (PR) for 12 weeks, followed by PR. Liver biopsy or non-invasive tests showing severe fibrosis were required at entry. This interim (ITT) report describes the 16 week data for the first 128 patients enrolled on to the program who were over the age of 65.

Results : For patients > 65 years old, mean age was 68 years and mean weight 73kg ; 47% were Male and 99% Caucasian, 66% had HCV RNA levels \geq 800,000 IU/mL, 39%/61% had severe fibrosis/ cirrhosis, 11% had genotype 1a. Fifteen patients were aged 70 years or older (protocol violators), 20% were treatment naïve, 27% prior relapsers, 50% prior non-responders and 2% had prior viral breakthrough. Up to week 16, 35/128 patients (27%) discontinued TVR (29 (23%) for adverse events). 80% of patients developed grade 1-4 anemia (Hb < 11 g/dL or > 2.5 g/dL reduction), with 45% severe cases (Hb < 9 g/dL or > 4.5 g/dL reduction) ; 86 patients (67%) dose reduced ribavirin ; 11 (9%) discontinued treatment for anemia. In multivariate analysis of the whole trial, patients aged > 65 years were significantly more likely to develop anemia ($p < 0.001$). By week 16, 41% of patients developed grade 1-3 rash versus 32% in the overall trial population. There were 2% severe rash cases (all grade 3) and one Stevens-Johnson syndrome ; 4 patients (3%) discontinued treatment for rash. 31 patients (24%) developed a serious adverse event (SAE) versus 12% in the overall trial population. HCV RNA responses at weeks 4 and 12 (ITT analysis) are shown below for patients > 65 (n = 128) and the overall trial population (n = 1573) :

Percent HCV RNA undetectable	Week 4		Week 12	
	> 65 years N = 128	Overall N = 1573	> 65 years N = 128	Overall N = 1573
Naïve (n = 26)	65%	65%	85%	85%
Relapser (n = 35)	54%	70%	83%	88%
Partial responder (n = 15)	73%	59%	80%	80%
Null responder (n = 44)	43%	46%	77%	72%
Overall (n = 128)	55%	60%	81%	82%

* There were 8 patients (non-responders and viral breakthrough) not included in the main four categories.

Conclusions : In this telaprevir early access program for patients with severe fibrosis or compensated cirrhosis, 81% of patients over the age of 65 had undetectable HCV RNA by Week 12 (ITT), which was comparable to the overall trial population. Patients > 65 years had a significant increased risk of anemia on treatment, compared to the overall population.

TEMPORAL DYNAMICS AND THERAPEUTIC POTENTIAL OF THE UNFOLDED PROTEIN RESPONSE IN HCC. Y.P. Vandewynckel (1), D. Laukens (1), A. Geerts (1), I. Colle (2), E. Bogaerts (1), A. Paridaens (1), X. Verhelst (1), L. Devisscher (1), C. Vansteenkiste (1), B. Descamps (1), C. Vanhove (1), L. Libbrecht (1), B. Lambrecht (3), S. Janssens (3), H. Van Vlierberghe (2). (1) Ghent University, Gent, Belgium ; (2) Ghent University Hospital, Gent, Belgium ; (3) VIB, Gent, Belgium.

Introduction : Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality. The unfolded protein response (UPR) is involved in the consecutive steps of carcinogenesis. Little is known about the temporal alterations of the UPR during carcinogenesis. We examined the kinetics and effect of UPR modulation in HCC.

Methods : The UPR was evaluated in a diethylnitrosamine-induced mouse model. Mice were sacrificed every 5 weeks until week 30. Hematoxylin/eosin, Sirius red and reticulin staining and electron microscopy were performed. Expression analysis was performed in a cohort of human HCC. The effect of UPR modulation on cell viability was assessed in HepG2, Hepa1-6 and BWTG3 cells and in the mouse model.

Results : The expression of (co-)chaperones peaked during tumor initiation and their expression increased further, predominantly in the nodules. Regarding the Ire1 pathway, a peak in phospho-Ire1 levels and Xbp1 splicing, concomitant with its targets, was observed at week 10. The Perk pathway was activated in both surrounding and tumor tissue and Chop expression was elevated from week 5 and continued to rise, especially in the tumors. The Atf6 pathway fine-tunes the UPR during tumor progression. Electron microscopy revealed expansion and reorganization of the endoplasmic reticulum in HCC cells. In human HCC, etiology and differentiation were key determinants of UPR activation. PERK inhibition reduced cell viability *in vitro* under stressed conditions.

Conclusions : We provide the first evaluation of the temporal dynamics of the UPR in a long-term cancer model. Finally, these data suggest that PERK is a promising candidate target for HCC therapy.

ALT LEVELS ARE NOT USEFUL FOR IDENTIFYING PERSONS WITH INSULIN RESISTANCE. S. Feloni (1), O. Descamps (1), M. De Vos (1), B. De Vroey (1), H. Vandembulcke (1), J. Henrion (1), P. Deltenre (2). (1) Hôpital de Jolimont, Haine-Saint-Paul, Belgium ; (2) Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

Introduction : The range of ALT currently defined as “normal” may underestimate the prevalence of NAFLD. It is unsettled if lower cut-offs could better identify people with insulin resistance (IR), the main determinant of NAFLD.

Aim : To assess, in a general population sample, if ALT is correlated with IR and could provide information in addition to BMI for identifying IR.

Methods : IR was defined by HOMA-IR > 2.5. The performances of ALT and BMI for identifying IR were assessed by their AUROC curves.

Results : 289 non-diabetic participants were prospectively included. 100 (35%) had IR. Persons with IR had higher ALT than those without (23 vs. 17 U/L, $p = 0.001$). In multivariate analysis, BMI was the only factor associated with IR ($p = 0.005$) whereas age ($p = 0.07$), cholesterol ($p = 0.06$) and ALT ($p = 0.06$) did not reach statistical significance. The predictive accuracy of ALT for the diagnosis of IR was confirmed by its AUROC curve (0.615 ± 0.035 , $p = 0.001$) but the AUROC curve of ALT was lower than the one of BMI (0.751 ± 0.030 , $p < 0.001$, and $p < 0.001$ for the comparison with ALT). The cut-off of BMI with the best diagnostic performance for identifying IR was 29. ALT was independently associated with IR in persons with a BMI > 29 ($p = 0.05$) but not in those without. No cut-off value of ALT disclosed high sensitivity and high specificity, or high negative predictive value in a significant proportion of participants, even in those with a BMI > 29.

Conclusions : Although associated with IR, ALT did not bring additional information to BMI for identifying people with IR.

BELGIAN HCV CIRRHOSIS REGISTRY : PRELIMINARY RESULTS. H. Vandenbulcke. Ucl Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Knowledge of the interactions between alcohol and hepatitis C virus (HCV) is essential to patients' care. Alcohol abuse is associated with liver fibrosis and cirrhosis in HCV patients, which results in higher liver-related deaths. However, light alcohol intake may have some beneficial cardiovascular effects and has not been clearly associated with worsening of the liver disease.

Aim : To determine the effect of different amounts of alcohol consumption on survival and incidence of hepatocellular carcinoma (HCC) and liver failure.

Patients and methods : Detailed data on alcohol consumption of compensated HCV-related cirrhotic patients were prospectively collected. Survival and HCC-free survival were estimated by the Kaplan-Meier method.

Results : 257 patients (156 men, median age 60 years) were included. Median follow-up was 2.7 years (95% CI : 2.5-3). At inclusion, data on past alcohol consumption were available in 239 patients (109 abstainers, 68 who had discontinued alcohol before inclusion and 62 who were active consumers). Among past and active consumers at inclusion, 64 (49%) consumed < 30 g/day. During follow-up, data on alcohol consumption were available in 228 patients. Among these patients, 155 were abstainers, 57 consumed < 30 g/day and 16 consumed > 30 g/day. Overall, 177 patients received antiviral therapy before inclusion (22 were reported as sustained responders) and 66 during follow-up (11 were reported as sustained responders). Abstainers were as frequently treated as consumers, either before inclusion or during follow-up. At 4 years, there was no statistical difference between abstainers and patients who consumed alcohol before inclusion in survival ($93.8 \pm 3.0\%$ vs. $87.3 \pm 4.5\%$, $p = 0.5$) and HCC-free survival ($90.1 \pm 4.5\%$ vs. $84.7 \pm 3.8\%$, $p = 0.2$). There was no statistical difference between abstainers and patients who consumed alcohol during follow-up in survival ($88.1 \pm 4.0\%$ vs. $80.4 \pm 8.3\%$, $p = 0.9$) but abstainers had higher probability of HCC-free survival than consumers ($90.0 \pm 3.1\%$ vs. $72.0 \pm 8.9\%$, $p = 0.05$). This difference remained significant when abstainers were compared only to patients consuming < 30 g/day ($90.0 \pm 3.1\%$ vs. $70.7 \pm 9.8\%$, $p = 0.05$). At the end of follow-up, there were no differences between abstainers and patients who consumed < or > 30 g/day during follow-up in serum bilirubin (0.8 vs. 0.9 vs. 0.8, $p = 1.0$), INR (1.1 vs. 1.0 vs. 1.1, $p = 0.1$), albumin (3.9 vs. 4.0 vs. 3.8, $p = 0.6$), Child-Pugh (5 vs. 5 vs. 5, $p = 0.1$) and MELD scores (8.5 vs. 8.0 vs. 8.0, $p = 0.1$). Similar results were observed when considering alcohol consumption before inclusion.

Conclusion : Alcohol consumption is common in patients with compensated HCV-related cirrhosis. In this short-term interim analysis, alcohol consumption did not significantly impact survival or liver function tests. However, alcohol consumption < 30 g/day had already an impact on the incidence of HCC. Longer follow-up is required to determine if a lower cut-off of alcohol consumption may be considered as safe.

THE IMPACT OF BETA-BLOCKERS ON THE COURSE OF SEVERE ALCOHOLIC HEPATITIS. T. Sersté (1), E. Trepo (2), J. Schreiber (2), D. Degré (2), T. Gustot (2), C. Moreno (2). (1) ULB Saint-Pierre, Brussels, Belgium ; (2) Erasme University Hospital, Anderlecht, Belgium

Aims : Non selective Beta-blockers (NSBB) have been demonstrated to decrease survival in patients with cirrhosis and refractory ascites. This deleterious effect could be related to potentially poor tolerance of beta-blockers in cases of acute deterioration of liver function. We aimed to test the impact of NSBB on the outcome of severe Alcoholic Hepatitis (AH).

Patients and Methods : All cirrhotic patients admitted for severe (Maddrey score ≥ 32) biopsy-proven AH from July 2006 to July 2013 were retrospectively studied. Patients with positive HbS antigen, anti HCV antibody and/or porto-systemic shunt were excluded. Day 0 was defined as the day of liver biopsy. Patients were divided into 2 groups (with and without NSBB) and compared according to ascites, Maddrey score, MELD score (day 0 and day 28), Lille score, occurrence of hepatorenal syndrome (HRS), occurrence of infection (bacterial/fungal) and survival times.

Results : 109 patients were included. Eighty-nine (81.7%) received corticosteroids. Fifty-one patients (46.8%) had NSBB. Eighty-four patients (77.1%) had ascites. The median Maddrey score was 53 (32-200). Median MELD score was 25 (15-48) at day 0 and 20 (9-48) at day 28. Median Lille score was 0.36 (0.01-1.00). Thirty-nine patients (35.8%) developed HRS and 77 patients (70.6%) had an infection during 90 days of follow-up. The median survival time was 80 days (95% CI 22-137). Lille score ≤ 0.45 (HR : 8.17, 95%CI : 2.4-27.8, $p = 0.001$) was predictor of survival at day 28. When the 2 groups were compared, the presence of ascites, the Maddrey and MELD scores at day 0 and day 28 were not significantly different. Response to corticosteroids (22/51 = 43.1% with NSBB versus 21/58 = 63.2%, $p = 0.7$) and rate of HRS (20/51 = 39.2% with NSBB versus 19/58 = 32.8%, $p = 0.3$) were not different between groups. The rate of infection was similar between groups (36/51 = 70.6% with NSBB versus 41/58 = 70.7%, $p = 1.00$). The median survival time was not different between groups : 61 days with NSBB (95%CI : 25-96), 97 days (95%CI : 0-256) without NSBB ($p = 0.18$).

Conclusion : The use of NSBB has no impact on the outcome and survival of AH.

P311 MODULATES HEPATIC STELLATE CELLS MIGRATION. E.L. Guimarães, L. Stradiot, I. Mannaerts, B. Schroyen, L.A. Van Grunsven. Laboratory of Liver Cell Biology, VUB, Brussels, Belgium, Brussels, Belgium.

Introduction : Liver fibrosis is induced by the accumulation of extracellular matrix, deposited mainly by activated hepatic stellate cells (HSCs). One key characteristic of stellate cell activation is the directional migration to the site of injury during the wound-healing process.

Aim : P311 is a protein that has been shown to play a role in migration and we aimed to study a possible role for this protein during stellate cell migration.

Methods : Mouse stellate cells were isolated and cultured *in vitro* in order to investigate P311 protein and gene expression during HSC activation by immunocytochemistry and RT-qPCR, respectively. Expression of P311 during *in vivo* activation was evaluated in CCl₄ and bile duct ligation-induced liver fibrosis. Production of reactive oxygen species was determined using the fluorescent probe DCFH-DA. By siRNA-mediated knockdown of P311 we investigated a possible effect on proliferation by incorporation of EdU and on migration by Boyden chamber assays.

Results : P311 gene expression was increased during both *in vitro* and *in vivo* activation of HSCs. siRNA-mediated knockdown led to a decrease in reactive oxygen production and cell proliferation. Migration induced by different chemokines, such as PDGF-bb and MCP-1 was inhibited by knockdown of P311.

Conclusions : P311 is central to reactive oxygen species-mediated HSC migration induced by different chemokines.

EMT REGULATORS ZFHX1A AND ZFHX1B ARE ESSENTIAL FOR REGULATION OF E-CADHERIN IN HEPATIC STELLATE CELL. I. Mannaerts (1), N. Eysackers (1), K. Maes (2), L. Van Grunsven (1). (1) Vrije Universiteit Brussel, Jette, Belgium ; (2) Laboratory of Liver Cell Biology, VUB, Brussels, Belgium, Brussels, Belgium.

Introduction : Upon liver injury, hepatic stellate cells become activated myofibroblast-like cells. They lose their vitamin A storing capacity and become collagen producing cells with migratory capacities. This transdifferentiation has been compared to an epithelial to mesenchymal differentiation or EMT. EMT is a strictly orchestrated process, important regulators are E-box repressors including Snail family members and Zfhx1/ZEB proteins and a role for the miR-200 family has been established. Both transcriptional repressors and microRNAs regulating EMT are TGF β sensitive regulators.

Aim : In this study we tested the role of Zfhx1 proteins and a miR-200 family member during HSC activation and migration.

Methods & Results : In *in vitro* activated primary HSCs and in HSCs isolated from CCl₄ treated mice low levels of miR-200c and E-cadherin were detected. We observe that the down-regulation of E-cadherin is paralleled by an increase of Zfhx1a and Zfhx1b. An siRNA mediated silencing of these repressors induced a strong up regulation of E-cadherin and resulted in a mild decrease of HSC activation marker Acta2. In contrast, silencing of snail1 and 2 did not affect E-cadherin expression. Ectopic expression of miR-200c on the other hand induced E-cadherin mRNA and protein expression and reduced HSC migration in a transwell migration assay.

Conclusions : We show that Zfhx1/ZEB proteins regulated E-cadherin expression during HSC activation and that forced miR-200c expression is sufficient to partially hamper HSC activation and reduce their invasive phenotype.

LIVER STIFFNESS BY SHEAR-WAVE ELASTOGRAPHY IN ALCOHOLIC LIVER DISEASE. L. Vonghia (1), W. Verlinden (1), L. Cauwemberghs (2), A. Steinhauser (2), J. Van Dongen (2), V. Van Marck (3), P. Pelckmans (1), P. Michielsen (1), S. Francque (1). (1) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium ; (2) Department of Gastroenterology and Hepatology, University Hospital Antwerp, Antwerpen, Belgium ; (3) Department of Pathology, Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Liver stiffness (LS) measurement is a validated tool in the non invasive assessment of liver fibrosis in several chronic liver diseases including alcoholic liver disease (ALD). Cut-off values may differ according to the aetiology of the disease and the technique used. Real-time shear wave elastography (SWE) is an emerging ultrasound guided technique that allows a real time visualization of liver elastography that needs validation.

Aim : To study the correlation between liver stiffness measured by SWE and liver histology in patients affected by ALD.

Methods : Patients affected by ALD who were scheduled for a liver biopsy after clinical evaluation were consecutively enrolled. Twentytwo patients met the inclusion criteria and underwent abdominal ultrasound, real-time SWE using Airexplorer (Supersonic Shear Imagine S.A., Aix-en-Provence, France) and liver biopsy at one single time point. Liver histology was assessed using the NASH CRN scoring system.

Results : The patients enrolled (mean age 52 ± 11 years, mean BMI 24.7 ± 5 Kg/m²) presented a diagnosis of cirrhosis in 45.5% of the cases. The mean alcohol intake in the study population was 73 ± 88 g/day ; 27% of the patients were abstinent at the moment of the liver assessment. LS measures increased concomitantly with the severity of fibrosis (Friedman test $p < 0.0001$). LS was significantly higher in the F4 group versus F0-F3 (Mann-Whitney test $p : 0.018$). There was a positive correlation between LS and Child-Pugh and MELD scores (respectively : $r : 0.63, p : 0.001$ and $r : 0.54, p : 0.09$, Spearman test). The analyses of receiver operating characteristics (ROC) curves for F0-F3 versus F4 showed an area under the ROC of 0.8 (95% CI : 0.6-0.9). The cut off value of 13.1 kPa showed a sensitivity of 0.9 and a specificity of 0.6, a NPV of 0.8 and a PPV of 0.7. The intraobserver agreement was 0.9 (95%CI : 0.86-0.96).

Conclusions : SWE is a useful tool in the assessment of liver fibrosis in patients with ALD. A cut-off of 13.1 kPa has a high NPV and hence can reliably be used to exclude cirrhosis.

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HEPATITIS B INFECTION IN PREGNANT WOMEN A STUDY OF ITS CLINICAL PROFILE IN A TERTIARY CARE HOSPITAL. A. Krishnan, J. Venkataraman. Stanley Medical College, Chennai, India.

Introduction : Viral hepatitis during pregnancy is associated with high risk of maternal complications and has become a leading cause of foetal death. The main transmission route of the hepatitis B virus (HBV) is mother to child transmission and contributes significantly to chronic HBV infection if untreated. The controversies and possible hazards during pregnancy is also an issue of concern.

Aim : To determine the prevalence of hepatitis B infections among pregnant women.

Methods : This was a hospital based cross-sectional study that pregnant women who attended the liver clinic of the hospital during the periods of June 2011 – May 2012. Clinical examination was followed by screening for liver disorders by liver function tests that included. Serum Bilirubin, Alkaline Phosphatase, Alanine Transaminase, Aspartate Transaminase. Serum HBsAg was tested in all pregnant women. HBVDNA and other serum HBV markers including hepatitis B e antigen (HBeAg), hepatitis B core antibody (anti-HBc) and hepatitis B surface antibody (anti-HBs) were tested among HBsAg positive pregnant women.

Results : Total patients : 35.22 were detected in 1st trimester, 13 were 2nd trimester, and 10 were 3rd trimester. 26 patients were primigravida, 9 multigravida 4 patients known HBsAg positive in first pregnancy. One patients was diagnosed with acute hepatitis B in the 12th week of pregnancy, was managed conservatively and seroconverted in the 36 the week. The average value in the 35 patients was 17.5. in 24 patients, HBV DNA was less than 20 IU/ mL. Among the remaining 11, two HBeAg positive patients had viral loads of 2, 23,200 IU/ mL and 88,000 IU/mL. They advised lamivudine in the last trimester of pregnancy. Post partum follow-up : 6 of 5 patients-FTND ; 1 patient- emergency LSCS average birth weight was 2.65 kgs, 1 LBW. No documented gestational diabetes or hypertension. All neonates vaccinated with HBsAg vaccine and two neonates were given HBIG.

Conclusion : Antenatal HBV infection is generally begin course, with no significant maternal or fetal events.

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NATURAL HISTORY OF IDIOPATHIC NON-CIRRHOTIC PORTAL HYPERTENSION : A PROSPECTIVE LONG TERM FOLLOW-UP. A. Krishnan, J. Venkataraman. Stanley Medical College, Chennai, India.

Introduction : Idiopathic portal hypertension(IPH) is characterized by a long-standing non-cirrhotic portal hypertension(NCPH) because of the intrahepatic block of small portal vein branches. NCPH is due to various causes that generally are extrahepatic, involving the prehepatic or the post hepatic circulation. NCPH includes Extra Hepatic Portal Vein Obstruction (EHPVO) and Non-Cirrhotic Portal Fibrosis (NCPF). The natural history of NCPH is not clear.

Aim : To determine prospectively the changes in the portal venous system in patients with NCPH.

Method : Patients with a diagnosis of NCPF and EHPVO registered since 2001 were serially followed at an yearly interval for changes in liver size, its echotexture, and in the intra and extrahepatic portal venous system. Baseline demographic details, LFT, and co-morbid illness including virological profile were noted. Patients with co-morbid illness and those with known etiology of cirrhosis were excluded from the study.

Results : There were 34 patients with NCPF (M: F 1:1.8) and 30 patients with EHPVO (M : F ratio 1.6:1). The mean age was 24.9 yrs and 41.2 yrs respectively. During follow up, 20 out of 34 and 16 out of 30 patients with NCPF and EHPVO

respectively had no progression of disease. 14 patients with NCPF progressed to cirrhosis over a mean period of 5.21 years. Eight patients developed ascites and required diuretics. 14 patients with EHPVO progressed to NCPF over the mean period of 8.6 years, 12 patients further progressed to cirrhosis over a mean period of 5.1 years. Overall 40% of patients with EHPVO progressed to cirrhosis over a mean period of 13.7 years.

Conclusion : NCPH is a spectrum wherein EHPVO progresses to NCPF and further to cirrhosis over a period of 13.7 years at least in a proportion of patients.

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SIMULTANEOUS LIVER AND THORACIC TRANSPLANTATION : INDICATIONS AND RESULTS AT A SINGLE-CENTER. L.J. Ceulemans (1), S. Strypstein (1), D. Monbaliu (1), J. Vanhaecke (2), B. Meyns (3), G. Verleden (4), D. Van Raemdonck (5), F. Nevens (6), J. Pirenne (7). (1) Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium ; (2) Cardiology, University Hospitals Leuven, Belgium, Leuven, Belgium ; (3) Cardiac Surgery, University Hospitals Leuven, Leuven, Belgium ; (4) Pneumology, University Hospitals Leuven, Leuven, Belgium ; (5) Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium ; (6) Hepatology, University Hospitals Leuven, Leuven, Belgium ; (7) Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium.

Introduction : Combined liver/thoracic transplantation (cLiThTx) is a theoretically life-saving transplant procedure for patients suffering from complex diseases involving liver, heart or/and lung. cLiThTx are complex and lengthy procedures performed in sick patients who are subsequently at higher risk for postTx morbidity and mortality. To avoid futile use of multiple scarce life-saving organs in single recipients, indications and results of cLiThTx are to be analyzed with scrutiny.

Aim : To report indications/results of cLiThTx at one center.

Methods : All cLiThTx performed at a single-center were reviewed. The following prospectively collected (*ad hoc* database) parameters were studied: recipient/donor characteristics, indications, immunosuppression, early/late (< 3mo/> 3mo post-Tx) acute rejection, chronic rejection, patient/graft survival.

Results : Between 4/2000 and 10/2013, 9 patients (1,2% of isolated LiTx-activity) received cLiThTx : 5 liver-lungTx (cLiLuTx), 3 liver-heartTx (cLiHTx), 1 liver-lung-heartTx (cLiLuHTx). In another patient who was listed for cLiLuHTx, LiTx was eventually not performed due to difficult LuHTx. He succumbed 2 months postTx. Mean age was 38 y (20-62). Male/female ratio was 4/5. Organs were retrieved from the same donor with a mean age of 37 y (27-53). Donor death was due to : intracranial bleeding (n = 5) and trauma (n = 4). **Liver** indications were : cystic fibrosis (CF)-induced cirrhosis (3), familial amyloidosis (2), cardiac cirrhosis (1), HCV cirrhosis (1), tuberculostatics-induced (Isoniazid) liver failure (1), epitheloid hemangio-epithelioma (1). Mean MELD was 15 (6-44). **Pulmonary** indications included : CF (3), COPD GOLD IV (1), epitheloid hemangio-epithelioma (1). **Cardiac** indications were : familial amyloidosis (2), restrictive cardiomyopathy (1). Indication for **pulmonary+cardiac** replacement was portopulmonary hypertension (1).

Heart and/or lungs were transplanted first, followed by the liver, except in one case of acute liver failure in a LuTx candidate in whom the liver was replaced first, to correct the poor coagulation and allow sequential LuTx. All organs were preserved by static cold storage, except in the latter case where the lungs were normothermally oxygenated/perfused *ex vivo* (OCS lung device, *Transmedics*). All patients received tacrolimus-based immunosuppression with MMF (n = 8) or azathioprine (n = 1) and low dose steroids (n = 9). Anti-thymoglobulin induction was administered in the 3 cLiHTx. 1 liver (cLiLuTx) was lost to hepatic artery thrombosis and successfully retransplanted 3 months postTx. 1 patient (cLiHTx) died to acute myocardial infarction 4 months postTx. Overall recipient survival is 89% with a mean follow-up of 5 years and 8 months (3mo-13.5y). At last follow-up, 2 early mild acute liver rejections, 3 mild late pulmonary rejections and no acute heart rejection have occurred. No chronic liver or heart rejection has been diagnosed. The cLiLuHTx recipient developed bronchiolitis obliterans stade 2 but is clinically well 13 years and 6 months postTx.

Conclusions : cLiThTx can provide excellent long-term results in selected recipients with complex dual liver and heart/lung disease and this with a low risk of immune graft loss.

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ASSESSMENT OF RISK FACTORS AND OUTCOME PREDICTORS ASSOCIATED WITH INHOSPITAL MORTALITY IN CIRRHOTICS. A. Krishnan, S. K, J. Venkataraman. Stanley Medical College, Chennai, India.

Introduction : Cirrhosis is a major global health problem. The scoring system has been extended to prognosticate for patients with complications of cirrhosis. However CTP score and MELD are associated with many limitations not used in assessing prognosis during hospitalization. Assessment of prognosis during hospitalization mortality can have important role in triaging for level of care.

Aim : was to find out the causes of hospital mortality in patients admitted with decompensated cirrhosis and to evaluate for the biochemical and hematological parameters that are related to mortality during hospitalization.

Material and Methods : Cirrhotic patients admitted between April 2010 and May 2011 were studied. Patients with decompensated cirrhosis liver who died during admission were selected as cases and patients who improved with treatment followed by discharge were selected as controls. Data collected included demographics ; etiology ; indication for hospital admission ; presence or absence of decompensation and portal hypertension ; the corresponding Child-Pugh, MELD and MELD-Na scores. Other hematological and biochemical markers were studied. The clinical diagnosis of cirrhosis was made by a history of portal hypertension, LFT, radiology. Exclusion criteria were patients with portal hypertension not due to primary cirrhosis, liver cirrhosis complicated by HCC were excluded.

Results : Total number of cases was 140 (70 each for cases and controls). The Mean age was 46.33 years The mean duration of disease in cases was 20.01 and 12.76 months for controls. The most common cause was ethanol. The most common cause of admission was hepatic encephalopathy. 11.4% of patients in both groups had Child's A. 48.6% had Child's B while 52.9% of controls. 40.0% and 35.7% controls had Child's C cirrhosis. The mean MELD and MELD-Na was significantly (< 0.001) higher for the cases group compared to the control group. The most common causes of death are due to cirrhosis related complications associated with decompensation like hepatic encephalopathy, hepatorenal syndrome, UGI bleeding and infections. On univariate analysis revealed that increasing levels of MELD, MELD-Na, serum creatinine, INR, WBC, albumin, neutrophilia and duration of disease were significantly (< 0.0001) associated with increased risk of death. On multivariate forward stepwise logistic regression, an elevated WBC count ($p = 0.02$, OR 1.2) and creatinine ($p = 0.003$, OR 1.2) were the only factors significantly associated with death.

Conclusion : Once patients are admitted with hepatic decompensation, clinical parameter like duration of disease, hematological parameters like leukocyte count, neutrophilia, biochemical parameters like creatinine, albumin, SGPT and INR can help predict short term or in hospital mortality along with MELD and MELD sodium. Child score did not help in predicting short term mortality in hospitalized patients.

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SIMEPREVIR EFFICACY/SAFETY IN DIFFICULT-TO-CURE HCV GT 1-INFECTED PT GROUPS : PROMISE PHASE 3 TRIAL. X. Forns (1), E. Lawitz (2), S. Zeuzem (3), E. Gane (4), J.P. Bronowicki (5), P. Andreone (6), A. Horban (7), A. Brown (8), M. Peeters (9), O. Lenz (9), S. Ouwkerk-Mahadevan (10), J. Scott (11), R. Kalmeijer (12), G. De La Rosa (12), R. Sinha (9), M. Beumont-Mauviel (10). (1) Liver Unit, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain ; (2) Texas Liver Institute, University of Texas Health Science Center, San Antonio, United States ; (3) J.W. Goethe University Hospital, Frankfurt, Germany ; (4) Auckland Hospital Clinical Studies Unit, Auckland, New Zealand (Aotearoa) ; (5) INSERM U954, Université de Lorraine, Centre Hospitalier Universitaire de Nancy, Vandœuvre-lès-Nancy, France ; (6) Dipartimento di Scienze Mediche e Chirurgiche, University of Bologna, Bologna, Italy ; (7) Medical University of Warsaw, Warsaw, Poland ; (8) Imperial College Healthcare NHS Trust, London, Great Britain (UK) ; (9) Janssen Infectious Diseases BVBA, Beerse, Belgium ; (10) Janssen Research & Development, Beerse, Belgium ; (11) Janssen Global Services LLC, High Wycombe, Great Britain (UK) ; (12) Janssen Global Services, LLC, Raritan, United States.

Introduction : The double-blind, multicentre, Phase III PROMISE study evaluated simeprevir (SMV), a once-daily (QD), hepatitis C virus (HCV) NS3/4A protease inhibitor, plus peginterferon alpha-2a/ribavirin (PR) in HCV genotype 1 patients with prior relapse. This analysis focuses on safety and efficacy of SMV/PR in difficult-to-cure patient subgroups (*IL28B* TT, METAVIR F4, and HCV genotype 1a, including those with baseline Q80K polymorphism).

Methods : Patients who had received IFN-based therapy for ≥ 24 weeks and relapsed ≤ 1 year thereafter were randomised 2 : 1 to SMV 150 mg QD (12 weeks) plus PR (response guided 24/48 weeks) or to placebo (PBO, 12 weeks) plus PR (48 weeks). The primary efficacy endpoint was sustained virological response at Week 12 (SVR12).

Results : The ITT population comprised 393 patients. SVR12 was significantly higher with SMV/PR vs PBO/PR overall (79.2% vs 36.8% ; $p < 0.001$). SVR12 was 88.7%, 85.9% and 82.0% in patients with *IL28B* CC genotype, HCV genotype 1b and METAVIR F0-F2, respectively, receiving SMV (all $p < 0.001$ vs PBO). SVR12 was also statistically significantly higher with SMV/PR vs PBO/PR in difficult-to-cure *IL28B* TT genotype (64.5% vs 18.8%), METAVIR F4 (74.4% vs 26.3%) and genotype 1a (70.3% vs 27.8%) patients, and higher in genotype 1a patients with baseline Q80K (46.7% vs 30.0% ; PBO genotype 1a/other). Rapid virological response (RVR) rates were also higher with SMV/PR versus PBO/PR, both overall (77.2% vs. 3.1% ; $p < 0.001$) ; and in patients with *IL28B* TT genotype (90.3% vs 0%), METAVIR F4 (76.9% vs 0%), and genotype 1a (68.2% vs 3.9%), including those with baseline Q80K (44.8% vs 5.9%). Of those patients who achieved RVR in the SMV/PR arm, 86.5% achieved SVR12. RVR was predictive of high SVR12 in the overall population as well as in difficult-to-cure subgroups. The incidence and profile of AEs was generally similar between the SMV and PBO treatment groups. Bilirubin increases with SMV/PR were mild, transient, and without concomitant increases in alanine aminotransferase/aspartate aminotransferase (ALT/AST) and the incidence and severity (mostly low grade) of rash and anaemia AEs were similar for SMV/PR and PBO/PR. Patient-reported fatigue and impairment in work and daily activities confirmed the safety and efficacy of SMV.

Conclusions : SMV conferred clinical benefit and was well tolerated across different patient sub-populations with prior relapse on IFN, including patients with *IL28B* TT and METAVIR score F4. SVR12 was higher in all subgroups treated with SMV/PR.

This study was supported by Janssen Research & Development, Beerse, Belgium.

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SUCCESSFUL LIVER TRANSPLANTATION IN HYPERORNITHINEMIA-HYPERAMMONEMIA-HOMOCITRULLINURIA SYNDROME. P. De Bruyne, S. Van Biervliet, M. Van Winckel, S. Vande Velde, P. Verloo, R. De Bruyne. Ghent University Hospital, Gent, Belgium.

Introduction : Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is a rare urea cycle defect, caused by a deficient ornithine transporter of the mitochondrial membrane (SLC25A15). Liver transplantation has been used as treatment for other urea cycle disorders, but has not yet been reported in HHH syndrome. We report the successful outcome eight years after liver transplantation in a 15 year old boy with HHH.

Case report : Our patient presented at four weeks with a hyperammonemic coma (one week after switching to a more energy and protein dense infant formula). Serum amino acid profile suggested HHH syndrome ; which was proven by sequencing of the *SLC25A15* gene. Two mutations were found : p.R275Q and a novel mutation p.A76D. Although immediate treatment with sodium benzoate, sodium phenylacetate and L-arginine resulted in a normalization of plasma ammonia levels within 24 hours, he developed cerebral oedema, coma, convulsions and subsequent neurological sequelae.

Since the age of two, his metabolic disease became more difficult to control with persistent mild hyperammonemia and recurrent metabolic decompensations. Despite dietary protein restriction, continuous feeding by gastrostomy and continuous supplementation with sodium benzoate and L-arginine, frequent hospitalisations were needed. Furthermore, there was a severe psychomotor delay with right sided spasticity, stereotypic behaviour, drooling, aggressivity, disturbed sleep and severe discomfort.

Despite this substantial developmental delay, he was transferred to our centre for liver transplantation which was considered to maintain improved metabolic control and avoid recurrent hospitalisation. Transplantation with a split liver graft of a cadaveric donor at the age of seven resulted in complete normalization of his plasma ammonia and serum amino acid levels whilst protein intake was normalized. His medication was reduced to oral tacrolimus only. Following the transplantation, the excellent metabolic control improved his general condition, mood and behaviour ; and further developmental achievements have been observed.

Conclusion : Liver transplantation corrects biochemical defects in a boy with HHH.

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SOX4 AND SOX9 CONTROL BILE DUCT DEVELOPMENT. A. Poncy. Université Catholique de Louvain, Brussels, Belgium.

Introduction : Abnormal development of intrahepatic bile ducts can lead to perinatal cholestasis and potentially to cirrhosis. Therefore, characterizing mechanisms of bile duct development is relevant to understand pathophysiology of biliary malformations and of bile duct regeneration. The transcription factor Sox9 controls the timing of bile duct morphogenesis (Antoniou *et al.*, 2009) : in the absence of Sox9 bile duct development is delayed, eventually leading to normal biliary function.

Aim : This transient phenotype prompted us to consider potential redundancy with other Sox factors in biliary development.

Methods : We investigated Sox factor expression in developing biliary cells and identified Sox4 as a possible candidate for controlling biliary development. We then investigated the effect of combined or single targeted disruption of Sox4 and Sox9 specifically in hepatoblasts on liver development using AlfpCre, Sox4^{loxpl/loxp} and Sox9^{loxpl/loxp} mice.

Results : Liver-specific inactivation of Sox4, like liver-specific depletion of Sox9, leads to delayed biliary differentiation. However, Sox4 depletion induces persistent defects in cell polarity and cell morphology. Combined inactivation of Sox4 and Sox9 in the liver is associated with strong defects in biliary morphogenesis : cells lining the ducts permanently co-express hepatoblast/hepatocyte and biliary markers, cell morphology is affected and apico-basal polarity is not established.

Conclusion : We conclude that Sox4 and Sox9 are key factors in bile duct development. Sox9 is transiently required for establishing differentiation, morphogenesis and polarity, while Sox4 is permanently required for apico-basal polarity. We will provide evidence that Sox4 and Sox9 are effectors/modulators of signaling pathways regulating biliary morphogenesis.

SEVEN YEARS OF TREATMENT WITH TENOFOVIR DF FOR CHRONIC HEPATITIS B VIRUS INFECTION. P. Marcellin (1), E. Gane (2), N. Tsai (3), R. Flisiak (4), J. Petersen (5), S. Gurel (6), I. Kotzev (7), J. Flaherty (8), P. Dinh (9), A. Gaggar (9), K. Kitrinis (8), M. Subramanian (9), J. Mchutchison (8), J. GEORGE (10), M. BUTI (11), J. Piessevaux (12). (1) Hôpital Beaujon, Clichy, France ; (2) Auckland City Hospital, Auckland, New Zealand (Aotearoa) ; (3) University of Hawaii, Honolulu, United States ; (4) Medical University of Bialystok, Bialystok, Poland ; (5) Asklepios Klinik St. George, University of Hamburg, Hamburg, Germany ; (6) Uludag Universitesi Tip Fakultesi, Bursa, Turkey ; (7) University Hospital Sveta Marina, Varna, Bulgaria ; (8) Gilead Sciences, Foster City, United States ; (9) Gilead Sciences, Foster City, United States ; (10) Westmead Hospital, Sydney, Australia ; (11) Department of Hepatology, Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain ; (12) Gilead Sciences, City of Brussels, Belgium.

Introduction : We previously reported that 5 years of tenofovir DF (TDF) therapy in treatment naïve patients results in sustained viral suppression with no development of resistance and was associated with either the halting or regression of fibrosis in 96%, and reversal of cirrhosis in 74% of previously cirrhotic patients.

Aim : Here we present 7 year results from these two ongoing 8 year studies (Studies 102 and 103).

Methods : After 48 weeks of double-blind comparison of TDF to adefovir dipivoxil, all patients undergoing liver biopsy were eligible to continue open-label TDF. Patients were assessed every 3 months for safety and efficacy with annual resistance surveillance. Annual assessments of bone mineral density (BMD) by DXA were added to both studies starting at year 4.

Results : In a total 641 patients who were initially randomized and treated, 585 (93%) entered the TDF extension phase at Year 1, and at Year 7, 437 (68%) remain on study. Efficacy results at Year 7 are shown in the table. Overall (both studies combined), TDF was well tolerated over the 7 year evaluation period. Less than 2.5% of patients discontinued TDF due to an adverse event, and ≤ 1.7% experienced a confirmed renal event (≥ 0.5 mg/dL increase in serum creatinine from baseline, or phosphorus < 2 mg/dL, or CrCL < 50 mL/min). BMD assessments (lumbar spine and hip T scores) were stable over 3 years of evaluation. No resistance to TDF has been detected through Year 7.

	HBeAg- Patients (Study 102) N = 375	HBeAg+ Patients (Study 103) N = 266
HBV DNA < 400 copies/mL (mITT) ^a	77% (269/348)	60% (149/247)
HBV DNA < 400 copies/mL ^b	99% (271/273)	99% (159/160)
ALT Normalization ^b	84% (213/255)	74% (115/155)
HBeAg loss ^b	-	54% (84/154)
HBeAg seroconversion ^b	-	40% (61/154)
HBsAg loss (KM%) ^c	- ^d	12% (n=26)
HBsAg seroconversion (KM%) ^c	- ^d	10% (n=21)

^aMissing-failure (LTE-TDF analysis set) ;^bMissing = excluded (On treatment analysis set) ; ^c Kaplan-Meier % ; ^dOne patients experienced HBsAg loss and seroconversion at Week 240.

Conclusions : In these two trials, TDF remains safe and effective over a 7 year treatment period with no detectable resistance to TDF ; a relatively low rate of renal events and no evidence of clinically relevant bone loss were also observed.

MITIGATING THE COST BURDEN OF HEPATITIS C VIRUS (HCV) IN BELGIUM. W. Laleman (1), C. Moreno (2), P. Starkel (3), P. Van Damme (4), H. Van Vlierberghe (5), S. Hindman (6), H. Razavi (6), D. Vandijck (7). (1) University Hospitals Leuven, Leuven, Belgium ; (2) Erasme Hospital, Brussels, Belgium ; (3) Clin universitaires St-Luc, UCL, Brussels, Belgium ; (4) University of Antwerp, Antwerpen, Belgium ; (5) Ghent University Hospital, Gent, Belgium ; (6) Center for Disease Analysis (CDA), Louisville, United States ; (7) Ghent University, Gent, Belgium.

Introduction & aim : Chronic HCV-infection heralds a significant clinical and economic burden. Long-term disease progression, inefficacious therapies, and an aging population contribute to increasing cost burden on the Belgian

healthcare system. However, as novel direct acting antiviral agents (DAAs) become widely available with higher sustained viral response (SVR) rate, better compliance, and fewer side-effects, they bring the opportunity to decrease the disease and cost burden of HCV.

Methods : Based on literature review, expert opinions, and historical assumptions, HCV-disease progression and cost burden for Belgium were modeled towards 2030. Historical healthcare costs associated with HCV related disease sequelae were modeled without considering costs of antiviral treatment and inflation. Monte Carlo simulation and bootstrap analysis were performed using Oracle Crystal Ball to generate 95% confidence intervals. Potential scenarios aimed to reduce the cost burden of HCV were modeled.

Results : The total number of HCV infections peaked in 2003 but the number of cases with (decompensated) cirrhosis, and HCC will continue to increase until 2028. This increase in advanced stage disease is found to be associated with increasing healthcare costs, which are expected to peak in 2024 at €83M (95% CI : € 25M-137M). By 2025, an estimated 30% of HCV-related healthcare costs will be attributable to advanced liver disease.

A strategy aimed to control HCV-disease burden by 2030 was developed. By increasing SVR to 85-90% beginning in 2018 and treating 1,990 F2-F4 cases annually as compared with 710 currently, an estimated € 314M in total healthcare cost savings could be achieved by 2030. A two-year delay in access to higher SVR treatment, will decrease potential cost savings by €70 M, while a two-year acceleration of access will allow for an additional €81 M in healthcare cost savings.

Conclusions : Reducing the burden of advanced stage disease sequelae of HCV will not only positively impact HCV-related morbidity, but will also significantly reduce the HCV cost burden on the Belgian healthcare system. Cost figures presented are conservative and do not include the current or projected future cost of treatment. This and future analysis will further assist with the development of rational strategies to minimize the HCV-related disease burden in Belgium.

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ACTIVE GENUINE HBV REPLICATION IN HUMAN LIVER CHIMERIC UPA+/+NOG MICE. M. Van De Garde, P. Biesta, K. De Groot-Kreefft, A. Boonstra, T. Vanwollegem. Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : Although new compounds to treat HCV infections come to the clinic in a rapid pace, there remains an unmet need to perform viral hepatitis pathogenesis studies for which animal models are essential. We have previously shown that both the human hepatitis B and C virus can replicate in uPA-SCID mice after reconstituting the murine liver with transplanted human hepatocytes. However, these experiments are hampered by high colony maintenance costs, as only uPA-heterozygous animals are fertile, while transplantation is exclusively successful in uPA+/+ mice. In addition, half of these fragile animals die during the 1st 6 weeks after transplantation, leading to low overall transplantation and infection efficacies.

Aim : To reduce costs associated with this valuable animal model, we aimed to set up a new mouse colony with lower liver-specific uPA-transgene levels on a profound immune deficient Nod-SCID-IL2R γ ^{-/-} (NOG) background.

Methods : uPA-NOG mice were rederived from cryopreserved embryo's. The mouse colony was expanded by both uPA-homozygous and -heterozygous matings. Pups were genotyped with an end-product multiplex PCR for Apolipoprotein A1 and human growth hormone and a subsequent copy number qPCR specific for the mouse uPA gene. Six to 9 week old uPA^{+/+}NOG mice were transplanted with upto 1 million cryopreserved human hepatocytes via intra-splenic injections. Human Albumin (hAlb) levels in serum were measured 5 to 8 weeks post transplantation (pTx) via ELISA. Well repopulated chimeric mice were infected intraperitoneally with 7 log IU HBV genotype A, 8 weeks pTx . HBV DNA levels were measured in mouse serum using the COBAS TaqMan HBV Test. HBV-specific immunohistochemistry for both the HBcAg and HBsAg was performed on formalin fixed livers 12 weeks after inoculation.

Results : Mixed uPA^{+/+} male and uPA^{+/-} female matings yielded a mean of 6 to 7 pups per nest. Genotyping of neonates revealed a pure Mendelian inheritance. Transplantation of human hepatocytes resulted in excellent survival rates of 97,9% of acceptor mice within 6 weeks pTx and no spontaneous deaths thereafter. The fraction of mouse liver occupied by human hepatocytes as shown with HE staining, correlated with hAlb levels in mouse plasma. HBV was inoculated in 4 mice with hAlb levels ranging from 580-1120 μ g/ml. HBV DNA levels increased in mouse serum from 4 weeks post infection to a maximum of 7 log IU at 12 weeks post infection, indicating active viral replication. HBV viremia at sacrifice correlated with HBsAg and HBcAg detection in human clusters . No changes were observed in hAlb levels during active HBV replication.

Conclusions : Based upon a higher breeding efficacy, higher survival rate after hepatocyte transplantation and subsequent susceptibility to HBV, uPA-NOG mice are a more cost-efficient model to perform translational in vivo studies for viral hepatitis.

OUTCOME OF COMBINED HEPATO- AND CHOLANGIOCELLULAR (cHCC-CCC) TUMORS AFTER LIVER TRANSPLANTATION (LT). J. Navez, C. Sempoux, Q. Lai, P. Goffette, J. Lerut. Clin universitaires St-Luc, UCL, Brussels, Belgium.

Introduction : LT is an established treatment for hepatocellular cancer. The value of LT in the treatment of cHCC-CCC is not yet well defined.

Aim : The aim of this study was to compare outcome of HCC and cHCC-CCC after LT.

Methods : During the period January 2005 and December 2012, 135 cirrhotic patients were transplanted for HCC. Examination of the total hepatectomy specimen revealed a mixed tumor in 11 pats. Recurrence and survival rates of HCC and cHCC-CCC groups were studied after a median follow-up of 34 (range 0-96) and 12 (range 2-18) months respectively. The possible role of adjuvant locoregional treatment in the tumor dedifferentiation was especially looked for.

Results : One-year overall survival rates for HCC and cHCC-CCC pats were respectively 86 and 64% (p 0,009). Five (45%) cHCC-CCC patients had tumor recurrence after a median of 7 months (range 2-17). This incidence markedly differed from the HCC pat group in which the rate of recurrence, diagnosed after a median of 14 months (range 6-24), only reached 8% (10/124 pats) (p < 0,001). Ten (90%) cHCC-CCC patients received pre-LT transarterial chemoembolization (TACE) vs. 88 (71%) of HCC patients (p 0,169).

Conclusions : Outcome of cHCC-CCC following LT is poor. The possible role of pre-LT cancer treatment in the occurrence of combined liver tumors needs to be further investigated.

ALCOHOL WITHDRAWAL. R. Henneberry, J. Freer, A. Rochford. Newham University Hospital, London, Great Britain (UK).

Background : In England, 33% of men and 16% of women consume alcohol in a 'harmful' way. This presents huge disease, public health, and financial burdens.

Aim : To audit the immediate management and subsequent follow up of acute alcohol withdrawal in patients presenting to the Emergency Department at Newham University Hospital, London, against local guidelines.

Methods : We reviewed the case notes of 32 patients presenting with alcohol withdrawal, either as a primary or non-primary diagnosis. We audited : 1) the prescription of chlordiazepoxide, Pabrinex, and oral B vitamins 2) the appropriateness of admission against local policy 3) documentation of alcohol use at admission, and 4) referral to community services.

Results : 94% of patients presenting in acute alcohol withdrawal were admitted ; 94% were admitted appropriately according to guidelines. Pabrinex was prescribed correctly in 7/30 = 23% of patients. Vitamin B Co-strong and thiamine were prescribed correctly in 4/26 = 15%. Chlordiazepoxide was prescribed correctly to 28% of patients.

Conclusions : The results show a poor adherence to the policy. Although the vast majority of our sample had alcohol use recorded, in the most part there was no quantity recorded and no risk stratification through CAGE or AUDIT-C questionnaires. We therefore recommend changes to the admission proforma and more detailed written information to be available to clinicians.

AN UNUSUAL CAUSE OF FAILURE TO THRIVE AND DIARRHEA IN INFANCY. R. Uwera, F. Haerynck, V. Bordon, S. Van Biervliet, S. Vande Velde, M. Van Winckel, R. De Bruyne. Ghent University Hospital, Gent, Belgium.

A 9 month old boy with a previous history of recurrent infections, failure to thrive and chronic diarrhea was referred because of high fever and suspicion of meningitis. Clinical examination revealed a sick, irritable child with hepatosplenomegaly without lymphadenopathy. The initial blood test revealed leucopenia, thrombocytopenia, coagulopathy with low fibrinogen, elevated transaminases, moderately elevated ferritin and mildly elevated triglycerides. The boy was admitted in the intensive care unit because of inappropriate ADH secretion and systemic inflammatory response syndrome with increasing respiratory distress and progressively worsening ascites. No haemophagocytosis was seen in the bone marrow cytology. Further diagnostic work-up initially revealed normal natural killer (NK)-cell activity, normal perforin activity but a high soluble CD25 (7404-à 24200 pg/ml). Brain MRI showed demyelination of the white matter. Treatment for Hemophagocytic Lymphohistiocytosis (HLH) was started following HLH-2004 protocol, consisting of

dexamethasone, cyclosporine and etoposide. In the further course, NK-cell activity was repeated and found to be slightly abnormal. To differentiate primary from secondary HLH, further genetic testing was performed with mutation analysis of STXBP2 in view of the antecedents of failure to thrive and intractable diarrhea. Two heterozygous mutations have been identified : 902+5G > A and c.421 del G (the latter mutation has not yet been described.) The patient underwent hematopoietic stem cell transplantation (HSCT) with good response, however he continued to have diarrhea requiring parenteral nutrition.

HLH is characterized by an uncontrolled, ineffective proliferation and activation of T lymphocytes, NK cells and macrophages infiltrating multiples organs (including liver, spleen, lymph nodes, central nerve system) with increased production of inflammatory cytokines . According to the HLH-2004 protocol, 5 of the 8 following diagnostic criteria need to be fulfilled : fever, splenomegaly, bicytopenia, high triglycerides/low fibrinogen, hemophagocytosis, high ferritin, low NK-cell activity and high soluble CD 25. Primary or familial HLH (FLH) is a rare autosomal recessive disorder presenting in infancy in 70-80% of the patients. The only curative treatment is HSCT.

Mutation in STXBP2 is causes FLH type 5 (FLH-5). The encoded protein, (Munc/Syntaxin) is involved in intracellular trafficking, control of SNARE (soluble NSF attachment protein receptor) complex assembly, and the release of cytotoxic granules by natural killer cells. FLH-5 often presents with early onset chronic diarrhea and failure to thrive. The pathophysiology of diarrhea is not clearly understood. Severe diarrhea mostly persists even after transplantation. Hearing defects, hypogammaglobulinemia and bleeding tendency can also be associated with FLH-5.

Conclusion : FLH type 5 should be considered in the differential diagnosis of a child presenting with HLH in a context of chronic diarrhea and failure to thrive. In these cases, partial conservation of NK cell function can be observed.

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SUCCESSFUL TREATMENT OF TYPE II ACHALASIA USING PERORAL ENDOSCOPIC MYOTOMY (POEM) IN A CHILD. I. Scheers, C. Wanty, X. Stephenne, H. Piessevaux, E. Sokal. Clin universitaires St-Luc, UCL, Brussels, Belgium.

Introduction : Allgrove or 4A syndrome is a very rare autosomal recessive disease mainly characterized by achalasia, alacrimia, ACTH insensitivity and autonomic nervous system disturbance. In these patients achalasia can be managed by successive pneumatic dilatations or Heller myotomy. Since recently, per oral endoscopic myotomy (POEM) has been proposed as a novel, minimal invasive, modality for the treatment of achalasia. This intervention, first developed in Japan by Inoue *et al.* (Endoscopy 2010), has since be increasingly performed. Meanwhile, pediatric cases who underwent the POEM procedure remains scarce. Described early complications include oesophageal perforation with pneumomediastinum and infections ; in the long-term, reflux disease can occur.

Case Report : We report here the case of a fourteen years old girl who developed dysphagia and odynophagia. She was diagnosed with Allgrove syndrome at the age of seven as she developed signs of adrenocortical insufficiency and neurological disturbance. An esophagus-stomach-duodenum contrast X-ray confirmed esophageal dysmotility. Subsequently, a manometry was performed, which was compatible with type II achalasia. Per oral endoscopic myotomy (POEM) was proposed as a treatment intending a permanent cure of esophageal achalasia. The technique was performed under general anesthesia according to the description of Inoue *et al.*

Results : The procedure was uneventful with no immediate complications recorded. Cefazolin antibioprophyllaxis was administrated for 48h. POEM quickly and significantly reduced signs of dysphagia in the patient, who recovered a normal eating pattern within the first week following the surgery. One year after the intervention, the patient is symptom free and does not present GERD.

Conclusions : POEM represents an interesting alternative approach to pneumatic dilatation and Heller myotomy for the treatment of achalasia also in children. The short and long-term outcomes of the procedure are excellent.

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GASTRIC EMPTYING AND GASTRO-OESOPHAGEAL REFLUX IN CHILDREN WITH CYSTIC FIBROSIS. B. Hauser (1), K. Keymolen (1), I. De Schutter (2), E. De Wachter (1), A. Malfroot (1), E. De Greef (1), T. Devreker (1), G. Veereman (1), Y. Vandenplas (1). (1) UZ Brussel, Jette, Belgium ; (2) UZ Brussel, Jette, Belgium.

Introduction : According to the literature gastric emptying (GE) can be normal, decreased or increased in children with cystic fibrosis (CF).

Aim : We studied GE in children with CF with symptoms suggestive for gastro-oesophageal reflux (GOR) (group 1) and children with CF without chronic gastro-intestinal symptoms (group 2).

Methods : Group 1 : 24 children, 13 boys, age 5.8 ± 4.2 (0.5-17.1) years. Group 2 : 22 children, 14 boys, age 8.8 ± 2.8 (5-14) years. Impedance-pH monitoring for detection of GOR (Sleuth, Sandhill Scientific Inc, Highlands Ranch, CO, USA) was performed in group 1. Acid reflux parameters were regarded as increased if the total oesophageal acid exposure was above the 95 th percentile of normal data obtained in healthy subjects (Vandenplas, 1991). ^{13}C -octanoic acid breath test to measure GE of solids (pancake) using Non Dispersive Infrared Spectrometry (Wagner Analysen Technik, Bremen, Germany) was performed in both groups. GE was considered delayed if the gastric half emptying time was above the 95 th percentile of normal data obtained in healthy subjects (Hauser, unpublished data).

Results : Group 1 : 11/24 children (45.8%) had increased acid GOR ; 7/24 children (29.2%) had delayed GE ; 3 patients had increased GOR and delayed GE (12.5%), 8 patients had increased GOR and normal GE (33.3%), 4 patients had normal GOR and delayed GE (16.7%), and 9 patients had normal GOR and normal GE (37.5%) ; delayed GE was present in 27.3% of children with increased acid GOR and in 30.8% of children with normal acid GOR. Group 2 : 2/22 children (9.1%) had delayed GE.

Conclusions : Increased acid GOR is present in about half of children with CF and symptoms suggestive of GOR. Delayed GE is documented in about 30% of these children with or without increased acid GOR. Delayed GE is only present in about 10% of children with CF without chronic gastrointestinal symptoms.

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PROPRANOLOL TO PREVENT BLEEDING IN CHILDREN WITH PORTAL VEIN THROMBOSIS. R. Hijazeen (1), A. Ghanma (2), R. Al-Dajah (2), B. Hauser (3), Y. Vandenplas (3). (1) Royal Medical Services, Amman, Jordan ; (2) Royal Medical Services, Amman, Jordan ; (3) UZ Brussel, Jette, Belgium.

Introduction : Prophylaxis with propranolol to prevent variceal bleeding is routinely performed in adults but not in children.

Aim : We performed a retrospective analysis on propranolol effectiveness in the prevention of variceal bleeding in children with portal vein thrombosis over a period of 15 years (1999-2013).

Methods : The population consisted of 28 patients with a portal vein thrombosis shown by Doppler ultrasound and CT angiogram . Upper endoscopy was performed in addition to full detailed medical history and thorough clinical evaluation. Laboratory tests were also performed including complete blood count, liver function tests, albumin, prothrombin time, INR, and thrombophilia screening (Methylene Tetra Hydro Folate Reductase MTHFR, Factor V Leiden, protein C and protein S). Propranolol was started at a dose of 1 mg/kg/day after measuring the basal heart rate during sleep the night before. The heart rate during sleep was then checked after 2 doses of propranolol. If there was no decrease of 25% of the heart rate during sleep, the dose of propranolol was increased with 0.5 mg/kg/day until the targeted heart rate was reached.

Results : Age range was 6 months to 14 years with a mean of 7 years and 3 months. Male/female ratio was 1.3 to 1. The main presenting signs were splenomegaly in 89% and upper gastrointestinal bleeding in 68% of the patients. The most common hematologic abnormality was decreased platelets in 50% of the patients. Seven % had elevated liver enzymes, 21% had increased prothrombin time and all patients had normal albumin. MTHFR gene mutation was found in 60% of the patients. Finally, 17 patients out of 24 (71%) of those on secondary prophylactic propranolol developed re-bleeding.

Conclusions : We conclude that propranolol is not effective in prophylaxis for secondary bleeding, and upper gastrointestinal bleeding is the most presenting symptom in children with portal vein thrombosis.

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EFFECT OF TAUROURSODEOXYCHOLIC ACID ON ENDOPLASMIC RETICULUM STRESS IN CHOLESTATIC LIVER DISEASE. A. Paridaens (1), E. Bogaerts (1), Y.P. Vandewynckel (2), L. Thoen (3), H. Van Vlierberghe (4), L. Van Grunsven (3), A. Geerts (4), I. Colle (1). (1) Ghent University, Gent, Belgium ; (2) Department of Hepatology and Gastroenterology, Universiteit Gent, Ghent, Belgium ; (3) Free University (VUB), Brussels, Belgium ; (4) Ghent University Hospital, Gent, Belgium.

Introduction : Ursodeoxycholic acid (UCDA) has been used for many years in the treatment of cholestatic liver disease. The taurine-conjugated form of UDCA (TUDCA) is a chemical chaperone known to reduce endoplasmic reticulum (ER) stress and unfolded protein response (UPR) signalling.

Aim : In this study, we investigated the effects of TUDCA on ER stress and degree of fibrosis in secondary hepatic fibrosis.

Methods : Liver fibrosis was induced by common bile duct ligation (CBDL) in male Sv129 mice. TUDCA or saline was administered intraperitoneally (500mg/kg) in sham and CBDL mice every other day from 14 days until 6 weeks after

surgery, after which the mice were euthanized. The UPR was assessed through RT-qPCR of different UPR markers and histology was performed to determine the degree of fibrosis.

Results : The CBDL+TUDCA group (n = 10), had significantly higher liver and significantly lower spleen weights (p < 0.001) compared to the CBDL control group (n = 8). Markers from the PERK, IRE1 and ATF6 pathway were up-regulated in the CBDL group in comparison to the sham groups. The CBDL+TUDCA group showed significantly lower XBP1s mRNA levels than the CBDL control group, while CHOP was upregulated in the CBDL+TUDCA group (p < 0.05). ATF4, GADD34 and Pdia3 were elevated, although not significantly, in the CBDL+TUDCA group compared to the CBDL control group. Interestingly, Herpud1, Derlin3 and Pdia4, three markers of the ATF6 pathway were upregulated in the sham+TUDCA group but not in the CBDL groups. Fibrosis was more diffuse but not reduced in the CBDL+TUDCA group.

Conclusions : Except for the IRE1 pathway, we did not observe a decrease in UPR markers after TUDCA treatment. In contrast, we show a clear trend to an upregulation of the ATF4 and ATF6 pathways, as also seen by significantly increased CHOP mRNA expression. Possibly, this TUDCA mediated activation of the UPR could account for the lack of expected decrease in fibrosis.

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SIMEPREVIR WITH PEGINTERFERON/RIBAVIRIN FOR TREATMENT OF CHRONIC HCV GENOTYPE 4 INFECTION IN TREATMENT-NAÏVE OR -EXPERIENCED PATIENTS : INTERIM RESULTS OF A PHASE III TRIAL. C. Moreno (1), C. Hezode (2), P. Marcellin (3), S. Bourgeois (4), S. Francque (5), D. Samuel (6), F. Zoulim (7), J.-D. Grange (8), W. Jessner (9), O. Lenz (9), S. Ouwerkerk-Mahadevan (10), M. Peeters (9), M. Beumont-Mauviel (9). (1) ULB Hôpital Erasme, Brussels, Belgium ; (2) Hôpital Henri Mondor, Créteil, France ; (3) Hôpital Beaujon, Clichy, France ; (4) ZNA Campus Stuivenberg, Antwerp, Belgium ; (5) UZ Antwerpen, Antwerp, Belgium ; (6) Hôpital Paul-Brousse, Villejuif, France ; (7) Hôpital de la Croix Rousse, Lyon, France ; (8) Hôpital Tenon, Paris, France ; (9) Janssen Infectious Diseases BVBA, Beerse, Belgium ; (10) Janssen Research & Development, Beerse, Belgium.

Objectives : Simeprevir (SMV) is a potent, once-daily investigational, HCV NS3/4A protease inhibitor. In Phase III studies, SMV 150 mg once daily (12 weeks) plus peginterferon/ribavirin (PR) significantly improved sustained virologic response (SVR) rate allowing shorter 24-week overall treatment duration versus PR alone in HCV genotype 1-infected patients. We present interim results (cut-off 09/16/2013) from a Phase III, multicenter, uncontrolled, open-label study evaluating SMV/PR in treatment-naïve/-experienced HCV genotype 4-infected patients (RESTORE ; NCT01567735).

Methods : Treatment-naïve patients and prior relapsers received SMV 150 mg QD with PR (12 weeks), plus PR (12/36 weeks) by response-guided therapy (RGT) criteria. Prior non-responders received SMV 150 mg once daily with PR (12 weeks) plus PR (36 weeks). Primary efficacy endpoint : SVR 12 weeks after planned end of treatment (EOT, SVR12).

Results : 107 patients received study treatment (78.5% male, median age 49 years, Black 28.0% ; METAVIR F4 28.8% ; treatment-naïve n = 35, relapsers, n = 22 ; partial responders, n = 10 ; null-responders, n = 40). 57 patients were still ongoing and 48 had completed the study (2 discontinuations not due to AEs). 52/61 (85.2%) patients achieved SVR12 (treatment-naïve, 87.5% ; relapsers, 90.5% ; partial responders, 33.3% ; null responders, 80.0%). SVR12 was achieved in 66.7% and 83.0% of patients with METAVIR F4 and *IL28b* CT/TT, respectively. 69/104 (66.3%) patients achieved RVR (treatment-naïve, 80.0% ; relapsers, 90.0% ; partial responders, 40.0% ; null responders, 48.7%). Of 57 treatment-naïve/relapser patients, 51 (89.5%) met RGT criteria and were eligible to complete PR at Week 24. Of these, 47 (92.2%) achieved SVR12. 25/84 (29.8%) patients experienced on-treatment failure (treatment-naïve, n = 4 ; relapsers, n = 2 ; partial responders, n = 2 ; null responders, n = 17). 0/29 (0%) treatment-naïve and 1/20 (5.0%) relapser patients with undetectable HCV RNA at EOT experienced viral relapse I suggest to simply provide the number of relapse and not the relapse among those with undetectable HCV RNA at more than 1 follow-up measurement. This sentence is quite confused . Adverse events (AEs, SMV/PR phase) were mainly grade 1/2 ; serious AEs were infrequent (5 patients [4.7%], none at least possibly SMV-related) ; no fatal AEs occurred. AEs of clinical interest were pruritus/rash (20.6%/13.1%), anemia (9.3%), neutropenia (4.7%) and hyperbilirubinemia (1.9%).

Conclusion : SMV 150 mg once daily for 12 weeks with PR was well tolerated and showed promising efficacy in treatment-naïve/-experienced HCV genotype 4-infected patients, consistent with observations in Phase III trials in HCV genotype 1-infected patients.

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- B01 -

CHEMOSENSORY SIGNALLING PATHWAYS INVOLVED IN THE EFFECTS OF AMINO ACIDS ON GHRELIN RELEASE. L. Vancleef, T. Van Den Broeck, J. Tack, I. Depoortere. Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium.

Introduction : A high-protein diet has beneficial effects on both weight loss and weight maintenance. Sensing of amino acids, as breakdown products of proteins, by specific receptors on enteroendocrine cells.

Results : in the release of anorexigenic peptide hormones such as peptide YY and glucagon-like peptide-1. However, there is still no consensus on the effect of protein-enriched meals on ghrelin release, an octanoylated 28 amino acid peptide produced in the stomach, which stimulates food intake. The sensing of amino acids in the gut involves different taste receptors : calcium sensing receptor (CaSR), G-protein coupled receptor C6A (GPRC6A), taste receptor 1 member 1 - taste receptor 1 member 3 (TAS1R1-TAS1R3).

Aim : To investigate the physiological role of amino acids in the regulation of octanoyl ghrelin secretion and to elucidate which amino acid taste receptors and intracellular signalling pathways are involved.

Methods : The effect of amino acid taste receptor antagonists on amino acid-induced octanoyl ghrelin secretion was studied by radioimmunoassay in the ghrelinoma cell line, MGN3-1. Changes in intracellular Ca²⁺ release were studied in cells loaded with, Fluo-4. *in vivo*, C57BL/6 mice were either gavaged with 8% peptone or 100mM L-Phe or injected intravenously with 100mM L-Phe. Plasma ghrelin levels and stomach ghrelin content were determined after 40 minutes.

Results : The ghrelinoma cell line expresses all 3 known amino acid taste receptors : CaSR, TAS1R1-TAS1R3 and GPRC6A. Stimulating ghrelinoma cells with 10 mM L-Phe, 40 mM MSG, 10 mM L-Ala or 3% peptone significantly increased octanoyl ghrelin secretion with $51 \pm 0,1\%$, $134 \pm 4\%$, $65 \pm 0,1\%$ and $209 \pm 59\%$, respectively. The effect of L-Phe and L-Ala was blocked by the CaSR antagonist, Calhex-231. Peptone-induced ghrelin release was decreased ($P < 0.01$) After preincubation with a GPRC6A antagonist, Calindol. Co-administration of a TAS1R1-TASR3 enhancer, iosine 5'-monophosphate (IMP, 2.5mM), significantly enhanced msg-induced ghrelin secretion. stimulation of MGN3-1 cells with 40mM MSG or 10mm l-ala, but not with L-Phe, significantly increased the intracellular Ca²⁺ release. L-Ala- and MSG-induced ghrelin release was blocked after preincubation of cells with a L-type calcium channel antagonist, nifedipine, and a PLC_{beta} antagonist, U-73122 but not with the SERCA pump antagonist, thapsigargin. *In vivo*, intra-gastric administration of 8% peptone decreased plasma octanoyl ghrelin levels and stomach ghrelin content ($p < 0,01$). Intravenous but not intragastric administration of L-Phe decreased stomach ghrelin content with 54%. Ghrelin mRNA expression levels were increased in the stomach after intravenous injection of L-Phe ($p < 0.05$).

Conclusions : The release of ghrelin in response to several amino acids is finely tuned by different amino acid taste receptors coupled to different signalling pathways. the sensing of L-Phe by the ghrelin cell *in vivo* is polarized and occurs via the bloodstream.

- B02 -

SEVENTH HEAVEN ? - SINGLE TRACK 2PE MICROSCOPY FOR SIMULTANEOUS IMAGING OF MULTIPLE FLUOROPHORES. J.M. Vanderwinden, P. Hague. ULB Faculty of Medicine, Anderlecht, Belgium.

Introduction : Two-photon excitation (2PE) confocal microscopy is a method of choice for imaging «deep» into tissues. Immunofluorescence has only limited depth of penetration, while smaller molecules, like DNA stains, may reach deeper into fixed tissues after mild permeation. Conversely, endogenous signals, namely autofluorescence (AF) and second harmonic generation (SHG), and, in transgenic animals, fluorescent proteins (FPs), are intrinsically present throughout the tissues. Confocal imaging of large volumes is time-consuming, hence.

Methods : to expedite data collection are in demand.

Aim : Here, we took advantage of the broad 2PE cross-excitation properties, tunable 2PE lasers and sensitive spectral detectors to develop a robust single excitation wavelength 2PE method for collecting simultaneously multiple fluorescent signals.

Materials and Methods : KitCreERT2 (Klein S *et al.* Nat Commun 2013) and keratin 14 (K14)-CreER(tam) (Vasioukhin V. *et al.* PNAS 1999) mice were interbred with multicolor Cre-reporter R26R-Confetti mice (Snippert H.J. *et al.* Cell 2010), then fed orally with tamoxifen (tam) in corn oil. A few weeks later, ear skin and gut were harvest, fixed overnight in 4% paraformaldehyde and stored in PBS/azide. Wholemounts were observed in PBS on an inverted Zeiss LSM780N-LO confocal microscope equipped with a Coherent 2PE tunable laser. Several classical DNA stains (DAPI, PI, Topro-3, 7-AAD) and various Triton-X permeation protocols were tested.

Results : As predicted in our models, mutually exclusive expression of one of four FPs (namely CFP, GFP, YFP, RFP) was readily detected in individual epidermal cells (< K14-Cre) or ICCs in the gut (< KitCre) in tam-treated Confetti

mice, while no FP was detected in untreated mice. 2PE excitation fingerprinting revealed that all four FPs could be excited simultaneously around Ex. 900nm. Using a single tract configuration with single excitation wavelength and spectral detection (lambda mode), six endogenous signals, namely the four FPs, AF and SHG could be readily individualized by linear unmixing. In addition, 7-Aminoactinomycin D (7-AAD), a DNA stain commonly used in flow cytometry, proved to be compatible with the configuration used.

Conclusion : In our models, single wavelength 2PE excitation and emission spectral unmixing allowed simultaneous imaging of up to seven distinct signals (4 FPs, SHG, AF, 7-AAD) in a single track. Compared to multitrack configurations, this configuration leads to a drastic reduction in acquisition time and also abrogates the issue of chromatic aberrations occurring with different excitation wavelengths. Prospective applications of the method in the GI tract will be discussed.

- B03 -

THE ADULT ZEBRAFISH AS ANIMAL MODEL TO STUDY INFLAMMATORY BOWEL DISEASE. L. Uyttebroek (1), C. Pype (2), I.T. Shepherd (3), J.P. Timmermans (4), L. Van Nassauw (1). (1) Laboratory of Human Anatomy and Embryology, Faculty of Medicine & Health Sciences, University of Antwerp, Antwerpen, Belgium ; (2) Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Antwerpen, Belgium ; (3) Emory University, Atlanta, United States ; (4) Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerpen, Belgium.

Introduction : Inflammatory bowel disease (IBD) is a gastrointestinal disease with a high impact on modern society. At present, therapeutic management of IBD is problematic because of the incomplete understanding of disease pathogenesis. Development of new drugs in IBD requires experiments in animal models. The zebrafish (*Danio rerio*) has emerged as a powerful model organism of human diseases, mainly due to the high genetic and organ homology to humans. Some studies on chemically-induced colitis were already performed on zebrafish larvae using immersion exposure. The purpose of this study was to establish a protocol for chemically-induced colitis in adult zebrafish mimicking the acute phase of inflammation in IBD.

Materials and Methods : We used TNBS (trinitrobenzene sulphonate), a chemical compound commonly used in mammals to induce colitis, that was intraluminally administered in the distal intestine of anesthetized adult zebrafish. We made a histological study of the time course of the intestinal inflammatory response (3 h to 7 days post-induction) to establish the optimal TNBS concentration and the acute phase of inflammation. Using immunofluorescence and whole mounts, the effect of inflammation on cholinergic, nitregeric and serotonergic neurons was evaluated.

Results : Based on the thickening of the intestinal wall, the disruption of epithelial folds, a reduction of the number of goblet cells and the infiltration of eosinophils, the histological analysis indicated an optimal TNBS concentration (320 mM in 25% ethanol) inducing a non-lethal inflammation reaching its peak at 6 h post-induction. The inflammatory response returned to baseline values at 3 days post-induction. At the acute phase of inflammation, it was observed in the intestinal wall, that inflammation had no influence on the distribution and the proportion of nitregeric neurons, while the proportion of cholinergic neurons was significantly reduced in the distal intestine. The proportion of serotonergic neurons significantly increased in the entire intestine during inflammation.

Conclusion : This study described a method of chemically-induced colitis in the distal intestine of the adult zebrafish. At the acute phase, inflammation was accompanied by an imbalance in neuronal content comparable to neuronal changes observed in humans and other animal models. It is concluded that the zebrafish is a valuable animal model to study IBD.

- B04 -

DENDRITIC CELLS AND NEURO-IMMUNE INTERACTIONS IN THE MOUSE ILEUM : VIP AS A PRIME CANDIDATE MEDIATOR. K. Alpaerts (1), R. Buckinx (1), N. Cools (2), Z. Berneman (2), L. Van Nassauw (3), J.P. Timmermans (1). (1) Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium ; (2) Laboratory of Experimental Hematology, Vaccine and Infectious Disease Institute, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium ; (3) Laboratory of Human Anatomy and Embryology, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium.

Introduction : The gastrointestinal tract is continuously exposed to innocuous as well as potentially pathogenic antigens and must therefore maintain a state of delicate immune homeostasis. The intestinal dendritic cell (DC) is a key regulator in this immune response. Two main DC subtypes are found in the lamina propria (LP) of the mouse ileum, *i.e.*, CD11c⁺CX3CR1⁺ LP-DCs and CD11c⁺CD103⁺ LP-DCs. The CD103⁺ subtype is able to migrate to the mesenteric lymph nodes (MLNs) and induce Foxp3⁺ regulatory T cells or interferon- γ -producing T cells. The CX3CR1⁺ subtype was regarded as residential, but was recently proven to be partly migratory. They continuously sample the gut lumen as well as

the circulation which places them in a unique position in immune surveillance. *In vitro* studies on bone marrow-derived DCs have shown that DC functions can be affected by substances released by enteric neurons ; however, this has not yet been demonstrated for the LP-DC subtypes *in situ*.

Aim : To study which LP-DC subtypes are affected in *schistosomiasis*-inflamed mouse ileum compared to healthy ileum and to assess their interaction with intestinal innervation.

Methods : LP-DC subsets were quantified in the ileum and MLNs using multi-parametric flow cytometry (FACS) and immunohistochemistry (IHC). Using FACS and the CX3CR1^{+/GFP} C57BL/6 mouse strain, the intestinal CX3CR1⁺ cell fraction was sorted to investigate neurotransmitter receptor expression by PCR.

Results : We were able to determine both LP-DC subtypes in the mouse ileum using a combined FACS-IHC strategy, hereby demonstrating the complementarity of both techniques. Using FACS, we showed that the CX3CR1⁺ population is approximately five times larger than the CD103⁺ population. In the MLNs, this ratio is inversed. IHC was applied to compare the LP-DC subsets under normal conditions and during intestinal *schistosomiasis*. Our Results : clearly revealed a further increase in the number of CD11c⁺CX3CR1⁺LP-DCs during inflammation, but this increase did not result in significant changes in density because of the broadening of the villi during intestinal *schistosomiasis*. Using IHC and 3D reconstructions, we were further able to show that VIP-expressing neuronal fibers of the enteric nervous system (ENS) lie in very close proximity to mouse intestinal DCs, indicating a possible neuro-immune communication. The presence of such an interaction pathway was further strengthened by the expression of VIP receptor 1 (VIPR1) by intestinal CX3CR1⁺ cells.

Conclusions : Our findings provide, for the first time, morphological support for the existing theory that neurotransmitters of the ENS, in particular VIP, are able to exert a modulatory effect on intestinal DCs. Our Results : highlight the need for further research, with special focus on *in vivo* experiments, to assess the effects of VIP on DC functions. Targeting these receptors might prove to be an interesting novel therapeutic approach in specific intestinal inflammatory diseases.

- B05 -

SYSTEMIC AND LOCAL IMMUNE FINGERPRINT DURING SEPTIC ILEUS INDUCED BY CECAL LIGATION AND PUNCTURE. S. Nullens (1), M. Heylen (1), N. Ruysers (1), J. De Man (1), P. Pelckmans (2), I. Van Brussel (3), D. Schrijvers (3), B. De Winter (4). (1) Laboratory of Experimental Medicine and Pediatrics, Division of Gastroenterology, University of Antwerp, Antwerp, Belgium ; (2) Universitair Ziekenhuis Antwerpen, Antwerpen, Belgium ; (3) Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium ; (4) Laboratory of Experimental Medicine and Pediatrics, University of Antwerp, Antwerp, Belgium.

Introduction : Sepsis remains a leading cause of mortality in modern day Intensive Care Units. Ileus not only Results : from sepsis but also maintains it by facilitating gastrointestinal (GI) barrier function and bacterial translocation. In order to characterize the immune environment during septic ileus we studied the cytokine profile and T lymphocytes subsets systemically in serum and spleen, and locally in the colon and mesenteric lymph nodes (MLN).

Methods : Sepsis was induced in Swiss-OF1 mice by cecal ligation and puncture (CLP). Sham-operated animals served as controls. Mice were sacrificed at day 2 (CLPd2) and 7 (CLPd7) following CLP. Occurrence of ileus was confirmed by determining the geometric center (GC) of beads. Blood serum samples were obtained by cardiac puncture and analyzed for the presence of cytokines with a *Cytometric Bead Array* (CBA). Colons were incubated for 24 h in complete RPMI, and supernatants were analyzed with CBA. Single cell suspensions of spleens and MLN were prepared for characterization of different subsets of T lymphocytes (T helper, cytotoxic and regulatory T cells) using a flow cytometer.

Results : The presence of sepsis was confirmed by the manifestation of clinical symptoms and hypothermia (sham 35.8 ± 0.2°C, CLPd2 34.7 ± 0.3°C, CLPd7 30.7 ± 0.5°C*). CLP resulted in a significant prolongation of GI transit time (GC : sham 4.9 ± 0.5, CLPd2 2.3 ± 0.5*, CLPd7 2.0 ± 0.2*). In the serum, a profound secretion of proinflammatory cytokines (IL-2, TNFα and IL-6) was measured at day 2, whereas the immune profile was skewed towards a predominantly Th17 profile at day 7. In colon supernatants, IL-10, IL-17A and IL-6 tended to increase only at day 7. Flow cytometric analysis of the spleen showed an increase in the percentage of CD4⁺ cells at day 2 (sham 23.9 ± 1.8%, CLPd2 33.6 ± 2.3%*), followed by a decrease at day 7 (10.8 ± 2.5%*). Interestingly, we found an increase in CD4⁺CD25⁺ cells that reached significance on day 7 (sham 8.9 ± 0.7%, CLPd2 11.9 ± 0.6, CLPd7 13.5 ± 1.3%*) and of CD4⁺CD25⁺Foxp3⁺ cells (sham 7.6 ± 0.7%, CLPd2 9.9 ± 0.8, CLPd7 8.9 ± 0.8%), albeit not significantly. The same is true for MLN, with a drop in CD4⁺ cells on day 7 (sham 49.6 ± 1.5%, CLPd2 52.3 ± 2.1, CLPd7 32.1 ± 2.4%*) and increase in CD4⁺CD25⁺ cells (sham 7.9 ± 0.3%, CLPd2 10.8 ± 0.9, CLPd7 11.6 ± 0.9%*). We also measured a rise in the percentage of CD4⁺CD25⁺Foxp3⁺ cells, most likely regulatory T cells, reaching significance at day 7 (sham 6.6 ± 0.2%, CLPd2 9.0 ± 1.0%, CLPd7 9.6 ± 0.7%*).

Conclusions : CLP-induced sepsis resulted in the clinical development of sepsis characterized by an initial proinflammatory phase in the serum, but not in the colon, that subsequently skewed towards a predominantly Th17 profile in blood with increased relative numbers of most likely regulatory T cells in spleen and MLN. The immunological fingerprint during CLP-induced sepsis in the systemic circulation cannot be extrapolated directly towards the local tissues.

- B06 -

Invited Lecture

Postoperative bowel inflammation : unmasking enteric glia and neurons as non-classical immunocytes.
J. Kalff, Bonn, Germany.

- B07 -

MONOCYTE AND NEUTROPHIL RECRUITMENT INTO MUSCULARIS RESTORE GUT MOTILITY IN POST-OPERATIVE ILEUS. G. Farro (1), P.J. Gomez-Pinilla (1), M. Di Giovangiulio (1), N. Stakenborg (1), D. Laoui (2), Y. Elkrim (2), J.A. Van Ginderachter (2), G.E. Boeckxstaens (1), G. Matteoli (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) Free University (VUB), Brussels, Belgium.

Introduction : Patients undergoing an abdominal surgical procedure develop a transient episode of impaired gastrointestinal (GI) motility known as postoperative ileus (POI). POI has been recognized as a consequence of a local inflammatory response within the muscularis externa (ME) induced by intestinal handling. The influx of neutrophils and monocytes has been proposed to be a key mechanism leading to impaired motility of the handled gut. In the current study using novel experimental approaches, we further dissect if these cells invading the ME contribute to the development of POI.

Methods : POI was induced in mice via standardized intestinal manipulation (IM). The severity and the progression of POI were evaluated by assessing GI transit and ME inflammation (myeloperoxidase (MPO)-positive cells, cytokine mRNA expression and flow cytometry analysis of recruited immune cells at different time points (24 to 120 hours). Selective in vivo ablation of neutrophils using a specific anti-Ly6G monoclonal antibody (clone 1A8) and ablation of migratory monocytes via CCR2^{-/-} mice were used to define their contribution to the pathogenesis of POI.

Results : Wild type C57BL/6 mice injected respectively with two doses of 10 ug/kg of a specific anti-Ly6G antibody or with an isotype control were subjected to IM. Specific neutrophil depletion was confirmed by flow cytometry 24 hours after intestinal manipulation in the blood and in the ME. Interestingly, neutrophil depletion did not prevent POI 24 hrs after IM (Geometric Center (GC) : isotype-WT mice, 6.7 ± 0.4 ; Ly6G-WT mice, 6.3 ± 0.4 , no statistical difference (ns), T-student test) nor did it decrease the infiltration of MPO+ cells (isotype-WT mice, 131 ± 12 ; Ly6G-WT mice, 107 ± 21 , ns). Similarly, the lack of circulating monocytes did not protect CCR2^{-/-} mice from POI (GC : CCR2^{+/+} 4.5 ± 0.3 ; CCR2^{-/-} 4.2 ± 0.4 and MPO cells : WT 182 ± 17 ; CCR2^{-/-} 116 ± 17 , ns). Interestingly, simultaneous depletion of neutrophils in CCR2^{-/-} mice using anti-Ly6G caused a significant worsening of POI with aggravation of GI transit delay and increased inflammation in the ME (GC : Ly6G-CCR2^{+/+} 5.8 ± 0.6 ; Ly6G-CCR2^{-/-} 2.9 ± 0.1 ; MPO Ly6G-CCR2^{+/+} 126 ± 28 ; Ly6G-CCR2^{-/-} 164 ± 23 , $p < 0.5$). Analysis of the migratory CD11b⁺Ly6G⁺Ly6C⁺MHCII⁺ immature macrophages and CD11b⁺Ly6G⁺Ly6C⁺MHCII⁺ mature macrophages isolated from the ME of WT mice 1, 3 and 5 days after IM showed a predominant expression of anti-inflammatory markers (Arg1, IL10, VEGF, CD163, MMR, YM1, Lyve1, Stab1) and a lower expression of pro-inflammatory markers (IL1 β , TNF α , IL12, iNOS). These findings suggest that monocytes acquired a M2-like phenotype after migration in the ME promoting tissue regeneration.

Conclusions : In vivo specific depletion of neutrophils and migratory monocytes did not prevent IM-induced ME inflammation or POI. On the contrary, absence of these cells worsened the severity and the duration of POI. Overall our data suggest that in contrast to earlier believes, migration of monocytes and neutrophils in the ME after IM may have a beneficial effect promoting tissue recovery.

- B08 -

NOVEL MOUSE MODEL REVEALS NO ROLE FOR MAST CELLS IN THE PATHOGENESIS OF POSTOPERATIVE ILEUS. P.J. Gomez-Pinilla (1), G. Farro (1), M. Di Giovangiulio (1), N. Stakenborg (1), A. Nemetova (1), A. De Vries (1), A. Liston (2), G.E. Boeckxstaens (1), G. Matteoli (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) VIB, Leuven, Belgium.

Introduction : Intestinal manipulation (IM) during abdominal surgery Results : in intestinal inflammation leading to hypomotility or ileus. Mast cell (MC) activation is thought to play a crucial role in the pathophysiology of postoperative ileus (POI). However, this conclusion was mainly drawn using mouse models deficient in MCs due to abnormal Kit signaling. In particular, Kit is necessary for the development of several immune cell subsets and interstitial cells of Cajal (ICC), with both Kit^{W^Wv} and Kit^{W^{sh}/W^{sh}} strains having abnormalities in ICC leading to severely disturbed gut motility and immune deficiencies. To avoid this experimental bias, we used a novel genetically modified mouse strain (Cpa3^{Cre/+}) with a targeted insertion of Cre-recombinase into the carboxypeptidase A3 (Cpa3) locus. This intervention leads to specific MC ablation leaving Kit-signaling unaffected, giving us the opportunity to specifically evaluate the role of MCs in the pathogenesis of POI.

Methods : POI was induced in mice lacking MCs, Kit^{W-sh/W-sh} and Cpa3^{Cre/+} via intestinal manipulation (IM). The severity of POI was evaluated by assessing GI transit and muscularis externa inflammation (number of myeloperoxidase (MPO)-positive cells, cytokine mRNA expression and flow cytometry analysis of recruited neutrophils and monocytes) 24 hours after IM. Immunostaining of ICCs was performed in Kit^{W-sh/W-sh} and Cpa3^{Cre/+} mice.

Results : Kit^{W-sh/W-sh} mice lack ICC networks and revealed significantly delayed GI transit under basal conditions (Geometric Center (GC) : Kit WT 10.5 ± 0.3, Kit^{W-sh/W-sh} 5.7 ± 0.3). IM did not further delay intestinal transit (GC IM : Kit WT 5.9 ± 0.15, Kit^{W-sh/W-sh} 5.5 ± 0.4), but induced infiltration of myeloperoxidase positive cells (MPO IM : Kit WT 143 ± 17, Kit^{W-sh/W-sh} 106 ± 29), expression of inflammatory cytokines (*il1b*, *il6*, *tnfa*, *cxcl1* and *ccl2*) and recruitment of monocytes and neutrophils into the muscularis externa to a similar extent as in Kit WT mice.

In contrast, we found that Cpa3^{Cre/+} mice have a normal network of ICCs and regular GI transit (GC : Cpa3^{+/+} 10.2 ± 0.2, Cpa3^{Cre/+} 10.1 ± 0.2). Surprisingly, IM in Cpa3^{Cre/+} mice caused significant delay in gut motility comparable to their littermates (Cpa3^{+/+}) (GC IM : Cpa3^{+/+} 4.2 ± 0.5, Cpa3^{Cre/+} 4.1 ± 0.3). In addition, IM in Cpa3^{Cre/+} mice led to influx of MPO-positive cells and expression of inflammatory cytokines similar to littermate controls (MPO IM : Cpa3^{+/+} 113 ± 11, Cpa3^{Cre/+} 94 ± 13). In line, flow cytometry analysis of the immune cells recruited after IM in the muscularis externa showed no differences in the percentage and in the absolute number of monocytes and neutrophils between Cpa3^{Cre/+} and littermate Cpa3^{+/+} mice.

Conclusions : Our study demonstrates that mast cells, at least in mice, do not play a crucial role in the pathogenesis of POI, and provide a further example of experimental discrepancies comparing mast cell- and Kit double-deficient mutants versus mast cell-deficient mice without defects in Kit signaling.

- B09 -

EFFECT OF EXOGENOUS NITRITE IN POSTOPERATIVE ILEUS. S. Cosyns, R.A. Lefebvre. Heymans Institute of Pharmacology, Gent, Belgium.

Introduction : Exogenous nitrite has shown to protect heart, liver, kidney and brain from ischemia/reperfusion injury. A possible mechanism of action is activation of soluble guanylate cyclase (sGC) by NO, produced from nitrite under hypoxic conditions, with reduction of platelet aggregation and opening of mitochondrial inner membrane K_{ATP} channels. Another proposed mechanism of action is reversible inhibition of mitochondrial complex I by S-nitrosation, reducing reactive oxygen species (ROS) generation. Impairment of gastrointestinal motility after abdominal surgery (postoperative ileus, POI) is mainly due to intestinal inflammation with leukocyte infiltration, triggered by surgical handling. As the latter will also lead to repetitive momentary ischemia/reperfusion of the bowel, the Aim : of this study was to investigate the effect of nitrite in a model of POI.

Methods : C57Bl6J mice were anesthetized (isoflurane) and after laparotomy, POI was induced by compressing the small intestine with cotton applicators (intestinal manipulation ; IM) for 5 min. Sodium nitrite (48 nmol) or its solvent (PBS) was administered in bolus in the inferior vena cava just before IM. Intestinal transit was assessed 24h postoperatively using fluorescent imaging, 90 min after fluorescein gavage (geometric centre, GC of intestinal fluorescein progression). Contractile activity to bethanechol (cumulative 0.3 μM-300 μM) was evaluated in circular mucosa-free mid-jejunal muscle strips, obtained 24h after IM. Additional mucosa-free muscularis segments were stored at -80°C for later analysis of myeloperoxidase (MPO) activity as an index of leukocyte infiltration, of the inflammatory cytokine TNFα, of ROS with the luminol derivate L-012, of mitochondrial complex I activity and of cGMP. TNFα and cGMP were assayed by enzyme immunoassay, MPO and complex I activity by spectrophotometric monitoring and ROS by chemiluminescence.

Results : Pre-treatment with nitrite partially prevented the delayed transit seen after IM, and restored the reduced contractile response of the circular muscle strips to bethanechol. Administration of nitrite tended to increase reduced cGMP levels and tended to decrease elevated TNFα and ROS (L-012 luminescence) levels. However, it did not have an influence on elevated leukocyte infiltration (MPO) and on reduced mitochondrial complex I activity in the muscularis of operated mice (Table 1).

Table 1. — Influence of nitrite on intestinal changes induced by IM

	Control	IM	IM+nitrite
GC	8.7 ± 0.3	3.2 ± 0.3***	7.4 ± 0.8***
E _{max} bethanechol (g.s/mg tissue)	16.8 ± 2.1	6.7 ± 3.1*	16.6 ± 2.1*
MPO (U/mg protein)	2.7 ± 0.4	9.1 ± 1.0***	10.3 ± 0.9
TNFα (pg/mg protein)	5.4 ± 0.6	12.0 ± 1.7**	8.5 ± 1.1
L-012 luminescence (AU/mg protein)	311.0 ± 38.2	544.0 ± 70.2**	376.3 ± 39.9
Complex I activity (μmol/min/mg protein)	179.0 ± 14.1	113.2 ± 10.1**	117.1 ± 11.5
cGMP (pmol/g tissue)	25.1 ± 3.5	14.6 ± 1.9*	19.0 ± 2.3

*P < 0.05, **P < 0.01, ***P < 0.001 vs. control ; *P < 0.05, ***P < 0.001 vs. IM ; n = 6-10.

Conclusions : The present study indicates that pre-treatment with exogenous nitrite partially prevents delayed intestinal transit upon IM, although not preventing leukocyte infiltration. Still, nitrite tended to reduce the inflammatory response, as judged from the TNF α levels, and the oxidative stress response to IM. The effects of nitrite are not related to inhibition of complex I activity, but activation of sGC in the muscular layer might play a role.

- B10 -

PRUCALOPRIDE ACTIVATES THE INTESTINAL CHOLINERGIC ANTI-INFLAMMATORY PATHWAY AND PREVENTS ILEUS. P.J. Gomez Pinilla (1), M. Di Giovangiulio (1), A. Nemethova (1), N. Stakenborg (2), G. Farro (3), G. Bosmans (2), G. Matteoli (1), G. Boeckxstaens (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) University Hospitals Leuven, Belgium, Leuven, Belgium ; (3) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium.

Introduction : We recently showed that vagus nerve stimulation prevents post-operative ileus (POI) and manipulation-induced intestinal inflammation through interaction with cholinergic myenteric neurons. Acetylcholine released by these neurons then dampens the activation of resident macrophages through interaction with $\alpha 7$ nicotinic receptors ($\alpha 7$ nAChR). Prucalopride is a highly selective serotonin receptor 4 agonist (5HT $_4$) with enterokinetic effects. As this compound acts through activation of 5HT $_4$ receptors on cholinergic enteric neurons enhancing the release of acetylcholine (ACh), we hypothesized that pre-operative administration of prucalopride would mimic the anti-inflammatory effect of vagus nerve stimulation, and hence would be more efficient to shorten POI compared to its post-operative use as prokinetic agent.

Aim : The Aim : of the current study was therefore to compare the effect of pre- vs post-operative administration of prucalopride on gastrointestinal transit and intestinal inflammation in our model of POI.

Methods : POI was induced by intestinal manipulation (IM) of the small bowel. Prucalopride (1 and 5 mg/kg) was orally administered pre-operatively (1.5 hours before IM) or post-operatively (22.5 hours after IM). 22.5 hours after surgery, mice were gavaged with FITC-Dextran (70KD) and gastrointestinal transit was determined 90 min later by calculation of the geometrical center (GC). Intestinal tissue was collected to assess the number of myeloperoxidase (MPO) positive cells and pro-inflammatory level of cytokine expression (*Il6*, *Il1a* and *Il1b*) in the muscularis externa.

Results : Post-operative administration of prucalopride (1 and 5 mg/kg) did not prevent POI (IM+placebo, GC = 5.0 ± 0.5 , IM+prucalopride (1 mg/Kg), GC = 7.4 ± 1.2 , IM+prucalopride (5 mg/Kg), GC = 6.5 ± 0.7 , ns) and failed to reduce intestinal inflammation. However, pre-operative administration of 5 mg/kg of prucalopride, but not 1 mg/kg of prucalopride, significantly reduced the influx of MPO positive cells and the expression level of *Il6*, *Il1a* and *Il1b*. In addition, 5 mg/kg prucalopride significantly (* $p < 0.05$) increased intestinal transit compared to placebo (IM+placebo, GC = 4.0 ± 0.2 , IM+prucalopride (1 mg/Kg), GC = 4.4 ± 0.4 , IM+prucalopride (5 mg/Kg), GC = $8.1 \pm 0.6^*$). To evaluate the role of $\alpha 7$ nAChR, $\alpha 7$ nAChR $^{-/-}$ mice that underwent IM were pre-operatively treated with 5 mg/kg of prucalopride. Of note, prucalopride failed to prevent the development of POI (IM+placebo, GC = 3.8 ± 0.3 , IM+prucalopride (5 mg/Kg), GC = 3.8 ± 0.2) and did not reduce intestinal inflammation in $\alpha 7$ nAChR $^{-/-}$ mice.

Conclusions : We showed that pre-operative, but not post-operative administration of prucalopride has an anti-inflammatory effect and prevents the development of POI, an effect that is dependent on the presence of $\alpha 7$ nAChR. Most likely, this effect Results : from prucalopride-induced activation of cholinergic myenteric neurons dampening the activation of resident macrophages via $\alpha 7$ nAChR, similar to vagus nerve stimulation. These data suggest that prucalopride is an interesting compound to prevent POI, but mainly when administered prior to surgery.

- B11 -

Invited Lecture

The hypoxia-inducible factor pathway as target for inflammatory bowel disease.

C. Taylor, Dublin, Ireland.

SOURCES OF ROS DURING TNFALPHA-INDUCED OXIDATIVE STRESS IN MURINE INTESTINAL EPITHELIAL MODE-K CELLS. D. Babu (1), G. Leclercq (2), R.A. Lefebvre (1). (1) Heymans Institute of Pharmacology, Gent, Belgium ; (2) Ghent University, Gent, Belgium.

Introduction : Intestinal epithelial cell lines are used as a model to investigate the response of the intestinal mucosa towards acute inflammation. TNF-alpha/cycloheximide (CHX)-induced apoptosis of the mouse intestinal epithelial cell line MODE-K corresponds with the production of reactive oxygen species (ROS ; Babu *et al.*, Curr. Pharm. Des. 2012). Activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and dysregulation of mitochondrial oxidative phosphorylation are considered to be the two major endogenous sources of ROS production in cells ; additionally, the interaction xanthine/xanthine oxidase can yield ROS.

Aim : The aim of the study is to investigate the sources of ROS production during TNF-alpha/CHX-induced oxidative stress in MODE-K cells by use of pharmacological inhibitors of the various ROS-producing systems (NADPH oxidase, electron transport chain and xanthine/xanthine oxidase). In comparison, the influence of the ROS scavenger butylated hydroxyl anisole (BHA) and the antioxidant N-acetyl cysteine (NAC) was studied.

Methods : Total intracellular ROS and mitochondrial superoxide production were measured using Carboxy-H₂DCFDA and MitoSOX Red, respectively. Carboxy-H₂DCFDA is a widely used cell-permeable indicator for total intracellular ROS. MitoSOX Red is a novel fluorogenic dye used for highly selective detection of superoxide in the mitochondria of live cells. Sytox Red Dead cell stain stains the nucleic acids of dead cells and exhibits bright red fluorescence. Mode-K cells (passage 10-35) were grown for 36 h, serum starved overnight and then exposed to 1 ng TNF-alpha/10 µg CHX for 6 h ; simultaneous detection of ROS production and cell death has been performed using either carboxy-H₂DCFDA or MitoSOX Red together with Sytox Red in a single experimental setup using flow cytometric analysis. For experiments involving the inhibitors, the confluent cells were pretreated with the test drugs for 1h followed by its co-treatment with TNF-alpha/CHX for 6h.

Results : Treatment with TNF-alpha/CHX increased mean fluorescence intensity (MFI) of carboxy-H₂DCFDA derived fluorescence in a time-dependent manner with concomitant increase in cell death as measured with Sytox Red positivity. The flavoprotein inhibitor diphenyleneiodonium (DPI), considered to be a non-selective NADPH oxidase inhibitor, did not affect total ROS and cell death, while the novel NADPH-oxidase inhibitor VAS-2870 partially decreased both effects. All the inhibitors of the respective electron train chain complexes I-V decreased both total ROS and cell death significantly, but the effects were most pronounced for complex I and II. Moreover, TNF-alpha/CHX also increased MFI of MitoSOX Red-derived fluorescence in a time-dependent manner. The xanthine oxidase inhibitor allopurinol did not affect the responses to TNF-alpha/CHX. NAC decreased ROS levels significantly with moderate reduction of TNF-alpha/CHX-induced cell death while BHA completely abolished both.

Conclusions : NADPH oxidases and mitochondria (most importantly complex I and II) both contribute to ROS produced in intestinal epithelial cells in response to TNF-alpha/CHX.

DUODENAL ACID PERFUSION IN HEALTHY VOLUNTEERS RESULTS : IN MAST CELL ACTIVATION AND INCREASED PERMEABILITY. H. Van Heel (1), T. Vanuytsel (1), M. Vicario (2), E. Deloose (1), J. Tack (1), R. Farré (1). (1) KU Leuven, Leuven, Belgium ; (2) Hospital Universitario Val d'Hebron, Barcelona, Spain.

Introduction : Functional dyspepsia (FD) is characterised by a variety of symptoms localised in the epigastric region. We recently reported (Vanheel *et al*, Gut 2013) that patients with FD show impaired duodenal integrity, associated with low-grade inflammation. A potential cause underlying impaired duodenal barrier function in FD may be the increased duodenal acid exposure that has been demonstrated in these patients (Lee *et al*, AJG 2004).

Aim : To evaluate the effect of duodenal acid perfusion on duodenal integrity and immune activation in healthy volunteers. To assess activation of duodenogastric reflex pathways (Vanuytsel *et al*, NMO 2013), we also measured intragastric pressure (IGP).

Methods : Ten healthy volunteers (3 men ; age 34.6 ± 4.2 years) participated in the study. An infusion tube was positioned in the second part of the duodenum and a high resolution manometry probe was positioned in the stomach to measure IGP. HCl 0.1 N or 0.9% saline was infused in the duodenum during 30 min at a rate of 5 mL/min in a randomized, double-blind manner. Thirty minutes after infusion, endoscopic duodenal biopsy specimens were obtained to measure transepithelial electrical resistance and paracellular permeability for fluorescein-labeled dextrans (MW 4 kDa) in Ussing chambers. Expression of tight junction proteins in biopsy specimens was evaluated at gene level by real-time RT-PCR (claudin 1-4, occludin, zonula occludens 1-3 and myosin light-chain kinase) and at protein level by western blot (claudin 1-4). Activation of mast cells and eosinophils was assessed by measuring tryptase and eosinophilic major basic protein respectively, both at mRNA and protein level. Differences between groups were analyzed using paired t tests or Wilcoxon signed rank tests when appropriate.

Results : Compared with saline infusion, acid perfusion significantly lowered transepithelial electrical resistance ($17.8 \pm 0.7 \Omega \cdot \text{cm}^2$ vs. $23.0 \pm 1.0 \Omega \cdot \text{cm}^2$, $p = 0.006$) and increased passage of fluorescein-labeled dextrans ($57.4 \pm 7.5 \text{ pmol}$ vs. $31.8 \pm 4.6 \text{ pmol}$, $p = 0.01$) of duodenal biopsy specimens. No difference in gene expression of tight junction proteins, tryptase and eosinophilic major basic protein was detected. However, compared with saline infusion, protein expression of claudin 3 was lower (0.55 fold ; $p = 0.002$) and tryptase expression was higher (1.86 fold ; $p = 0.0008$) after acid perfusion. In addition, IGP was significantly lower during acid perfusion compared with saline infusion ($p = 0.02$).

Conclusions : Duodenal acid perfusion in healthy volunteers disrupts epithelial integrity and activates mucosal mast cells. This response could underlie altered permeability and low-grade inflammation observed in FD. Hence, increased duodenal acid exposure in patients with FD is a potential pathophysiological mechanism contributing to dyspeptic symptom generation.

- B14 -

MUCOSAL PERMEABILITY IS ALTERED IN BOTH TYPE-1 AND TYPE-2 DIABETIC PATIENTS WHEN COMPARED TO MATCHED CONTROLS. C. Vanormelingen (1), T. Vanuytsel (1), H. Vanheel (1), S. Salim Rasoel (1), J. Toth (1), P. Vanden Berghe (2), J. Tack (2). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) KU Leuven, Leuven, Belgium.

Introduction : Increased small intestinal permeability and ultrastructural alterations in the duodenum have been reported in diabetic patients. To date, alterations in permeability have been studied by carbohydrate absorption testing (e.g. lactulose/mannitol), but this does not allow to establish the site of involvement and the underlying molecular changes. Increased duodenal permeability and local immune activation have been demonstrated in functional dyspepsia, can be associated with delayed gastric emptying and dyspeptic symptoms (1,2).

Aim : The aim of this study is to establish whether duodenal permeability is altered in Type-1 and -2 diabetes patients compared to controls and whether this is associated with their symptom burden.

Methods : Duodenal biopsies from Type-1, Type-2 and age- and gender-matched control patients without diabetes undergoing endoscopy ($N = 7$ in each group) were mounted in Ussing-chambers to measure transepithelial resistance (TER) and passage of a fluorescein-labeled 4 kDa dextran (FD4). They also filled out a dyspeptic symptom severity scale (DSS) and the visceral sensitivity index (VSI) questionnaire.

Results : Compared to controls ($22.2 \pm 1.0 \text{ } \Omega \cdot \text{cm}^2$), a significant decrease in TER was observed in both Type-1 and Type-2 diabetics (Type-1, 18.3 ± 0.8 ; Type-2, 16.9 ± 0.6 , $p < 0.05$ for both). Additionally, increased passage of FD4 was observed in the duodenum of diabetics (Type-1, 35.0 ± 14.0 vs. $13.0 \pm 1.7 \text{ pmols}$; Type-2, 28.4 ± 4.7 vs. $13.0 \pm 1.7 \text{ pmols}$; $p < 0.05$). No significant difference in TER and FD4 values was found between Type-1 and Type-2 diabetics. A significant negative correlation between TER and passage of FD4 confirms the functional relevance of the measurements ($r^2 = 0.23$; $p < 0.05$). Both VSI and DSS did not differ significantly between diabetics and matched controls and did not correlate significantly with alterations of duodenal permeability.

Conclusions : Duodenal mucosal integrity is impaired in both Type-1 and Type-2 diabetes patients. Further studies will be needed to unravel the relevance of this observation to symptom generation, gastric sensorimotor dysfunction and to low-grade duodenal inflammatory changes, all of which have been shown to be present in FD.

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2. Kindt S., Tertychnyy A., de Hertogh G. *et al.* Intestinal immune activation in presumed post-infectious functional dyspepsia. *Neurogastroenterol. Motil.*, 2009, 21 : 832-e56.

- B15 -

FRUCTOSE CONSUMPTION IMPAIRS NEURONAL SENSITIVITY AND SEROTONERGIC SIGNALING TO THE SUBMUCOUS PLEXUS. K. Lowette, J. Tack, P. Vanden Berghe. Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium.

Introduction : Since the development of high fructose corn syrup (HFCS) 50 years ago, the amount of fructose consumption in the western world has increased from 20 to 80 gram per day. Not only has fructose consumption been associated with an increased prevalence of the metabolic syndrome, recent studies also suggest a link with mild cognitive impairment.

Aim : Regarding the fructose-induced consequences in neuronal processes, we Aim : to investigate the effects of fructose in the enteric nervous system of mice with a focus on serotonin signaling.

Methods : Male C57/B16 mice were put on a 23% fructose drink diet for 4 weeks. Drink and food consumption was measured daily, and body weight and serum glucose concentration was assessed weekly. Next, duodenal mRNA was extracted from total mucosa and isolated submucous plexus for RT-PCR. In addition, Ca²⁺-signaling ($\Delta F/F_0$) in duodenal submucous plexus neurons, loaded with Fluo-4, was measured in response to high K⁺ and 5-HT (10⁻⁵ M) stimulation.

Results : Mice on the fructose diet showed a significantly increased drink and a decreased food consumption compared to control mice. Body weight and serum glucose concentrations did not differ between groups. GLUT5 mRNA expression increased significantly after fructose consumption in duodenal mucosa (4.75 ± 1.58 vs 36.24 ± 6.04 , $p < 0.01$) and submucous plexus (1.38 ± 0.51 vs 17.96 ± 6.47 , $p < 0.01$). Fructose consumption resulted in decreased mRNA expression in total mucosa tissue of both synaptophysin (0.01 ± 0.002 vs 0.005 ± 0.0004 , $p < 0.01$) and synaptobrevin (0.17 ± 0.03 vs 0.07 ± 0.007 , $p < 0.01$) two important synaptic vesicle proteins as well as the serotonin receptor 5-HT_{3a} (0.057 ± 0.022 vs 0.006 ± 0.003 , $p < 0.05$). Expression of the Ca²⁺ channels Ca_v2.1 (0.10 ± 0.05 vs 0.03 ± 0.003 , $p < 0.05$) and Ca_v2.2 (0.026 ± 0.01 vs 0.007 ± 0.0004 , $p < 0.05$) was significantly decreased after fructose consumption in total mucosa. In addition, the fructose-induced decrease of Ca_v2.2 (0.016 ± 0.0064 vs 0.008 ± 0.0004 , $p < 0.05$) was also present in the submucous plexus. Stimulation with high K⁺ (19 ± 2 vs $18 \pm 2\%$) and 5-HT (5 ± 0.4 vs $4 \pm 0.4\%$) did not induce significant differences in Ca²⁺-response amplitudes between both groups of mice. However, the number of high K⁺ responsive neurons per field of view (0.13 mm²) (13.35 ± 1.33 vs 7.88 ± 0.91 , $p < 0.01$) and the percentage of neurons identified by high K⁺ responding to 5-HT (54.5 ± 8.67 vs $32.98 \pm 8.47\%$, $p < 0.05$) was significantly decreased after fructose consumption.

Conclusions : We conclude that excessive fructose consumption Results : in a down regulation of important components of the serotonergic signaling pathway to the submucous neurons of mouse duodenum, as well as a reduction in the number of neurons that can be depolarized and respond to serotonin.

- B16 -

A-317491, A SELECTIVE P2X3 RECEPTOR ANTAGONIST, REDUCES POST-INFLAMMATORY VISCERAL HYPERSENSITIVITY. A. Deiteren (1), A. De Wit (1), L. Van Der Linden (1), J. De Man (1), T. Moreels (2), P. Pelckmans (2), B. De Winter (1). (1) University of Antwerp, Antwerpen, Belgium ; (2) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium.

Introduction : P2X3 receptors (P2X3Rs) are believed to contribute to visceral hypersensitivity. To date, most experiments have been conducted in P2X3 knock-out mice or using *non-selective* P2X receptor antagonists in animal models of acute *inflammatory* visceral hypersensitivity. Besides, only limited data are available on the role of P2X3Rs in post-inflammatory hypersensitivity.

Aim : The Aim : of our study was to investigate the effect of the *selective* P2X3R antagonist A-317491 in a rat model for *post-inflammatory* visceral hypersensitivity.

Methods : Colitis was induced in Sprague-Dawley rats by a trinitrobenzenesulphonic acid enema (TNBS, 15 mg in 50% ethanol). The extent of colitis was confirmed by colonoscopy on day 3. From day 10 onwards, convalescence was monitored by repeated colonoscopy that was performed every 4 days to determine the exact timepoint of endoscopic healing in each individual animal. Three days after endoscopic resolution of inflammation, visceral sensitivity was assessed by quantifying visceromotor responses (VMRs) to colorectal distension and expressed as total area under the curve (AUC). A-317491 (10-25 mg/kg) or saline was administered 30 min prior to VMR testing. Compliance was evaluated by applying graded volumes to the colorectally inserted balloon and recording the corresponding colonic pressures. Afterwards remaining signs of inflammation were assessed by endoscopy, macroscopy, histology and myeloperoxidase activity. P2X3R mRNA expression was determined in colon specimens and dorsal root ganglia (DRGs ; Th13-L2 and L6-S1) by RT-PCR.

Results : Endoscopy demonstrated that colitis was present 3 days after the TNBS enema and had resolved by day 12 (range 10-14) ; inflammatory markers were normal in control and post-colitis rats at the time of VMR testing. After endoscopic healing of colitis, post-colitis rats displayed significant visceral hypersensitivity compared to controls (3763 ± 554 vs 1795 ± 240 μ V for controls ; $n = 7$; $p < 0.05$). Post-inflammatory hypersensitivity was dose-dependently reduced by A-317491 : the 10 mg/kg dose attenuated increased VMRs (2140 ± 422 vs 3763 ± 554 μ V for vehicle ; $n = 7$; $p < 0.05$), whereas 25 mg/kg fully reversed visceral hypersensitivity (1590 ± 191 vs 3763 ± 554 μ V for vehicle ; $n = 7$; $p < 0.05$) without affecting VMRs in controls (2030 ± 526 vs 1795 ± 240 μ V for vehicle ; $n = 5-7$; ns). Colonic compliance was comparable in both groups and remained unaltered by A-317491 ($n = 5-7$). P2X3R mRNA was similarly expressed in the colon and DRGs of control and post-colitis rats.

Conclusions : The selective P2X3R antagonist A-317491 dose-dependently reduced post-inflammatory visceral hypersensitivity, highlighting the modulatory role of P2X3Rs in visceral nociception. Our findings validate this receptor subtype as a potential target in the treatment of abdominal pain syndromes such as irritable bowel syndrome and inflammatory bowel disease.

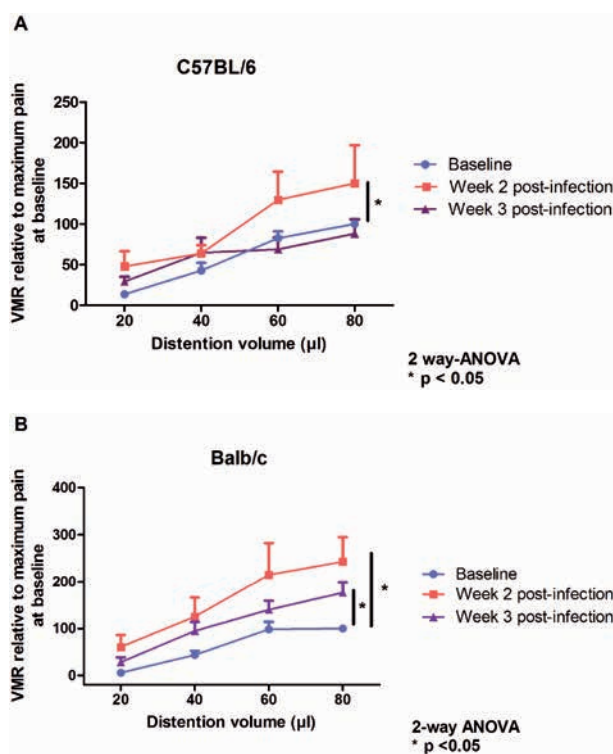
TH1 VERSUS TH2 GENETIC BACKGROUND AS RISK FACTOR TO DEVELOP POSTINFECTIOUS VISCERAL HYPERSENSITIVITY. S. Mondelaers, G. Boeckxstaens, M. Wouters. KU Leuven, Leuven, Belgium.

Introduction and Aim : An acute gastrointestinal infection, especially in the presence of psychological co-morbidity, is a well-defined risk factor to develop irritable bowel syndrome, a condition characterized by visceral hypersensitivity (VHS). As mast cell activation, a typical Th2 mediated phenomenon, is involved in the development of IBS, we reasoned that a Th2 genetic background would favor the development of post-infectious IBS. Therefore, we compared two different mouse strains, i.e. Th1 predominant C57BL/6 and Th2 predominant Balb/c mice, with regard to the development of post-infectious VHS and their response to stress.

Methods : C57BL/6 and Balb/c mice were implanted with ETA-F10 electrodes (Data Science International) for electromyographic (EMG) recording of colorectal distention (20-80 μ L)-induced contractions of the abdominal musculature (visceromotor response or VMR). After recovery, baseline VMR recordings were performed. Then, mice were infected with 3×10^{10} CFU *Citrobacter rodentium* and VMR was recorded from week 2-6 post-infection. At week 5, mice were exposed to 1 hour water avoidance (WA) 24 hours before colorectal distention. EMG signals were analyzed with Acknowledge 3.2.6 software. Results : are presented as % VMR response \pm SEM ; relative to maximum pain at baseline recording (i.e. maximum pain at 80 μ l distention is set at 100%). Statistical analysis was performed using 2-way ANOVA, p values < 0.05 were considered statistically significant.

Results : Colorectal distention induced a volume-dependent increase in VMR with maximal response at 80 μ L. The visceral pain response was significantly more pronounced in Balb/c mice compared to C57BL/6 mice. The VMR was significantly increased at week 2 post-infection in both C57BL/6 (80 μ l : $150 \pm 47\%$ compared to baseline ; p = 0.02 ; n = 5, figure 1A) and Balb/c mice (80 μ l : $243 \pm 52\%$ compared to baseline ; n = 7 ; p = 0.03, figure 1B). The VMR returned to basal levels in week 3 in C57BL/6 mice, but was still present in Balb/c mice (80 μ l : $176 \pm 23\%$ compared to baseline ; n = 7 ; p = 0.02). Exposure to WA did not re-introduce VHS in C57BL/6 (80 μ l $109 \pm 27\%$ compared to baseline, n = 4) or Balb/c mice (80 μ l : $146 \pm 21\%$ compared to baseline ; n = 7).

Conclusions : *Citrobacter rodentium* induced transient visceral hypersensitivity in both Th1 predominant C57BL/6 and Th2 predominant Balb/c mice. Of note, the increased pain response lasted longer in the Th2 predominant Balb/c mice compared to the Th1 predominant C57BL/6 mice. Acute stress in the post-infectious phase however failed to induce VHS in both C57BL/6 and Balb/c mice. Based on these data, we propose that a Th2 background may be a risk factor to develop post-infectious symptoms during a longer period of time. To what extent this risk is further increased by psychological co-morbidity such as depression will be further studied in maternal separated mice.



EVIDENCE FOR HISTAMINE-MEDIATED SENSITIZATION OF TRPV1 SIGNALING IN SENSORY NEURONS IN MICE AND IBS PATIENTS. D. Balemans (1), Y. A. Alpizar (2), Y. Nasser (3), E. Valdez Morales (3), A. Moonen (1), C. Cirillo (1), S. Vanner (3), K. Talavera (2), P. Vanden Berghe (1), M.M. Wouters (1), G.E. Boeckstaens (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), University of Leuven, Belgium ; (2) Laboratory of Ion Channel Research, University of Leuven, Belgium ; (3) Gastrointestinal Diseases Research Unit (GIDRU), Queen's University Kingston, Canada.

Background & Aims : Mast cell activation and neuronal sensitization through TRPV1 have been proposed to underlie visceral hypersensitivity in patients with irritable bowel syndrome (IBS). We recently showed that 12 weeks of treatment with the H₁R antagonist ebastine significantly improved abdominal pain and global relief compared to placebo. As histamine released by mast cells activates afferent nerves through histamine 1 receptors (H₁R), and we recently showed increased pain responses to rectal application of capsaicin in IBS, we hypothesized that histamine could be one of the mediators sensitizing TRPV1, a mechanism prevented by H₁R blockade and potentially contributing to the therapeutic effect of ebastine.

Methods : The submucosal plexus was isolated from rectal biopsies taken from 16 healthy volunteers (HV) and 12 IBS patients and responses to exogenously applied capsaicin (0.1, 1 and 10 nM) and histamine (1, 10 and 100 μM) were monitored using calcium (Ca²⁺)-imaging. In addition, the effect of 10 μM histamine pre-incubation on the capsaicin response was studied in HV. In parallel, murine dorsal root ganglia (DRG) were isolated to study the calcium response to capsaicin (10-250 nM) in the presence of histamine or after overnight incubation with mucosal biopsy supernatant from HV and IBS patients. Pyrilamine (1 μM) was used as H₁R antagonist.

Results : Application of histamine and capsaicin evoked significantly higher peak amplitudes in submucosal neurons from IBS patients compared to HV (Table 1). Pretreatment with histamine significantly increased the peak amplitudes in response to capsaicin in submucosal neurons from HV (Table 1). This sensitization of TRPV1 by histamine was confirmed in mouse DRG neurons, an effect that was prevented by preincubation with pyrilamine (Table 2). In parallel studies, overnight incubation with HV supernatant spiked with 10 μM histamine significantly increased the response to capsaicin compared to control HV supernatant. Similarly, overnight exposure of DRG neurons to IBS supernatant significantly increased the capsaicin response, an effect that was reduced by pyrilamine (Table 2).

Conclusion : Using 3 different approaches, we provide clear evidence for the involvement of histamine in the increased sensitivity to capsaicin, a mechanism that most likely contributes to increased visceral pain perception in IBS. The therapeutic effect of the H₁R antagonist ebastine in IBS patients can at least partly be explained by interference with this pathway.

Table 1. — Submucosal neuronal activation in rectal biopsies (% response)

Histamine	HV	IBS	Unpaired T-test
1 μM	0.66 ± 0.19 (n = 8)	0.54 ± 0.10 (n = 9)	p = 0.77
10 μM	0.60 ± 0.10 (n = 14)	1.44 ± 0.45 (n = 12)	p = 0.03
100 μM	1.02 ± 0.26 (n = 15)	1.70 ± 0.48 (n = 12)	p = 0.11
Capsaicin	HV	IBS	Unpaired T-test
0.1 nM	0.31 ± 0.11 (n = 8)	0.97 ± 0.21 (n = 6)	p = 0.01
1 nM	0.47 ± 0.09 (n = 8)	0.90 ± 0.13 (n = 6)	p = 0.01
10 nM	0.65 ± 0.07 (n = 8)	0.83 ± 0.10 (n = 6)	p = 0.28
TRPV1 activation	Before Histamine	After Histamine	Paired T-test
	0.42 ± 0.08 (n = 7)	1.08 ± 0.26 (n = 7)	p = 0.016

n = number of subjects.

Table 2. — Activation of mouse DRG sensory neurons

TRPV1 activation	Krebs	+ Histamine	+ Histamine + Pyrilamine
(Δ[Ca ²⁺] (μM))	0.03 ± 0.01 (n = 126) (p = 0.0004) ¹	0.11 ± 0.02 (n = 85)	0.02 ± 0.01 (n = 109) (p = 0.0016) ²
Rectal biopsy supernatant	HV supernatant	+ Histamine	Unpaired T-test
(F340/380)	2.45 ± 0.24 (n = 32)	3.98 ± 0.24 (n = 46)	p < 0.0001
	IBS-D supernatant	+ Pyrilamine	Unpaired T-test
	3.90 ± 0.18 (n = 63)	2.73 ± 0.20 (n = 65)	p < 0.0001

n = number of neurons

¹ Krebs vs. + Histamine

² +Histamine vs. + Histamine + Pyrilamine.

BELGIAN PANCREATIC CLUB (BPC)

- P01 -

Invited Lecture

Epithelial to mesenchymal transition in pancreatic cancer.
V. Hervieu, CHU Hôpital Edouard Herriot, Lyon, France

- P02 -

SONIC HEDGEHOG AND GLI1 EXPRESSION PREDICT OUTCOME IN RESECTED PANCREATIC ADENOCARCINOMA. R. Maréchal (1,2), A. Calomme (1,2), F. Puleo (1,2), P. Demetter (3), L. Verset (3), J.B. Bachet (4), J.L. Van Laethem (1). (1) Department of Gastroenterology and Gastrointestinal cancer Unit, Erasme Hospital, Université Libre de Bruxelles, Belgium ; (2) Laboratory of Experimental Gastroenterology, Erasme Hospital, Université Libre de Bruxelles, Belgium ; (3) Department of Pathology, Erasme Hospital, Université Libre de Bruxelles, Belgium ; (4) Department of Hepato-Gastroenterology, Pitié Salpêtrière Hospital, APHP, Paris, France.

Background : Aberrant activation of the hedgehog (Hh) pathway is implicated in pancreatic ductal adenocarcinoma (PDAC) tumorigenesis. We investigated the prognostic and predictive values of four Hh signaling proteins and of the tumor stromal density.

Methods : Using tissue microarray and immunohistochemistry, we assessed four hedgehog signaling proteins in 567 patients from three independent cohorts of patients who underwent surgical resection for PDAC. In 82 patients, the tumor stromal index (SI) was calculated and its association with overall survival (OS) and disease-free survival (DFS) was investigated.

Results : Shh and Gli1 protein abundance were independent prognostic factors in resected PDACs ; low expressors for those proteins experiencing a better OS and DFS. The combination of Shh and Gli1 levels was the most significant predictor for OS and defined 3 subgroups of patients with different prognosis (Gli1 and Shh low ; HR set at 1 v 3.08 for Shh or Gli1 high v 5.69 for Shh and Gli1 high ; $P < 0.001$). The two validating cohorts recapitulated the findings of the training cohort. After further stratification of patients with lymph node status, the prognostic significance of combined Shh and Gli1 was maintained. The tumor SI was correlated with Shh levels and showed significant association with OS ($P = .023$).

Conclusions : Shh and Gli1 are prognostic biomarkers for patients with resected PDAC. They should be used in combination for an adequate selection of patients for anti-stromal therapies.

- P03 -

PREVALENCE AND DETERMINANTS OF HYPEROXALURIA IN CHRONIC PANCREATITIS. N. Demoulin (1), Z. Issa (1), F. Ausloos (1), R. Crott (1), M. Jadoul (1), P. Deprez (2). (1) Clin universitaires St-Luc, UCL, Brussels, Belgium ; (2) Cliniques Universitaires St Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Chronic pancreatitis (CP) has anecdotally been linked to enteric hyperoxaluria and acute oxalate nephropathy, both in native and transplanted kidneys, mainly due to loss of pancreatic function and steatorrhea.

Aim : to evaluate the prevalence and determinants of hyperoxaluria in a cohort of patients with chronic pancreatitis.

Methods : The study was approved by the Ethical Committee and proposed to all adult patients with CP seen at the gastroenterology day clinic in our hospital from 01/03/2012 to 31/10/2012. The diagnosis of CP was based on CT or MRI imaging. Pancreatic exocrine function was evaluated in all patients by fecal elastase-1 (a pancreatic enzyme) and fecal acid steatocrit (a reliable and inexpensive measure of steatorrhea on a spot stool specimen). Oxaluria was measured at two separate visits. Baseline characteristics (demographics, medications, physical examination) were recorded.

Results : 48 patients accepted to participate. The cohort was 96% Caucasian, 50% male and 40% diabetic (63% on insulin). Median BMI was 23.3 (14.5-36.2) kg/m². Etiology of CP was alcoholic in 29%, autoimmune, obstructive or hereditary in 19% of patients and idiopathic in 52%. 5/48 patients had a newly diagnosed CP. CC was diagnosed a median of 4.5 (0-27) years before inclusion and was/had been complicated (pseudocysts, bile duct or duodenal obstruction, pancreatic ascites, pleural effusion) in 18/48 patients. Estimated GFR was > 60 ml/min/1.73m² in all patients, 2/48 patients had ACR between 30 and 300 mg/g. 17% of patients had clinical steatorrhea and 48% were taking pancreatic enzyme supplementation.

11/48 (23%) of patients had hyperoxaluria (> 32 mg/g of creatininuria, median 49 (35-75)). Oxaluria was moderately correlated with clinical steatorrhea ($\rho = 0.46$), pancreatine treatment ($\rho = 0.32$) and fecal steatocrit ($\rho = 0.44$) and

inversely correlated with fecal elastase ($\rho = 0.42$). The 2 oxaluria measures were well correlated (correlation $\rho = 0.68$). Age, presence of diabetes, smoking and alcohol habits, BMI, HbA1c, vitamin A, D and E levels, INR and calciuria were not correlated with oxaluria.

Conclusions : To our knowledge, our study is the first to define the prevalence of hyperoxaluria in a cohort of patients with CP. Approximately $\frac{1}{4}$ patients had hyperoxaluria. These patients are possibly at risk of developing acute oxalate nephropathy and nephrolithiasis, especially in case of intercurrent hypovolemia. Clinical and fecal indices of steatorrhea were moderately correlated with oxaluria. Intensifying pancreatic enzyme supplements and treatment with calcium carbonate could be proposed in patients with hyperoxaluria.

- P04 -

SHORT TIME DRAINAGE OF PANCREATIC PSEUDOCYSTS. C. Ionita, G. Mavrogenis, A. Sibille, N. Kouinche, P. Warzee. Notre Dame, Charleroi, Belgium.

Endoscopic cystogastrostomy and cystoduodenostomy are now considered the gold standard in the management large, complicated, accessible pancreatic collections. Usually, a pair of double pigtail stents are positioned under endoscopic ultrasound (EUS) and fluoroscopic control and left in place for several months or years. The best time for stent removal is not yet established. Recently, fully covered self-expandable metallic stent (CSEMS) have been used in the drainage of pancreatic collections ; their large calibre may offer the possibility of a better drainage and allows revision for washing and necrosectomy if needed. Metal stent are usually removed 1 or 2 weeks after placement and replaced by plastic stents for weeks or months. **Material and Methods :** Between 2009 and 2013, 22 patients were considered for endoscopic drainage of pancreatic collections. In two cases no drainage was performed due to spontaneous resolution of the collection and the diagnosis of a serous cystadenoma, respectively. 20 complicated cysts were managed endoscopically. Transpapillary drainage was performed in 2 cases, transgastric or transduodenal drainage using double pig tail stents in 10 cases, simultaneous transgastric and transduodenal drainage using double pig tail stents in 4 cases. The drainage was successful in all cases but one (pseudo papillary solid pancreatic neoplasm). Since November 2012, CSEMS (Taewoong Nagi Stent Nitis 14x20mm) was used for drainage of pancreatic collections in 4 patients (sepsis in 3 cases, biliary compression in 1 case). In the first case, the stent was left in place for 20 days. In the last 3 patients the stent was removed after 8 days. No additional drainage was performed. Patients dramatically improved after metal stent placement, all collections disappeared and no complications was encountered. No recurrence of the collection was noticed at 7 days and 3 months follow up. These preliminary data suggest that in selected cases (large or infected collections) a short time endoscopic drainage with wide metal stents may be sufficient and safe. Larger studies are required to confirm these data.

- P09 -

Invited Lecture

Belgian consensus on chronic pancreatitis. Medical treatment : pain, malnutrition, pancreatic insufficiency, complications.

V. Putzeys, CHR La Citadelle, Liège, Belgium.

V. Putzeys (1), P. Gast (2), P. Pelckmans (3), E. van der Wijst (4), G. Hans (5), L. Plat (6), P.H. Deprez (7). (1) Dept of Gastroenterology and Hepatology, CHR Citadelle, Belgium ; (2) Department of gastroenterology, CHU Sart Tilman Liège ; (3) Division of Gastroenterology and Hepatology, UZA ; (4) Division of Gastroenterology and Hepatology - University Hospital of Antwerp ; (5) Multidisciplinary Pain Center – University Hospital of Antwerp ; (6) Erasme Hospital ; (7) Dpt of Hepato-gastroenterology, Cliniques univ St-Luc, Université catholique de Louvain.

There is no medical treatment specific for chronic pancreatitis, except the treatment of pain and of exocrine and endocrine insufficiency. All efforts should be done to support the patient to stop drinking and smoking.

Pain is the most disabling symptom in chronic pancreatitis but its treatment can be quite a challenge. The “Pain Ladder” approach is recommended for the treatment of pain, although paracetamol alone or in combination with NSAID’s is rarely sufficient. A weak opioid could be added as a next step. Anti-oxidants may be beneficial, but clinical studies have shown mixed results in clinical practice.

The treatment of pancreatic exocrine insufficiency is nutritional and medical. There is no specific diet that patients should be advised to, but they should keep on a well-balanced diet following the “food pyramid chart” with at least a normal fat intake. Enzyme replacement therapy benefits to symptomatic patients, but also in subclinical insufficiency. Enteric coated preparations should be taken during all meals or snacks containing fat. In adults, the initial dose recommended is 25,000-40,000 units lipase with every meal, and 25,000 units of lipase for snacks, to titrate up to a maximum of 10,000 units/Kg/day. Weight gain and improvement in symptoms are correlated to adequate enzyme use.

Data are lacking for evidence-based practice in patients with pancreatogenic diabetes. Insulin therapy is the preferred treatment for most patients but many T3cDM patients could also be initially treated with metformin as a drug of first choice.

- P10 -

Invited Lecture

Belgian consensus on chronic pancreatitis. Endoscopic treatment : pain, complications.
M. Delhay, Erasme University Hospital, ULB, Brussels, Belgium

M. DELHAYE, M. ARVANITAKIS. Erasme Hospital, ULB, Brussels, Belgium.

Pain is the symptom in chronic pancreatitis (CP) that most often requires treatment. It may be related to : 1) outflow obstruction of the main pancreatic duct (MPD) with resulting increased pressure within the ductal system and/or parenchyma or to : 2) complications including mainly pseudocyst(s), common bile duct (CBD) obstruction or cancer development.

Endoscopic treatment can be proposed as an initial approach, early in the course of painful CP.

Patients with uncomplicated painful CP associated with at least one calcification ≥ 5 mm located in the pancreatic head or body with upstream dilatation of the MPD can be selected for treatment by extracorporeal shock wave lithotripsy alone.

Pancreatic duct stenting is recommended in cases with a dominant stricture of the MPD located in the head of the pancreas and should be pursued for at least 12 months in patients with persistent pain relief.

It is recommended to assess the clinical response to MPD drainage within 6 to 8 weeks and to discuss therapeutic alternatives in a multidisciplinary team in case of unsatisfactory outcome.

Treatment of chronic pseudocysts should be attempted by EUS-guided transmural (TM) drainage as the first-line therapy, if drainage is required and if TM access is technically feasible.

For CP-related symptomatic biliary strictures, current recommendation includes the insertion of multiple 10F plastic stents for approximately 12 months in order to obtain stricture calibration in about 2/3 of patients.

In patients with an inflammatory mass in the head of the pancreas associated with a double stricture of the CBD and the MPD, pancreatic cancer should always be adequately searched for, including brush cytology/biopsy of strictures during ERCP and fine-needle aspiration during EUS.

POSTERS

- P13 -

EUS-GUIDED PANCREATIC PSEUDOCYST DRAINAGE : AN ASSESSMENT OF EFFICACY, SAFETY, LONG-TERM FOLLOW-UP. A. Krishnan, R. Ramakrishnan. Fortis Malar Hospitals, Chennai, India.

Introduction : Pancreatic pseudocyst is common complication of acute and chronic pancreatitis. While surgery is associated with significant complications and mortality, percutaneous drainage is associated with prolonged hospitalization and often times the need for other adjunctive treatment.

Aim : To Assess the safety and efficacy of single-step EUS-guided pseudocyst drainage, evaluate the technical Feasibility.

Methods : 69 patients who had undergone Single-step EUS guided drainage of pancreatic pseudocyst were included. Controlled radial expansion wire guided balloon dilation of the puncture tract was performed followed by insertion 10 Fr double pigtail stents were inserted into the pseudocyst from either the stomach or the duodenum in adults and 7F stents in children.

Results : The mean age of 39 years. Median size was 12.5 cm in diameter. 56 patients had infected and rest had non-infected pseudocyst. Stent placement was successful in all. The technical success rate was 100%, and the treatment success rate was 98.5%. 54 patients had cystogastrostomy and rest of the patients had cystoduodenostomy with cyst drainage. There was one case with perforation and required an emergency operation. 98.5% patients had complete resolution of a pseudocyst. The double pigtail stent was removed in all cases after median duration of 10 weeks. Regarding long-term outcomes, recurrence of a pseudocyst was not observed over a median follow-up of 58 weeks.

Conclusions : Single-step EUS-guided transmural drainage is safe and associated with high success rate. It can be the first choice for therapy of pancreatic pseudocyst with good technical feasibility, efficacy, and safety with long-term results are acceptable.

EUS GUIDED NECROSECTOMY AND CYSTOGASTROSTOMY WITH FCSEM STENT FOR PANCREATIC INFECTED NECROSIS. A. Krishnan, R. Ramakrishnan. Fortis Malar Hospitals, Chennai, India.

Introduction : Pancreatic pseudocyst with infected necrotic tissue is associated with a high rate of complications and death. Standard treatment is open necrosectomy but is associated with significant morbidity, mortality, and prolonged hospital stay. Endoscopic cyst drainage with necrosectomy is an alternative and less invasive technique.

Aim : To evaluate pseudocyst drainage with cystogastrostomy, endoscopic necrosectomy for infected pancreatic necrosis with fully covered self-expanding metallic stents (FCSEMS).

Methods : Patient details, disease severity scores, treatment procedures, length of hospital stay, and outcome were recorded. Patients proceed to intervention if infection is strongly suspected on clinical and radiological backgrounds or bacteriologically. After the necrosis cavity had been accessed with EUS, a large orifice was created and necrotic debris was removed using 15mm FCSEMS with large flares was deployed across the tract under radiological control. Completeness of the necrosectomy procedure was ascertained by endoscopy.

Results : A total of 12 patients with median age 39 were treated successfully. Median APACHE 2 score on presentation was 11. Two patients presented with organ failure and needed intensive care. Necrosis was successfully treated in all with median of 2 interventions. Complication included superinfection in one who made an uneventful recovery. After median of 5 weeks the metal SEMS was extracted by endoscopy. The patients have remained asymptomatic and median follow-up was 4 months.

Conclusion : Endoscopic necrosectomy and temporary cystogastrostomy with self-expanding metallic stent approach is feasible, safe, and effective in patient with infected pancreatic necrosis. The benefits of this approach, terms of less morbidity is conceivable and our report demonstrates that such an approach is feasible.

**BELGIAN SOCIETY FOR GASTROINTESTINAL ENDOSCOPY (BSGIE)
AND SMALL BOWEL GROUP**

- G01 -

Invited Lecture

Endoscopic ampullectomy : what, how and when or... not at all ?
W. Laleman, Leuven, Belgium

- G02 -

EUS-GUIDED FNA USING A 25-GAUGE PROCORE HISTOLOGY NEEDLE VERSUS A 22-GAUGE STANDARD CYTOLOGY NEEDLE. G. Mavrogenis (1), A. Sibille (1), B. Weynand (2), H. Hassaini (3), P. Deprez (4), C. Gillain (3), P. Warzee (1). (1) Notre Dame, Charleroi, Belgium ; (2) UCL, Mont-Godinne, Belgium ; (3) Grand Hopital de Charleroi, Charleroi, Belgium ; (4) Université Catholique de Louvain, Brussels, Belgium.

Introduction : A new 25-Gauge endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) needle has been developed which features a hollowed-out reverse bevel to trap core (ProCore™, Cook Medical). However, there are limited data on the differences in diagnostic yield between 25-Gauge procore needle and 22-Gauge standard EUS needle.

Materials and Methods : 29 patients were referred during a 10-month period (between December 2012 and September 2013) for EUS-guided FNA of solid lesions and lymph nodes adjacent to the upper GI tract. All patients signed informed consent forms. Each lesion was punctured once by both 25-Gauge Procore needle and 22-Gauge standard needle, using capillary sampling : 10-15 to-and-fro movements with minimal negative pressure by pulling the stylet slowly. Blinded pathologic and cytological analyses were conducted. Final diagnosis was based on cytological findings of malignant cells, pathological analysis of the surgical specimen, and/or radiological and clinical follow up. For comparison of continuous data, a paired t-test was performed if normal distribution was shown, and the Wilcoxon rank sum test was carried out if normality could not be demonstrated. McNemar's test was used for dichotomous categorical data (SPSS 17.0).

Results : A total of 33 EUS FNA procedures targeting masses of the pancreas (n = 19), liver (n = 2), bile ducts (n = 1), mediastinum (n = 1), stomach (n = 1) and lymph nodes (n = 9) were performed in 29 patients. No complications were encountered. Sensitivity and specificity for malignancy of the 25-Gauge Procore needle was 83.3% (95% CI, 64.5-93.6) and 100% (95% CI, 30.9-100) respectively, while for the 22-Gauge standard needle it was 80% (95% CI, 60.8-91.5) and 100% (95% CI, 30.9-100) respectively (Table 1). There was a reported difference between the two types of needles in terms of EUS visualization (p = 0.031), with the 22-G needle being significantly better ; however, no relevant difference regarding the amount of blood (p = 0.705), the quantity of material (p = 0.861), quality of cytology (p = 0.776) and pathological specimen (p = 0.273) was observed between needle types.

Conclusion : Both needles were safe and successful in terms of high diagnostic yield, with similar histo-cytological results.

Table 1. — Performance comparison of the two needles

	25-Gauge Procore Needle Median (Range)/Frequency	22-Gauge Standard Needle Median (Range)/Frequency	P
Cytological score	2 (0-3)	1 (0-3)	0.776¶
Histology score	1 (0-2)	1 (0-3)	0.273¶
Total score	3 (0-5)	3 (0-6)	0.709§
Macroscopic quantity	2 (1-3)	2 (1-3)	0.861¶
Blood amount	1 (1-2)	1 (1-3)	0.705¶
Difficulties in visualization	20%	0%	0.031*
Difficulties in use	12.1%	6.1%	0.687*
Sensitivity for malignancy	83.3%	80%	1.000*
Specificity	100%	100%	

¶ = Wilcoxon rank sum test, § = Paired t-test, * = McNemar's test.

EUS-FNA FOR LIVER LESIONS : COMPARATIVE RESULTS IN A LARGE SERIES OF MORE THAN 150 PATIENTS WITH PRIM. B. Alexandre (1), I. Borbath (2), C. Galant (2), C. Sampoux (2), J. Wallon (2), B. Weynand (3), T. Aouattah (4), C. Gillain (5), R. Yeung (6), C. Hubert (2), C. Remue (2), P. Goffette (2), P. Deprez (2). (1) Clinique Saint-Luc, Namur, Belgium ; (2) Clin universitaires St-Luc, UCL, Brussels, Belgium ; (3) UCL, Mont-Godinne, Belgium ; (4) Clinique St. Pierre, Ottignies, Belgium ; (5) Grand Hôpital de Charleroi, Hôpital Saint-Joseph, Gilly, Belgium ; (6) Centre Hospitalier de Jolimont-Lobbes., La Louvière, Belgium.

Introduction and aims : A large part of the liver is now accessible for EUS-FNA, but there is limited data in the literature evaluating efficacy, clinical relevance and safety of the technique.

Methods : We performed a 5-y review (2008-12) of all EUS-FNAs performed in primitive and secondary hepatic lesions. Results are shown as mean [range] and simple proportions for continuous and dichotomous variables respectively. Student's t test and χ^2 were used to test for differences in comparisons between continuous and dichotomous variables, respectively.

Results : 152 patients (81 M and 71 F) with a median age of 63 years (range 29-90) with 158 lesions were examined either for primitive masses (n = 50) or for metastases (n = 102). Liver EUS-FNA's were relevant with clinical impacts by providing cytohistological confirmation for 137 patients (90%) for both benign and malignant conditions. Results were significantly better (P < 0.05) in secondary vs primitive masses. It provided diagnosis for 15 patients without evident primary location, confirmed all suspected recurrences, upstaged pancreatic cancer TNM for 4 patients and provided positive strain culture in 4 abscess situations. 25 gauge needles were significantly less sensitive (P < 0.01) than 22 gauge needles. Single lesions (P < 0.05), smaller size and hilar location (P < 0.01) were more frequently associated with false negative results. Bleeding was the sole complication observed in 2.5% of patients, mainly in primitive lesions, and statistically associated with histology needles (P < 0.001) without significant impact from clinical risk factors including portal hypertension, abnormal coagulation, anticoagulant therapy and cirrhosis.

Conclusions : EUS-FNA in the liver is highly sensitive for the diagnosis of malignancy in both primitive and secondary masses with better outcomes in the second category and is helpful in determining the primary origin of liver metastases even in negative specimens from primary locations. Histology needles might be preferred for diagnosis but their used was associated with a small but significant increase of bleeding complications.

COMBINED ENDOSCOPIC AND ENDOBRONCHIAL ULTRASOUND FOR EVALUATION OF MEDIASTINAL LYMPH NODES. A. Badaoui (1), M. Delos (2), S. Ocak (2), T. De Ronde (3), B. Weynand (2), P. Deprez (4), P. Eucher (2), F. Duplaquet (2). (1) UCL, Namur, Belgium ; (2) UCL, Namur, Belgium ; (3) UCL, Namur, Belgium ; (4) UCL, Brussels, Belgium.

Introduction : Combined endoscopic ultrasound (EUS) and endobronchial ultrasound (EBUS) guided fine needle aspiration (FNA), is an accurate technique for evaluation of mediastinal lymph nodes and stadification of lung cancer. The combined technique is considered less invasive than mediastinoscopy.

Aim : The aims of the study are to evaluate the feasibility and the efficacy of the combined technique compared to mediastinoscopy for the diagnosis of mediastinal lesions.

Methods : All patients with suspected malignant mediastinal lymph nodes and/or lung lesion identified by PET-CT underwent combined EUS-EBUS-FNA in a single time procedure or separately. All patients underwent previously diagnostic flexible bronchoscopy and in 6 patients computed tomography guided-transsthoracic needle aspiration. The combined procedure was performed in outpatients under general anesthesia for EUS and sedation by intravenous Midazolam for EBUS when performed separately, using linear-array echoendoscopes (Hitachi/EG 3830UT, Pentax for EUS and Olympus UC-180F for EBUS). The mediastinal lesions were punctured during the EUS and EBUS-FNA procedures respectively with a 22-g (Cook Medical or Boston Scientific) and 21-g needles (Olympus).

Results : A total of 30 patients underwent prospectively EUS and EBUS-FNA between September 2011 and April 2013 (7 women and 23 men with a mean age of 66.3 y, range 51-83). Combined EUS-EBUS-FNA was performed in a single time procedure in 22 patients and in two different times in 8 patients (mean delay between procedures 3 days). Of the 30 patients, 13 were diagnosed non-small cell lung cancer (11 adenocarcinomas and 2 squamous cell carcinomas), 2 had small cell lung cancer, 1 had composite lung cancer and 1 had adenocarcinoma of unknown origin. Five patients presented metastatic diseases from colorectal adenocarcinoma, sarcoma, neuroendocrine tumor, renal carcinoma and lymphoma. Finally, we observed 1 sarcoidosis and 7 inflammatory lesions. Mediastinoscopy was performed in 7 patients and confirmed in 6 patients the initial combined EUS-EBUS-FNA diagnosis. In the remaining patient, mediastinoscopy diagnosed a neoplasia (renal carcinoma node metastasis). The diagnosis was obtained in 86.7% with EUS-FNA, 70% with EBUS-FNA and 96.7% when combined procedure was performed. The overall sensitivity, specificity, positive and

negative predictive values of EUS-EBUS-FNA for diagnosing malignancy were 95.65%, 100%, 100% and 87.5% respectively. Finally, the mean time of the procedure was 50 min and no complications related to the procedure were observed.

Conclusions : Combined EUS-EBUS-FNA is an accurate technique in the management and diagnosis of mediastinal lesions. Furthermore, these technique can be done in a single time procedure, has the advantage of being less invasive than mediastinoscopy and is associated with reduced costs.

- G05 -

Invited Lecture

Diagnosis and treatment of cholangiocarcinoma.
M. Giovannini, Marseille, France

- G06 -

EMR OF SPORADIC DUODENAL POLYPS IS ASSOCIATED WITH A HIGH RISK OF COMPLICATIONS. P. Martens (1), I. Demedts (2), S. Van Gool (3), H. Willekens (2), R. Bisschops (2). (1) University of Leuven, Leuven, Belgium ; (2) Gastroenterology, University Hospitals Leuven, Leuven, Belgium ; (3) AZ Sint-Jozef, Turnhout, Belgium.

Introduction : Endoscopic mucosal resection (EMR) is an established technique for the treatment of early neoplastic lesions in the colon, esophagus and the stomach. Sporadic duodenal adenomas (SDA) are a rare finding on endoscopy. Little data is available about the safety and efficacy of EMR for SDA in larger case series.

Aim : To report our experience with regard to the safety and efficacy of duodenal EMR for SDA.

Methods : Prospectively collected data of fifty nine patients (31 men, 28 female, mean age 61) referred for duodenal EMR to our center between 2006 and 2013, were analyzed. Only duodenal polyps were included in the study. Data regarding polyp size, location, endoscopic morphology, EMR technique, procedure time, complications, pathology result and periodical follow up were recorded. All patients underwent day after endoscopy to detect and treat delayed bleeding.

Results : Seventy-one duodenal EMRs were performed in fifty nine patients during the study period. The median polyp size was 15mm (range 7-40 mm). The success rate of complete endoscopic removal after a single EMR was 83%. Complete remission was achieved with 2 and 3 EMRs in 9 and 3 patients respectively. Complications occurred in 26% of the procedures. We encountered 10 cases of early bleeding (< 4 hours after EMR) and 10 cases of delayed bleeding (> 4 hours after EMR) with need of additional hemostatic measures, transfusion or radiological intervention and admission to intensive care. In one patient, a small perforation could be managed conservatively with clips. No patients were referred for rescue surgery. Except for 2 neuro-endocrine tumors, all lesions were adenomas with low grade dysplasia in 82% and high grade dysplasia in 18%. Long-term histological follow up (median : 18 months, range 12-50 months) was available in 30 patients, complete histologic remission was achieved in 25 patients (83%). Five patients revealed histologic arguments of residual adenomatous tissue, all showing low grade dysplasia. No tumor related deaths were reported.

Conclusions : This study is one of the largest available series confirming the efficacy of EMR for SDA. Duodenal EMR is efficient (83%) in achieving long-term complete histological remission. However morbidity (26%) seems higher for duodenal EMR as compared to EMR in other location within the gastrointestinal tract and in comparison to other smaller series. Our systematic approach of day after follow-up endoscopy could contribute to the higher morbidity rate with detection of non-significant late bleeding.

- G07 -

ENDOSCOPIC MANAGEMENT OF BARIATRIC SURGERY COMPLICATIONS : A SINGLE CENTER EXPERIENCE. N. Kouinche, C. Ionita, G. Mavrogenis, A. Sibille, Y. Hoebeke, B. Calicis, F. Corbisier, P. Warzee. Notre Dame, Charleroi, Belgium.

Introduction : Complications of bariatric surgery include anastomotic leaks, stenosis and fistulae. Initial management of post-surgical leaks consists in percutaneous drainage and antibiotics. Second line treatment includes a multimodal endoscopic approach by using a variety of clips, glues and stents. Anastomotic stenoses are usually managed by iterative balloon dilation and or stent placement. Biliary leaks are managed by stent placement however treatment is challenging due to the altered anatomy.

Methods : A total of 974 patients underwent bariatric surgery between 2008 and 2012 (Sleeve gastrectomy and Roux-en-Y gastric bypass). Among them, 27 patients were referred for endoscopic management of the following complications : stenosis (n = 11), anastomotic leaks (n = 12) and biliary leaks (n = 2).

Results : One patient with anastomotic leak was managed successfully with an Ovesco clip (Life Partners). 11 patients were treated with partially or full covered metal stents. A total of 15 stents (Ultraflex 18 x 150 mm (4), Endoflex (6), Taewoong Medical Niti-S, 20 x 100 mm(4), Life Partners Megastent (Barthet) (1)) were successfully placed. Two patients were lost at follow up. Fistula closure was observed in 8/9 patients (88%) after a mean duration of 36.4 days (range 5-70). Persistent blind fistula with secondary closure was seen in 1/9 patients (12%). Early migration occurred in 4/14 cases (28.5%). Longstanding nausea, vomiting, retrosternal pain and gastro oesophageal reflux occurred in all patients. Twenty-seven balloon dilations were performed in 12 patients with post-bariatric anastomotic stenoses. A mean of 2.2 sessions was required (range 1-4). Perforation occurred in 5/27 dilations (18%) : 1/5 at 11 mm balloon diameter, 2/5 at 12 mm, 2/5 at 15 mm. Perforations were managed by endoclips (Boston Medical) alone (1/5), endoclips and percutaneous drainage (1/5), endoclips and stent placement (1/5) and stent alone (2/5). One case of refractory stenosis was managed by metal stent placement. Migration occurred in 3/5 cases (60%). Treatment of biliary leaks was performed in 2 patients, 8 days after an access gastrostomy. Plastic biliary stents were placed for 49 days and 88 days respectively, leading to complete healing of the leak. One patient developed stenosis of the principal biliary duct, that was treated successfully by placement of a fully covered metal stent (Taewoong, Kaffes) for 91 days.

Conclusion : Metal stents are effective for treatment of post-bariatric anastomotic leaks and stenoses. Early migration rate is high, especially for stenoses but may be overwhelmed by clip stent stowage. Adverse effects of stent placement are common but can be managed by drugs and psychological support. Balloon dilation of anastomotic stenosis is effective, but perforation is frequent even in experienced hands. However, this later maybe managed endoscopically. A temporary gastrostomy access consists of an alternative method of biliary access for the management of post-bariatric biliary leaks.

- G08 -

UTILITY OF THE SINGLE OPERATOR CHOLANGIOSCOPY SYSTEM SPYGLASS : A SINGLE-CENTER COHORT STUDY. W. Laleman (1), K. Verraes (2), S. Van Der Merwe (1), W. Vansteenbergen (1), C. Verslype (1), F. Nevens (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium.

Background : Biliary strictures of uncertain clinical significance and failed conventional therapeutic endoscopic retrograde cholangiopancreatography (ERCP) remain challenging clinical situations with often no other option than referral for surgery. We previously reported on feasibility of the single operator cholangioscopy (SOC)-system SpyGlass in a small group of patients undergoing SOC for the purpose of optically guided biopsy or elektrohydraulic lithotripsy (EHL).

Aim : To investigate the clinical value of SOC by means of SpyGlass in a larger follow-up cohort.

Methods : All SOC-procedures were reviewed with regard to procedural success defined as the ability to 1) visualize target lesions/stone (primary success), 2) to collect biopsy specimens or initiate stone fragmentation by EHL, if indicated (secondary success) and 3) diagnostic/therapeutic accuracy. Additional evaluation was made with regard to occurrence of adverse events.

Results : Since 3-2010 until 10-2013, 74 SOC-procedures (median age 59, 36 males, on average 2 previous ERCPs before SOC) were performed either for diagnostic (n = 36, 10 primary sclerosing cholangitis (PSC) – 26 non-PSC) or therapeutic reason (n = 38, 36 biliary and 2 pancreatic stones). Overall primary and secondary success and accuracy were 97%, 87% and 83% respectively. More specifically, for difficult stones a therapeutic accuracy of 82% was obtained with a primary and secondary success of 95% and 85%, respectively. For “indeterminate stenosis” (n = 36), diagnostic accuracy aggregated to 85% : 10 patients were immediately re-classified (initially missed common bile duct stone, pseudo-stenosis or extrinsic compression), leaving 26 patients of which 22 could be correctly classified based on either visual impression (enforced by follow-up of 6 at least months), targeted biopsy or a combination of both. Neovascularization appeared the only macroscopic predictor of malignancy ($P < 0.05$). The accuracy rate of optically-guided biopsy was 79%. Clinical significant complication were observed in 13.5% of the patients : cholangitis (n = 6), mild pancreatitis (n = 3) and EHL-related intraductal bleeding (n = 1). All adverse events were reversible and treated conservatively.

Conclusions : Single operator cholangioscopy with the Spyglass-device was of therapeutic value in 80% of the patients that failed conventional interventional ERCP for large bile duct stones and improved the diagnostic success in more than 80% of the patients with an indeterminate stenosis. SOC with SpyGlass is therefore of clinical use and added value in these previously challenging situations.

(DIS)ADVANTAGES OF SINGLE-BALLOON ENTEROSCOPE TO PERFORM ERCP AFTER BILLROTH II GASTRECTOMY. T. Moreels, E. Macken, H. De Schepper, P. Pelckmans. Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium.

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 cannulation of the intact papilla.

Aim : Analysis of ERCP procedures performed with the single-balloon enteroscope (SBE) in Billroth II patients.

Methods : 17 Billroth II patients underwent SBE ERCP between 2009 and 2013. Technical aspects, outcome and complications were registered.

Results : All ERCP indications were common bile duct stones. Male / female ratio was 10/7 with mean age of 80 ± 2 and 74 ± 2 years respectively. Overall success (cannulation, papillotomy with or without papiloplasty and stone extraction) was 88% using dedicated accessories. The papilla was reached in all patients but cannulation was unsuccessful in 2 because of its location inside a duodenal diverticulum. In all other cases cannulation was possible with the forward viewing SBE. After cannulation, papillotomy was performed followed by progressive balloon dilation (from 8 to 16 mm final diameter) when necessary. Papiloplasty allowed direct cholangioscopy using the SBE in most patients. Transient non-serious complications (bleeding in papillotomy and biochemical post-ERCP pancreatitis) occurred in 2 patients each (24%) without clinical implications. However, 1 (6%) serious complication (fatal perforation of gallbladder) occurred because of a barotrauma during direct cholangioscopy and air insufflation in a closed limb system.

Conclusions : SBE is suitable to perform ERCP in patients with Billroth II gastrectomy. With the SBE the papilla is easily reached and using dedicated accessories papillotomy and stone extraction is feasible. However, perforation may occur due to increased air pressure inside the closed afferent limb and/or biliary tree. Deflating the overtube's balloon while in the afferent limb and the use of CO₂ may be necessary in order to prevent barotrauma in the closed limb system.

Invited lecture

The NBI-international endoscopic classification (NICE) of colorectal polyps. Interactive session on the recognition and classification of polyps.

T. Ponchon, Lyon, France

POSTERS

CLINICAL PROFILE AND NATURAL HISTORY OF SYMPTOMATIC ILEO-CECAL ULCERS. J. Toshniwal (1), R. Chawlani (1), M. Kumar (1), A. Arora (1), M. Sachdeva (1), K. Mönkemüller (2). (1) Sir Ganga Ram Hospital, New Delhi, India ; (2) Marienhospital, Bottrop, Germany.

Introduction : Ileo-cecal (IC) ulcers represent 15-50% of the total ulcers diagnosed during colonoscopy. Ulcerations in the IC region have a plethora of etiologies and outcomes with a unknown natural history.

Aim : This prospective study was intended to evaluate the etiology, clinical presentations and natural history of IC ulcers.

Methods : Patients diagnosed to have ulcerations in the IC region during colonoscopy were enrolled. Their clinical presentation and outcome at first visit were recorded. These patients were followed at every three month interval for presence of symptoms for atleast one year ;and colonoscopy was repeated in symptomatic patients.

Results : Out of 1632 colonoscopies performed in a tertiary care hospital from May 2010 to October 2011, 104 patients had ulcerations in the IC region. This population represents the study group. The median age was 44.5 years (range 18-85)and 59% were male. The predominant presentation was lower GI bleed (55.5%), pain abdomen +/-diarrhea (36.3%) or diarrhea alone (9.9%). Associated fever was present in 32 (31%) patients. The etiology of ulcers was classified into infective causes (43%), non-infective causes (29%), and non-specific ulcers (28%) at first visit. Three patients (3%) died (all had presented with bleed and had non-specific ulcers). These remaining 101 patients were followed at every 3 months for atleast one year. All patients with infective etiology were asymptomatic at one year follow-up. Of the 14 patients with crohn's disease 3 were symptomatic at 1 year follow-up ; all were on treatment with steroids. Of the 6 patients with malignant IC ulcers 3 expired ; while 3 were asymptomatic. Of the 29 patients with non-specific ulcers ; 3 were diagnosed

as tubercular IC ulcers at follow-up and responded to anti-tubercular treatment ; while 1 was diagnosed as Non-Hodgkins lymphoma and responded well to chemotherapy ; while 2 were diagnosed as Crohn's disease during follow-up and were asymptomatic at end of 1 year on medications

Conclusions : Infection was the most common (> 40%) cause of ulcerations of the IC region in the study. Cecal involvement and fever were important clues to infective cause. Majority of the patients (95/101) were asymptomatic at the end of one year ; while 3 patients with Crohn's disease were symptomatic at the end of one year. 50% of patient with malignant IC ulcers experienced mortality at end of one year. While 20%(6/29) patients with non-specific ulcers were reclassified in terms of etiology during the follow-up period of one year.

- G12 -

EXPANDING THE HORIZONS OF EUS : DIAGNOSIS OF NON-DIGESTIVE PATHOLOGIES. G. Mavrogenis (1), H. Hassaini (2), A. Sibille (1), S. Feloni (2), P. Deprez (3), C. Gillain (2), P. Warzee (1). (1) Notre Dame, Charleroi, Belgium ;(2) Grand Hopital de Charleroi, Charleroi, Belgium ;(3) Université Catholique de Louvain, Brussels, Belgium.

Introduction : Endoscopic ultrasound (EUS) is mainly used for the evaluation and sampling of mediastinal and abdominal lymph nodes, luminal and submucosal lesions of the upper and lower gastrointestinal tract as well as in the diagnostic approach of pancreatic, biliary and liver disease. However, several non-digestive pathologies may be encountered as well, expanding the diagnostic yield of EUS.

Aim : The purpose of this article is to review the capabilities of EUS beyond routine evaluation of gastrointestinal organs.

Methods : Retrospective review of our prospectively maintained image and video database of all EUS procedures performed between January 2011 and July 2013. The selection was restricted to cases with a clinically relevant endoscopic ultrasound observation concerning a non-digestive structure excluding lesions of the adrenal glands. Among cases with similar findings, the case with the best or most representative images was chosen.

Results : The following pathologies were diagnosed by means of EUS : Thyroid nodules, lung atelectasis, pleural effusion, lung mass, pulmonary embolism, tumoral invasion of the pulmonary artery, linitis plastica of the urinary bladder, aortic aneurysm, right heart failure.

Conclusion : With this study we would encourage endoscopic endosonographers to expand the horizons of EUS, beyond the limits of current practice. Diagnosis of non-digestive pathologies during endoscopic ultrasound requires a methodical and careful approach to EUS examination, but can yield important benefits for the patient.

- G13 -

SINGLE BALLOON ENTEROSCOPY ASSISTED ERCP : INDICATIONS AND RESULTS IN PATIENTS WITH SURGICALLY ALTERED ANATOMY. T. Aouattah, R. Yeung, H. Piessevaux, P. Deprez, Cliniques Universitaires Saint Luc, Bruxelles, Belgium.

Introduction : ERCP may be difficult to perform in patients with post-surgical altered anatomy. Various techniques have been described to access the bile ducts including EUS assisted bile duct access, laparoscopic and endoscopic hybrid procedures and double balloon assisted enteroscopy. Experience with single balloon assisted enteroscopy is more limited. The aim of our study was to report feasibility, safety and success rate with this latter technique.

Patients and methods : retrospective review including examinations performed between 2008 and 2013. The endoscope used was a SIF180 (Olympus Belgium). The procedures were performed under general anesthesia with intubation, supine or prone, and CO₂insufflation. Indications for ERCP were either biliary problems (cholestasis, choledocolithiasis, anastomotic strictures or tumors) or pancreatic indications (pancreatitis on anastomotic stenosis, suspected ampulloma or obstruction).

Results : A total of 76 procedures were performed in 62 patients, mean age 60 y (range 21-85) including 34 men and 28 women. The mean procedure time was 88 min (22-230). The papilla, biliary or pancreaticenteric anastomosis was reached in 62/76 procedures (81.5%). The inability to reach the bile ducts or pancreas were caused by tumor infiltration, intestinal adhesions and length of the Roux-en-Ylimb. Biliary and/or biliopancreatic catheterization was obtained in 54 cases (71%). Various therapeutic procedures were performed : sphincterotomy (n = 9), balloon dilations (n = 17), stone extractions (n = 14), and plastic or metal biliary stent placement (n = 20). Nine examinations proved to be normal. Two immediate complications (perforation of the small intestine) were successfully treated by surgery.

Conclusion : ERCP using a single balloon enteroscope in patients with post-surgery altered anatomy is feasible for diagnostic and therapeutic procedures in more than 70% of cases, showing that it may be considered as the first-line technique to get access to the bile or pancreatic ducts.

IBD RESEARCH GROUP (BIRD)

- I01-

Invited Lecture

Role of epithelial barrier / permeability in IBD.

L. Pastorelli, Milan, Italy

- I02 -

SILENCING ENDOTHELIAL PHD1 PREVENTS ENDOTHELIAL DYSFUNCTION AND DAMPENS MURINE COLITIS. S. Van Welden (1), D. Laukens (1), L. Devisscher (1), H. Devlies (1), K. Olievier (1), C. Correale (2), S. D'alessio (2), S. Danese (2), M. De Vos (1), P. Hindryckx (1). (1) Ghent University, Ghent, Belgium ; (2) Istituto Clinico Humanitas, Milano, Italy.

Introduction : Active inflammatory bowel disease (IBD) is characterized by extensive mucosal angiogenesis. However, these newly formed blood vessels are likely dysfunctional, as they are unable to resolve the inflammation-induced mucosal hypoxia. Prolyl hydroxylases (PHD1-3) are oxygen sensing enzymes that are actively involved in tumoral vascular dysfunction. We previously showed that the expression of PHD1, but not PHD2 and 3, is increased in inflamed biopsies of IBD patients.

Aim : The aim was to characterize endothelial dysfunction in IBD patients and to investigate the role of PHD1, 2 and 3 in the vascular endothelium during experimental colitis.

Methods : The expression of endothelial dysfunction markers was analyzed by qRT-PCR in inflamed and non-inflamed colonic biopsies from IBD patients and compared to samples from healthy controls and infectious colitis patients. Human colonic microvascular endothelial cells were isolated from resection specimens and subjected to TNF to mimic inflammatory angiogenesis. The expression of endothelial dysfunction markers and PHD isoforms was analyzed. We then generated endothelial specific PHD1, PHD2 and PHD3 knock-out mice and subjected these mice to dextran sulfate sodium (DSS)-induced colitis.

Results : Inflamed colonic biopsies from both UC and CD patients showed a significant up-regulation of the endothelial dysfunction markers ICAM-1, VCAM-1, vWF and VEGFR-2 (all $p < 0.0001$). Moreover, these markers all displayed a strong positive correlation with PHD1 ($r = 0.667$, $r = 0.792$, $r = 0.731$ and $r = 0.747$ respectively). TNF-stimulated endothelial cells showed a significant up-regulation of PHD1 ($p < 0.01$). In accordance, PHD1^{-/-} cko mice had significantly less weight loss ($p < 0.0001$), reduced colon shortening ($p < 0.01$) and a lower histological inflammation score ($p < 0.001$) during DSS-induced colitis, when compared to the littermate controls. Furthermore, the PHD1^{-/-} cko mice demonstrated a significant down-regulation of the endothelial dysfunction markers ICAM-1, VCAM-1, vWF and VEGFR-2 (all $p < 0.05$) in colonic lysates. Genetic inhibition of endothelial specific PHD2 and PHD3 had no effect on the course of DSS-colitis.

Conclusions : Our findings characterize a dysfunctional endothelial phenotype in IBD and show that selective silencing of PHD1 in microvascular colonic endothelial cells is sufficient to restore endothelial function and to dampen experimental colitis.

- I03 -

HELMINTH EGG ANTIGENS AND THEIR PROTECTIVE EFFECT ON COLITIS IN A MURINE TRANSFER MODEL. M. Heylen (1), N. Ruysers (1), S. Nullens (1), J. De Man (1), D. Schrijvers (2), I. Van Brussel (2), G. Schramm (3), P. Pelckmans (4), T. Moreels (4), B. De Winter (1). (1) Laboratory of Experimental Medicine and Pediatrics, Division of Gastroenterology, University of Antwerp, Antwerp, Belgium ; (2) Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium ; (3) Cellular Allergology, Research Center Borstel, Borstel, Germany ; (4) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Helminth-based therapy as a treatment strategy for inflammatory bowel disease is a well-established concept by now, however the search for immunomodulatory helminth-derived compounds and their mechanisms of action is still going on.

Aim : To determine whether *Schistosoma mansoni* Egg Antigens (SEA) could influence the course of colitis using a murine colitis transfer model.

Methods : Colitis was induced in immunodeficient SCID mice by the adoptive transfer of CD4⁺CD25⁻CD62L⁺ T cells ; controls were injected with PBS (Heylen *et al.*, *Inflam. Bowel Dis.* 2013 ;19 :967-976). Animals were treated twice a

week during 3 weeks with vehicle or SEA in a dose of 20 $\mu\text{g}/\text{injection}$ (i.p.) starting 2 weeks after adoptive transfer. The included groups were : control mice treated with vehicle (CONTROL n = 6) or SEA (CONTROL+SEA n = 6) and colitis mice treated with vehicle (COLITIS n = 7) or SEA (COLITIS+SEA n = 8). From the beginning of the treatment (week 2), colonic inflammation was assessed at different time points by clinical outcomes (bodyweight, stool consistency, mobility, piloerection) and an endoscopic scoring system. After sacrifice at week 4, lamina propria mononuclear cells (LPMC) were isolated from 2 pooled colons for flow cytometric T cell characterization.

Results : CONTROL and CONTROL+SEA mice gained weight during the experiment and showed no inflammation signs. COLITIS mice, on the contrary, lost $7.8 \pm 1.7\%$ of their body weight, while COLITIS+SEA mice only lost $5.4 \pm 1.7\%$. Clinical scores of COLITIS mice increased rapidly over time (2.9 ± 0.5 , 5.6 ± 0.5 , 5.7 ± 0.5 respectively at week 2, 3 and 4) whereas clinical scores of COLITIS+SEA mice remained significantly lower compared to COLITIS mice from week 3 onwards (1.9 ± 0.6 (P = 0.172), 3.4 ± 0.6 (P = 0.004), 3.8 ± 0.5 (P = 0.004) respectively at week 2, 3 and 4). Colonoscopic scores of COLITIS+SEA mice (week 2 : 2.1 ± 0.5 and week 4 : 5.0 ± 0.8) decreased compared to colonoscopic scores of COLITIS mice (week 2 : 2.9 ± 0.5 and week 4 : 6.3 ± 1.0). Analyses of LPMC showed a significant higher upregulation of CD3⁺CD4⁺ T helper (Th) cells in COLITIS+SEA mice ($22.1 \pm 2.9\%$) compared to COLITIS mice ($11.8 \pm 1.6\%$ (P = 0.003)). No CD3⁺CD4⁺ Th cells were upregulated in CONTROL and CONTROL+SEA mice as expected ($0.1 \pm 0.0\%$ and $0.2 \pm 0.0\%$ respectively). Within the CD3⁺CD4⁺ T cell population, the amount of cells expressing CD25 and Foxp3, which we assume to be regulatory T cells, was comparable in COLITIS+SEA mice ($1.6 \pm 0.4\%$) and COLITIS mice ($2.1 \pm 0.6\%$). Interesting differences were seen in the amount of CD3⁺CD4⁺ T cells producing IL-17, IL-4 or IL-10 between COLITIS and COLITIS+SEA mice (IL-17 : $4.1 \pm 1.4\%$ vs. $0.9 \pm 0.1\%$ (P = 0.064) ; IL-4 : $22.9 \pm 3.2\%$ vs. $34.4 \pm 1.9\%$ (P = 0.022) ; IL-10 : $1.9 \pm 0.1\%$ vs. $0.9 \pm 0.1\%$ (P < 0.001) respectively).

Conclusions : Treatment with SEA seems to reduce the severity of colitis, induced by the adoptive transfer of CD4⁺CD25⁺CD62L⁺ T cells in SCID mice. The underlying immunological mechanism is based on the suppression of the Th17 response and an increased Th2 response.

- I04 -

STATUS OF ER STRESS AND AUTOPHAGY IN CROHN'S DISEASE : TRANSLATING GENETICS TO FUNCTIONAL RESPONSES. K. Nys (1), W. Vanhove (1), I. Cleyne (1), M. Ferrante (1), G. Van Assche (1), P. Rutgeerts (1), P. Agostinis (2), S. Vermeire (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium.

Introduction : The imminent **Introduction** of new therapeutic classes for inflammatory bowel disease patients emphasizes the need for efficient personalized medicine. Large-scale studies have identified several stress and inflammatory signaling pathways to be important during Crohn's disease (CD) and ulcerative colitis (UC) pathogenesis. However, it remains unclear if and how the identified genetic variants correlate with a functional response.

Aim : Therefore, the aim of this study was to evaluate if CD-associated genetic variants can be translated into a characteristic functional response and if this response can in turn be used for a better categorization of patients.

Methods : We studied CD-associated genetic variants in innate immunity (*NOD2*), ER stress (*XBPI1*, *ORMDL3*) and autophagy genes (*ATG16L1*, *IRGM*, *ULK1*, *MTMR-3*, *LRRK2*), and investigated if patients carrying a low or a high number of genetic risk alleles for these variants behave differently for the functional readouts. Peripheral blood-myeloid cells were isolated from 182 individuals (36 healthy controls and 146 CD patients), all genotyped by immunochip, and exposed to an inflammatory stimulus (LPS), ER stress (thapsigargin) or autophagy modulation (inhibition with chloroquine).

Results : Compared to healthy individuals, CD patients showed a significant increase of LPS-induced cytokine levels [TNF, IL-6/10/1beta secretion : 29.4-139% median increase, p = 0.01-0.04] ; increased levels of Bip (300% basal median increase, p = 0.02 ; 173.1% median increase with added stress, p = 0.02), an important ER chaperone, and 22.1% increased accumulation of p62 (p = 0.04), an autophagy-related tagging protein, after autophagy inhibition. When comparing patients according to their genetic risk load, "high genetic risk" patients (> 7 risk alleles, corresponding to Q4 of the distribution of risk alleles ; n = 98) had a 4.6-45.1% increased release of TNF and IL-6/10/1beta after LPS exposure, increased ER stress (21.7% basal ; 39.9% with added stress ; Fig. 1) and 14.6% decreased autophagic activity (p = 0.03 ; Fig. 2) compared to 'low genetic risk' patients (< 4 risk alleles, corresponding to Q1 of the distribution of risk alleles ; n = 57). For individual risk loci such as *ATG16L1*, *IRGM*, *MTMR-3*, *XBPI1*, *ORMDL3* and *NOD2* an augmented LPS-induced cytokine release was observed with increasing risk alleles.

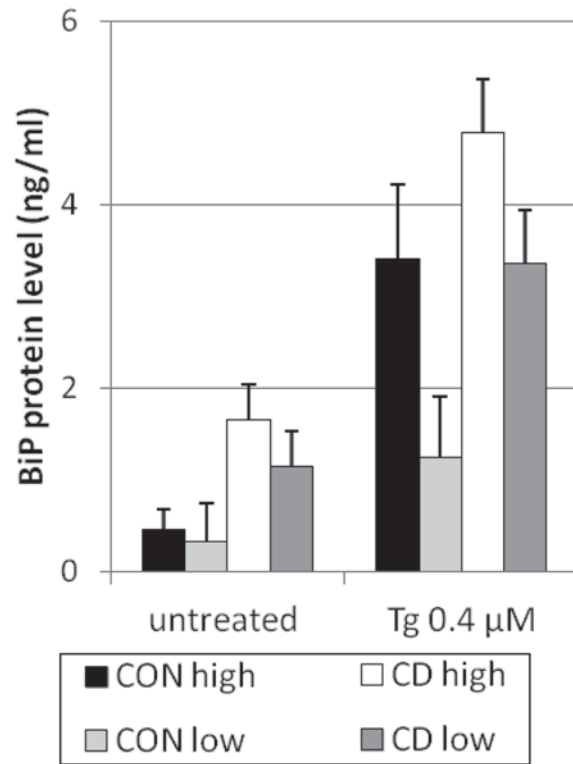


Fig. 1. — ER stress

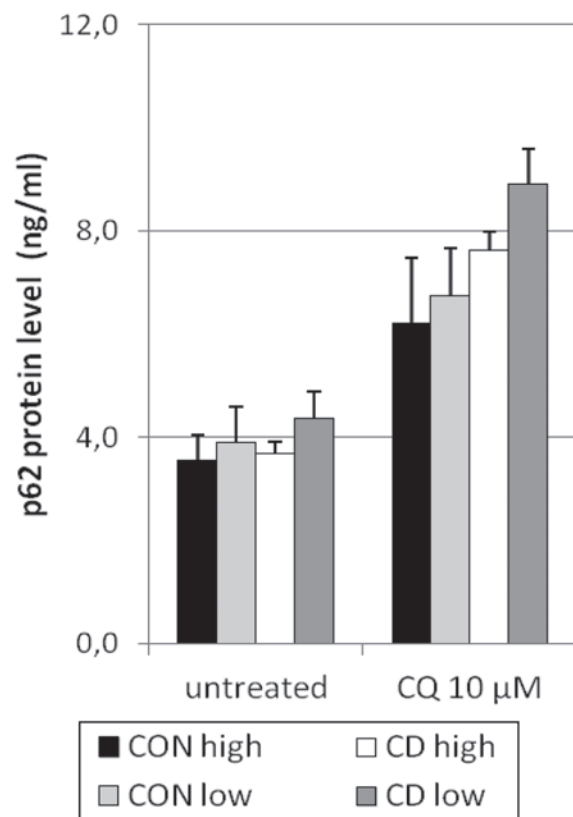


Fig. 2. — Autophagic rate

Conclusions : Our data suggest, for the first time, that blood-myeloid cells from CD patients typically show a more severe LPS-induced cytokine response, increased unresolved ER stress and increased autophagic demand. Our study also indicates that the burden of risk alleles, in these pathways or in individual susceptibility loci, correlates with the LPS-induced cytokine response, the level of ER stress and the autophagic rate. These findings highlight the promising potential of using these functional read-outs for personalized management of Crohn's disease.

Invited Lecture

Interplay between genetic and microbial factors in IBD.
I. Cleynen, KULeuven, Belgium.

REDUCED *B. PULLICAEORUM* LEVELS IN MUCOSA OF UC PATIENTS CORRELATE WITH ABERRANT CLDN1 EXPRESSION. S. Devriese, V. Eeckhaut, F. Van Immerseel, R. Ducatelle, M. De Vos, D. Laukens. Ghent University, Gent, Belgium.

Introduction : Butyrate maintains colonic homeostasis by modulating a wide variety of cellular functions including the control of intestinal epithelial integrity. *Butyrivibrio pullicaecorum* is a butyrate-producing bacterial strain that is found in reduced amounts in stool samples of patients with ulcerative colitis (UC) and is currently being investigated as a probiotic. Conditioned growth medium of *B. pullicaecorum* reduces TNF-induced colonic epithelial permeability *in vitro*, however its *in vivo* relevance is unknown.

Aim : To investigate the relationship between the presence of *B. pullicaecorum* in the colonic mucosa and the expression of tight junction protein 1 (TJP1), occludin (OCLN) and claudin 1 (CLDN1), essential components of the tight junction complex which are partially regulated by butyrate.

Methods : The expression of these genes was analyzed by quantitative real-time PCR (qPCR) in a collection of colonic biopsies from healthy controls (N = 21) and UC patients with active disease (N = 26). Next, the effect of the conditioned growth medium of *B. pullicaecorum* (strain 25-3^T) on the expression of these genes was investigated in HT-29 cells in the presence or absence of TNF. Finally, *B. pullicaecorum* bacteria were quantified in an extended cohort of colonic mucosa of UC patients (N = 36) and healthy controls (N = 31) using a genus-specific qPCR.

Results : TJP1 and OCLN were significantly downregulated in colonic biopsies of UC patients (both $P < 0.005$), whereas CLDN1 expression was increased ($P < 0.003$). The conditioned growth medium of *B. pullicaecorum* increased the baseline expression of TJP1 and OCLN but did not decrease CLDN1 levels in HT-29 cells. TNF did not affect expression of TJP1 or OCLN but increased CLDN1 expression which was counteracted by 21% after co-incubation with the conditioned growth medium. *B. pullicaecorum* could be detected in colonic biopsies of 71% of healthy controls and in only 42% of UC patients (Fisher exact $P = 0.026$). In addition, in samples where *B. pullicaecorum* was detected, the absolute amount was lower in UC samples ($P = 0.081$). Interestingly, the quantity of *B. pullicaecorum* correlated with the deregulated expression of CLDN1 ($R = -0.528$).

Conclusions : *Butyrivibrio pullicaecorum* is a mucus-adherent bacterium and is underrepresented in colonic biopsies of UC patients. Their reduced prevalence correlates with aberrant CLDN1 expression which can be reversed *in vitro* by the conditioned growth medium of *B. pullicaecorum*. Together, these data support a role for *B. pullicaecorum* in the preservation of colonic barrier integrity.

PREDOMINANT GUT MICROBIOTA PREDICT POUCHITIS FOLLOWING COLECTOMY AND IPAA IN ULCERATIVE COLITIS. K. Machiels (1), L. Vandermosten (1), M. Joossens (2), I. Arijs (1), I. Terrasson (3), V. Ballet (4), M. Ferrante (1), J. Verhaegen (4), K. Van Steen (5), A. De Buck Van Overstraeten (3), A. Wolthuis (3), A. D'hoore (3), P. Rutgeerts (1), S. Vermeire (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) VIB-Vrije Universiteit Brussel, City of Brussels, Belgium ; (3) University Hospitals Leuven, Leuven, Belgium ; (4) University Hospitals Leuven, Leuven, Belgium ; (5) Montefiore Institute, Liège, Belgium.

Introduction : Pouchitis is the most common complication after colectomy with ileal pouch anal anastomosis (IPAA) for ulcerative colitis (UC). The cumulative risk to develop pouchitis is approximately 50% and is the highest within the first year after surgery. The pathogenesis of pouchitis is not completely understood but the gut microbiota has been proposed to play a dominant role.

Aim : We hypothesized that the risk for pouchitis following IPAA can be predicted based on differences in the fecal microbial composition before colectomy.

Methods : We obtained 19 fecal samples from UC patients before colectomy and IPAA surgery. All patients were prospectively assessed post-IPAA for clinical and endoscopic activity at predefined time points : at month 1 ($N_{\text{pouchitis}} = 2$; $N_{\text{normal pouch}} = 17$), month 3 ($N_{\text{P}} = 4$; $N_{\text{NP}} = 15$), month 6 ($N_{\text{P}} = 6$; $N_{\text{NP}} = 12$) and month 12 ($N_{\text{P}} = 5$; $N_{\text{NP}} = 8$). The

predominant microbiota was analyzed using denaturing gradient gel electrophoresis (DGGE) analysis followed by sequencing of purified bands. Kaplan–Meier log-rank testing was performed to study occurrence of pouchitis with respect to presence and absence of the identified species. The R statistical package “pvclust” was used to detect hierarchical clusters.

Results : In patients developing pouchitis, an increase of *Ruminococcus gnavus* ($p = 0.002$), *Bacteroides vulgatus* ($p = 0.026$), *Clostridium perfringens* ($p = 0.017$) and a reduction of 2 genera (*Blautia* ($p = 0.077$), *Roseburia* ($p = 0.016$)) classified to the family Lachnospiraceae was seen compared to patients with normal pouches. The cumulative risk for pouchitis one year following IPAA was clearly increased in patients where *R. gnavus* (78% vs. 0%, $p = 0.007$), *B. vulgatus* (67% vs. 33%, $p = 0.071$) and *C. perfringens* (100% vs. 27%, $p = 0.001$) were present before colectomy when compared to absence of the species. Conversely, the cumulative pouchitis risk was decreased in presence of *Blautia* genus (20% vs. 100%, $p = 0.012$) and *Roseburia* genus (0% vs. 70%, $p = 0.024$) compared to absence of the genera. Hierarchical clustering revealed 5 subgroups of patients (p -values = 100%), each with a similar combination in presence/absence of the microbial predictors. Two clusters correspond well to patients developing pouchitis, 2 clusters to patients with normal pouches and 1 cluster to mixed outcomes after 1 year follow up ($p_{\text{Fisher's exact}} = 0.06$).

Conclusions : Predominant presence of *R. gnavus*, *B. vulgatus*, *C. perfringens* and absence of members of the Lachnospiraceae genera *Blautia* and *Roseburia* in fecal samples of UC patients before surgery is associated with a higher risk of pouchitis within the first year after IPAA. Our findings shed new light on the etiology of pouchitis and may lead to new predictive and therapeutic strategies.

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IDENTIFICATION OF MICRORNA LANDSCAPE OF INFLAMMATION-INDUCED COLORECTAL CARCINOGENESIS. N. Bouznad, C. Josse, P. Geurts, A. Irrthum, V.A. Huynh-Thu, A. Hego, L. Servais, P. Delvenne, V. Bours, C. Oury. University of Liege, Liège, Belgium.

Introduction : MicroRNAs are short non-coding single-stranded RNAs which are highly conserved among mammals. MiRNAs modulate gene expression by destabilizing mRNA and/or inhibiting translation. MiRNAs are transcribed and processed in response to extracellular stimuli, or during developmental stages, in a tightly regulated manner. They are also implicated in many processes, including inflammation and cancer. Patients diagnosed with inflammatory bowel disease (IBD) have an increased risk of developing a colorectal cancer. Inflammatory conditions are increasingly being acknowledged to contribute to tumor formation ; however, there is a limited understanding of mechanisms that are involved in the transition from intestinal inflammation to cancer.

Aim : We used the well-established Azoxymethane (AOM)/Dextran Sulfate Sodium (DSS) mouse model of colitis-associated cancer, to analyze microRNAs modulation accompanying inflammation-induced tumor development and to determine whether inflammation-triggered miRNA alterations affect the expression of genes or pathways involved in cancer.

Methods : Male C57Bl/6J mice (five-weeks-old) were given a single intraperitoneal injection of Azoxymethane (12 mg/kg). Chronic inflammation was then induced by three cycles of DSS (2%) administration via the drinking water for five days, followed by 16 days of regular water. In this model, colorectal tumors develop after 65 days in all animals.

Results : A miRNA micro-array experiment was performed to establish miRNA expression profiles in mouse colon at early and late time points during inflammation and/or tumor growth. Chronic inflammation and carcinogenesis were associated with distinct changes in miRNA expression. Nevertheless, prediction algorithms of miRNA-mRNA interactions and computational analyses based on ranked miRNA lists consistently identified putative target genes that play essential roles in tumor growth, or that belong to key carcinogenesis-related signaling pathways. We identified PI3K/AKT and the insulin growth factor-1 (IGF-1) as major pathways being affected in the AOM/DSS model.

DSS-induced chronic inflammation downregulates miR-133a and miR-143/145, which is reportedly associated with human colorectal cancer and PI3K/AKT activation. Accordingly, conditioned medium from inflammatory cells decreases the expression of these miRNA in Human colorectal adenocarcinoma Caco-2 cells. Overexpression of miR-223, one of the main miRNA showing strong Upregulation during AOM/DSS tumor growth, inhibited AKT phosphorylation and IGF- 1R expression in these cells.

Finally, cell sorting from mouse colons delineated distinct miRNA expression patterns in epithelial and myeloid cells during the periods preceding and spanning tumor growth.

Conclusions : Based on our microarray data, networking, and target prediction analyses, as well as *in vitro* and *in vivo* validation experiments, we propose a model in which chronic inflammation could induce cell-type specific miRNA dysregulation and subsequent PI3K/AKT activation may be involved in the transition from intestinal inflammation to cancer.

- I09 -

Invited Lecture

Interplay between inflammation and carcinogenesis in IBD.
G. van den Brink, Amsterdam, The Netherlands.

- I10 -

Invited Lecture

How can I optimize screening colonoscopy in UC ?
E. Louis, ULiège, Belgium.

- I11 -

Interactive case 1 on endoscopy / pathology.
P. Bossuyt, Bonheiden, Belgium.

- I12 -

Interactive case 2 on endoscopy / pathology.
Denis Franchimont, Erasme Hospital, ULB, Brussels, Belgium.

- I13 -

NATURAL HISTORY OF NON-SEVERE INFLAMMATORY BOWEL DISEASES AT DIAGNOSIS. C. Pirard (1), E. Louis (2), C. Reenaers (2). (1) Centre Hospitalier Universitaire de Liège, Liège, Belgium ; (2) CHU Sart Tilman, Liège, Belgium.

Introduction : Crohn's disease (CD) and ulcerative colitis (UC) are progressive diseases characterized by the occurrence of complications requiring immunomodulators and surgery. Few data are available for the prevalence and the factors associated with long-term non-severe (NS) inflammatory bowel diseases.

Aim : Our aim was to assess the natural history of NS CD and NC UC at diagnosis and to identify predictive factors of mild evolution over the long term.

Methods : A retrospective study of the IBD patients registered in the database of the university hospital of Liège, Belgium. NS CD was defined as the absence of stricturing, penetrating or perianal disease, no treatment with immunomodulators and anti-TNF, no need for surgery in the course of the disease. NS UC was defined as no requirement for immunomodulators, anti-TNF and colectomy. Patients were assessed at 1 year, 5 years and at the maximum follow-up. Patients with less than 5 years of follow-up were excluded.

Results : Among 887 patients, a subgroup of 439 CD and 173 UC were included with a mean follow-up of 19 and 15 years respectively. One year after the diagnosis 147 CD patients had NS CD. At 5 years and at the maximum follow-up respectively, 83/147 (56%) and 15/147 (10%) patients still had NS CD. Complications were strictures (29%), fistulizing disease (18%), perianal disease (37%). Immunomodulators and anti-TNF were required in 79% and 54% of patients respectively. Prognostic factors for persistent NS CD were older age at diagnosis (38 vs 26 years, $p = 0.005$), no corticosteroid during the first year ($p = 0.036$). In UC, 142 patients had NS disease one year after the diagnosis. 102/142 (72%) and 62 patients (44%) had NS UC after 5 years and at the maximum follow-up respectively. Surgery occurred in 19 patients (13%) after a mean time à 164 months. Immunomodulators were needed in 66 patients (47%) and anti-TNF in 37 patients (26%). NS UC was associated with absence of hospitalization for active UC over the first 5 years ($p = 0.009$) and during the total course of UC ($p < 0.0001$), no intake of corticosteroid during the first year ($p = 0.03$).

Conclusions : In our cohort representing referral centre recruitment, nearly all CD patients and 2/3 of UC with NS disease at diagnosis became severe with time. Old age at diagnosis was associated with NS CD outcome while absence of hospitalisation during the first year was associated with NS UC outcome. Absence of steroid use during the first year was associated with NS outcome in both diseases.

THE MODIFIED MAYO ENDOSCOPIC SCORE (MMES): A NEW SCORE FOR THE ASSESSMENT OF EXTENSION AND SEVERITY. T. Lobaton (1), T. Bessissow (2), G. De Hertogh (3), G. Van Assche (1), S. Vermeire (1), P. Rutgeerts (1), R. Bisschops (1), A. Bitton (2), W. Afif (2), M. Ferrante (1). (1) UZ Leuven, Leuven, Belgium ; (2) McGill University, Montreal, Canada ; (3) Pathology, UZ Leuven, Leuven, Belgium.

Introduction : Complete mucosal healing (MH) has become a major endpoint in clinical trials. However, in Crohn’s disease, partial mucosal healing has also been associated with a better long-term outcome. Since current endoscopic activity scores for ulcerative colitis (UC) do not take into account the extent and distribution of mucosal inflammation, the effect of partial MH in UC has not been assessed.

Aim : As a first step, we developed a simple score for UC endoscopic activity, taking into account extent and distribution of mucosal inflammation.

Methods : During endoscopy, the colon was divided into five segments and for each one the operator reported the Mayo endoscopic subscore. The Mayo endoscopic subscores for each segment separately were added to give a Modified Score (MS). The Extended Modified Score (EMS) was obtained by multiplying the MS by the maximal extent of inflammation (in decimeters). The Modified Mayo Endoscopic Score (MMES) was obtained by dividing the EMS with the number of segments with active inflammation (excluding cecal patch). Colon biopsies were obtained from rectum and sigmoid, as well as from all inflamed segments. Clinical activity was scored according to clinical Mayo partial score and symptomatic remission was defined as Mayo stool frequency subscore of 0 or 1 and Mayo rectal bleeding subscore of 0. Biological activity was scored according to C-reactive protein (CRP) and fecal calprotectin levels (FC). Histological activity was scored according to the Geboes’ score (GS). Cutt-off values for active disease were CRP > 5 mg/dL, FC ≥ 250 µg/g and GS ≥ 3.1 (presence of neutrophils in the epithelium).

Results : 98 UC patients from 2 hospitals were included. Patient’s characteristics are collected in Table 1. Correlations between endoscopic activity scores, clinical Mayo score, FC, CRP and GS are shown in Table 2. Mean MMES scores were significantly higher in patients with clinical activity, CRP > 5 mg/dL, FC ≥ 250 µg/g and GS ≥ 3.1 (Table 3).

Table 1. — **Patient’s characteristics: median (IQR)**

Age (years)	47 (35.8-56.3)
Disease duration (years)	11 (5-21)
Clinical activity (Mayo subscore)	0 (0-3)
Mayo endoscopic subscore	1 (0-2)
Modified Mayo Subscore	1 (0-3)
Extended Modified Score	0.6 (0-12)
Modified Mayo Endoscopic Score	0.6 (0-6)
CRP (mg/L)	2.1 (0.6-6)
Fecal calprotectin (µg/g)	180 (100-357)
Histological activity (Geboes score)	3 (0-5)

Table 2. — **Spearman correlations between clinical, biologic, endoscopic and histologic activity**

	Clinical Activity (Partial Mayo score)	Fecal Calprotectin (µg/g)	CRP (mg/L)	Histological activity (Geboes Score)
Mayo endoscopic subscore	0.618 p < 0.001	0.549 p < 0.001	0.319 p = 0.002	0.734 p < 0.001
MS	0.629 p < 0.001	0.554 p < 0.001	0.244 p = 0.020	0.689 p < 0.001
EMS	0.551 p < 0.001	0.535 p < 0.001	0.250 p = 0.017	0.652 p < 0.001
MMES	0.524 p < 0.001	0.534 p < 0.001	0.276 p = 0.008	0.665 p < 0.001
Histological activity (Geboes Score)	0.567 p < 0.001	0.525 p < 0.001	0.178 p = 0.199	
CRP (mg/L)	0.205 p = 0.051	0.481 p < 0.001		
Fecal Calprotectin (µg/g)	0.533 p < 0.001			

Table 3. — Association of MMES scores with clinical, biological and histological activity

	Mayo endoscopic subscore	MS	EMS	MMES
Geboes score (mean ± SD)				
< 3.1	0.3 ± 0.8	0.5 ± 1.1	2.1 ± 8.8	0.7 ± 1.9
≥ 3.1	1.9 ± 1.2 (p < 0.001)	3.3 ± 2.9 (p < 0.001)	15.3 ± 22.8 (p = 0.002)	6.0 ± 6.5 (p < 0.001)
FC (µg/g) (mean ± SD)				
< 250	0.6 ± 0.8	0.9 ± 1.4	3.9 ± 9.9	1.8 ± 4.2
≥ 250	1.9 ± 1.3 (p < 0.001)	3.5 ± 3.1 (p < 0.001)	17.6 ± 24.6 (p = 0.009)	6.4 ± 6.6 (p = 0.002)
CRP (mg/L) (mean ± SD)				
< 5	0.9 ± 1	1.4 ± 1.8	5.8 ± 11.6	2.5 ± 4.4
≥ 5	1.9 ± 1.3 (p = 0.003)	3.9 ± 3.7 (p = 0.006)	22.3 ± 29.8 (p = 0.023)	6.3 ± 5.9 (p = 0.012)
Clinical activity (mean ± SD)				
No	0.6 ± 0.8	0.9 ± 1.4	4.5 ± 9.9	2.2 ± 4.5
Yes	2 ± 1.1 (p < 0.001)	3.9 ± 3.1 (p < 0.001)	18.5 ± 25.2 (p = 0.003)	6.3 ± 5.9 (p = 0.001)

Conclusions : Correlations between the new endoscopic activity scores and the clinical, biological and histological activity were as good as those with the Mayo endoscopic subscore. A longitudinal analysis that assesses the accuracy of evolution in MMES scores to predict long-term response to treatment is ongoing.

- I15 -

CLINICAL OUTCOME OF PERIANAL CROHN'S DISEASE AND IMPACT OF TREATMENT STRATEGIES OVER THE TIME. A. Natalis (1), E. Louis (2), C. Vankemseke (1), L. Seidel (1), J. Belaiche (2), C. Reenaers (2). (1) CHU Liege, Liège, Belgium ; (2) CHU Sart Tilman, Liège, Belgium.

Introduction : Perianal Crohn's disease (pCD) is associated with complications leading to recurrent surgery and tissue damage. Immunosuppressive drugs (IS) including anti-TNF have changed the management of pCD.

Aim : Our aim was to describe the management and the natural history of a cohort of patients with active pCD and to identify predictive factors of poor evolution.

Methods : A retrospective study of pCD patients registered in the database of the university hospital of Liège, Belgium. Perianal lesions included abscess, fistulae, anal fissure, anal strictures. pCD treatments included antibiotics, surgical drainage (with or without seton), stoma. Medical treatments including IS and anti-TNF were recorded at pCD diagnosis and over follow-up. pCD relapse was defined as antibiotherapy for recurrent abscess, the need for surgical drainage or stoma. The subgroups of patients followed before (old cohort) and after (young cohort) the year 2000 were compared in a subanalysis.

Results : 181 patients with pCD were included. Mean follow-up was 7.9 years Mean time between CD and pCD diagnosis was 6.3 years. Lesions at pCD diagnosis were abscess in 93/181 (51%), fistula in 91/181 (50% ; 77/93 of complex fistulae), anal fissure in 28/181 (15%), anal stricture in 18/181 (10%). At diagnosis abscess drainage was performed in 31/181 (17%), drainage + seton in 44/181 (24%), stoma in 18/181 (10%). 132/181 (74%) and 83/181 (47%) had IS and anti-TNF respectively at pCD diagnosis. Relapse rate was 51% within a mean time of 33 months. During follow-up 15% required a stoma. Predictive factors of relapse were perianal abscess (p < 0.0001, HR = 4.4), fistula (p < 0.0001, HR = 4.5) or surgical drainage at diagnosis (p < 0.0001, HR = 4.5), young age at pCD diagnosis (28 versus 31 yo, p = 0.02), short time between CD and pCD diagnosis (5.7 versus 7 years, p = 0.01), IS (p = 0.04, HR = 1.8) and anti-TNF (p = 0.01, HR = 1.5) at pCD diagnosis. Anti-TNF during follow-up, time to introduce them and duration of anti-TNF treatment were not predictive of relapse. The young and old cohort had the same characteristics at pCD diagnosis except a higher use of IS (87% vs 48%, p < 0.0001) and anti-TNF (3% vs 68%, p < 0.0001) in the young cohort. Clinical outcome including the time to relapse, type of relapse, need for surgery and stoma was similar in both cohorts.

Conclusions : In our cohort of pCD patients half of them had a perianal relapse over the time requiring surgery in more than 2/3 of them. At pCD diagnosis perianal abscess, fistula, surgical drainage, young age, treatment with IS or anti-TNF were associated with a higher risk of relapse. Although higher prescription of anti-TNF and IS in the last years new treatment strategies have not impacted the outcome of pCD.

Invited Lecture

Thiopurine Metabolites “Coming of Age” – When, How, In Whom ?

X. Roblin, Department of Gastroenterology, University Hospital of St-Etienne, France

In the big picture, we should be questioning the role of thiopurine monotherapy given recent data. Two recent randomized control trials concluded that early azathioprine was no more effective than placebo (AZTEC study) or conventional management for newly diagnosed Crohn’s Disease (RAPID study). Moreover, Colombel et al reported that combination therapy (IFX-azathioprine) was more effective than IFX or thiopurines alone in CD patients naïve to immunosuppressive therapy. Finally, in a large prospective, observational cohort study of 19,486 patients (CESAME study), the authors have confirmed an increased risk of lymphoma and non-melanoma skin cancer (NMSC) in IBD patients treated with Thiopurines. But when we look at Thiopurines in IBD in the local picture, we need them and need to know how best to use them. In a recent meta-analysis, treatment with thiopurines reduced the need for first surgical resection in Crohn’s Disease. Moreover, the team of Saint Antoine reported long-term outcomes of Crohn’s Disease (CD) patients who respond to azathioprine. In this single-centre, case-control study of 220 CD patients, 38% of patients maintained clinical remission at 10 years. We know, actually, the metabolism of thiopurines. In a recent meta-analysis, 6-TGN levels above 230-260 pmoles was associated with 2-fold higher rate of clinical remission in IBD. Moreover, 6-MMP levels correlate with hepatotoxicity.

Firstly, when should you measure thiopurine metabolites ? Certainly, in patients failing an adequate weight-based dose and duration (3 months) of thiopurine therapy, in patients experiencing adverse events to thiopurine therapy and finally in patients when non-adherence is suspected. In the future, it could be interesting to analyse a role for earlier metabolite testing to proactively optimise initial dose escalation rather than reactively act to treatment failures or adverse events. If we have two randomized control trials which concluded that 6-thioguanine nucleotide-adapted azathioprine therapy does not lead to higher remission rates than standard therapy, these two studies presented strong bias and so, we can’t conclude really that an adapted dose according to 6-TGN is not useful. Secondly, When *Isn’t* There Always Time to Optimise Thiopurine Monotherapy ? Probably when predictors of a severe disease phenotype mean prompt anti-TNF therapy is needed. Finally, we must measure both 6TGN and 6MMP and ideally we must repeat after 4 weeks to confirm optimization. Thirdly, is it interesting to analyse TPMT prior beginning treatment ? The percentage of leucopenia is significantly decreased with AZA dose depending of TPMT genotype. If TPMT activity could be interesting to isolate patients with low TPMT activity corresponding to genotype mutation, we thought that moreover, TPMT activity isolated shunters (refractory to thiopurines) but, in fact, high TPMT activity is not the major reason of « shunters ». So, we can suggest an algorithm based on 6-TGN and 6MMP levels (Table 1).

A lot of data reported that we can optimise outcome by co-prescription of allopurinol with Thiopurines specially in «shunters». Moreover, adjunctive allopurinol may improve thiopurine tolerability. If the 6-TGN levels increased and conversely the 6-MMP levels strongly decreased under allopurinol, we know recently that the mechanism of 6MMP decreasing is not linked to a decrease TPMT activity by allopurinol but by 6-Thioxanthine (6-TX) which reduces 6-MMP Levels. In the future, it could be very interesting to analyse the impact of metabolism of thiopurines in IBD patients treated with combination therapy.

	Metabolite Result*	Interpretation	Action Recommended	Approximate Prevalence
Group 1	No/very low 6TGN (< 50) No/very low 6MMP (< 50)	Non-adherence	Educate	10%
Group 2	Low 6TGN (< 260) Low 6MMP (< 5,700)	Underdosed/ Rapid metabolizers	Dose escalate	30%
Group 3	Low 6TGN (< 260) High 6MMP (> 5,700)	Thiopurine shunter	Add allopurinol and dose reduce to 25% of original thiopurine dose	10%
Group 4	Therapeutic 6TGN (260-450) Low or high 6MMP	Refractory	Change therapy	40%
Group 5	High 6TGN (> 450) Low or high 6MMP	Over-dosed or refractory	Consider dose reduction or change in therapy	10%

PREDICTORS OF IBD EXACERBATIONS AFTER LIVER TRANSPLANTATION IN PATIENTS WITH PSC AND IBD. N. Schoofs, R. Aerts, D. Monbaliu, J. Pirenne, W. Van Steenberghe, W. Laleman, C. Verslype, F. Nevens, G. Van Assche, J. Fevery. University Hospitals Leuven, Leuven, Belgium.

Introduction : In patients with primary sclerosing cholangitis (PSC) and concomitant inflammatory bowel disease (IBD), a more aggressive course of IBD after orthotopic liver transplantation (OLT) has been reported. Several hypotheses have been put forward to explain these findings, but so far, definite risk factors have not been established.

Aim : We aimed to identify risk factors for worsening of IBD after OLT in a single referral/transplant center cohort.

Methods : Between 1983 and 2012, 46 (36 males) patients with PSC underwent OLT. Three had a total colectomy before OLT and 2 died in the early post-OLT period, leaving 41 patients at risk. Of these, 23 had pre-existing or concomitant IBD. Median age at diagnosis of PSC was 38 years (range 12 to 65). The IBD disease course and all immunosuppressive therapy was prospectively recorded after OLT. Significant IBD exacerbations were defined as symptomatic flares within 2 years of OLT requiring change in medical therapy and/or hospitalization. Possible contributing factors for exacerbation of IBD were investigated by a retrospective analysis of our database.

Results : After OLT, 10 of 23 patients (43.5%) had a significant exacerbation of IBD, 2 others developed de novo clinical IBD after transplantation. Patients with exacerbations or de novo IBD after OLT were significantly younger ($p = 0.0113$), had a significantly higher alanine transaminase (ALT) value ($p = 0.0447$) and more features of auto-immunity at the diagnosis of PSC. At the time of transplantation, these patients were also younger ($p = 0.0475$), had slightly higher values of ALT and alkaline phosphatase, more often positive perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) serology ($p = 0.07$). The IBD was totally inactive at the time of OLT in 4 of the 13 who did not experience an exacerbation, in contrast none of those with a later upsurge had inactive IBD. The use of immune suppressive therapy in the affected patients was not significantly different from those not having exacerbations.

Conclusions : Patients with a young age at diagnosis of PSC, having signs of auto-immunity and a positive p-ANCA, are at increased risk for exacerbation of IBD after OLT. They are also younger and have more severe liver disease (increased ALT) at time of transplantation. Our data do not support the use of specific immunosuppressive regimens to avoid IBD flares after OLT.

MICHELASSI STRICTUREPLASTY FOR STENOTIC TERMINAL ILEAL CROHN'S DISEASE : A PROSPECTIVE COHORT SERIES. A. De Buck Van Overstraeten (1), S. Vermeire (1), D. Vanbeckevoort (1), G. Vanassche (1), M. Ferrante (1), P. Rutgeerts (1), A. Wolthuis (1), A. D'hoore (2). (1) University Hospitals Leuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium.

Introduction and Aim : Side-to-side isoperistaltic strictureplasty has been proposed as an alternative to resectional surgery. It has mostly been performed in patients at risk for short bowel syndrome ; however, we have extended this procedure to patients with primary terminal ileal disease. This prospective cohort series assesses the outcome of this technique in (primary) stenotic ileal disease.

Method : All patients with stenotic small bowel disease were proposed for side-to-side isoperistaltic strictureplasty. Data were retrieved from a prospectively maintained institutional IBD database. MRE and colonoscopy were performed during the medium term follow up.

Results : Twenty-one patients (9 males) with a median age of 33 years (range 16-71 y) underwent side-to-side isoperistaltic strictureplasty. This was the primary procedure in 13 patients. Median length of the strictureplasty was 50 cm (range 11-90) and was extended over the ileocecal valve in 16 patients. One patient developed an early leak for which re-suturing was performed. 17 patients underwent a postoperative colonoscopy at a median interval of 6.4 months (range : 3.25-22 months) and showed at least partial healing in all cases with a median SES-CD score of 4 (range : 0-8). Only one stricture, although asymptomatic, was reported. Early MRE, performed in 18 patients, at a median follow up of 13.3 months (range : 3.29-36.6) shows significant regression of inflammation and a normal luminal patency. Clinical recurrence was reported in 7 patients at a median interval of 14 months. No patients developed any surgical recurrence.

Conclusions : The Michelassi strictureplasty can be used safely in primary stricturing ileal disease with increased mucosal and bowel wall healing assessed by colonoscopy and MRE.

LONG-TERM CLINICAL OUTCOME AFTER INFLIXIMAB CESSATION IN CROHN'S DISEASE PATIENTS IN REMISSION. K. Papamichail (1), N. Vande Castele (2), S. Tops (2), A. Gils (2), V. Ballet (1), M. Ferrante (1), G. Van Assche (1), P. Rutgeerts (1), S. Vermeire (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) Laboratory for Therapeutic and Diagnostic Antibodies, Leuven, Belgium.

Introduction : The STORI trial showed that in Crohn's disease (CD) patients who stop infliximab (IFX) for remission but continued immunomodulators (IMM), half will relapse within one year after discontinuation. In this study, biologic or endoscopic features of persistent inflammation were predictors of relapse.

Aim : To investigate the long-term outcome of CD patients who discontinued IFX while in clinical remission (CR) and search for predictors of sustained clinical remission (SCR) after IFX cessation.

Methods : Observational, retrospective, single-center study. CD patients who discontinued IFX therapy due to steroid-free CR (excluding pregnancy) with a follow up > 2 years were included in the study. Primary outcome was SCR after discontinuation of IFX and secondary objectives included the identification of predictors for SCR. SCR was defined as maintained disease remission without the need to re-introduce medical therapy (biologics, corticosteroids, thiopurines or methotrexate) or surgical interventions (ileocolonic resection, stricturoplasty, fistulotomy) until the end of follow up. Serial IFX trough levels (TL) in prospectively collected serum samples were analyzed using an in house developed and clinically validated ELISA.

Results : 100 CD patients [40 males, median age at diagnosis 25 (IQR 18-34) years, 43 treated mainly for fistulising disease] were able to discontinue IFX therapy when having CR after a median of 7.3 (IQR 1.4-16.2) months and a median number of 3.5 (IQR 2-6) infusions. The majority (n = 65) had been treated with episodic IFX therapy ; 15 had only received the induction phase ; and 20 received maintenance therapy. After stopping IFX, 84 patients continued on IMM while 16 received 5-ASA or no therapy. With a median follow up of 9.9 (IQR 7.9-11.7) years, 52 had SCR while 48 relapsed after a median of 4.7 (IQR = 3.1-7.1) years. The cumulative probability of CD patients to relapse within the first year after discontinuation of IFX, as showed by the Kaplan-Meier curve, was only 4% (SE = 0.02). COX regression univariate analysis showed that age at diagnosis > 25 years (HR = 2.039, 95%CI : 1.146-3.625, Log-Rank p = 0.013), IFX TL < 6µg/ml at the time of IFX discontinuation (n = 19) (HR = 4.602, 95%CI : 1.005-21.068, Log-Rank p = 0.031) and mucosal healing at the time of IFX discontinuation (n = 18) (HR = 4.509, 95%CI : 0.887-22.908, Log-Rank p = 0.048) predicted sustained remission. Of interest, CRP, platelets, white blood count, neutrophils and hemoglobin levels at the time of discontinuation, did not influence relapse rates.

Conclusion : In this single-center study, 52% of CD patients who discontinued IFX therapy due to CR remained in SCR and mainly with continued IMM treatment. This implies that within the variable responses to IFX some patients may achieve indefinite remission after IFX discontinuation.

Invited Lecture

Which new drugs will I be using in the near future ?
G. D'Haens, AMC, Amsterdam, The Netherlands

POSTERS

PREDICTION OF CLINICAL RESPONSE TO ANTI-TNF THERAPY BY CIRCULATING T CELL SUBSETS BUT NOT BY B CELLS. Z. Li (1), S. Vermeire (1), D. Bullens (1), M. Ferrante (1), K. Van Steen (2), M. Noman (1), P. Rutgeerts (1), J.L. Ceuppens (1), G. Van Assche (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) University of Liege, Liège, Belgium.

Introduction : Infliximab (IFX) therapy increases circulating Foxp3 (+) regulatory T cells (Treg) in patients (pts) with Crohn's disease (CD), ulcerative colitis (UC), rheumatoid arthritis (RA), psoriasis and Behçet's disease. Co-expression of CD45RA & Foxp3 distinguishes resting & active Treg (rTreg & aTreg) from Foxp3 (+) effector T cells. IFX also up-regulates blood total memory and pre-switched memory B cells in RA. In IBD, IgM (+) memory B cells are decreased. CD19(+) B cells in the inflamed intestinal mucosa predicts long lasting remission to IFX in CD. Simultaneously restoration of Treg, Foxp3(-) Type 1 Regulatory-Like T Cells (Tr1L) and some subsets of B cells correlated with clinical

response and biological response(CRP) to IFX. But it is unknown if IFX pharmacokinetics correlates with these cells. IFX is an effective treatment for IBD, yet about 30% of pts have a weak or no response.

Aim : To investigate if clinical response to IFX for IBD can be predicted by circulating subsets of Foxp3(+)/Tregs, Foxp3(-)/Tr1L, and B cells.

Methods : Blood was taken from IBD pts before and during therapy (IFX, 5 mg/kg IV 0-2-6 and q8 wks). These cells were assessed by flow cytometry after staining for CD4, CD45RA, Foxp3, CD25, CD127 and CD19. Assessment of symptoms, endoscopic healing & histological improvement was used to distinguish responders (RS) from non-responders (NRS) at 4 to 12 weeks after start of therapy. These cells at baseline before therapy were assessed as potential predictors of clinical response to the therapy after 14 or 22 wks in 40 IBD pts (RS : 25, NRS : 15). Trough levels of IFX determined in serum obtained immediately before the next infusion beyond week 14 of treatment (n = 20).

Results : Trough levels of IFX positively correlated with aTreg($r = 0.52, P = 0.01$) and total Treg($r_{\text{Treg+aTreg}} = 0.52, 0.02$), Tr1L (0.55, 0.01) and B cells (0.63, <0.01), but not with rTreg(0.30,0.20) (all n = 20). ROC curves showed clinical response was predicted by rTreg (area under ROC : 0.81, $P < 0.001$, cut off value : 0.43 as % of CD4 T cells), aTreg (0.74, = 0.015, 0.75), total Treg (0.82, <0.0001, 1.25) and Tr1L(0.73,0.02,0.82) respectively, but not by B cells (0.58,0.46) (all n = 40 ; RS : 25, NRS : 15)

Conclusions : Trough level of IFX pharmacokinetics correlates with aTreg, totalTreg, Tr1L and B cells, suggesting IFX could manipulate these populations by direct functions. Prediction of clinical response to IFX by baseline Treg and Tr1L but not by B cells would allow for better patient selection and increase the benefit to risk ratio and the cost effectiveness of these drugs.

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MRI T2 RELAXOMETRY TO IMAGE FIBROSIS IN PATIENTS WITH CROHN'S DISEASE. C. Breynaert (1), T. Dresselaers (2), R. Peeters (3), I. Roebben (3), K. Van Steen (4), T. Vanuytsel (1), V. Ballet (1), M. Ferrante (1), S. Vermeire (1), P. Rutgeerts (1), U. Himmelreich (2), S. Dymarkowski (3), D. Vanbeckevoort (3), G. Van Assche (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KU Leuven, Leuven, Belgium ; (2) Biomedical MRI, KU Leuven, Leuven, Belgium ; (3) Radiology, UZ Leuven, Leuven, Belgium ; (4) University of Liege, Liège, Belgium.

Introduction : Non-invasive imaging tools that can assess connective tissue changes and can be applied repetitively, would be a major asset for the management of IBD, especially for the study of treatment efficacy and to predict treatment response. *In vivo* μ MRI T_2 relaxometry, a non-invasive imaging tool, allows to discriminate between acute and chronic phases of bowel wall inflammation and fibrosis in murine DSS colitis (Breynaert C. *et al.* PLoS One 2013 ; 8 : e68876.).

Aim : To assess the value of MRI T_2 relaxometry in patients with Crohn's disease (CD).

Methods : The study was approved by the Ethics Committee of the University Hospitals of Leuven (S53186 – Belgian number B322201111559). To define the normal value of T_2 intensity of the rectum, healthy volunteers had a pelvic MRI with acquisition of high resolution T_2 weighted images and T_2 relaxometry. CD patients in whom a pelvic MRI was indicated for assessment of CD activity were recruited after informed consent for an additional T_2 relaxometry. On the T_2 map of the pelvis, the rectum was identified on 3 cross-sections per patient and delineated. Within these regions of interest the distribution (histogram) of the T_2 times between 0 and 250 ms was determined using ImageJ.

Results : In total, 18 healthy volunteers (10 M) and 17 patients (4 M) were included. Six patients were excluded because of claustrophobia or early discontinuation of scanning. The mean T_2 of the rectum was not significantly different ($p = 0.119$) between patients (113.0 ms (109.0-120.4)) and volunteers (118.3 ms (115.4-121.3)). However, analysis of the T_2 map of the rectum of CD patients showed a broadening of the histogram to lower values compared to healthy volunteers ($p = 0.002$).

Conclusions : T_2 relaxometry of the pelvis in patients with CD identifies a histogram shift compared to healthy volunteers consistent with the changes observed in chronic DSS colitis. The data suggest that MRI T_2 relaxometry is a promising tool to assess fibrosis in CD. Further investigation of this non-invasive, radiation and IV contrast free imaging tool is warranted.

EFFICACY AND SAFETY OF ANTI-TNF THERAPY IN ELDERLY PATIENTS WITH INFLAMMATORY BOWEL DISEASE. T. Lobaton, M. Ferrante, V. Ballet, P. Rutgeerts, G. Van Assche, S. Vermeire. UZ Leuven, Leuven, Belgium.

Introduction : The general increased life expectancy is reflected in the age of patients with inflammatory bowel disease (IBD). The knowledge about efficacy and safety of anti-tumor necrosis factor (TNF) therapy in elderly is scarce and conflicting.

Aim : Our objectives were to assess the efficacy and safety of anti-TNF therapy in elderly patients taking into account eventual comorbidity.

Methods : Retrospective single-centre study where 63 IBD patients initiating anti-TNF treatment at age ≥ 65 years (cases) were compared to 118 IBD patients initiating anti-TNF at age < 65 years (control 1) and 70 anti-TNF naïve IBD patients treated with azathioprine (AZA) and/or corticosteroids (CS) ≥ 65 years (control 2). Both control groups were matched to the case-group for type of IBD (Crohn's disease or ulcerative colitis), follow up, disease duration and type of anti-TNF (adalimumab or infliximab) in the case of control 1. Comorbidity was assessed using the Charlson Comorbidity Index (CCI). Both efficacy and safety of treatment were analyzed.

Results : Baseline characteristics are collected in Table 1. The short-term clinical response (STCR) to anti-TNF (4-10 weeks) was significantly lower in the elderly than in younger patients (67% vs. 87% ; $P < 0.001$). At long-term (≥ 6 months) the differences were not significant. Considering all patients with a CCI = 0, age of ≥ 65 years remained a risk factor for lower STCR (62% vs. 87% ; $P < 0.001$). Comparing cases with control 1, age ≥ 65 was a risk factor for severe infection and any Severe Adverse Event (SAE). In the multivariate analysis age remained as an independent risk factor for lower clinical response at short-term : OR 3.4 (1.4-6.6 95% CI, $P = 0.005$), severe infection (OR 4.2 (1.2-14.4 95% CI, $P = 0.025$)) and SAE (OR 2 (1.1-3.7 95% CI, $P = 0.029$)). Considering the 3 groups, age ≥ 65 and CCI > 0 were risk factors for malignancy and mortality.

Table 1

	Cases : ≥ 65 anti-TNF (n = 63)	Control 1 : < 65 anti-TNF (n = 118)	Control 2 : ≥ 65 IS-CS (n = 70)
Baseline Characteristics			
Female (%)	60	60	36 ($p = 0.005$)**
Median age (years) (IQR)	69 (67-73)	65 (65-68)	35 (23-47) ($p < 0.001$)**
Comorbidity (% pat CCI > 0)	46	20 ($p < 0.001$)*	41
Median Dis.Duration (years) (IQR)	7 (2-22.3)	5 (1-13)	13 (3-22)
Median follow-up (months) (IQR)	59 (19-93)	74 (37-87)	41 (19.5-85)
UC/CD (%)	51/49	48/52	56/44
Adverse events			
Infection with hospitalization (%)	13	3 ($p = 0.026$)*	16
Any Severe Adverse Event (%)	56	39 ($p = 0.028$)*	49
Need for surgery (%)	19	10	14
Death (%)	6	1	11
Malignancy (%)	6	2	14

* P value < 0.05 comparing cases-control 1 ; ** P value < 0.005 comparing cases-control 2.

Conclusions : Elderly patients treated with anti-TNF have a lower rate of clinical response (regardless of their comorbidity). The rate of adverse events is higher in elderly patients but especially in those with a higher comorbidity.

DIFFERENT INFLAMMASOME SUBTYPES ARE ACTIVATED IN HUMAN INFLAMMATORY BOWEL DISEASES. W. Vanhove (1), D. Staelens (1), P. Peeters (2), J. Van Der Goten (1), S. De Schepper (1), E. Wouters (2), G. Van Assche (1), M. Ferrante (1), P. Rutgeerts (1), S. Vermeire (1), K. Nys (1), I. Arijis (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) Maastricht University Medical Centre, Maastricht, Netherlands.

Introduction : Recently, intracellular danger – and pathogen receptor activation has been described in the gut. Inflammasome activation in the cytosol of myeloid and epithelial cells leads to potent caspase-1 mediated pyrogene and alarmin secretion (such as IL-1 β and HMGB1) that might be involved in the pathogenesis of IBD (Becker *et al.*, Gastroenterology, 2013, 144-2, pp. 283-293). Moreover, inflammasome activation has been shown to be pivotal in a murine model of IBD colitis (Bauer *et al.*, Digestive diseases, 2012, 30s1, pp. 82-90). However, the functional importance of the combined inflammasome subtype activation during IBD pathogenesis remains unknown.

Aim : This study investigated whether there are different inflammasomes activated in colonic mucosa of active IBD patients before and after anti-TNF- α therapy.

Methods : Gene expression of genes involved in inflammasome activation was investigated in colonic mucosa from 43 active IBD patients [24 ulcerative colitis (UC) and 19 Crohn's disease (CD)] before and 4-6 weeks after their first infliximab (IFX) infusion and from 6 normal controls. Total RNA was used to analyze gene expression via Affymetrix Human Genome U133 Plus 2.0 Arrays and qRT-PCR. Data was analyzed using Bioconductor software. Protein localisation of AIM2, IFI16, cleaved IL-1- β , CASP1 and HMGB1 was determined by immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded mucosal biopsies of IBD patients and controls. dsDNA-induced intracellular IL-1 β cleavage was determined by western blot in protein lysates from intestinal FHC and HT-29 cells.

Results : Gene expression analysis showed a significant (false discovery rate < 5%) increase in colonic expression of *AIM2*, *IFI16* and a borderline significant increase of *NLRP3* in active IBD vs. controls. Moreover, after IFX therapy, a significant decrease in expression of *AIM2* and *IFI16* was observed in IBD responders showing complete mucosal healing when compared to their baseline samples. Interestingly, IHC demonstrated an epithelial expression pattern for AIM2 with minor expression in inflammatory cells. In contrast, we observed positive staining for IFI16 in lymphocytes and epithelial cells. Finally, we confirmed the presence of active IL-1- β , CASP1 and HMGB1 in tissue sections of inflamed mucosa and we endorsed the epithelial presence/activation of an AIM2/IFI16-mediated inflammasome since dsDNA (AIM2/IFI16 specific stimulus) could induce IL-1 β cleavage in intestinal FHC and HT-29 cells.

Conclusions : For the first time we have shown combined transcriptional and functional activation of the different inflammasome subtypes NLRP3, AIM2 and IFI16 in the inflamed colon of active IBD patients, accompanied with expression levels of activated CASP1, IL-1 β and HMGB1.

MANAGEMENT OF POST-OPERATIVE RECURRENCE IN CROHN'S DISEASE : HAVE WE IMPACTED ON OUTCOME ? C. Amicone, C. Coimbra, C. Reenaers, C. Van Kemseke, J. Belaiche, L. Seidel, E. Louis. Centre Hospitalier Universitaire de Liège, Liège, Belgium.

Introduction : Clinical recurrence after first surgical resection for ileo-caecal Crohn's disease (CD) is around 70% after 8 years. Endoscopic recurrence rate is around 80% after one year and the intensity of this early endoscopic recurrence is predictive of later clinical recurrence. Hence controlling for early endoscopic recurrence aiming at adapting preventive treatment has become common practice since more than 10 years.

Aim : Our aim was to assess if this practice of early post-operative endoscopic exploration had impacted on the clinical recurrence rate.

Methods : We performed a retrospective study on the cohort of CD patients followed up at our university hospital. We first assessed clinical and surgical recurrence rates in two successive cohorts of patients operated between 1992 and 2002 and between 2002 and 2012. We then analyzed the clinical, demographic, endoscopic (also assessing the impact of performing an early endoscopic exploration 6-12 months after surgery) and therapeutic factors associated with the time-to-relapse in the whole cohort of patients.

Results : Between 1992 and 2012, we identified in our records 168 patients having undergone a first ileo-caecal resection (70 in the 1992-2002 period and 98 in the 2002-12 period). While the proportion of early post-operative endoscopic exploration was significantly higher in the recent (69/98) than in the older period (12/70), there was no significant difference in the time-to-clinical or surgical recurrence. In univariate analysis, performing the post-operative endoscopic control was not associated with time-to-clinical or surgical recurrence, but in patients having undergone this exploration, the Rutgeerts score was the strongest predictor of clinical and surgical recurrence. On the whole cohort, in multivariate analysis, the factors associated with clinical recurrence were an immunosuppressant before surgery ($p < 0.001$; HR = 2.9) and smoking ($p = 0.03$; HR = 1.6) while the factors associated with surgical recurrence were

female gender ($p = 0.01$; HR = 3.8) and smoking ($p = 0.01$; HR = 2.9). In the 2002-2012 period, 65 patients had an early post-operative endoscopy with a Rutgeerts score assessed : 33/65 had a score $> i1$ and among them only 7 received anti-TNF, 9 immunosuppressant, 10 mesalazine and 7 no treatment.

Conclusions : The time-to-clinical or surgical recurrence after first ileo-caecal resection has not changed over the last 20 years in our practice despite a more frequent use of early post-operative endoscopic exploration. The reason is probably the insufficient efficacy of purines or mesalazine, prescribed after the endoscopy, to impact on this recurrence rate. Recent results suggest that anti-TNF may be more effective in this setting.

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POSTOPERATIVE MORBIDITY AFTER POUCH SURGERY IN ULCERATIVE COLITIS AND FAMILIAL ADENOMATOUS POLYPOSIS. C. Snauwaert, M. De Visschere, P. Pattyn, M. De Vos. Ghent University Hospital, Gent, Belgium.

Introduction : There is some controversy about whether immunosuppressant agents (specifically anti tumour necrosis factor- α (TNF α) therapy) increase the risk of postoperative complications in ulcerative colitis (UC) patients undergoing restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA). In this context, we wanted to evaluate the post-operative outcome of UC and familial adenomatous polyposis (FAP) patients undergoing IPAA.

Aim and Methods : Our aim was to review the outcomes after IPAA in UC and FAP patients. We retrospectively analysed the medical records of all UC and FAP patients who underwent IPAA-surgery at our institution between 2002 and 2012. Clinical data including pre-operative indications, type of surgery, pre-operative use of immunosuppressive drugs (e.g. corticosteroids (CS) and anti-TNF α therapy) and postoperative course and complications were assessed. Postoperative complications were divided into early (< 30 days after restoration of ileoanal continuity) and late (> 30 days after restoration of ileoanal continuity) complications. Early complications were subdivided into infectious and non-infectious complications.

Results : UC. Indications were refractory disease with failure of medical management (17/37), acute severe colitis not responding to medical therapy (12/37), dysplasia or adenocarcinoma found on screening biopsy (4/37), tubular colon (2/37), toxic megacolon (1/37) and uncontrolled rectal bleeding (1/37). Twenty-seven patients underwent 1-stage surgery (proctocolectomy and IPAA without ileostomy), 9 patients underwent 2-stage surgery (proctocolectomy and IPAA + ileostomy) and 1 patient underwent 3-stage surgery (emergency colectomy and subsequent proctectomy/IPAA + ileostomy). 10 patients were operated laparoscopically. Early infectious complications were identified in 30% of patients (11/37), early non-infectious complications in 43% of patients (16/37) and 57% experienced late complications (21/37). 57% of patients received anti-TNF α therapy (21/37) and 68% (25/37) received CS within 12 weeks prior to surgery. No statistically significant difference in early (infectious and non-infectious) and late complications between treatment groups was observed (anti-TNF α versus non-anti-TNF α and CS versus non-CS treated patients respectively). Among the 1-stage surgery patients, 3 required reoperation for pouch salvage.

FAP. One-stage surgery was performed in 5 patients and 2-stage surgery in the other 11 patients. 8 patients were operated laparoscopically. Early infectious complications were found in 19% of patients (3/16), early non-infectious complications in 31% of patients (5/16) and late complications in 31% of patients (5/16). 1 patient required reoperation for pouch salvage.

Conclusions : This limited study shows that ileoanal pouch surgery has relatively high morbidity rates in both UC and FAP patients, underscoring the technically demanding nature of this surgical procedure. Furthermore, in the UC group, we found no significant correlation between preoperative use of CS or anti-TNF α therapy and infectious and non-infectious morbidity rates.

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THE EFFECT OF INFLIXIMAB THERAPY ON THE TRYPTOPHAN CATABOLISM PATHWAY IN INFLAMMATORY BOWEL DISEASE. I. Arijis (1), W.J. Wollants (1), G. Clarke (2), P. Persoons (3), W. Vanhove (1), L. Van Lommel (4), K. Machiels (1), G. Van Assche (1), M. Ferrante (1), F. Schuit (4), S. Vermeire (1), P. Rutgeerts (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) Laboratory of Neurogastroenterology, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland, Cork, Ireland ; (3) University Hospitals Leuven, Leuven, Belgium ; (4) Gene Expression Unit, Department of Molecular Cell Biology, KULeuven, Leuven, Belgium.

Introduction : Indoleamine 2,3-dioxygenase (IDO) is expressed in innate immune cells and acts as the first rate-limiting step in the tryptophan (TRP) catabolism along the kynurenine pathway. Decreased serum TRP levels have been associated with active inflammation in Crohn's disease (CD) due to induction of IDO (Gupta *et al.*, Inflammatory Bowel Diseases, 2012, 18(7), pp. 1214-1220).

Aim : We studied the effect of infliximab-induced downregulation of inflammation on serum levels of TRP and kynurenine, as well as mucosal *IDO* expression in patients with inflammatory bowel disease (IBD).

Methods : Serum samples and endoscopically-derived ileal or colonic mucosal biopsies were obtained from controls and patients with active IBD, and this before and after their first treatment with infliximab. Short-term clinical response and mucosal healing were assessed by experienced clinicians at 4 or 10 weeks after a single infusion or induction schedule, respectively (Ferrante *et al.*, *Inflammatory Bowel Diseases*, 2007, 13(2), pp. 123-128 ; Schnitzler *et al.*, *GUT*, 2009, 58(4), pp. 492-500). Serum TRP and kynurenine were measured by high performance liquid chromatography, and *IDO* mucosal gene expression was measured by Affymetrix Human Genome U133 Plus 2.0 Arrays. Data were analyzed with SPSS software using non-parametric tests and p-values of < 0.01 were considered significant.

Results : Overall, 156 IBD (76 CD and 80 ulcerative colitis) patients and 71 controls were included. In both ileum and colon, *IDO* mucosal expression was > 9-fold increased in active IBD patients before infliximab therapy vs. controls ($p_{\text{ileum}} = 0.001$ and $p_{\text{colon}} < 0.001$). Although the *IDO* mucosal expression levels significantly decreased after infliximab therapy in IBD responders when compared to their baseline samples ($p_{\text{short-term response}}$ and $p_{\text{mucosal healing}} < 0.001$), the colonic expression still remained significantly higher than controls ($p_{\text{short-term response}} < 0.001$ and $p_{\text{mucosal healing}} = 0.002$). Serum TRP levels were significantly decreased ($p < 0.001$) and the kynurenine/tryptophan (K/T) ratio was significantly increased ($p = 0.001$) in active IBD patients when compared to controls. However, we found no significant effect of infliximab therapy on both the TRP levels and K/T ratio.

Conclusions : This study demonstrates that mucosal *IDO* expression levels are increased and serum TRP levels decreased in IBD patients with active disease. Of note, after controlling the inflammation with infliximab therapy, both levels remained impaired in IBD responders showing a complete mucosal healing. This persistent upregulation of *IDO* in patients with complete colonic healing may explain why mucosal ulcers recur very early if patients do not receive maintenance therapy with infliximab.

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MUCOSAL MICRORNA AND GENE EXPRESSION AS BIOMARKER OF RESPONSE TO INFLIXIMAB IN ULCERATIVE COLITIS. J. Van Der Goten (1), I. Arijs (1), L. Van Lommel (2), W. Vanhove (1), M. Ferrante (1), G. Van Assche (1), P. Rutgeerts (1), F. Schuit (2), S. Vermeire (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) Gene Expression Unit, Department of Molecular Cell Biology, KULeuven, Leuven, Belgium.

Introduction : MicroRNAs (miRNAs) are increasingly recognized as major regulators of gene expression in many processes, including inflammation and tissue remodeling. Recently, altered expression of miRNAs has been reported in association with ulcerative colitis (UC).

Aim : In this study, we investigated the effect of controlling inflammation with infliximab (IFX) on the miRNA expression in UC and we correlated our findings with mucosal gene expression.

Methods : Colonic mucosal biopsies were obtained during endoscopy from 7 UC patients before and after IFX induction therapy (infusions at weeks 0, 2 and 6) and from 10 normal controls. Endoscopic response was assessed at 14 weeks after start of IFX and was defined as a Mayo endoscopic subscore of 0 or 1. Endoscopic non-response was defined as a Mayo endoscopic subscore of 2 or 3. Total RNA, including small RNA, was extracted from the biopsies and used to analyze the miRNA expression via Affymetrix GeneChip® miRNA 2.0 arrays. To assess gene expression, total RNA was analyzed via Affymetrix Human Gene 1.0ST arrays. Data was analyzed with Bioconductor and Ingenuity Pathway Analysis software. A false discovery rate < 5% combined with > 2-fold change was considered as significant. Predicted target genes were identified using the miRWalk software tool. Microarray data were validated by qRT-PCR analysis.

Results : Four out of 7 patients showed endoscopic response. In these 4 responders, expression of 893 gene probe sets (200↗ and 693↘) was significantly different after IFX therapy when compared to their baseline samples. We also identified 4 miRNAs that were significantly upregulated and 6 downregulated. By contrast, in the 3 non-responders IFX did not significantly affect gene or miRNA expression. Five miRNAs were selected for validation. In responders, we could confirm the upregulation after IFX therapy of miR-375 ($p = 0.07$), and the downregulation of miR-21-5p ($p = 0.14$), miR-31-5p ($p = 0.07$) and miR-155-5p ($p = 0.07$). Upregulation of miR-422a could not be confirmed. Next, we identified potential target genes in responders before and after IFX treatment using *in silico* analysis of the significantly dysregulated miRNAs and genes. The target genes encode proteins that were predominantly involved in hematological system development and function, immune cell trafficking and tissue development (f.e. *CARD11*, *CXCL1*, *FCGR3B*, *IL1R1*, *JAK2*, *S100A9*, *TLR4*, *TNFRSF9*). Out of 39 pairs of miRNAs and target genes of clinical interest in UC, a highly significant inverse correlation was observed between the expression of miR-378a-5p and *IL1R1*, identified as one of the UC susceptibility genes (Spearman $\rho = -0.84$; FDR = 0.003).

Conclusions : IFX therapy has a profound effect on the mucosal gene and miRNA expression. Integrated analysis of miRNA and gene expression profiles revealed potential mucosal biomarkers, such as miR-378a-5p and its target *IL1R1*, for response to IFX therapy.

DISEASE SEVERITY AFTER 3 YEARS OF TREATING NEWLY DIAGNOSED PEDIATRIC CROHN'S DISEASE PATIENTS (THE BELCRO COHORT). E. Degreef (1), J. Mahachie (2), I. Hoffman (3), F. Smets (4), S. Vanbiervliet (5), P. Bontems (6), I. Paquot (7), P. Alliet (8), K. Vansteen (2), G. Veeremanwauters (1). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) Montefiore Institute, Liège, Belgium ; (3) University Hospitals Leuven, Leuven, Belgium ; (4) Université Catholique de Louvain, City of Brussels, Belgium ; (5) Department of Hepatology and Gastroenterology, Universiteit Gent, Ghent, Belgium ; (6) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (7) CHC Clinique de l'Esperance, Liège, Belgium ; (8) Virga Jesse Hospital, Hasselt, Belgium.

Introduction : The BELCRO cohort was initiated in 5/2008 to prospectively study newly diagnosed pediatric Crohn's disease patients. We here report on disease outcome at 3y follow up.

Methods : Data from the BELCRO database were evaluated at diagnosis (M0), after 24 (M24) and 36 months (M36). Cross sectional analysis at M36 and longitudinal analysis from M0 to M36 were performed on the outcome data obtained. Hypothesis were tested at 5% significance.

Results : At M 36, consecutive data were available on 84 patients. From the initial 65%, 56% remained under pediatric care at M36 with an unchanged proportion (70%) at tertiary care hospitals. Between time point M0 and M36, disease severity evolved from 5% inactive to 70%, from 19% mild disease to 24% and from 76% moderate to severe disease to 6%. No positive associations were found with disease severity as outcome. Especially none of the following variables were associated with disease severity as outcome at M36 : cumulative treatment, disease location at diagnosis, sex nor age. Over time, adult physicians followed active patients (p-value = 0.03 moderate-severe vs inactive ; p = 0.007 mild vs inactive). There were no deaths or cancers reported. Treatment changed as follows : immunomodulator (IM) monotherapy from 49 to 29%, steroids from 78 to 6%, combination therapy (IM+biologicals) from 1 to 17% and biologicals monotherapy from 0 to 43%. The median disease duration before initiating biologicals was 5 m (range 5d-2.3 y) and 60% of patients had biological as part of their treatment. Six % never received IM or biologicals and 6% had no therapy at M36. Disease related surgery was performed in 13%. In 91% of patients, BMI z-scores and in 97% height z-score were > -2SD and < 2SD.

Conclusions : In the BELCRO cohort disease activity appears very well controlled at M36 with the current treatment strategies. The majority of patients received biologicals as part of their treatment and are followed in tertiary care hospitals. Further follow-up is planned and will be crucial to confirm this favourable outcome.

NOD2 POLYMORPHISMS AND INTESTINAL TRANSPLANTATION. L.J. Ceulemans (1), K. Lenaerts (2), I.H. Hundscheid (2), S.W. Olde Damink (2), D. Monbaliu (1), G. De Hertogh (3), S. Vermeire (4), J. Pirenne (1). (1) Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium ; (2) Maastricht University Medical Centre, Maastricht, Netherlands ; (3) Pathology, University Hospitals Leuven, Leuven, Belgium ; (4) Gastroenterology, University Hospitals Leuven, Leuven, Belgium.

Introduction : In healthy people, NOD2 polymorphisms occur in 4-10% but the prevalence increases to 50% in patients with Crohn's Disease (CD). Intestinal Transplantation (ITx) is a treatment for irreversible life-threatening intestinal failure but its application is limited by the high susceptibility to rejection. A link between NOD2 polymorphisms and intestinal rejection has been suspected as a high prevalence of NOD2 polymorphisms was reported earlier among ITx recipients (*Fishbein, Gut* 2008, 57 : 323-30).

Aim : To analyze the prevalence of NOD2 polymorphisms in ITx recipients/donors and its relation with rejection/clinical outcome.

Methods : From 10/2000 to 12/2012, 12 ITx were performed at our institution. In a postTx cross-sectional analysis, NOD2 genotype could be determined in 10 donors and 8 recipients/survivors. Demographics, indications, early/late (< 3 mo/ > 3 mo postTx) allograft rejection were reviewed. Recipients/donors were genotyped for the three most common Single Nucleotide Polymorphisms (SNPs) in the NOD2/CARD15 gene : missense mutation 2104C- > T (SNP8) ; missense mutation 2722G- > C (SNP12) ; frameshift C-mutation 3020insC (SNP13).

Results : Median donor age was 14y (3y-17y). Male/female ratio was 6/4. Cause of death was : trauma (6), anoxia (1), intracranial bleeding (1), suicide (1), CO intoxication (1). Median recipient age was 32y3mo (2y8mo-56y8mo). Male/female ratio was 2/6. Indication for ITx was intestinal failure secondary to volvulus (4), CD (2), ischemia (1), chronic intestinal pseudo-obstruction (1). Median follow-up was 6y (2 y-11 y4 mo). Two (20%) of the *donors* had at least one NOD2 polymorphism (1 homozygous mutant for SNP8 ; 1 heterozygous for SNP13) and four (50%) *recipients* had at least one NOD2 polymorphism (3 heterozygous for SNP8 ; 1 homozygous mutant for SNP13). One of the CD patients carried one NOD2 polymorphism. The incidence of NOD2 polymorphisms in recipients without CD was 50% (3/6). Two out of 4 (50%) recipients with NOD2 polymorphism developed rejection *versus* none in the 4 patients without

NOD2 polymorphism. Severe early (18d postTx) and late (46mo postTx) acute rejection developed in one of the 2 CD patients in whom a NOD2 polymorphism was present in both donor (SNP8) and recipient (SNP8). Another late rejection developed in a recipient (volvulus) with a NOD2 polymorphism (SNP8), but whose graft had no NOD2 polymorphism. All rejections were reversible. The second donor graft with NOD2 polymorphism (SNP13) was transplanted in a recipient without NOD2 polymorphism and no rejection was diagnosed so far.

Conclusions : This limited patient cohort indicates that NOD2 polymorphism incidence is high (50%) in ITx recipients, irrespective of the intestinal disease. This suggests that NOD2 polymorphism predisposes to development of severe intestinal failure necessitating ITx. The high incidence of NOD2 polymorphisms in this patient group may also account -at least partly- for the high frequency of rejection described after ITx (vs other organ Tx), albeit the donor/graft NOD2 status may also play a role. Larger patient cohorts are required.

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PREVENTION OF RECURRENT CLOSTRIDIUM DIFFICILE INFECTION BY MONOCLONAL ANTIBODIES IN A HAMSTER MODEL. D. Staelens (1), M. Van De Wouwer (2), E. Brouwers (2), S. Caluwaerts (3), P. Rottiers (3), P. Vanhoenacker (3), N. Geukens (4), P. Declerck (2), M. Ferrante (1), S. Vermeire (1), P. Rutgeerts (1), G. Van Assche (1). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) Laboratory for Therapeutic and Diagnostic Antibodies, Leuven, Belgium ; (3) ActoGeniX NV, Gent, Belgium ; (4) PharmAbs, Leuven, Belgium.

Introduction : In recent years, the incidence and severity of *Clostridium difficile*-associated disease (CDAD) is increasing with significant morbidity and mortality. Moreover, IBD patients are more sensitive to CDAD compared to the non-IBD population. Unfortunately, current treatment regimes are far from optimal with relapse rates around 30%.

Aim : Therefore, we aim to develop *Clostridium difficile* (*C diff*) toxin-antigen binding antibody fragments for targeted mucosal delivery as a novel treatment modality in CDAD.

Methods : Monoclonal antibodies (mAbs) against both *C diff* toxins (TcdA and B) were generated, produced and purified by a standard protocol using SJL/J mice, a CeLLine system and protein A affinity chromatography. mAbs were evaluated for their *in vitro* neutralization capacities of TcdA or TcdB at 570 pM or 56 pM, respectively, using human fibroblasts (IMR-90) and selected for *in vivo* tests. Male Syrian Golden hamsters (95-100 gram) received 30 mg/kg oral clindamycin on day -5 and were infected with *C diff* by oral gavage (1000 spores, 630 strain) at day 0. Next, hamsters were treated with vancomycin for 4 consecutive days (5 mg/kg, n = 25). Fourteen of these hamsters were treated with a combination of mAbs against both toxins (injected on day 5 and 7, 20 mg/kg, intraperitoneal). Hamsters were monitored for weight loss and disease symptoms (wet tail, hunched posture, raised ears, loss of interest) up till day 22. Serum mAb concentrations were measured using a mouse IgG-specific sandwich-ELISA. Statistical analysis was performed using GraphPad Software.

Results : We observed potent *in vitro* neutralizing activity for two mAbs, exhibiting EC50 values of 59 pM and 182 pM, for the anti-TcdA and anti-TcdB antibody, respectively. Next, when testing these mAbs in the well-established hamster model for recurrent *C diff* infection, survival curves showed a significant reduction in mortality rate of hamsters treated with the mAbs (Log-rank test, p = 0,0075) as compared to vancomycin treatment only. The proportion of surviving animals increased from 27,3% in hamsters receiving vancomycin only to 78,6% in the mAbs-treated group. This coincided with a complete disappearance of disease symptoms, such as weight loss and wet tail, after mAb therapy. Additionally, we were able to detect antibody levels up to 61 µg/ml in serum of hamsters 15 days after administration of the last mAb dose.

Conclusions : This study demonstrates high *in vitro* as well as *in vivo* neutralizing properties for anti-TcdA/B mAbs in the well-established Syrian Golden hamster model of recurrent CDAD. Therefore we will further develop a system for local delivery of antigen binding fragments of the selected mAbs using the ActoGeniX *Lactococcus*-based mucosal delivery system.

FEASIBILITY OF CACHEXIA SCREENING IN AMBULATORY CANCER PATIENTS : A MULTICENTER PILOT-STUDY. K.J. Cheung (1), E. Meulemans (1), K. Wouters (1), M. De Clercq (1), L. Bartholomeussen (1), M. Sels (1), S. Pallemans (1), C. Wellens (1), D. Ysebaert (1), S. De Keersmaecker (1), C. Verschueren (1), L. De Clerck (1), D. Baert (2), C. Vandoninck (2), S. Kindt (2), S. Schelfaut (2), M. Vankerkhove (2), A. Troch (3), L. Ceulemans (3), H. Vandenberg (3), S. Leys (3), J. Pauwels (3), T. Rondou (4), E. Dewitte (4), K. Maes (4), B. De Winter (5), M. Vandewoude (1), L. Van Gaal (1), P. Van Aken (1), M. Peeters (1). (1) Universitair Ziekenhuis Antwerpen, Antwerpen, Belgium ; (2) Maria Middelaars Ziekenhuis, Gent, Belgium ; (3) heilig hart ziekenhuis, Lier, Belgium ; (4) St Jozefkliniek, Bornem, Belgium ; (5) University of Antwerp, Antwerpen, Belgium.

Introduction : Malnutrition and cachexia impact treatment options and ultimately also the outcome of patients with (advanced) cancer. However, malnutrition and cachexia are often under- or misdiagnosed especially in ambulatory cancer patients.

Aim : In this regard, the aim of this multicenter pilot study is to investigate the usefulness of evaluation tools for the early detection of cachexia in an ambulatory cancer population.

Methods : Ambulatory patients with digestive, lung, breast and head/neck cancer were recruited and followed in 4 academic / non-academic medical centers. Subjects were monitored for one year and malnutrition and cachexia were evaluated at 5 consecutive time points, corresponding with 2- to 3-monthly visits. Nutritional status was evaluated by means of body mass index (BMI), nutritional risk score (NRS 2002) and patient-generated subjective global assessment (PG-SGA). Muscle metabolism was measured using bio-electric impedance analysis (BIA) and handgrip strength, while serum albumin, pre-albumin and C-reactive protein (CRP) were measured routinely. Data was recorded in Remecare (Remedus, Belgium). Descriptive statistics were conducted by means of univariate (i.e. t-test test) and mixed effects model (i.e. linear mixed effects) analyses. Cachexia was defined as follows : weight loss > 5% within last 6 months OR weight loss > 2% and BMI < 20 OR weight loss > 2% and sarcopenia (Fearon *et al.*, 2011).

Results : 176 participants (33.5% female) with a median age of 65 years (57 – 71) (Q1 – Q3), were recruited across the different centers (2012 – 2013). The population consisted of predominantly stage IV (67.6%) cancer disease of digestive origin (66.5%). 167 patients (94.8%) had effectively undergone baseline nutrition screening. Data concerning weight prior to baseline were obtained from 150 patients (85.2%). Intermediate results showed that 103/150 patients (68.7%) developed cachexia over time with 71 (68.9%) already cachectic at study-entry. No significant difference was observed in incidence of cachexia between centers, sex or tumour types. Mixed effects models showed decrease in BMI , fat free mass and muscle strength in cachectic patients ($p < 0.0001$). Questions concerning symptoms during food intake, weight loss, quality of life and physical functioning showed significant differences between cachexia and non-cachexia ($p < 0.0001$). Routine blood analyses showed a decrease in albumin ($p = 0.0006$) and pre-albumin ($p = 0.0007$), and an increase in CRP ($p < 0.0001$) during cachexia. According to univariate analyses age and tumour type were not significantly associated with cachexia.

Conclusions : Many different screening tools correlate excellent with the occurrence of cachexia in this interim analysis. This holds the promise to find the most accurate screening tool to predict early cachexia, and to identify those patients that could profit from an enhanced nutritional or medical intervention.

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RESULTS AND TRENDS IN INTESTINAL TRANSPLANTATION : 2013 INTERNATIONAL INTESTINAL TRANSPLANT REGISTRY. L.J. Ceulemans (1), D. Monbaliu (1), G. Van Helleputte (2), I.H. Hundscheid (3), K. Lenaerts (3), S.W. Olde Damink (3), M. Marquez (4), D. Grant (4), J. Pirenne (2). (1) Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium ; (2) Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium ; (3) Maastricht University Medical Centre, Maastricht, Netherlands ; (4) Intestinal Transplant Registry, Toronto, Canada.

Introduction : Intestinal Transplantation (ITx) is a life-saving treatment for patients suffering from short bowel syndrome (SBS) and TPN-complications. ITx is perceived as a challenging procedure with inferior outcome to other solid organTx because of higher rejection rates and need for more profound immunosuppression with its associated side-effects. Except for a few large North-American centers, only small-size single-center ITx series have been reported and do not provide gastroenterologists with a clear view of the current indications and results of ITx.

Aim : The international Intestinal Transplant Registry (ITR) aims to collect multi-center data and to determine the scope, success/failures and global trends in ITx worldwide.

Methods : ITx performed between 04/1985 and 02/2013 were prospectively reported to a centralized international registry. Demographics, indications, results and variables influencing outcome were retrospectively analyzed. Patient/graft survival estimates were obtained (Kaplan-Meier).

Results : Since 1985, 82 centers (47 currently active) provided data on 2887 ITx in 2669 patients. 1309(45.3%) are isolated ITx ; 898(31.2%) combined Liver-ITx (cLITx) and 680(23.5%) Multivisceral Tx (MVTx). 56% are children ; 44% adults. Indications for pediatric-ITx : SBS(63%), motility disorder(18%), malabsorption(8%), others(11%). Etiologies of pediatric-SBS : gastroschisis(35%), volvulus(25%), necrotizing enterocolitis(22%), atresia(6%), others(12%). Indications for adult-ITx : SBS(64%), tumor(13%), motility disorder(11%), others(12%). Etiologies of adult-SBS : ischemia(39%), Crohn's disease(17%), volvulus(14%), trauma(12%), others(18%). Four global trends are seen : (1) decrease in annual number of ITx from 250 (2008) to 110 (2012) ; (2) more patients at home pre-Tx ; (3) relative decrease of cLITx ; and (4) increase in colon graft inclusion (30%). The latter implicates a higher rate of TPN-independence post-Tx ($p = 0.047$) and a superior graft survival ($p = 0.05$). Currently, 1416(53%) of all patients are alive and 70% are TPN-free. The Karnofsky performance score (quality-of-life surrogate) is 61-100% in 70%. Graft-survival has improved in successive eras. During the last 5y, actuarial 1y graft survival remains stable at 75%. 1y adult recipient survival is 80% (ITx), 75% (cLITx) and 70% (MVTx). 1y pediatric recipient survival is 90% (ITx), 85% (cLITx) and 75% (MVTx). Leading causes of death within the first year postTx in 2012 : sepsis (37%), technical (22%), lymphoma (5%), various (36%). Variables independently associated to better outcome are : pediatric vs. adults ($p = 0.045$) ; preTx status home vs. hospital ($p = 0.01$) ; liver inclusion ($p = 0.006$) ; and m-TOR inhibitor immunosuppression ($p < 0.001$).

Conclusions : During the last three decades, ITx has evolved into a life-saving (and quality-of-life restoring) procedure for SBS patients with severe TPN-complications and should be considered *standard treatment* in those. Excluding moribund recipients, inclusion of liver, pediatric Tx, colon inclusion and m-TOR inhibition contribute to improved results. Simultaneously, the annual number of ITx has decreased worldwide probably reflecting improved management of TPN and intestinal failure. With a decrease in number, multi-center collaboration and research is needed to further optimize the results and expand the criteria for ITx.

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PHARMACO-NUTRITION IN ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS): SYSTEMATIC REVIEW AND META-ANALYSIS. C. Santacruz (1), D. Orbeago Cortes (1), J.L. Vincent (1), J.C. Preiser (2). (1) Erasme Hospital, Brussels, Belgium ; (2) Hôpital Erasme, City of Brussels, Belgium.

Introduction : ARDS is a complex disease with a pro-inflammatory profile and high mortality rate. Immuno-modulation is an attractive therapeutic option of treatment for patients with ARDS. However, many doubts still persist on the effectiveness of pharmaco-nutrition.

Aim : We aimed to perform a systematic review and meta-analysis on the effects of pharmaco-nutrition to treat ARDS patients when compared to a control diet on the outcomes of all-cause mortality, ventilator-free days (VFD), intensive care unit (ICU-LOS) and ICU-free days until day 28-30.

Methods : We independently search for randomized clinical trials (RCTs) from electronic databases with no date or language restrictions (PubMed, OVID, EMBASE, the Cochrane Systematic Review database) and hand searched the selected articles for additional references. The quality of the trials was assessed using the Jadad scale and we calculated the Q of Cochrane and I² to assess heterogeneity. We performed a sensitivity analysis excluding trials with different methodological quality. Forrest plots were constructed to graphically show the effects of pharmaco-nutrition on mortality, VFD, ICU-free days and ICU-LOS. A funnel plot was constructed to evaluate publication bias of the mortality outcome.

Results : No effect on mortality with the use of immunonutrition when all trials were included could be demonstrated (RR 0.83,95%CI 0.55 to 1.25 ; $p = 0.37$; I₂ : 57.6%). In the sensitivity analysis, there was a significant reduction in the risk of mortality (RR 0.68,95%CI 0.50 to 0.91 ; $p = 0.009$, I₂ : 6.5%) with the use of immunonutrition when the Rice *et al.* trial was excluded. Low to moderate heterogeneity for the mortality outcome was found among the included trials for the mortality outcome. No benefit could be shown on the outcomes of VFD (mean effect 0.32 ; 95%CI -0.35 to 0.98 ; I₂ = 90.1%), or ICU-free days (mean effect 0.29 ; 95% CI -0.24 to 0.82 ; I₂ = 98.9%). A benefit was shown in the outcomes of ICU-LOS (mean difference -0.5 ; 95% CI -0.85 to -0.16 ; I₂ = 30.9%). High heterogeneity was founded among the included trials.

Conclusions : There is insufficient evidence to recommend the use of pharmaco-nutrition for ARDS patients to reduce mortality, VFD or ICU free days. Although a benefit was shown in the outcome of ICU-LOS, these was a secondary outcome among trials with high heterogeneity. In the sensitivity analysis, excluding better methodologically performed trials changed the direction of the effect towards a reduction in the risk of mortality.

CURRENT KNOWLEDGE AND PRACTICE OF NUTRITION IN INTENSIVE CARE. M. De Ryckere (1), J.C. Preiser (2). (1) Erasme Hospital, Brussels, Belgium ; (2) Hôpital Erasme, City of Brussels, Belgium.

Introduction : The issue of nutritional management of critically ill patients can be overlooked by the nursing team.

Aim : A survey aimed to analyse the current knowledge and practice of French-speaking nurses working in intensive care units (ICUs) in the area of nutrition therapy.

Methods : An on-line questionnaire was sent to the members of the Nursing section of the Belgian Society of Intensive Care (SIZ-Nursing), in partnership with the Société Belge de Nutrition Clinique. The issues addressed included professional experience, theoretical knowledge, and the practical aspects of the management of nutrition.

Results : Two hundred and twenty nurses working in 52 ICUs answered the survey. The duration of professional experience of the respondents ranged from less than 2 years (7%) to more than 20 years (30%). A local protocol for the management of nutrition is used by 61% ; a dietician is dedicated to the ICU in 66% of the cases. Theoretical knowledge, assessed by 8 questions was sufficient for 57% of the respondents. In practice, enteral nutrition is administered continuously by 95%, started within the first 24h and 48 h of the ICU stay by 45 and 85% of the respondents, respectively. Complementary parenteral nutrition is started by 67% of the respondents before the fifth day of the ICU stay and 6% start parenteral nutrition together with enteral nutrition. Gastric residual volumes are measured by 95%, with a threshold of intolerance ranging from 150 to 300 ml. In case of poor tolerance, pro-motility agents are used by 27%.

Conclusions : These results indicate that there is an ample room for improvement of the theoretical knowledge. Current practice is heterogeneous and rarely based on the most recent recommendations. These findings pave the way for educational training accessible to ICU nurses.

SUCCESS OF A LOW FODMAP DIET IN PATIENTS WITH IBS. J. Arts (1), J. Tack (2), T. Lanssens (1), R. Van Schaik (1), S. Vanooteghem (1), C. Beyls (1), A. Holvoet (1), S. Decock (1), P. Van Hootegem (1). (1) AZ Sint-Lucas, Brugge, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium.

Introduction and Aim : The irritable bowel syndrome (IBS) is a highly prevalent condition with unclear underlying pathophysiology and for which no standard effective therapy is established. A number of recent studies have shown that fermentable oligo-, di-, mono-saccharides and polyols (FODMAPS) alter intestinal physiology and can trigger gastrointestinal symptoms, while a low FODMAP diet improves symptoms in tertiary care IBS patients. Our aim was to determine whether a low FODMAP diet improves symptoms in IBS patients in the setting of a regional hospital in Belgium.

Methods : Consecutive IBS patients, seen at the gastroenterology outpatient clinic, were explained a low FODMAP diet by an experienced dietician. All patients were asked to score their symptom severity before and 8 weeks after the implementation of a low FODMAP diet and at the time of the evaluation using a questionnaire sent by mail. After a period of 8 weeks patients were offered a dietician guided re**Introduction** of selected FODMAPS. The intensities of 5 symptoms (bloating, abdominal cramps, borborygmi, stool disturbances, fatigue) were evaluated using 0- to 100-mm visual analogue scales (VAS). The global effect of the low FODMAP diet was also evaluated by means of a VAS (0 mm = no improvement to 100 mm = complete symptom resolution). VAS are reported as mean \pm standard deviation. Responses were compared using the Wilcoxon signed rank test was used. Results were significant if $p < 0.001$.

Results : Thirty patients of a total of 39 patients (77%) returned the questionnaire. After 8 weeks of low FODMAP diet, we found a significant reduction of all IBS symptoms (abdominal pain, 7.9 ± 1.9 vs. 4.3 ± 2 mm ; bloating 7.0 ± 1.8 vs. 3.8 ± 1.9 mm ; flatulence 6.5 ± 1.9 vs. 3.4 ± 2.0 and stool disturbances (7.0 ± 2.0 vs. 4.6 ± 2.2 mm) significantly improved (all $p < 0.001$). Also fatigue improved significantly better with a low FODMAP diet (6.9 ± 2.1 vs. 4.8 ± 2.8 mm, $p < 0.001$).

Global symptom score (sum of all evaluated symptoms) was also significantly improved with a strict low FODMAP diet (35.5 ± 5.8 vs. 21.0 ± 8.9 ; $p < 0.001$). Evaluation after re-**Introduction** of FODMAPs showed a persistent improvement (total symptom score 17.7 ± 9.1 ; $p < 0.001$).

Conclusions : A diet low in FODMAPs effectively reduces IBS symptoms and fatigue in secondary care practice. Robust symptom improvement occurs with a strict low FODMAP diet and persists with selected re-**Introduction** of FODMAPs. This retrospective study confirms the results obtained in tertiary care and supports dietary intervention in IBS in general clinical practice.

BELGIAN GROUP FOR DIGESTIVE ONCOLOGY (BGDO)

- O01 -

Clinical implications of RAS testing.
Sabine Tejpar, KUL, Belgium.

- O02 -

European experience in RAS testing and quality control.
Han van Krieken, Nijmegen, The Netherlands.

- O03 -

Belgian perspective.
Anne Mourin, UCL, Belgium.

- O04 -

USE OF SOM230 AS A PREVENTIVE AND THERAPEUTIC TOOL IN CHEMICALLY INDUCED HCC IN RATS. I. Borbath (1), M.P. Berghmans (1), V. Lebrun (2), B. Delire (1), I. Leclercq (3). (1) Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium ; (2) Université catholique de Louvain, Hepatogastroenterology unit, Woluwe-Saint-Lambert, Belgium ; (3) Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium.

Introduction : Hepatocellular carcinoma (HCC) is linked with poor prognosis. Patients with cirrhosis are at the highest risk, making the liver a pre-neoplastic organ. Preventive treatments are not available, and therapy of advanced HCC is to be improved, sorafenib being the only treatment available. We have previously shown that lanreotide, a somatostatin analogue targeting receptor subtype (sstr) 2, is active in preventing HCC appearance in chemically induced HCC in rats. Those tumours expresssstr 2 and 3. SOM230 is a new somatostatin analogue that targetssstr 1, 2, 3 and 5, making it an appealing candidate in this setting.

Aim : to assess the potential effect of SOM230 in prevention and treatment of HCC in rats and the value of animal MRI for HCC assessment and follow-up.

Methods : Male Wistar rats (n = 42) were given the genotoxic diethylnitrosamine (DEN) in drinking water for 6 weeks. SOM230 treatment (IP, 80 mg/kg, 1 dose every 6 weeks) was initiated week 7 (prior to tumour development) (n = 12), or at week 9 (when preneoplastic lesion are present) (n = 12), and incidence, number of size of HCC were evaluated at week 13. In addition, In this last group, 6 DEN control rats and 6 DEN/SOM rats (SOM230 treatment initiated week 9) underwent repeated MRI (11.7 Tesla Birker device, using T2 sequence) at weeks 12, 16, 20 and 25 for longitudinal follow-up of tumour development and progression.

Results : Early administration of SOM230 (week 7) was associated with a decrease in incidence (80% vs 100%), number (2.2 vs 8.6 per liver, p < 0.001) and size of tumours (median size 7.5 mm vs 11.5 mm) compared to untreated (DEN only) rats. Administration of SOM230 at a later time point (week 9) decreased more drastically the incidence (15% vs 100%), number 0.16 vs 5.16, p < 0.01) of tumour compared with untreated corresponding controls. At MRI examination performed at week 12, there was no difference in tumour number and size whether DEN rats were treated with SOM230 or not. However, at week 20, the number of tumours (but not their size) was significantly lower in SOM-treated rats than in controls (median 1 vs 6.5 tumours, p < 0.05). This difference was maintained at examination at week 25 (median 3.5 vs 10 tumours/liver, p < 0.01).

Conclusions : SOM230 prevents the occurrence of HCC in a model of DEN-induced carcinogenesis, when given at 2 different timing in HCC induction (prior or after development of foci of preneoplastic hepatocytes). Incidence and MRI data suggest that SOM230 prevents tumour occurrence. In vitro studies are ongoing to understand mechanisms by which SOM230 inhibit HCC.

TGF β -INDUCED PROTEIN IG-H3 IS ESSENTIAL FOR THE GROWTH OF HUMAN LIVER METASTASES. V. Castronovo (1), A. Blomme (1), P. Delvenne (1), O. Detry (2), A. Turtoi (1). (1) University of Liege, Liège, Belgium ; (2) Centre Hospitalier Universitaire de Liège, Liège, Belgium.

Introduction : Transforming growth factor-beta-induced protein ig-h3 (TGFBI) is extracellular matrix component known to be important for cell-collagen interaction. We and others have reported elevated expression of TGFBI in several human cancers, where its role remains controversial. **Aim** Current study aims at clarifying the function of TGFBI in colorectal carcinoma liver metastases (CRC-LM), a frequently encountered malignancy with no satisfactory treatment to date.

Methods & Results : Employing immunohistochemistry we have confirmed that TGFBI is highly expressed in human CRC-LM and in liver metastases originating from breast, lung and pancreatic tumors. We have next focused on functional aspects and have silenced TGFBI expression in SW1222 human colorectal carcinoma cells. The suppression of TGFBI protein led to a marked decrease in cell migration (-70%) and proliferation (-30%) *in vitro*. To study the effects *in vivo* we have developed a novel animal model of colorectal carcinoma based on chicken chorioallantoic membrane (CAM) that mimics human CRC-LM. TGFBI silencing resulted in 50% reduction of tumor volume in the CAM tumor model. Notably, the tumors displayed a marked inhibition of vascularization, suggesting an additional anti-angiogenic effect. Indeed, SW1222 cells silenced for TGFBI expression secreted lower levels of VEGFA *in vitro*. Finally, we have investigated if TGFBI can be used as systemically reachable target for antibody-drug delivery. For this purpose we have produced a fluorescently labeled anti-TGFBI monoclonal antibody and have injected it in mice bearing liver metastases. The *in vivo* data demonstrated that TGFBI is an accessible tumor target.

Conclusions : Taken together, the present study shows that TGFBI is essential for promoting the development of CRC-LM and therefore represents a promising target for designing novel therapeutic approaches.

STUDY OF GENOMIC ALTERATIONS IN COLON CARCINOMA BY ARRAY COMPARATIVE GENOMIC HYBRIDIZATION. E. Mampaey (1), A. Fieuw (2), T. Van Laethem (2), N. Van Roy (2), L. Ferdinande (1), W. Ceelen (1), P. Pattyn (1), Y. Van Nieuwenhove (1), K. De Ruyck (2), K. Geboes (1), S. Laurent (1). (1) Ghent University Hospital, Gent, Belgium ; (2) Ghent University, Gent, Belgium.

Introduction : Colorectal cancer is characterized by the presence of deletions and amplifications of several genes. Large genomic profiling studies however, have not been conducted in colorectal carcinoma. Since the number of genetic aberrations in a tumour sample can be correlated with recurrence or survival, these analyses can be used as biomarker for therapeutic decision making or even restaging of patients.

Methods : High resolution DNA copy number alterations of 102 colon carcinoma samples were studied using array CGH analysis. These data were combined with other parameters such as *KRAS* mutation status, MSI status and clinical data for data mining purposes.

Results : Both large and small (SRO) chromosomal deletions, gains and amplifications were identified in our sample cohort. Our results were consistent with data presented in the literature, but we also identified new recurrent chromosome alterations in interesting regions. The deletion of the *RBFOX1* gene is recently being described as associated with a poor clinical outcome in one study. We could not confirm this finding, but we found a significant correlation between *RBFOX1* deletion and *KRAS* mutation status ($p = 0,05$). We also found a correlation between the number of chromosomal alterations and the overall survival of patients with colon cancer ($p = 0,001$). The presence of chromothripsis in a number of patients was significantly correlated with the colon disease stage ($p = 0,038$).

Conclusions : In this study, we identified a number of new recurrent chromosome alterations in colon cancer. Furthermore, we found a significant correlation between the number of copy number alterations and the 3-year overall survival of colon cancer patients.

INDUCING, UNDERSTANDING AND OVERCOMING RESISTANCE TO EVEROLIMUS IN PANCREATIC NEUROENDOCRINE TUMORS. T. Vandamme (1), K. Op De Beeck (1), M. Beyens (1), G. Mortier (1), W. De Herder (2), G. Van Camp (1), P. Pauwels (1), L. Hofland (2), M. Peeters (1). (1) University of Antwerp, Antwerpen, Belgium ; (2) Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : Pancreatic neuroendocrine tumors (PNETs) are tumors arising from the endocrine glands of the pancreas. The phosphoinositide-3-kinase/Akt/mammalian target of rapamycin (PI3K-Akt-mTOR) signaling pathway plays a major role in PNET by regulating cell growth, proliferation, cell survival and protein synthesis. mTOR proves to be an interesting target for therapy of PNET and mTOR-inhibiting rapamycin and analogs (rapalogs) such as everolimus have been developed. Recent phase III trials with everolimus show an improved progression-free survival in progressive advanced PNET. However, adaptive resistance to mTOR inhibition with rapalogs is described.

Methods : To study resistance to everolimus therapy in PNET, two human PNET cell line models, QGP-1 and BON-1, were used. Genetic background of the cell lines was evaluated through short tandem repeat profiling, karyotyping, SNP array and whole exome sequencing. The everolimus concentration that reduces growth by 50% (IC₅₀) was determined in both cell lines. Starting from this IC₅₀ concentration, QGP-1 and BON-1 were cultured in increasing concentrations of everolimus during 20 to 22 weeks until a final concentration of 1000-fold and 250-fold of the initial IC₅₀ was reached for BON-1 and QGP-1, respectively. In parallel, untreated and vehicle-treated conditions of both cell lines were cultured. To evaluate the mechanisms underlying everolimus resistance in PNET, the BON cell lines with induced resistance are compared with the initial, sensitive, cell lines using whole exome sequencing. To overcome the resistance to everolimus, the cell lines with induced everolimus resistance were treated with BEZ235, a combined PI3K-Akt inhibitor.

Results : Redetermination of the IC₅₀ concentration in both cell lines revealed a strong reduction in everolimus sensitivity in the everolimus-treated cell lines in comparison to the vehicle treated cell lines, thus confirming an induced resistance in those cell lines. Initial results of the whole exome sequencing of the BON-1 untreated, vehicle and everolimus-treated cell lines revealed predicted protein-damaging nucleotide alterations in 19 genes in the everolimus-treated but not in the other cell line conditions. Interesting genes that were mutated in the everolimus-treated cell line include the pro-apoptotic *BNIP2*, the DNA polymerase-associated *POLDIP2* and the chemotherapy-resistance-associated *FPGS* genes. Initial results using BEZ-235 on sensitive and resistant cell lines showed to have an effect on cell proliferation in both cell lines and this way was able to overcome the resistance.

Conclusions : Two PNET cell lines with induced everolimus resistance were established. Exome sequencing unravels 19 possible resistance related genes in BON-1. Similar analysis is ongoing for QGP-1. Everolimus resistance might be overcome by using another approach to block the PI3K-Akt-mTOR pathway.

Highlights of the year in GI oncology - Impact for clinicians

Pancreatic and hepatobiliary tumors.
Jean-Luc Van Laethem, Erasme Hospital, ULB, Brussels.

Oesophago-gastric tumors.
Marc Peeters, UZA, Belgium.

Colorectal tumors.
Eric Van Cutsem, Gasthuisberg, KUL, Belgium.

POSTERS

- O11 -

EARLY METABOLIC RESPONSE ASSESSMENT OF SORAFENIB AND CAPECITABINE IN CHEMO-REFRACTORY METASTATIC CRC. A. Hendlisz (1), A. Deleporte (2), C. Garcia (2), T. Delaunoit (3), R. Marechal (4), M. Peeters (5), M. Van Den Eynde (6), S. Holbrechts (7), G. Houbiers (8), B. Filleul (9), J.L. Van Laethem (10), C.D. Rolfo (11), M. Diaz (7), R. Lhommel (6), G. Demolin (8), M. Moreau (2), L. Ameye (2), M. Paesmans (2), M. Piccart-Gebhart (2), P. Flamen (2). (1) Institut Jules Bordet, City of Brussels, Belgium ; (2) Institut Jules Bordet, Brussels, Belgium ; (3) Hôpital de Jolimont, Haine-Saint-Paul, Belgium ; (4) Hopital Erasme, Dilbeek, Belgium ; (5) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium ; (6) Université Catholique de Louvain, Brussels, Belgium ; (7) CHU Ambroise Paré MONS, Mons, Belgium ; (8) Clinique St-Joseph, Liège, Belgium ; (9) Centre Hospitalier de Jolimont-Lobbes, La Louvière, Belgium ; (10) Hôpital Erasme, City of Brussels, Belgium ; (11) University of Antwerp, Antwerpen, Belgium.

Introduction : The Sorafenib (SOR)-capecitabine (CAP) combination has shown clinical activity in several phase I-II trials involving metastatic breast cancer and metastatic colorectal cancer (mCRC) patients. SoMore aims to substantiate the combination's effects in mCRC refractory to all medications and the predictive value of early metabolic response (MR) on survival.

Aim : SoMore (EUDRACT 2010-023695-91) has 2 co-primary objectives : 1) to demonstrate an overall survival (OS) rate at 6 months > 30%, and 2) to compare OS between patients with and without MR.

Methods : SoMore is a non-randomized prospective open-label phase II. There were no stopping rules. CAP was given at 1700 mg/m²/day, 2 weeks out of 3. SOR was administered at 600 mg/day for the first cycle, then at 800 mg/day until progression or unacceptable toxicity. FDGPET-CT was performed at baseline and before the second cycle. MR analysis was centralized and blinded for the investigators.

Results : From February to October 2011, 92 eligible patients were prospectively recruited in 6 Belgian centers : M/F : 54%/46% ; ECOG PS 0/1 : 55%/45% ; median age : 61. A median of 5 treatment cycles were given (0-28+). Grade 3-4 toxic reactions were reported in 61.2%, mainly fatigue (18%), hand-foot skin reaction (14%) and diarrhea (11%), but no toxic death. 6.9% of the patients stopped therapy due to toxicity. 6 months OS was 71% (95% CI : 61%-79%), significantly > 30% (p < 0.001). 47% of the 79 patients evaluable for metabolic assessment showed homogeneous MR (HMR) of all metastatic lesions, 32% mixed MR and 21% homogeneous non-MR. Median overall OS and PFS of the intent-to-treat population and of patients with and without HMR are shown in the table below. Hazard ratio for HMR was 0.34 (95% CI, 0.21 to 0.56) p-value < 0.001 for PFS and 0.59 (95% CI, 0.37 to 0.96) p-value 0.03 for OS.

	All pts (92)	MR (37)	Non-MR (42)
mOS (months)	8.15 (95%CI: 6.77-10.45)	9.9 (95% CI, 7.6 to 16.3)	6.6 (95% CI, 4.8 to 8.3)
mPFS (months)	4.27 (95% CI: 3.48-4.83)	5.0 (95% CI, 4.0 to 8.9)	2.3 (95% CI, 1.4 to 3.1)

Conclusions : These data suggest robust efficacy for the SOR-CAP combination in heavily pretreated mCRC, associated with high but manageable toxicity. Early MR assessment, by detecting unresponsive lesions within the whole body tumoral load, is able to capture the patients' likelihood of benefit, opening the path to personalized medicine.

- O12 -

PRAVASTATIN EFFICACY FOR PATIENTS WITH CHILD-PUGH A/B CIRRHOSIS AND ADVANCED HCC. M. Grandjean (1), P. Starkel (2), Y. Horsmans (3), I. Borbath (4). (1) University Hospitals Leuven, Leuven, Belgium ; (2) Cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (3) Cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (4) Cliniques Universitaires St Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Second-line treatments after sorafenib resistance or intolerance are currently unavailable for patients with advanced hepatocellular carcinomas (HCC). Pravastatin has shown anti-tumoural effects, *both in vitro* and *in vivo* as well as in a clinical phase 2 trial.

Aim : to assess the effect of pravastatin for patients with advanced HCC.

Methods : We retrospectively reviewed the files of all consecutive HCC patients treated with pravastatin (40 mg/d) between 07/2005 and 03/2013. HCC was diagnosed histologically (n = 10) or with non-invasive AASLD criteria (i.e. the presence of arterial enhancement and venous wash-out on MRI/CT). The Barcelona Clinic Liver Cancer (BCLC) staging system was used to assess the oncologic staging. We analysed overall survival (OS), progression-free survival (PFS), biologic and radiologic response rate and toxicity.

Results : Fourteen patients, median age 80 y, were treated for a median time of 6.2 months (range : 1.6-44.7 months). Twelve patients were cirrhotic (4 with Child-Pugh A, 8 with Child-Pugh B), due to alcohol (n = 4), HCV (n = 8). Thirteen had a BCLC C score (metastatic in 2, vascular invasion in 8, diffuse tumours in 3), one BCLC B patient progressed after loco-regional therapy. Pravastatin was given as the only treatment in 2 patients, before sorafenib in 2 (pre-sorafenib era) and after sorafenib in 10. Treatment was stopped upon clear radiologic progression in 5 patients, or death. Median overall survival (OS) was 8.9 months (range : 2.5-50.7 months) ; OS at 24 months was 20%. Median progression-free survival (PFS) was 6 months. Median OS after pravastatin stop was 1 month. Subanalysis done for the 12 patients treated by pravastatin after sorafenib (n = 10) or only by pravastatin (n = 2) showed a median OS of 8.8 months and an 8.8 months PFS. Two patients had radiologic (decrease > 30% of largest diameter) and biologic (decrease of > 50% of AFP) partial response, lasting for 39 and 50 months. The drug was well tolerated with only one patient with minor side effect (muscle cramps).

Conclusions : In our study, pravastatin was well tolerated and was associated with promising PFS and OS. Given the small size of the cohort and the retrospective design, these results need to be confirmed by a prospective randomized study, comparing pravastatin with placebo after sorafenib failure/intolerance.

- O13 -

SIMULTANEOUS DETECTION OF KRAS, NRAS AND BRAF HOTSPOT MUTATIONS ON ION TORRENT PGM PLATFORM. C. De Rop, G. Beniuga, J.L. Dargent, P. Vannuffel. Institut de Pathologie et de Génétique, Charleroi, Belgium.

Introduction : Colorectal cancer is the third most common cause of cancer and cancer death, and approximately 20% of all patients will present with metastatic disease. Treatment has become increasingly complex with the emergence of new chemotherapy drugs and targeted agents.

To date, in metastatic colorectal cancer (mCRC), KRAS testing has been restricted to codons 12 and 13. However, phase II and III trials now suggest including both KRAS and NRAS codons 12, 13, 61, 117, and 146. Searching for these additional codons could help screen 20% more patients with mCRC for treatment with EGFR inhibitors. Other finding suggests that testing for the BRAF V600 mutation complements KRAS and NRAS mutations analysis and may be as important for treatment decisions.

Aim : Here we present a multiplexed strategy enabling to test simultaneously these 11 hotspot mutations.

Methods : A custom panel (CP-KRAS), covering exons 2, 3 and 4 of KRAS and NRAS and exon 15 of BRAF, was designed using the Ion AmpliSeq Designer module. FFPE tissue sections were macro-dissected and DNA was extracted using the Maxwell FFPE Tissue LEV purification kit. Sequencing was performed according to the AmpliSeq protocol on the Ion Torrent PGM, starting from as less as 6 ng of DNA. Data were analysed with both Torrent Suite v4 and Next-GENe software v2.3 with a hotspot positions-targeting bed file. Sensitivity of the technique was assigned from 2.5% at 500 x coverage to 10% at 100 x coverage, with a forward to reverse reads ratio > 0.25.

Results : After validation of the CP-KRAS panel on well-characterized EQC samples, more than 120 FFPE specimens (corresponding to a 4 months of CP-KRAS testing) were sequenced. 35% of the samples were mutated for KRAS codon 12 and 5% for codon 13. Of the wild-type samples tested for additional mutations, 3% of samples were mutated for KRAS codon Q61, 3% for KRAS codon A146, 5% for NRAS G12 and 2% for NRAS Q61. 8% additional samples were mutated for BRAF V600. Each additional mutation was confirmed by pyrosequencing, mini-sequencing (SNaPshot) or Sanger sequencing. In total, 26% tumours wild type for KRAS exon 2 harboured a mutation in another of the RAS pathway genes.

Conclusions : This next generation and high throughput sequencing workflow on a single-tube multiplexed amplification of those KRAS, NRAS and BRAF hotspot mutations could help to identify additional patients who possibly fail to respond to anti-EGFR treatment. Moreover, this strategy is cost-effective, less fastidious and requires minimal DNA quantity from FFPE tissue as compared to standard analysis.

DETECTION OF C-KIT AND PDGFRA MUTATIONS IN GIST BY NEXT GENERATION SEQUENCING.
C. De Rop, G. Beniuga, P. Ngendahayo, P. Vannuffel. Institut de Pathologie et de Génétique, Charleroi, Belgium.

Introduction : Gastrointestinal stromal tumours (GIST) occur in the gastrointestinal track, most commonly in the stomach or small intestine. These sporadic tumours are associated with genetic changes. In GIST subsets, c-KIT or PDGFRA mutations correlate with distinct anatomical site, clinical phenotype and sensitivity to tyrosine kinase inhibitors.

Aim : Here we depict a multiplexed evaluation strategy based on Next Generation Sequencing (NGS) to investigate for the most relevant regions of c-KIT and PDGFRA in a GIST context.

Methods : A custom panel, covering exons 9, 11, 13 and 17 of c-KIT and exons 12, 14 and 18 of PDGFRA, was designed using the Ion AmpliSeq Designer module. FFPE tissue sections were macro-dissected and DNA was extracted using the Maxwell FFPE Tissue LEV purification kit. Sequencing was performed according to the AmpliSeq protocol on the Ion Torrent PGM, from as less as 6 ng of input DNA. Data were analysed with both Torrent Suite v4 and NextGENe software v2.3 with a whole exons-targeting bed file. Sensitivity of the technique was assigned from 2.5% at 500 x coverage to 10% at 100 x coverage, assuming a forward to reverse reads ratio > 0.25.

Results : DNA samples (10 providing from EQC schemes and 30 from our regular practice), for which c-KIT and PDGFRA mutations have been previously identified by Sanger sequencing, were tested with the designed panel. For all 40 samples, there is a perfect correlation between our multiplexed sequencing strategy and the «gold standard» Sanger sequencing. Mutations - point mutations, duplications, deletions, insertions or both, mainly located in exons 9 and 11 of c-KIT and in exon 18 of PDGFRA – were found in 32 samples by both techniques, while no critical variant was detected in 8 normal samples.

Conclusions : This multiplexed amplification of 7 exons in GIST samples demonstrates that custom NGS panels can be designed to identify mutations in genes or regions more relevant to diagnosis, prognosis or therapeutic choices. For any tumour setting or pathway, NGS panels allow more comprehensive sequence coverage than standard techniques and are scalable in term of regions of interest and number of samples.

ENDOSCOPIC FINDINGS IN CASE OF PET-CT INCIDENTAL COLONIC UPTAKE : HOW TO IMPROVE PET-CT SPECIFICITY ? M. Seivert, O. Plomteux, A. Colard, P. Leclercq, G. Houbiers, G. Demolin, P. Dupont, F. Fontaine, D. Jean Claude, G. Namur, N. Witvrouw, P. Gomez, B. Bastens. Clinique St-Joseph, Liège, Belgium.

Introduction : Unexpected colo-rectal 18FDG focal uptake (UCFU) in PET CT happens in about 1.4% of case. Due to their personal status, all oncologic patients are not able to undergo colonoscopy. In retrospective studies, when endoscopy was performed, 70% showed colonic abnormalities in the predicted colonic segment and 20% showed adenocarcinoma.

Aim : The purpose of this study was to evaluate the significance of UCFU encountered during PET CT. The secondary endpoint was to assess criteria improving specificity of PET CT for advanced adenoma (adenoma with villous histology, high grade dysplasia or ≥ 10 mm in size) and adenocarcinoma.

Methods : A prospective study was conducted in a single institution from April 2012 to September 2013. In the 2904 patients who underwent 18FDG PET-CT for oncologic, infectious or inflammatory disease, those with UCFU were referred for colonoscopy. We studied the lesion/liver SUVmax ratio and tomodensitometric imaging in PET CT, the colonoscopic and the pathological findings.

Results : 52 patients with an UCFU were referred and 43 underwent colonoscopy. Among them, eight patients had no colonic abnormality (18.6%), thirty three had lesions in the expected colonic segment (76.7%) : 18 had advanced adenoma (42.9%) and 10 had adenocarcinoma (23.3%)

21 patients with UCFU showed tomodensitometric endoluminal mass in the same colonic area. Among them, eighteen underwent a colonoscopy. 15 had lesions in the expected colonic segment (83.3%), 9 was neoplastic (50%). Sensibility and specificity for colonic neoplasia in case of associated pathological images (PET and tomodensitometry) are respectively 90% and 71.9%. The negative predictive value of this associated pathological image is 95.8%.

Comparing lesion/liver SUVmax ratio between adenocarcinoma group, advanced adenoma group and normal colonoscopy group, we showed statistically significant difference between the adenocarcinoma group and the others ($p < 0.05$) but no cut off value could be determined. No significant difference was found comparing advanced adenoma group and normal colonoscopy group.

Conclusions : Our prospective study confirms that UCFU is often associated to significant endoscopic findings and especially neoplastic lesions. So, patients with UCFU should undergo systematic endoscopic exploration, but in practice, not all oncologic patients are fit to undergo endoscopy due to their fragility. We need criteria improving PET CT specificity for advanced adenoma and adenocarcinoma to select patients who should undergo prompt colonoscopy and those

who can be delayed: We showed that associated tomodesitometric abnormalities and colonic FDG uptake are more predictive of a neoplastic finding. By this study, we could not determine lesion/liver SUVmax ratio cut off between normal colonoscopy, advanced adenoma and adenocarcinoma.

- O16 -

AGE- & GENDER-CORRECTED BMI : A NEW APPROACH TO MORTALITY RISK ASSESSMENT IN ESOPHAGECTOMY FOR CANCER. J. Moons, H. Van Veer, T. Lerut, W. Coosemans, G. Decker, D. Van Raemdonck, P. De Leyn, P. Nafteux. University Hospitals Leuven, Leuven, Belgium.

Introduction : Esophagectomy for cancer is often accompanied with severe preoperative weight loss. The magnitude of this weight loss is typically measured by weight-loss percentage (WL%) and Body Mass Index (BMI). Both measures are frequently used to identify negative outcomes. However BMI criteria, as defined by the World Health Organization, are both age- and sex-independent.

Aim : The aim of this study was to incorporate the latter two parameters in “age-gender specific BMI-percentiles” (AG-BMI) and evaluate this new model in relation to survival outcome.

Methods : Six hundred forty-two consecutive patients who received esophagectomy for esophageal cancer between 2005 and 2010, were analyzed. Length, weight before onset of symptoms and on the day before surgery were recorded. WL%, BMI and AG-BMI were calculated. Cut-off points to determine underweight were : WL% more than 10%, BMI < 18.5 kg/m² and AG-BMI less than 10th percentile. Overall survival (OS) was analyzed by means of Kaplan-Meier log rank test. A multivariate analysis was performed on all univariate significant variables.

Results : On univariate analysis, all three models showed significant OS differences, especially in the underweight groups. However AG-BMI showed a more homogeneous distribution and better correlation between weight-classes and OS. Multivariate analysis withheld five independent prognosticators for OS : age (OR 1.021), early versus advanced disease (OR 0.251), R-status (OR 1.879), number of positive lymph nodes (OR 1.059) and the AG-BMI-10th percentile (OR 1.540), but not BMI and WL%. Furthermore, AG-BMI-10th percentile identified a significant (p < 0.0001) higher number of non-esophageal-cancer- related deaths (i.e patients who died without any evidence recurrent esophageal cancer) at 1 and 3 years post-esophagectomy.

Conclusions : Age-Gender specific BMI percentiles are more performant compared to the current BMI-classes and WL% in predicting OS after esophagectomy. Furthermore our data suggest a more negative impact on OS from underweight and not from overweight. By Identifying preoperatively risk patients for poorer OS, especially the non-esophageal-cancer-related deaths, this can be a more precise tool to tailor peri-operative nutritional strategies and prevent further weight loss.

- O17 -

BEVACIZUMAB BEYOND PROGRESSION IN MCRC : A PHASE II, SINGLE ARM, BELGIAN MULTICENTER STUDY (AVASTAY). M. Peeters (1), A. Bols (2), J. Van Erps (3), R. Kalantari (4), W. Demey (5), M. Heijndijk (6), J.L. Canon (7). (1) Universitair Ziekenhuis Antwerpen, Antwerpen, Belgium ; (2) AZ Sint-Jan Brugge-Oostende, Brugge, Belgium ; (3) General Hospital Aalst, Aalst, Belgium ; (4) CH Peltzer-La Tourelle, Verviers, Belgium ; (5) KLINA, Brasschaat, Belgium ; (6) F.HOFFMANN-LA ROCHE, Brussels, Belgium ; (7) Grand Hopital de Charleroi, Charleroi, Belgium.

Introduction : The study ML18147 (TML study) already demonstrated, in a prospective trial design, the clinical benefit of continuing BEV + CT in mCRC patients who progressed after discontinuation of first-line (1L) BEV + CT. This is the first study to prove the feasibility of adding BEV to an oxaliplatin-containing regimen shortly after progression on 1L treatment with FOLFIRI + BEV, within the scope of local therapeutic options.

Methods : Patients with unresectable, histologically confirmed mCRC who progressed within 8 weeks after discontinuation of 1L BEV + FOLFIRI received XELOX or FOLFOX + BEV (2.5 mg/kg/week equivalent). The choice of CT regimen was at the investigator’s discretion. The primary endpoint was progression-free survival (PFS) ; secondary endpoints included time to PD2 from start of 1L therapy (TTP2, retrospectively documented), overall response rate, safety, and serum concentrations of proangiogenic cytokines (bFGF, HGF and placental GF, in a subset of patients).

Results : 75 patients entered the study between July 2009 and July 2011 : 50 received FOLFOX + BEV and 25 received XELOX + BEV. The intent-to-treat (ITT) population showed a median PFS of 5.8 mo (95% CI 4.1-6.2). Median TTP2 was 18.0 mo (95% CI 15.2 - 19.4). Overall response rate was 13%. The adverse event profile was consistent with previously reported data for BEV + CT. The most common grade 3+ adverse events were diarrhea (10.7%), neutropenia (9.3%), and fatigue (6.7%). Three treatment-related serious adverse events (anal fistula, febrile neutropenia, drug

intolerance) and 3 deaths (necrotizing fasciitis and general physical health deterioration [2x]) occurred during the study. Serum concentrations of proangiogenic cytokines did not correlate with PFS or tumor response.

Conclusions : PFS and safety profile associated with the studied BEV regimen in a local therapeutic setting were generally consistent with those reported by previous studies, such as the TML study (ML18147). The findings further support the continuation of BEV after PD during 1L BEV + CT.

PATHOLOGY CLUB, RADIOLOGY, NUCLEAR MEDICINE

- R01 -

Invited Lecture

HISTOPATHOLOGICAL ASSESSMENT OF TOTAL MESORECTAL EXCISION SPECIMENS : REFLEXIONS FROM THE PROCARE PROJECT. Pieter Demetter, Department of Pathology, Erasme University Hospital, Université Libre de Bruxelles.

The concept of total mesorectal excision (TME) is based on the notion that lateral mesorectal spread of small tumour foci, which are not removed in classic anterior resection, can lead to local recurrence after rectal cancer surgery. TME has been introduced in the 1980s, and has now become standard of care for rectal cancer.

PROCARE (PROject on CANcer of the REctum), a Belgian multidisciplinary national project to improve outcome in rectal cancer patients, was launched in 2006. In this project, pathologists from participating hospitals record histopathological information of the resected tumour on a standard minimal data form for all patients.

Central pathology review was organized to ensure consistent quality of all pathology data and procedures. Based on photographic material and histopathology slides, a review committee of gastrointestinal expert pathologists re-evaluated the mesorectal plane, the tumour differentiation grade, the (y)pT stage and the tumour regression grade in 444 cases previously routinely assessed by local pathologists.

The surgical plane was reported in 89% and the circumferential resection margin in 88% of cases by the local pathologist. The median number of lymph nodes harvested in patients undergoing neoadjuvant radiochemotherapy was 11, and 14 in the other patients. The review committee downgraded the surgical plane in 17% from (intra)mesorectal to intramuscular, and upgraded it in 27% from intramuscular to (intra)mesorectal. Tumour differentiation grade, T stage and tumour regression grade differed between local pathologists and review committee in 15%, 10% and 38%, respectively. T stage was upgraded in 8% of cases, mainly from T2 to T3. Tumour regression was judged by the review panel to be less advanced in 15% of cases.

This analysis shows that pathologists continue to fail to report key diagnostic features and are confronted with significant interobserver variation, and that there is still a long way to go with rectal cancer care.

- R02 -

RESTRICTED OVEREXPRESSION OF P53 IS PROGNOSTIC AFTER COLORECTAL CANCER (CRC) RESECTION. H.M. Lê (1), C. Nyiraneza (1), A. Jouret-Mourin (2), A. Kartheuser (1), R. Detry (1), K. Dahan (1), A. Van Maanen (1), C. Sempoux (2), M. Van Den Eynde (2). (1) Clin universitaires St-Luc, UCL, Brussels, Belgium ; (2) Cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Restricted overexpression of p53 in immunohistochemistry (p53IHC RO) was previously described as a different pattern from the negative or diffuse p53 IHC expression. p53IHC RO is characterized by a limited number of homogeneously scattered strongly positive CRC cells and is associated with some clinico-pathologic CRC feature. Nevertheless, the prognostic impact of p53IHC RO remains unknown.

Material and methods : p53 and MMR (MLH1, MSH2, MSH6 and PMS2) proteins IHC expression were performed on CRC tumor of an unselected cohort of patients who underwent surgery at the Cliniques universitaires St-Luc between 1995 and 2000. p53 IHC results were defined as negative, diffuse or restricted expression. Clinico-pathologic CRC characteristics and patient follow-up must be available for this retrospective analysis. Between p53 overexpression group comparison of numerical and categorical clinico-pathological data were evaluated respectively by ANOVA and Fisher exact tests. Progression-Free Survival (PFS) and Overall Survival (OS) analyses were performed using Kaplan-Meier method. Between group comparison was done using a log-rank test, with a Bonferroni correction applied for multiplicity. Uni and multivariate PFS and OS regression were performed using a Cox Proportional Hazard model to establish best fitting model.

Results : Among 100 CRC tumors analyzed from 99 patients, p53 IHC expression was categorized as restricted (n = 36), diffuse (n = 46) or negative (n = 18). p53IHC RO (total n = 36, defective MMR n = 20, proficient MMR n = 16) was significantly associated with age (≥ 60 y), proximal location and poorly differentiated CRC, and a lowest lymph node ratio (LNR) ($p < 0.05$). Among the defective MMR CRC, 20/24 was p53IHC RO ($p < 0.001$). After a mean follow-up of 13 years after CRC resection, median PFS and OS were significantly higher in the p53IHC RO group as compared with the diffuse and negative p53 IHC expression (PFS respectively not reached vs 40.9 vs 22.8 months, $p < 0.001$ and OS respectively not reached vs 70.7 vs 48.0 months, $p = 0.009$). LNR, synchronous metastases and p53IHC RO were significantly associated with PFS in multivariate analysis ($p < 0.05$).

Conclusion : p53IHC RO is a prognostic factor after CRC resection. These results must be confirmed on a largest cohort but could be clinically relevant for prognosis especially for proficient MMR CRC tumor.

ADAM-17/FHL2 COLOCALISATION SUGGESTS INTERACTION AND ROLE OF THESE PROTEINS IN COLORECTAL CANCER. L. Verset (1), S. Sauvage (2), J. Tommelein (3), X. Moles Lopez (2), C. Decaestecker (4), M. Mareel (3), M. Bracke (3), I. Salmon (1), O. De Wever (3), P. Demetter (1). (1) Department of Pathology, Erasmus University Hospital, ULB, Dilbeek, Belgium ; (2) DIAPATH, Center for Microscopy and Molecular Imaging (CMMI), Charleroi, Belgium ; (3) Department of radiation Oncology and Experimental Cancer research, Ghent University Hospital, Ghent, Belgium ; (4) Laboratory of Image Synthesis and Analysis, ULB, Brussels, Belgium.

Introduction : ADAM-17, a disintegrin and metalloprotease, is responsible for the ectodomain shedding of trans-membrane proteins, including Epidermal Growth Factor Receptor (EGFR) ligands.

Four-and-a-half LIM domains protein 2 (FHL2), a molecule involved in multiple protein-protein interactions, interacts with the cytoplasmic tail of ADAM-17 and regulates its localisation and activity. Previously, we demonstrated that high expression of FHL2 in colorectal cancer is associated with poor prognosis.

Aim : The present study aims to compare ADAM-17/FHL2 colocalisation in colorectal cancer cells and myofibroblasts versus normal colon epithelium.

Materials and methods : ADAM-17 and FHL2 expression were studied in several colon cancer cell lines (HT29, HCT8/E11, Caco-2, SW480, SW620, Colo320DM) and in an immortalised myofibroblast cell line (CT5.3htert) by immunocytochemistry. To highlight a possible colocalisation of ADAM-17 and FHL2, we used the Duolink[®] kit for a proximity ligation assay (PLA), performed on SW480, Colo320DM and CT5.3htert cells.

The PLA was also performed on biopsy specimens of 10 colorectal adenocarcinomas as well as on normal colonic mucosa from the same patients. To quantify ADAM-17/FHL2 colocalisation, numbers of PLA signals were counted. Comparison between matched PLA signal numbers was performed using the non-parametric Wilcoxon signed-rank test.

Results : ADAM-17 was detected by immunocytochemistry in all cell lines except in Colo320DM. All cell lines exhibited FHL2 positivity. The PLA revealed ADAM-17/FHL2 colocalisation in SW480 and in CT5.3htert, but not in Colo320DM cells.

PLA signals were 2 to 28-fold more frequent in neoplastic colorectal epithelium than in matched control epithelium ($p < 0.01$).

Conclusion : ADAM-17 colocalises with FHL2 in neoplastic epithelium in colorectal cancer. This colocalisation is more obvious in cancerous than in matched control tissue, suggesting a role for ADAM-17/FHL2 mediated ectodomain shedding in colorectal cancer.

EFFECT OF HAART ON GASTROINTESTINAL AND HEPATOBILIARY OPPORTUNISTIC INFECTIONS. J. Toshniwal (1), R. Chawliani (1), M. Sachdeva (1), K. Mönkemüller (2). (1) Sir Ganga Ram Hospital, New Delhi, India ; (2) MARIENHOSPITAL, Bottrop, Germany.

Introduction : Opportunistic infections (OI) of gastrointestinal (GI) and hepatobiliary system (HB) are common in HIV infected patients. Despite highly active antiretroviral therapy (HAART) GI OI have been reported in HIV infected patients. There is paucity of data describing occurrence of GI and HB OI in AIDS with use of HAART.

Aim : This prospective study aimed to study the effect of HAART on Gastrointestinal and Hepatobiliary opportunistic infections

Methods : Study population included 74 HIV seropositive patients (Male = 57, Female = 17) in the age group of 17 to 63 years admitted to a tertiary care referral center in North India from January 2011 through December 2012. Only subjects who presented with GI and HB system manifestations were enrolled in the study. 74 study subjects were stratified into HAART naïve (36) and HAART experienced (38) groups according to their HAART status on admission. HIV infection was confirmed with western blot test. Various gastrointestinal and hepatobiliary pathologies including OI were evaluated and diagnosed as per standard protocols.

Results : In HAART experienced group 33% and in HAART naïve group 52% patients were diagnosed with OI. Esophageal candidiasis was present in 10% patients in HAART experienced group and in 7% patients in HAART naïve group (p value > 0.05). Abdominal TB was present in 24% patients in HAART experienced group and in 33% patients in HAART naïve group (p value > 0.05). Drug induced liver injury was present in 10 patients in HAART experienced group and in 5 patients in HAART naïve group.

Conclusions : In our study we found that OI are also common in patients taking HAART especially esophageal candidiasis and tuberculosis, reason for this may include drug resistance, noncompliance. Immune restoration inflammatory syndrome and high prevalence of tuberculosis in this region.

Invited Lecture

Hereditary colorectal cancer syndromes : an update.

M. Novelli. University College London Hospitals, London, Great Britain (UK)

Until relatively recently our knowledge of hereditary colorectal cancer was limited to the autosomal dominantly inherited syndromes Familial Adenomatous Polyposis (FAP) and Lynch syndrome (formerly HNPCC). However, it has become increasingly apparent that the hamartomatous polyp syndromes Juvenile polyposis and Peutz-Jegher syndrome are actually cancer syndromes and over the last ten years or so a number of new colorectal cancer syndromes have also been described (including MYH-associated polyposis, Hereditary mixed polyposis, Polymerase proofreading-associated polyposis, serrated polyposis and congenital mismatch repair deficiency). These syndromes include a couple of autosomal recessively inherited conditions (MYH-associated polyposis and congenital mismatch repair deficiency) and serrated polyposis where the mode of inheritance remains unclear. The clinical features, genetics and histopathology of these syndromes will be described.

UNDIFFERENTIATED PLEOMORPHIC SARCOMA OF THE LIVER DEVELOPING IN THE CONTEXT OF LYNCH SYNDROME. H. Djedaimi (1), G. Delvaux (1), J. De Greve (1), A. Hoorens (2). (1) UZ Brussel, Brussels, Belgium ; (2) UZ Brussel, Brussels, Belgium.

Aim : To report the occurrence of an undifferentiated pleomorphic sarcoma of the liver that developed on the basis of Lynch syndrome (HNPCC).

Methods and results : A 36-year-old man presented with right upper quadrant pain. This patient had previously been shown to harbour a germ-line mismatch repair gene mutation. Since almost 10 years he had an annual screening colonoscopy, that each time revealed the presence of adenomatous polyps. His last colonoscopy dated back to 9 months before. At that time a flat adenomatous polyp had been removed from the colon. Radiological examination identified a large mass (9x7x6cm) pendulating from segment 6 of the liver, penetrating the liver capsule and infiltrating the parietal peritoneum and mesocolon. An en bloc resection of the liver mass was performed together with a right hemicolectomy and parietal peritoneal resection. The histological features, immunohistochemical profile and complementary molecular pathology findings of this liver mass were consistent with the diagnosis of an undifferentiated pleomorphic sarcoma. Immunohistochemistry in addition showed loss of expression of the MMR proteins MLH1 and PMS2. Expression of MSH2 and MSH6 appeared preserved. A peritoneal washing demonstrated the presence of tumour cells in the abdominal cavity

Conclusions : Lynch syndrome was originally described as a genetic syndrome predominantly causing colorectal cancer. Numerous other neoplasms however may be associated with HNPCC, mainly located in the endometrium, stomach, ovary, hepatobiliary and urinary tract. Sarcomas have only rarely been reported in Lynch syndrome. This case emphasizes that sarcomas indeed may develop in the context of Lynch syndrome and be caused by the underlying genetic defect as indicated by the loss of expression of MMR proteins in the liver sarcoma in this patient. It makes clear that sarcomas may not be disregarded when analysing pedigrees in the context of HNPCC, especially if they occur at an early age. Like for colorectal cancer, the prognosis compared to sporadic sarcomas might be more favourable.

ORGANIZED PROTEOMIC HETEROGENEITY IN COLORECTAL LIVER METASTASES AND IMPLICATIONS FOR THERAPIES. A. Turtoi (1), A. Blomme (1), D. Debois (1), J. Somja (2), E. De Pauw (1), P. Delvenne (2), O. Detry (2), V. Castronovo (1). (1) University of Liege, Liège, Belgium ; (2) Centre Hospitalier Universitaire de Liège, Liège, Belgium.

Introduction : Tumor heterogeneity is a major obstacle for developing effective anti-cancer treatments. Recent studies have pointed at large stochastic genetic heterogeneity within cancer lesions, where no pattern seems to exist that would enable a more structured targeted therapy approach.

Aim : Because to date no similar information is available at the protein (phenotype) level, we aimed at characterising the proteomic heterogeneity in human colorectal carcinoma (CRC) liver metastases.

Methods & Results : We employed MALDI imaging-guided proteomics and explored the heterogeneity of extracellular and membrane sub-proteome in a unique collection of eight fresh human CRC liver metastases. Monitoring the spatial

distribution of over 1000 proteins we found unexpectedly that all liver metastasis lesions displayed a reproducible, zonally delineated, pattern of functional and therapeutic biomarker heterogeneity. Peritumoral region featured elevated lipid metabolism and protein synthesis, the rim of the metastasis displayed increased cellular growth, movement and drug metabolism whereas the center of the lesion was characterized by elevated carbohydrate metabolism and DNA-repair activity. From the aspect of therapeutic targeting zonal expression of known and novel biomarkers was evident, reinforcing the need to select several targets in order to achieve optimal coverage of the lesion. Finally we highlight two novel antigens, LTBP2 and TGFBI, whose expression is a consistent feature of CRC liver metastasis.

Conclusions : In the current work we studied human CRC liver metastases and demonstrated for the first time that their proteome heterogeneity has a distinct, organized, pattern. This particular hallmark can now be used as a part of the strategy for developing rational therapies based on multiple sets of targetable antigens.

- R08 -

T-CELL INFILTRATION ON LIVER COLORECTAL METASTASES RESECTED AFTER TREATMENT IS PROGNOSTIC. M. Van Den Eynde (1), B. Mlecnik (2), J.P. Machiels (3), D. Debetancourt (3), C. Sempoux (1), A. Jouret-Mourin (1), J.F. Gigot (1), C. Hubert (1), Y. Humblet (1), N. Haicheur (2), F. Marliot (2), F. Pages (2), J. Galon (2). (1) Cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (2) Hôpital Européen Georges Pompidou, Paris, France ; (3) Clin universitaires St-Luc, UCL, Brussels, Belgium.

Background : Colorectal cancer T-cell infiltration (TCI) is a strong prognostic factor for survival after primary tumor resection. Curative surgery of liver colorectal metastases LCM is the only hope for cure of metastatic patients (pts). Nevertheless, 70% of them will relapse. TCI analysis of LCM is poorly characterized and could be a prognostic factor for disease-free survival (DFS) and overall survival (OS) as in primary tumor.

Material and methods : pts engaged for curative liver surgery after preoperative treatment with available FFPE blocks for all resected LCM, were included. An immunoscore (IS), defined by the TCI in the center (CT) and the invasive margin (IM) for each LCM, was determined using whole-slide quantitative immunohistochemistry (markers : CD3, CD8, CD45RO). The mean value of the 3 most infiltrated fields (0.8 mm²) for each markers was defined in the CT and IM for all LCM. The total number of high densities (Hi, above the cut-off at the median density) in CT and IM for each marker was used to stratify pts for the IS. The markers were combined 2 by 2 in CT and IM (CD3-CD8, CD3-CD45RO, CD8-CD45RO) and finally regrouped to an IS of 0-2 Hi (IS 0-2 : low TCI) or 3-4 Hi (IS 3-4 : high TCI). For pts with multiple LCM ; the median value of all densities, the less and the most infiltrated LCM/pt were analyzed. Cumulative DFS/OS analyses were performed using the Kaplan-Meier estimator. OS/DFS analyses were made using univariate Cox regression and compared by log-rank tests (IS0-2 vs 3-4).

Results : 59 patients (M/F 1.1, 203 LCM, mean 3.4 /pt, synchr/metachr 5.4) were included. IS 3-4 in the less infiltrated metastasis is significantly associated with OS and DFS for all markers combinations.

LCM/pt	Markers	Survival	HR (IS 0-2 vs 3-4;95%IC)	logrank p-value	months (IS 0-2 vs 3-4)
Median of all	CD3-CD8	DFS	1.2 (0.7-2.3)	0.48	
		OS	2.2 (0.7-6.9)	0.14	
	CD3-CD45RO	DFS	1.5 (0.8-2.9)	0.16	
		OS	2.2 (0.8-5.9)	0.11	
	CD8-CD45RO	DFS	1.0 (0.6-2.0)	0.82	
		OS	1.0 (0.4-2.8)	0.93	
Less infiltrated	CD3-CD8	DFS	1.8 (1.0-3.4)	0.05	8.0 vs 14.9
		OS	8.8 (2.0-39.1)	0.0007	27.9 vs NR
	CD3-CD45RO	DFS	2.9 (1.5-5.7)	0.0008	8.0 vs 17.0
		OS	4.5 (1.2-14.2)	0.009	31.8 vs NR
	CD8-CD45RO	DFS	2.3 (1.2-4.4)	0.006	8.4 vs 16.0
		OS	2.9 (1.0-8.4)	0.04	47.8 vs NR
Most infiltrated	CD3-CD8	DFS	1.0 (0.6-1.9)	0.93	
		OS	2.1 (0.7-6.6)	0.17	
	CD3-CD45RO	DFS	1.7 (0.9-3.2)	0.12	
		OS	3.7 (0.8-16.2)	0.06	
	CD8-CD45RO	DFS	1.6 (0.8-3.2)	0.16	
		OS	2.4 (0.7-8.4)	0.16	

Conclusions : The T-cell infiltration determined in the less infiltrated LCM/pt after resection is a prognostic factor.

FILIFORM POLYPOSIS IN A CYSTIC FIBROSIS PATIENT : A CASE REPORT. L. Bienfait (1), E. Farinella (1), P. Demetter (2). (1) Erasme Hospital, Brussels, Belgium ; (2) Hôpital Erasme, City of Brussels, Belgium.

Introduction : A 25-years-old woman suffering from cystic fibrosis and homozygous for delta F508 mutation presented with distal intestinal obstruction syndrome (DIOS) due to inspissated mucus. The patient was recently diagnosed with chronic myeloid leukemia ; medical history also included laparotomy for ileocaecal resection.

Methods : Partial transverse colectomy was performed because of a mass effected of the mucus. Macroscopically the specimen showed multiple pedunculated polyps exhibiting a "coral" shaped apparence reaching up to 6 cm in height. Microscopical examination revealed numerous polypoid projections consisting of fibromuscular stalks covered with inflammatory but otherwise normal colonic mucosa, without any nucleocytoplasmic atypia. A diagnosis of filiform polyposis was made.

Conclusion : Filiform polyposis is a rare disorder of uncertain pathogenesis. Although it can be seen in any portion of the large intestine, this form of pseudopolyposis is usually found in the transverse and descending colon. Most cases of filiform polyposis are associated with Crohn's disease, ulcerative colitis or granulomatous disease ; it is supposed that long-term inflammation of the colonic mucosa during chronic inflammatory bowel disease (IBD) with alternating periods of ulceration and healing may lead to the formation of finger-like projections. The present case is, however, associated to cystic fibrosis, without any argument for IBD

Invited Lecture

Differential diagnosis of benign ulcers and strictures in the small bowel.

G. Williams, Cardiff.

CLINICAL VALUE OF 18F-FDG-PET-CT IN THE PREOPERATIVE STAGING OF COLORECTAL PERITONEAL CARCINOMATOSIS. N. De Vos, I. Goethals, W. Ceelen. Ghent University, Gent, Belgium.

Introduction : In a subgroup of patients with limited colorectal peritoneal carcinomatosis (PC), extensive cytoreductive surgery (CRS) combined with hyperthermic intraoperative chemoperfusion (HIPEC) may offer a survival benefit. Since completeness of cytoreduction is one of the main prognostic indicators after surgery and HIPEC, an accurate iconographic prediction of the extent and location of PC is of paramount importance in these patients. However, imaging studies often fail to detect small peritoneal implants, leading to underestimation of the true disease burden.

Aim : To assess the value of locoregional staging using 18F-FDG-PET-CT in patients with colorectal PC planned for CRS and HIPEC.

Methods : Patients underwent staging including 18F-FDG-PET-CT. In the absence of systemic dissemination, CRS and HIPEC were performed. The extent of PC was quantified using the modified 7 region count (7RC). The correlation between imaging based estimation of PC extent and surgical 7RC was analyzed using Pearson correlation using both patient based and region based analyses.

Results : Fifty-five patients were included. The presence of PC was detected by 18F-FDG-PET-CT in 96% of patients with non-mucinous tumors and in 60% of mucinous tumors. In a region based analysis, overall sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 57%, 98%, 95%, 78% and 82% respectively for non-mucinous tumors and 32%, 100%, 100%, 55% and 63% respectively for mucinous tumors. Accuracy was less good in staging pelvic disease and small bowel disease. The correlation between imaging findings and the 7RC was better for non-mucinous histology ($r = 0.623$) than for mucinous histology ($r = -0.180$).

Conclusions : 18F-FDG-PET-CT shows acceptable performance in locoregional staging of PC from non-mucinous colorectal cancer. Imaging accuracy is less optimal for pelvic and small bowel disease.

CAN WE PREDICT MICROVASCULAR INVASION IN HCC ON FDG PET-CT IMAGING PARAMETERS ?
V. Agarwal, S. Pande, S. Krishan, D.R. Jangid. Medanta the Medicity Hospital, Gurgaon, India.

Aim : The purpose of this study is to correlate clinicopathologic and PET-CT parameters with the presence of microvascular invasion at histopathologic examination in patients with hepatocellular carcinoma (HCC) who have undergone liver transplantation

Methods : In this retrospective single-center study, we assessed 224 patients (187 men and 37 women ; mean age, 52 years) with HCC who underwent liver transplantation and pretransplant PET-CT (performed within 20 days before liver transplantation). Three physicians (two nuclear medicine specialist and one radiologist) analyzed the following tumor parameters in consensus : size, multi-focality, pattern of uptake, quantitative FDG uptake (SUV), pattern of enhancement and distance to closest vessel. The size and number of lesions, tumor differentiation and the presence or absence of microvascular invasion were determined at histopathologic examination. Histopathological findings were analysed vis-a-vis to the imaging parameters on PET-CT to determine any useful indicator for predicting microvascular invasion.

Results : None of the clinical parameters was predictive of microvascular invasion ; however on univariate analysis, MVI was statistically significantly associated with morphologic features of multi-focality, uptake pattern and distance to the closest vessel ; on FDG PET-CT. By applying multiple logistic regression analysis, uptake pattern (heterogeneous and peripheral FDG uptake) was found to be the only independent risk factor for MVI.

Conclusions : Heterogeneous and peripheral FDG uptake on FDG PET-CT was the only parameter that correlated significantly with microvascular invasion.

COMPARISON BETWEEN ELASTPQ AND FIBROSCAN FOR EVALUATION OF FIBROSIS IN CHRONIC LIVER DISEASES. M.A. Van Caulaert, N. Michoux, P. Starkel, Y. Horsmans, E. Danse. Clin universitaires St-Luc, UCL, Brussels, Belgium.

Introduction : Chronic fibrotic liver diseases represent a huge burden worldwide. Multiple potential causes are identified, with viral infections, alcohol and steatosis as the main causative factors. Accurate assessment of the level of fibrosis is a key point of the diagnostic evaluation, as it may influence prognosis and management. Shear wave ElastPQ (Philips IU22) is a new imaging technique allowing investigating the elasticity of tissue during standard ultrasound examination. However, its interest, compared to already widely used and validated Fibroscan remains to be fully evaluated. Therefore, in our study, we sought to compare ElastPQ to Fibroscan, considered as the standard reference, in liver fibrosis of different origins.

Methods : 132 patients were recruited and underwent liver examination for elasticity assessment using both ElastPQ (Philips IU22) and Fibroscan (Echosens, Paris, France) on the same day. Only patients with both a success rate > 60% at Fibroscan and a standard deviation < 30% were considered for comparing both techniques. 70 patients were thus evaluated. Patients were divided into 4 groups, based on the etiology of liver disease ; our study included 33 Hepatitis C (HCV) patients, 12 Hepatitis B (HBV) patients, 5 non alcoholic steatohepatitis (NASH) patients and 20 patients with other chronic liver diseases.

Inter-technique agreement on fibrosis gradation was assessed for each patients group with Cohen's weighted kappa (κ) Agreement was interpreted as follows : $\kappa \leq 0$ = poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial and ≥ 0.81 = almost perfect.

Inter-technique agreement on elasticity measurement (in kPa) was assessed for each patients group with Bland-Altman plot.

This study was conducted after approval from the local Ethics Committee.

Results : ElastPQ was found to be more accurate than Fibroscan in the evaluation of obese patients (BMI > 30). Inter-technique agreement for fibrosis gradation was almost perfect in HCV-related and "other chronic liver disease" group ($\kappa = 0.86$ and 0.89 , respectively). Results were poorly concordant in the HBV group ($\kappa = 0.20$ and < 0 , respectively). Concerning the 5 patients with NASH, agreement was found to be fair ($\kappa = 0.27$).

Bland-Altman plot showed that Fibroscan slightly overestimates elasticity measurements compared to ElastPQ (regardless of patients group) with no influence on stratification.

Conclusions : This study constitutes one of the first attempts to compare ElastPQ and Fibroscan in well-defined subgroups of patients. Our preliminary results, although based on a small number of patients, seem to indicate that ElastPQ cannot be considered as a suitable alternative for fibrosis assessment in HBV-related cirrhosis and NASH. However, it revealed accurate concerning HCV-related cirrhosis and "other causes" (including alcohol, Wilson disease, α 1-antitrypsin deficiency and steatosis)-related cirrhosis.

Invited Lecture

Early response prediction in metastatic colorectal cancer using FDG PET-CT : ready for implementation ?
P. Flamen, Institut Bordet, Brussels, Belgium

POSTERS

EGFR AND E-CADHERIN EXPRESSION IN RARE AGGRESSIVE COLORECTAL SIGNET RING CELL ADENOCARCINOMA. A.R.A. Foda, K. Zalata, A. El-Hawary. Mansoura Gastroenterology Center, Mansoura, Egypt.

Introduction : Colorectal mucinous adenocarcinoma (MA), a morphologic subtype of colorectal carcinoma (CRC), has more than 50% of the tumor composed of mucin, either extracellular with mucin lakes (colloid carcinoma) (CC) or intracellular with signet ring cells (signet ring cell adenocarcinoma) (SRA). SRA is a rare entity that accounts for 1% of CRC, with five times more often in Egypt for the rest of the world. SRA has specific clinicopathological features than non-mucinous adenocarcinoma (NMA) and even CC.

Aim : to compare SRA, NMA and CC regarding clinicopathological, histological parameters, survival, EGFR and E-cadherin expressions.

Methods : In this work, we studied tumor tissue specimens from 75 patients with MA (including 19 cases of SRA and 56 cases of CC) and another 75 patients with NMA for comparison, who underwent radical surgery from Jan 2007 to Jan 2012 at the Gastroenterology Centre, Mansoura University, Egypt. Their clinicohistopathological parameters and survival data were revised and analyzed using established statistical methodologies. High density manual tissue microarrays were constructed using modified mechanical pencil tips technique and immunohistochemistry for EGFR and E-cadherin was done.

Results : SRA was significantly associated with younger age, more depth of invasion, more lymph node metastasis, higher grade, advanced stage, more lymphovascular emboli, perineural invasion and worse survival than NMA and even CC. On the other hand, both NMA and CC were significantly associated with overlying adenomas than SRA. SRA expressed EGFR and E-cadherin in a significantly lower rates than NMA ($P < 0.001$ for both) and CC ($P = 0.01$ for EGFR and < 0.001 for E-cadherin). In a univariate analysis, neither EGFR nor E-cadherin expression showed a significant impact on disease-free or overall survival in patients with SRA.

Conclusions : SRA is a specific entity of CRC that has more aggressive clinicopathological features, worse survival and different molecular characteristics than NMA and CC. Targeted therapy against EGFR and E-cadherin isn't expected to be useful in patients with SRA. Further studies are needed to investigate molecular features of this entity to tailor successful targeted therapies for it.

EGFR, MMP-13 AND E-CADHERIN EXPRESSION IN COLORECTAL ADENOCARCINOMA WITH MUCINOUS ACTIVITY. A.R.A. Foda, A. Abdel Aziz, A. Hosni, A. Gado. Mansoura Gastroenterology Center, Mansoura, Egypt.

Introduction : Colorectal adenocarcinoma with mucinous activity (AWMA) is a vague entity of ordinary adenocarcinoma that shows intra or extracellular mucin secretion less than 50% of the tumor. Few studies were concerned with the clinicopathological and molecular criteria of this entity, and whether it resembles ordinary adenocarcinoma without mucinous activity (OA) or mucoid adenocarcinoma with more than 50% of the tumor composed of mucin (MA).

Aim : to compare NWMA, OA and MA regarding clinicopathological, histological parameters, survival, EGFR, E-cadherin and MMP-13 expressions.

Methods : In this work, we studied tumor tissue specimens from 28 patients with AWMA, 47 with OA and 56 with MA, who underwent radical surgery from Jan 2007 to Jan 2012 at the Gastroenterology Centre, Mansoura University, Egypt. Their clinicohistopathological parameters and survival data were revised and analyzed using established statistical methodologies. High density manual tissue microarrays were constructed using modified mechanical pencil tips technique and immunohistochemistry for EGFR, MMP-13 and E-cadherin was done.

Results : AWMA was significantly associated with more perineural invasion ($P = 0.027$), lower EGFR ($P = 0.007$), and MMP-13 expressions ($P = 0.005$) than OA, with no difference in E-cadherin expression ($P = 0.287$). Conversely, only microscopic abscess formation was significantly more with AWMA than MA ($P = 0.003$) with no difference in EGFR ($P = 0.876$), MMP-13 ($P = 0.287$) and E-cadherin ($P = 0.432$) expression between both groups. However, AWMA showed better survival than MA ($P = 0.015$) and no difference with OA ($P = 0.419$). In a univariate analysis, EGFR,

MMP-13 and E-cadherin expression didn't show a significant impact on disease-free or overall survival in patients with AWMA.

Conclusions : Based on the differences between AWMA, OA and MA in clinicopathological and molecular findings, AWMA resembles MA than OA. However, the contrary is true regarding survival. AWMA remains a vague entity that needs further molecular studies to be grouped with either OA or MA.

- R17 -

A BREAKTHROUGH IN GASTROINTESTINAL NEOPLASIA RESEARCH : COST-FREE MANUAL TMA CONSTRUCTION TECHNIQUE. A.R.A. Foda, K. Zalata. Mansoura Gastroenterology Center, Mansoura, Egypt.

Introduction : Manual tissue microarray (TMA) construction had been introduced to avoid the high cost of automated and semi-automated techniques. Although many researchers tried to develop simple techniques for TMA construction, these methods were complicated, difficult to apply, and expensive. Nowadays, TMA is mandatory in gastrointestinal neoplasia research.

Aim : We tried to introduce a new simple inexpensive method for manual TMA construction with high quality reaching that of expensive ones.

Methods : Two conventional mechanical pencil tips of different diameters (0.7 and 0.9 mm) were used. The smaller for preparing holes of the recipient block, and the larger for puncture of the desired cores from donor blocks. A source of mild heat was used during construction to ease puncture. Blocks were incubated at 38° C overnight, in order to increase stability and quality of the blocks.

Results : Three high density TMA blocks with 206 cores/block were constructed with high stability from 150 colorectal adenoma and carcinoma blocks in addition to normal controls of various tissues. We successfully performed H&E slides and immunostaining for EGFR, E-cadherin, MMP-13, glypican-3 and others without substantial tissue cylinder loss.

Conclusion : Our no-cost mechanical pencil tip technique raises the quality of manual TMA blocks, increase the number of cores per block and improve the stability of the cores within the paraffin block. This new modified technique is a good alternative of expensive machines in many laboratories. This technique can make a breakthrough in gastrointestinal neoplasia research.

CASE REPORT SESSION

- C01 -

SUCCESSFUL TREATMENT OF SPURTING PEPTIC ULCER BLEEDING : COMBINATION OF TWO MISCELLANEOUS METHODS. C. Snauwaert, D. De Looze. Ghent University Hospital, Gent, Belgium.

Case report : A 91-year-old female presented to the emergency department with hypotension and hematochezia. Her medical history included chronic renal failure and she was on chronic aspirin therapy. Despite her age, she still lived by herself at a sheltered housing complex and was doing relatively well. Urgent upper endoscopy revealed a deep ulcer (about 2.5 cm in diameter) in the antero-superior wall of the duodenal bulb with a large pulsating artery in the centre. Because of the large-caliber size of the vessel, endoscopic hemostatic therapy was deemed very difficult, but because the patient seemed not fit for any surgical or angiographic approach, an endoscopic attempt to treat this lesion was undertaken. The surrounding area was injected with an epinephrine 1:10000 mixture and the vessel was treated with bipolar electrocoagulation therapy. After the first attempt, oozing bleeding began, and multiple bicap-attempts were necessary to achieve homeostasis. After the procedure, the patient could be hemodynamically stabilized. However, one week later, the patient was found in shock status on the ward. A new endoscopy revealed a spurting bleeding in the antero-superior wall of the duodenal bulb. Because of the overwhelming nature of the bleeding, new endoscopic injection therapy and hemostatic clip placement was deemed impossible. Therefore, a decision was made to apply Hemospray (Cook Medical), which resulted in immediate hemostasis. The patient could again be hemodynamically stabilized. At repeat endoscopy, the stomach and duodenum were free of blood. Still, a deep ulcer was seen in the antero-superior wall of the duodenal bulb with a pulsating artery in the centre. As the patient was fit for no other treatment than endoscopic therapy, a decision was made to inject the vessel with N-butyl-2-cyanoacrylate (as used in the treatment of gastric fundal varices), taking into account the risk for post-injection embolization of cyanoacrylate. A total of 1.5 mL Histoacryl-solution was injected intra-arterially using a dilution ratio of 0.5 mL Histoacryl to 1.0 mL Lipiodol (1:2), immediately followed with saline to ensure full delivery of the mixture and clearing of the needle injector channel. The procedure went uneventful without development of bleeding. No signs of organ ischemia were noted in the immediate postprocedure period. At follow-up four weeks later, the patient was totally asymptomatic and at control endoscopy nearly complete healing of the ulcer was seen. All endoscopic procedures were recorded on video.

Conclusion : As demonstrated in this case, spurting peptic ulcer bleeding can be stopped by application of Hemospray, followed by definitive therapy with endoscopic embolization of the visible vessel with Histoacryl. However, endoscopic embolization to control peptic ulcer bleeding should be reserved for selected cases, because of possible embolic complications.

- C02 -

HEMATEMESIS FOLLOWING DOUBLE-BALLOON ERCP WITH RENDEZVOUS TECHNIQUE. L. Vandenaabeele, C. Snauwaert, L. Defreyne, M. De Vos. Ghent University Hospital, Gent, Belgium.

Case report : A 54-year old man was referred to our centre because of recurrent biliary pancreatitis and cholangitis with documented choledocholithiasis on MRCP. The patient had a history of complicated bariatric surgery, with a Scopinaro-procedure followed by conversion to gastric bypass.

At double-balloon ERCP (DBE-ERCP), the major papilla could be identified, but frontal view of the papilla (as with a side-viewing scope) could not be obtained. Due to difficult position of the endoscopic material, leading to a more difficult approach, cannulation of the common bile duct (CBD) was not possible and only the pancreatic duct could be opacified. Finally, cannulation of the CBD could be achieved by means of a DBE-ERCP rendezvous technique. Due to the presence of many adhesions and some looping of the scope, positioning of the endoscopic equipment was again difficult. Eventually, papillotomy and balloon extraction of sludge and stones could be performed. A percutaneous biliary drain was left in place. The postprocedural cholangiogram showed no residual CBD stones.

However, only a few hours later, the patient developed a severe septic shock with MOF and was admitted to the ICU. Control cholangiogram showed adequate drainage. After two weeks on the ICU the patient was transferred to the ward. Meanwhile, the biliary drain and all other indwelling catheters were removed. Rehabilitation therapy was initiated. In fact, he was on the road to recovery when one night he collapsed and was readmitted to the ICU, where he developed coffee ground vomiting. An urgent endoscopy was performed. Old blood was seen in the gastric pouch and the alimentary and biliary limb. However, no bleeding focus could be identified. A few days later the patient became hemodynamic unstable again with fever and hematemesis. After stabilisation, a new endoscopy was performed. A lot of fresh blood clots were seen in the gastric pouch and the biliary limb. No blood was seen in the distal alimentary limb. Despite the absence of jaundice or elevated liver enzymes, hemobilia was suspected. However, because of the altered anatomy and the use of a forward-viewing scope, direct visualisation of the major papilla was not possible. An urgent CT angiography

could not detect any active bleeding sites. The patient recovered quickly without any bleeding recurrence. Because of the unexplained fever episode, a MRCP was performed, which showed a pseudo-aneurysm in segment IV of the liver. This appeared to be the cause of the hemobilia, which caused the hematemesis. Duplex sonography confirmed active flow in this pseudo-aneurysm, which could be successfully coiled by means of an angiographic approach.

Conclusion : In case of overt (but obscure) gastrointestinal bleeding following rendezvous procedures, hemobilia (e.g. due to pseudo-aneurysm formation) has to be suspected.

- C03 -

CONGENITAL EXTRAHEPATIC PORTOSYSTEMIC SHUNT TYPE II IN AN ADULT WOMAN WAITING FOR A KIDNEY TRANSPLANT. W. Verlinden, S. Francque, P. Michielsen. University Hospital Antwerp, Antwerp, Belgium.

Case report : A 36 year-old-woman of Turkish descent with chronic renal failure on hemodialysis was referred to our hepatology unit. She was diagnosed at an age of 23 with a nephrotic syndrome during pregnancy in 1997. A diagnosis of postpartum thrombosis of the portal vein without any signs of portal hypertension was also noted. She was listed for kidney transplantation in 2009 because of progressive renal insufficiency of unknown origin. She recently moved to Belgium and was evaluated for listing here. Since her file mentioned an heterogeneous liver parenchyma without any further details, a hepatological evaluation was asked for. Past medical history further revealed multiple miscarriages and a psychiatric history consisting of mood disorders, an adjustment disorder with anxiety and an episode of decreased consciousness.

Clinical investigation showed no signs of liver disease or other physical problems, apart from a certain degree of psychomotor retardation. Laboratory tests showed normal liver enzymes. Ultrasound however, revealed a dysmorphic liver with an enlarged left lobe, a heterogeneous parenchyma and an aberrant hepatic vascularisation. The superior mesenteric vein and the splenic vein directly entered the caval vein. CT angiography confirmed a subtotal portocaval shunt with only one portal branch supplying the left liver lobe. There were no signs of thrombosis or large collaterals. According to the classification of Morgan and Superina, this is a congenital extrahepatic portosystemic shunt (CEPS) type II malformation.

Aminopyrine breath test showed a low function of the hepatocytes and ammonia blood levels were elevated. Liver biopsy showed siderosis with sinusoidal dilatation and nodular changes consistent with regenerative hyperplasia, without signs of cirrhosis.

After thoroughly investigating her file the diagnosis could already have been made on an MRI scan performed in 1999 during the postpartum period, but this was misinterpreted as a portal vein thrombosis, for which she received long-term anticoagulation.

Discussion : Congenital extrahepatic portosystemic shunt is a rare anomaly of the splanchnic venous system in which the portomesenteric blood drains into a systemic vein, bypassing the liver through a complete or partial shunt. Complete shunting (CEPS type I) is commonly detected at a young age and is often associated with concomitant congenital malformations. Partial shunting (CEPS type II) presents more often at an older age and is seldom associated with other malformations.

Type II CEPS can only be treated by ligation or coiling if the supplying portal branch can sufficiently take over the entire portal flow without developing portal hypertension. If treatment is not performed, follow-up is advised through ultrasound and biochemical tests.

In patients with encephalopathy without other signs of liver insufficiency, CEPS should be suspected and differentiated from portal vein thrombosis by the absence of concomitant signs of portal hypertension.

- C04 -

STAPHYLOCOCCUS AUREUS DISCITIS IN A CHILD WITH CROHN'S DISEASE TREATED WITH INFLIXIMAB. B. Hauser (1), D. Van Schaik (2), A. Laumen (1), E. De Greef (1), T. Devreker (1), G. Veerman (1), J. Van Der Werff Ten Bosch (3), F. Mana (4), D. Urbain (1), Y. Vandenplas (1). (1) UZ Brussel, Jette, Belgium ; (2) UZ Brussel, Jette, Belgium ; (3) UZ Brussel, Jette, Belgium ; (4) UZ Brussel, Jette, Belgium.

Introduction : Tumor necrosis alpha (TNF- α) inhibitors are increasingly administered to children and adolescents with inflammatory bowel disease. Adult studies indicate that TNF- α inhibitors lead to an increased risk of serious infections. Pediatric studies show that most frequently reported infections are mild and viral but that severe bacterial and fungal infections are less common.

Aim : We describe a case of a *Staphylococcus aureus* discitis in a 16 years old girl treated with Infliximab (IFX).

Case report : She was diagnosed with a Crohn's disease at the age of 12 years. She was treated initially with enteral feeding, antibiotics, corticosteroids and azathioprine, but finally underwent an ileocaecal resection at the age of 12.5 years because she developed an intestinal fistula with abdominal abscess. She was free of symptoms for 3 years with azathioprine. She presented with anorexia, vomiting and weight loss at the age of 16 years. Endoscopy showed important inflammation and ulcerations with narrowing of the antropyloric region. Biopsies showed gastritis but no presence of *Helicobacter pylori*. She was treated with enteral feeding, corticosteroids and proton pump inhibitors while azathioprine was continued. There was clinical but no endoscopic improvement so that IFX was started. IFX was administered at a dose of 5 mg/kg at 0, 2 and 6 weeks to obtain mucosal healing after having excluded tuberculosis. Gastrointestinal symptoms disappeared but she developed lower back pain that started after the second dose but became more pronounced after the third dose of IFX. Physical examination showed painful and limited lower back mobility. Inflammatory parameters were increased. Scintigraphy and MRI of the lumbar spine showed a discitis at the level of lumbar vertebrae 4 and 5 with destruction of the vertebral bodies and lower endplate of L4 and upper endplate of L5. Blood culture was negative. Culture of lumbar bone biopsy was positive for *Staphylococcus aureus* and negative for *Mycobacterium tuberculosis*. She was treated with flucloxacilline for 6 weeks and a corset for 3 months with clinical, biological and radiological improvement. Immunologic work up that was performed later showed a decreased number of natural killer cells but normal humoral immunity, number of B and T lymphocytes, and CD4/CD8 ratio.

Conclusion : We describe an adolescent with Crohn's disease who developed *Staphylococcus aureus* discitis of the lumbar vertebrae after the second dose of IFX. The concurrent intake of azathioprine and a lower number of natural killer cells could have facilitated the development of a serious infection under IFX. This case illustrates the fact that you should be aware of these infectious side effects.

- C05 -

ISOLATED ARTERIOPORTAL MALFORMATION PRESENTING WITH VARICEAL HEMORRHAGE.
W. Verlinden, S. Francque, P. Michielsens. University Hospital Antwerp, Antwerp, Belgium.

Case report : Our patient is a 42 year old male who was transferred from a nearby hospital to our tertiary centre. He presented to the referring hospital with hematemesis. Initial laboratory results showed mild anemia, low albumin level and slightly elevated INR. Bilirubin, alkaline phosphatase, transaminases, renal function and inflammatory parameters were normal. Medical history consisted of paranoid schizophrenia and alcohol abuse. Gastroscopy showed esophageal varices for which alcoholic liver cirrhosis with portal hypertension was assumed to be the cause. Several endoscopic variceal ligations were performed without sustained hemostasis. Hemodynamic instability, falling hemoglobin level and persisting hematemesis necessitated intubation and placement of a Sengstaken-Blakemore balloon. The patient was transferred for TIPSS (transjugular intrahepatic portosystemic shunt) placement, which was performed without complications. After the procedure hemodynamic stability was achieved with a stable hemoglobin level. Control by abdominal ultrasound showed a patent TIPSS in the right liver, but no signs of liver cirrhosis and unexpectedly a vascular malformation in the left liver. Angiography displayed an arteriportal malformation with shunting between the left hepatic artery and the left portal vein. The absence of a cirrhotic liver on imaging and the presence of the malformation indicate an incorrect initial diagnosis of alcoholic liver cirrhosis and suggest the intrahepatic arteriportal malformation as the cause of the esophageal variceal bleeding. Since these malformations are among other things associated with portal hypertension and biliary disease, treatment is indicated. Transarterial selective embolisation was performed and had to be repeated twice because of persistent shunting. Post-embolisation recovery of the patient has been uneventful and no additional episodes of upper gastrointestinal bleeding have been reported.

Discussion : Arteriportal fistulas are a rare but treatable cause of portal hypertension associated with gastrointestinal hemorrhage. Most fistulas are caused by precipitating (blunt or penetrating) trauma, surgery, congenital or hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) or hepatocellular carcinoma. Clinical presentation varies from asymptomatic to symptoms related to congestive heart failure, portal hypertension, biliary disease by ischaemia of the biliary tree and diarrhea with abdominal pain secondary to a 'steal phenomenon'. Ten percent of arteriportal fistulas are congenital, associated with portal hypertension, failure to thrive and gastrointestinal hemorrhage in infancy or early childhood. Treatment of arteriportal fistulas through shunt reduction is either surgical or minimally invasive through interventional radiology techniques.

Our patient had no history of trauma, surgery, malignancy or failure to thrive. Except some labial telangiectasias, there were no other signs of hereditary hemorrhagic telangiectasia, nor was there a family history. We believe that this is a case of an adult onset congenital arteriportal fistula that was successfully treated through interventional radiology.

COMBINED LIVER/LUNG TRANSPLANT FOR A COPD PATIENT WITH TUBERCULOSTATICS-INDUCED ACUTE LIVER FAILURE. L.J. Ceulemans (1), S. Strypstein (1), D. Monbaliu (1), P. De Leyn (2), W. Laleman (3), C. Verlseye (3), S. Van Der Merwe (3), R. Vos (4), G. Verleden (5), F. Nevens (3), J. Pirenne (6), D. Van Raemdonck (2). (1) Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium ; (2) Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium ; (3) Hepatology, University Hospitals Leuven, Leuven, Belgium ; (4) Pneumology, University Hospitals Leuven, Leuven, Belgium ; (5) Pneumology, University Hospitals Leuven, Leuven, Belgium ; (6) Centre of Microbial and Plant Genetics (CMPG), KU Leuven, Leuven, Belgium.

Introduction : Isolated Lung transplantation (LuTx) or Liver transplantation (LiTx) are established treatments of irreversible lung or liver failure. Combined Lung and Liver Tx (cLuLiTx) is an approved surgical therapy of combined lung/liver failure, mostly for patients suffering from progressive cystic fibrosis and advanced liver disease. In cLuLiTx, the lung is classically transplanted first, followed by the liver.

Aim : We report a patient primarily referred for LuTx because of end-stage Chronic Obstructive Pulmonary Disease (COPD), who developed tuberculostatics-induced Acute Liver Failure (ALF).

Case report : A 62-year-old female suffering from end-stage respiratory failure (FEV(1) : 31% and DLCO : 27%) secondary to COPD GOLD IV had been listed for LuTx. Because of latent tuberculosis infection, she received Isoniazid (250mg/day). To prevent infectious COPD exacerbations, she was also given Roxithromycine (150mg ; 3-5x/week). 4 months later, she was urgently admitted with anorexia and jaundice. Diagnosis of Isoniazid (and possibly Roxithromycine)-induced ALF {worsening encephalopathy, bilirubine 21 mg/dL, AST 3732U/L, INR 1.9} was made. Tuberculostatics are a well-known cause of ALF (and indication for LiTx), but therapeutic options for ALF in a LuTx candidate are virtually unknown. It was felt that this COPD patient would not survive LiTx without simultaneous LuTx. The only therapeutic option was Hyper-Urgent (HU) cLuLiTx, a procedure not described before in a patient with ALF and extremely poor coagulation. Liver and lungs from a 40-year-old male donor became available. The liver was transplanted first, to correct the extremely poor coagulation and to reduce the risk of excessive bleeding, facilitating sequential LuTx. In anticipation of the longer lung preservation time that this unusual liver-lung Tx sequence would entail, the lungs were normothermically oxygenated/perfused *ex vivo* (OCS lung device, *Transmedics*) instead of simply cold stored. After completion of LiTx, the lungs were transplanted *via* bilateral anterior thoracotomy. Cold ischemia time of the liver was 5hrs20' and preservation time of the lungs was 11hrs15'. Surgery was uncomplicated. 3 months postTx, the patient is doing well, rejection-free, with normal liver/lung function under immunosuppression (tacrolimus, Imuran, steroids).

Conclusions : This case makes 4 points : 1) Isoniazid is a rare, but well-known cause of ALF and indication for HU LiTx ; in this patient, Roxithromycin may also have played a role in ALF ; 2) A particularity of this case is that ALF in a LuTx candidate renders isolated LiTx probably futile and leaves HU cLuLiTx as the only life-saving option ; 3) To correct the extremely poor coagulation, LiTx was performed first (in contrast with previous cases of cLuLiTx) ; 4) To better tolerate a necessarily longer preservation period, the lungs were kept oxygenated at warm temperature *ex vivo*. In summary, this case suggests that HU cLuLiTx can be performed with success and that -with improved lung preservation technologies- the liver can be transplanted first.

A CHALLENGING CASE OF GRANULOMATOUS COLITIS. G. Moors, J. Nijs, J. Van Meerbeek, S. Vanstraelen, P. Geyskens, S. Verhamme, J. Wynants, L. Van Den Bergh. Sint-Trudo ziekenhuis, Sint-Truiden, Sint-Truiden, Belgium.

Introduction : We present a case of suspected sarcoidosis of the gastrointestinal tract in a patient with proven pulmonary sarcoidosis.

Case report : A 47-year old man was diagnosed with colonic Crohn's disease in September 2003. He remained nine years in remission under treatment with Azathioprine. In August 2012, he presented at the emergency department with abdominal pain, vomiting and constipation. C-reactive protein was elevated to 110 mg/L (< 5.0). Abdominal ultrasound revealed dilated right colon and small bowel loops. CT-scan of the abdomen showed a relapse of Crohn's disease particularly at the terminal ileum and caecum in combination with thickening of the appendix wall. Slow regression was seen after treatment with intravenous antibiotics and corticosteroids, although there were still seen abscesses around the appendix on abdominal CT. There were no signs of remaining obstruction on MRI enteroclysis. Treatment with Azathioprine was interrupted because of clinical and biochemical signs of pancreatitis. In September 2012, our patient was hospitalized again with an episode of pain in the right iliac fossa. CT-scan of the abdomen showed comparable changes around the appendix like one month before. Surprisingly, ileocolonoscopy, which was performed one day before, was completely normal. So our diagnosis of relapse of Crohn's disease in August 2012 was questioned. An appendectomy was performed after antibiotic treatment and drainage. Anatomopathological examination discovered an appendiceal

mucinous neoplasm with positive resection margins. So our patient underwent a second surgery with resection of the caecum. Because there was no evidence of peritoneal metastases, the prognosis was well. In the next year our patient presented twice with abdominal pain, diarrhea and red blood loss per anum. It was quite a diagnostic challenge : CT-scan of the abdomen showed submucosal fat deposition at the terminal ileum. Calprotectine in the stool was low and both times ileocolonoscopy didn't show any abnormalities. Those findings argue against a relapse of inflammatory bowel disease. Despite a macroscopically normal colon mucosa, blind biopsies showed the presence of granulomas. Based on the fact that our patient was diagnosed with pulmonary sarcoidosis in January 2013, a tentative diagnosis of sarcoidosis of the gastrointestinal tract was made. Corticosteroid therapy and Infliximab were started.

Conclusions : Sarcoidosis is a multisystemic granulomatous disease of unknown cause. Involvement of the gastrointestinal tract is uncommon. Especially the colon is an extremely rare location. So far, only a few case reports were published. Clinical trials are lacking but sarcoidosis of the gastrointestinal tract seems to respond well to corticosteroid therapy.

- C08 -

OLMESARTAN-INDUCED ENTEROPATHY. C. Snauwaert, L. Ferdinande, D. De Looze, M. De Vos. Ghent University Hospital, Gent, Belgium.

Introduction : Olmesartan is a widely prescribed angiotensin-II-receptor blocker approved for the treatment of hypertension. In 2012, the U.S. Food and Drug Administration (FDA) issued a warning relating to a celiac disease mimicking condition that can occur in patients taking olmesartan.

Aim : We describe two cases of severe, unexplained diarrhoea, necessitating hospitalisation, due to "olmesartan enteropathy".

Case reports : Patient 1, a 58-year-old male, was admitted to another hospital with 8 weeks of refractory diarrhoea, weight loss, hypokalemia and prerenal kidney failure (rapidly resolving after intravascular volume repletion). His medical history included atrial fibrillation and arterial hypertension (treated with propranolol and olmesartan). Multiple stool cultures and a colonoscopy with biopsies were non-diagnostic. Duodenal biopsies revealed moderate villous blunting, increased intraepithelial lymphocytes and eosinophilic and lymphoplasmacytic infiltration of the lamina propria, consistent with celiac disease (Marsh 3a). However, both IgA tissue transglutaminase and anti-gliadin antibodies were normal, HLA DQ2 and DQ8 were absent and no clinical response was noted to a gluten-free diet. A ¹⁴C-d-xylose breath test was suggestive of small intestinal bacterial overgrowth, but a course of ciprofloxacin and metronidazole did not result in any clinical improvement. Additional CT enterography showed some wall edema of the jejunum. The patient was subsequently referred to our hospital for further evaluation. Although no active inflammation was seen on deep enteroscopy, jejunal biopsies also revealed intraepithelial lymphocytosis. In view of these findings and the recent FDA safety alerts, olmesartan-induced enteropathy was suspected. After discontinuation, his symptoms gradually resolved. Interestingly, one positive rechallenge was seen after one accidental intake of olmesartan.

Patient 2, a 55-year old male, was admitted to our hospital with 9 weeks of watery diarrhoea, hypotension and prerenal azotemia. His medical history was significant for arterial hypertension (treated with olmesartan and amlodipine). All medications were stopped on admission and he rapidly improved with supportive therapy. Stool cultures were negative. Upper and lower endoscopies were non-diagnostic (apart from some mild duodenitis) and biopsies were taken. After discharge from hospital, he returned to the out-patient clinic 3 weeks later. He had experienced one episode of diarrhoea, after olmesartan was restarted (for hypertension), which was again stopped. Colonic biopsies showed some non-specific inflammation. Duodenal biopsies revealed active inflammation with increased intraepithelial lymphocytes (Marsh 2). Celiac serology was negative. In view of these results, and the positive rechallenge, olmesartan enteropathy was suspected.

Discussion : Olmesartan enteropathy is a sprue-like condition, consisting of diarrhoea, weight loss, dehydration and often necessitating hospitalization. It mimics celiac disease, but does not typically respond to a gluten-free diet. The underlying mechanisms are unknown. Considering the long delay between onset of olmesartan therapy and development of symptoms, a cell-mediated immune response is suggested.

Conclusions : Physicians who encounter patients with diarrheal syndromes using olmesartan, should consider a possible causal relationship.

POSTERS

- C09 -

GASTROENTERITIS AND FEEDING PRACTICES AMONG INFANTS IN A RURAL AREA OF NORTHERN BANGLADESH. M.A. Kalam. Siam Health Care, Dhaka, Bangladesh.

Introduction : Gastroenteritis is a cause of infant mortality all around the world, especially in developing countries. Many measures have been taken, but still a large number of children aged less than 5 years, suffer from gastroenteritis.

Aim : To find out the feeding practices of infants and influence of feeding on occurrence of gastroenteritis among infants.

Methods : This descriptive type of cross-sectional study was conducted among all 122 infants who completed 1 year of age living in Bagha Upazilla (sub-district) under Rajshahi district from 1st October to 31st October 2013. Respondents were mothers of the infants. They were interviewed using a pre-tested questionnaire. Frequency distribution was seen, chi-square tests were done to find out the influence of feeding practices on gastroenteritis.

Results : Twenty four percent of the infants (n = 122) were given prelacteal food as the first food after birth ; 99% at 1 year ; 43% of the mothers exclusively breast-fed their babies. Nearly two-thirds (62%) of the mothers started complementary feeding at the age of completed six months. Three-quarters (77%) of the infants suffered from gastroenteritis. Although no significant association was found between gastroenteritis and infant-feeding practices, it was observed that occurrence of gastroenteritis was higher ($x^2 = 20.11$, $df = 3$, $p < 0.001$) in infants whose family members were more than 3 compared to those having less than 3. The disease occurred mostly among infants residing in families with katcha latrine ($x^2 = 12.547$, $df = 2$, $p = 0.002$). The occurrence of gastroenteritis was very high when infant's stool was discarded in open space, while it was less common in infants whose mothers put the stool in sanitary latrines ($x^2 = 23.440$, $df = 2$, $p < 0.001$).

Conclusions : The prevalence of exclusive breastfeeding and introduction of complementary feeding at proper age was high compared to the national record. However, surprisingly, the proportions of gastroenteritis among infants were also high. By improving feeding practices of infants, hygiene practices by mothers, and improving the socio-economic-demographic condition with the help of the government, non-government and private organizations, this condition can be improved.

- C10 -

ANOMALOUSLY PLACED SUPRAHEPATIC GALL-BLADDER :A CASE DETECTED ON F-18 FDG PET/CT. V. Agarwal, S. Pande, S. Garg, D.R. Jangid. Medanta The Medicity Hospital, Gurgaon, India.

Aim : To appraise the imageologists of a possible mis-localization of tracer accumulation to anomalously placed gall-bladder during PET-CT examination. PET-CT is increasingly playing an important role in staging and restaging of the disease process in the cancer patients. With the advent of fusion imaging, the tracer accumulation can be correctly localized to a structure or lesion on CT.

Methods : We did a staging PET-CT scan of a patient with hepato-cellular carcinoma for liver transplant evaluation. F-18 FDG was used as a tracer and scan was performed on SEIMENS Biograph-mCT PET-CT machine.

Results : We noted the tracer accumulation at the superior surface of liver which was localized to the anomalously placed gall-bladder in supra hepatic sub-diaphragmatic location.

Conclusions : The anomalously placed gall-bladder can create localization confusion. Keeping the possibility of ectopically placed gall-bladder in mind, the imageologist can better localize the tracer uptake.

NLH-AN UNCOMMON MENIFESTATION OF A COMMON INFECTION. J. Toshniwal, R. Chawlani, M. Sachdeva, M. Kumar, S. Bhalla, N. Bansal. Sir Ganga Ram Hospital, New Delhi, India.

Case Report : 18 years old student presented with complaint of failure to gain weight over last six years, associated with pain in abdomen on and off for last 1 year. His clinical examination was normal. However, his weight was 36 Kg ; height was 1.6 meters ; with Body Mass Index of 14.06 Kg/m². His hematological and biochemical parameters were normal. Ultrasound examination was also normal. In view of pain in abdomen Esophago-gastroduodenoscopy was done ; which revealed multiple polypoidal nodules throughout length of visualised duodenum ; with normal stomach and esophagus. Multiple biopsies were taken from the nodules and were submitted for histopathological examination. Colonoscopy also revealed similar nodules in terminal part of ileum with normal colonic mucosa. Histopathology revealed nodular lymphoid hyperplasia with Giardia lamblia infestation. Serum levels of Immunoglobulin A were measured and were absent. Serum IgG, IgE, IgM, IgD levels were normal. So, the final diagnosis of isolated IgA deficiency with nodular lymphoid hyperplasia secondary to giardiasis was made. Patient was treated with oral metronidazole. Subsequently, he gained 6 kg weight over next 6 months. Thus, this case represents uncommon manifestations of giardiasis.

Enclosure : Esophago-gastroduodenoscopy video, Colonoscopy video, Histopathology photograph demonstrating NLH with Giardia.

PLENARY SESSION

- D01 -

Brohée Lecture

Genetics, immunity, inflammation or the gut in the pathogenesis of alcoholic liver disease :
and the winner is..

P. Stärkel, UCL, Belgium.

- D02 -

ENDOSCOPIC SUBMUCOSAL DISSECTION FOR BARRETT'S ESOPHAGUS ; RESULTS IN A LARGE TERTIARY CENTER. J.B. Chevaux (1), H. Piessevaux (2), A. Jouret-Mourin (2), C.P.R. Yeung (2), E. Danse (2), P. Deprez (2). (1) Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Woluwe-Saint-Lambert, Belgium ; (2) Université Catholique de Louvain, Cliniques universitaires St Luc, Woluwe-Saint-Lambert, Belgium.

Aims : To assess efficacy, safety and long-term results of endoscopic submucosal dissection (ESD) in Barrett's esophagus (BE).

Methods : We analysed charts from 75 consecutive patients with BE treated by ESD from January 2007 through November 2013. ESD was performed in case of visible lesions, poorly lifting tumours, multifocal neoplasia, previous radiotherapy, and esophageal varices. Efficacy of ESD was assessed on the basis of rates of complete endoscopic and histologic remission concerning BE and safety on the rate of short and long-term complications.

Results : Median age of patients was 68 years (IQR, 61-76) and 63 patients were male (85%). Median BE evolution was 41 months (IQR, 11-190) and median follow up was 18 months (IQR, 8-34). A mucosal defect was present in 92% of BE and classified 0-IIa (47%) and 0-IIc (42%) according to Paris classification. Median duration of procedure was 118 minutes (IQR, 100-146) and median circumference ablation was 75% (IQR, 66-80). Two procedures failed due to extensive submucosal fibrosis and a perforation. Five complications (6.6%) occurred during ESD including two haemorrhages and three perforations all treated endoscopically, no patients died. All patients were placed under proton-pump inhibitor regimen after ESD, 13 (17%) received oral corticosteroid therapy additionally. Median specimen size was 53 mm (IQR, 44-71) and good-to-moderate differentiation (G1/G2) was the more frequent encountered histologic type (75%). After ESD, endoscopic follow up was scheduled in all cases at 2 weeks. In case of increasing dysphagia or lumen narrowing, dilatation was performed on 45 patients (66%) with a median procedure number of 4 (IQR, 2-6). Additional endoscopic ablation of BE was compulsory for 40 patients (53%) and included mainly radiofrequency (28%). Median number of upper endoscopy per patient was 7 (IQR, 4-11) and median endoscopic procedure number for BE eradication was 2 (IQR, 1-2). Seven patients underwent surgery for vertical incomplete resection and/or undifferentiated lesions. At the latest control, BE endoscopic eradication was reached in 59% ; histologic eradication of intestinal metaplasia in 58% and histologic eradication of neoplasia in 83%. Overall mortality was 8% but only one tumor-associated death was observed corresponding to an undifferentiated lesion with incomplete endoscopic resection in a ninety-year-old patient not suitable for surgery.

Conclusions : We report the largest series of ESD performed in BE for specific indications such as mucosal or submucosal adenocarcinoma, multifocal neoplasia or poorly lifting epithelium. ESD appears to be safe and effective with complete remission of neoplasia similar to previously published data.

- D03 -

ROUX-EN-Y GASTRIC BYPASS ATTENUATES HEPATIC MITOCHONDRIAL DYSFUNCTION IN MICE WITH NASH. J. Verbeek (1), M. Lannoo (2), E. Pirinen (3), D. Ryu (3), P. Spincemaille (4), I. Van Der Elst (5), P. Windmolders (5), K. Thevissen (4), B.P. Cammue (4), J. Van Pelt (5), C. Ceuterick-De Groote (6), P.P. Van Veldhoven (7), P. Bedossa (8), F. Nevens (1), D. Cassiman (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium ; (3) Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland ; (4) KU Leuven, Leuven, Belgium ; (5) University Hospitals Leuven, Leuven, Belgium ; (6) University of Antwerp, Antwerpen, Belgium ; (7) KU Leuven, Leuven, Belgium ; (8) Hôpital Beaujon, Clichy, France.

Introduction : Nonalcoholic steatohepatitis (NASH) is associated with mitochondrial dysfunction. No therapy for NASH has been approved so far. Roux-en-y gastric bypass (RYGB) is emerging as a therapeutic option, although its effect on NASH remains subject to debate. Moreover, the effect of RYGB on NASH-related molecular pathways is a

highly underexplored research area that is hampered by the difficulty of obtaining human liver tissue once bariatric surgery has been performed. We studied the effects of RYGB on pre-existent NASH and hepatic mitochondrial dysfunction in a novel diet-induced mouse model nicely mimicking human disease.

Methods : (1) C57/B16-J mice were fed a high fat-high sucrose diet (HF-HSD) or normal chow diet for 4, 12 and 20 weeks. (2) RYGB/SHAM was done in mice fed a HF-HSD for 12 weeks in advance. HF-HSD was continued for 8 weeks after surgery till sacrifice. Detailed metabolic and hepatic mitochondrial profiling was performed.

Results : HF-HSD led to obesity, insulin resistance, hypercholesterolemia and consistently induced NASH (steatosis, ballooning, inflammation) and frequent fibrosis already after 12 weeks feeding. 20 weeks HF-HSD further aggravated these metabolic and histological features.

Hepatic mitochondrial dysfunction was present in NASH mice (decreased complex I-IV activity, ATP depletion, ultra-structural abnormalities), together with higher 4-hydroxynonenal (HNE) levels, increased tumor necrosis factor-alpha (TNF α) mRNA, increased uncoupling protein 2 (UCP2) mRNA and free cholesterol accumulation.

Subsequently, we performed RYGB or SHAM surgery in our model of NASH and acquired mitochondrial dysfunction (12 weeks HF-HSD in advance).

RYGB induced sustained weight loss, improved insulin resistance and inhibited progression of NASH, with a marked reversal of fibrosis. RYGB preserved hepatic mitochondrial complex-I activity and restored ATP levels. RYGB limited HNE production and decreased TNF α mRNA. UCP2 mRNA and free cholesterol accumulation were unaltered by RYGB.

Conclusions : Progression of NASH and NASH-related hepatic mitochondrial dysfunction can be prevented by RYGB. RYGB preserves respiratory chain complex activity, thereby restoring ATP production and limiting the amount of lipid peroxidation (HNE). Our data suggest that modulation of hepatic mitochondrial dysfunction contributes to the therapeutic effect of RYGB on established NASH, in a mouse model that displays every aspect of the clinical disease.

- D04 -

BIOMARKER PANEL PREDICTING MUCOSAL HEALING IN PATIENTS WITH CROHN'S DISEASE UNDER INFLIXIMAB THERAPY. M. De Bruyn (1), T. Bessisow (2), T. Billiet (3), I. Cleynen (3), R. Kirkland (4), X. Liu (4), S. Hauenstein (4), K. Drake (4), S. Singh (4), M. Ferrante (3), P. Rutgeerts (3), G. Van Assche (3), I. Arijs (3), G. Opendakker (1), S. Vermeire (3). (1) Rega Institute, Leuven, Belgium ; (2) McGill University, Montreal, Canada ; (3) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (4) Prometheus Laboratories Inc., San Diego, United States.

Introduction : Infliximab has led to new therapeutic goals in Crohn's disease (CD) such as complete mucosal healing and improvement of quality of life. The current standard for assessing mucosal healing is endoscopy. However, frequent assessments are costly and uncomfortable to the patient. Non-invasive, accurate surrogate serum markers would therefore be welcomed to aid clinicians in predicting mucosal healing.

Methods : In a retrospective study, 119 CD patients who started infliximab and who underwent serial endoscopies (before and during infliximab) were included. At each endoscopy, mucosal healing status was recorded as either not healed, having marked improvement or having complete healing. Serum samples were available at the time of endoscopy and levels of markers were correlated with the degree of healing. Thirty-five biomarkers were measured in 181 serum samples with the use of CEER, a proprietary highly sensitive protein micro-array, or homogenous mobility shift assays (Prometheus Laboratories Inc., San Diego, CA). These markers included growth and repair factors, pro- and anti-inflammatory markers and the IBD SGI serology panel. Infliximab and antibodies to infliximab were also measured. Clinical information regarding age at sampling, gender, age at diagnosis, location of disease, anal involvement and previous surgery was included. For statistical analysis SPSS and R were used and p-values < 0.05 were considered significant.

Results : From the 119 CD patients, 64 CD patients showed complete healing with no relapse, whereas 55 CD patients never showed mucosal healing. Explorative univariate analyses indicated that age and 12 serum markers (HGF, BTC, TWEAK, CRP, ICAM, SAA, VCAM, IL-2, IL-8, IFN- γ , IL-6 and IL-10) were significantly associated with mucosal healing (p < 0.1). Selection of the best subset of predictors through multiple logistic regression analysis retained age [Odds ratio 0.97 (95% confidence interval 0.94-0.99), p = 0.010], HGF [0.86 (0.79-0.94), p = 0.001], BTC [1.24 (1.07-1.43), p = 0.003], TWEAK [1.04 (1.01-1.07), p = 0.014] and VCAM [0.93 (0.87-0.98), p = 0.012] as independent markers associated with healing based on Bayesian information criterion (BIC). The results remained significant after internal validation with bootstrap. A cumulative prediction score was developed by combining 5 prediction factors (Age < 29.5 years, HGF < 11.42 CU/ml, BTC > 11.44 CU/ml, TWEAK > 20.62 CU/ml and VCAM < 4200 μ g/ml). Based on this cumulative prediction score, we could observe a significant and gradual increased prediction of mucosal healing (Chi Square linear-by-linear p < 0.001).

Conclusions : We have identified a surrogate marker panel consisting of four serum markers and one clinical parameter that could facilitate prediction of mucosal healing in the future.

HYPOXIA DRIVEN GENE EXPRESSION IS A PROGNOSTIC FACTOR IN STAGE II AND III COLON CANCER PATIENTS. J. Dekervel (1), D. Hompes (1), H. Van Malenstein (1), D. Popovic (2), X. Sagaert (1), B. De Moor (2), E. Van Cutsem (1), A. D'hoore (1), C. Verslype (1), J. Van Pelt (3). (1) University Hospitals Leuven, Leuven, Belgium ; (2) ESAT, Leuven, Belgium ; (3) KU Leuven, Leuven, Belgium.

Introduction : Colon cancer is a major cause of morbidity and mortality worldwide. The efficacy of adjuvant chemotherapy has already been proven in patients with a high risk of recurrence. In subgroups of stage II and III patients the AJCC staging is inadequate. As such, there is an urgent need for better prognostic tools.

From a scientific point of view, formalin fixed paraffin embedded (FFPE) tissue often presents a challenge for gene expression studies.

Aim : Hypoxia is considered a major microenvironmental factor influencing cancer behavior. Our aim was to develop a hypoxia-based gene score that could identify high and low risk within stage II and III colon cancer patients.

Methods : Differential gene expression of CaCo-2 colon cancer cells cultured in chronic hypoxia versus normoxia was tested for correlation with prognostic variables in published microarray data sets. These data sets were further used to downsize and optimize a gene score, which was subsequently determined in FFPE material of 126 patients with colon cancer treated in our center using the Nanostring nCounter platform. Additionally, the prognostic value of the hypoxia score was tested in an external patient cohort of 90 stage II patients.

Results : In the CaCo-2 cells, 923 genes with a 2-fold change and Limma corrected $p \leq 0.0001$ were found differentially expressed in hypoxia versus normoxia. We identified 21 genes with prognostic value and overlapping in three different training sets and ($n = 224$). With a fourth published data set ($n = 177$), the six gene Colon Cancer Hypoxia Score (CCHS) was developed. Validation in our patient cohort showed a significant better disease free survival (62.2 months, 95% CI 56.6-67.7) in low CCHS patients compared to high CCHS patients (42.7 months, 95% CI 33.4-52) ($p = 0.006$). This was independently confirmed in an external patient cohort of 90 stage II patients (102.5 months, 95% CI 93.1-111.9 vs. 55.4 months, 95% CI 38.7-72) ($p = 0.001$).

Conclusions : Hypoxia driven gene expression is an independent risk factor in colon cancer. A 6-gene score was found to be of independent prognostic value in stage II/III colon cancer patients. These findings should be incorporated in the molecular classification of colon cancer.

THE BELGIAN REGISTRY OF PEDIATRIC CROHN'S DISEASE (BELCRO) : GROWTH STATUS AFTER 3 YEAR FOLLOW UP. E. Degreef (1), J. Mahachie (2), I. Hoffman (3), F. Smets (4), S. Vanbiervliet (5), P. Bontems (6), I. Paquot (7), P. Alliet (8), K. Vansteen (2), G. Veeremanwauters (1). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) Montefiore Institute, Liège, Belgium ; (3) University Hospitals Leuven, Leuven, Belgium ; (4) Université Catholique de Louvain, City of Brussels, Belgium ; (5) Department of Hepatology and Gastroenterology, Universiteit Gent, Ghent, Belgium ; (6) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (7) CHC Clinique de l'Espérance, Liège, Belgium ; (8) Virga Jesse Hospital, Hasselt, Belgium.

Introduction : The BELCRO cohort was initiated in 5/2008 to prospectively study newly diagnosed pediatric Crohn's disease patients. Here we report on growth outcome at 3y follow up.

Methods : Data from the BELCRO database were evaluated at diagnosis (M0), after 12 (M12), 24 (M24) and 36 months (M36). Cross sectional analysis at M36, longitudinal analysis and profile analysis from M0 to M36 were performed on the growth data obtained. Hypothesis were tested at 5% significance.

Results : At M 36, consecutive data for BMI and height z-scores was available in 67 and 75 patients respectively. Disease severity went from 5% inactive, 19% mild and 76% moderate to severe at M0 to 70% inactive, 24% mild and 6% moderate to severe at M36. Median BMI z-score is -0,11 (range -3,38 to 2,01) and median height z-score is 0,13 (range -2,03 to 2,3). Five patients (7%) had height z-score and 19 patients (28%) BMI z-score $< -2SD$ at M0. At M36, 0/5 and 5/19 remained $< -2SD$. Even though 75% of BMI z-scores and 93% of height z-scores remained within normal ranges ($> -2SD < 2SD$) at diagnosis, 66% of patients improved their BMI z-score and 43% their height z-score over 36M resulting in 91% of BMI z-scores between normal ranges at M36 and 97% for height z-score. Patients diagnosed and followed by adult physicians had significantly better height z-scores at M0 which remained at M36 ($P = 0.027$). L3 or L4A involvement imply a worse height z-score at M 36 ($p = 0.02$; $p = 0.02$). Nor disease severity, nor treatment seemed to influence growth (BMI z-score/length z-score) over time, besides patients with inactive disease at M36 on Immuno-modulator monotherapy. They have a better height z-score at M 36 ($p = 0.006$).

Conclusions : The majority of patients had severe disease at diagnosis, but few of them had severe growth retardation. An increase in z-scores for BMI and height is noticed in a large group of patients. Disease location at diagnosis seems to influence height z-scores at M36.

- D07 -

State-of-the-art Lecture

The role of the gut microbiome in gastrointestinal and liver disease.
N. Delzenne, UCL, Belgium.

- D08 -

PROGNOSTIC VALUE OF FDG PET/CT IN LIVER TRANSPLANTATION FOR HEPATOCARCINOMA.
O. Detry, L. Govaerts, N. Bletard, N. Meurisse, G. Verset, A. Deroover, J. Delwaide, A. Lamproye, P. Honore, R. Hustinx. CHU Liege, Liège, Belgium.

Aim : FDG uptake has been shown to predict the outcome in large series of patients with hepatocarcinoma (HCC) in Asia, but few data are available regarding European populations. Our aim was to evaluate the prognostic value of pre-treatment FDG PET-CT in patients treated by liver transplantation.

Methods : We retrospectively analyzed the data of 27 patients (24 M and 3 W, mean age 58 ± 9 years). The mean follow-up was 26 ± 18 months (min 1 month, max 66 months). All patients had an FDG PET-CT before the transplantation. The FDG PET/CT was performed according to a standard clinical protocol : 4 MBqFDG/kg body weight, uptake 60 min., low-dose non-enhanced CT. We measured the SUVmax and SUVmean of the tumor and the normal liver. The tumor/liver activity ratios (RSUVmax and RSUVmean) were tested as prognostic factors and compared to the following conventional prognostic factors : MILAN, CLIP, OKUDA, TNM stage, alphafoetoprotein level, portal thrombosis, size of the largest nodule, tumor differentiation, microvascular invasion, underlying cirrhosis and liver function.

Results : The DFS was 87.2% at 1y and 72.1% at 3y. The OS was 85.2% at 1y and 80.7% at 3y. According to an univariate Cox model, RSUVmax, RSUVmean and healthy liver were predictors of DFS and RSUVmax, RSUVmean, size of the largest nodule, CLIP, liver involvement > 50%, and healthy liver predicted the OS. According to a multivariate Cox model, only RSUVmax predicted DFS and RSUVmax and liver involvement > 50% predicted OS. An ROC analysis of the ratios showed that the 1.15 cut-off for RSUVmax was best for predicting both the DFS (Cox regression : HR 14.4, $p = 0.02$) and OS (HR 5.6, $p = 0.049$). The Kaplan-Meier curves and Logrank tests confirmed those results. Even though the MILAN criteria alone were not predictive, it is worth noting that none of the patients outside the MILAN criteria and with RSUVmax < 1.15 relapsed.

Conclusions : The RSUVmax is a strong prognostic factor for recurrence and death in patients with HCC treated by liver transplantation with a cut-off value of 1,15. further prospective studies should test whether the metabolic index should be systematically included in the preoperative assessment.

- D09 -

PREOP. CHEMOSENSITIVITY TESTING AS PREDICTOR OF ADJUVANT BENEFIT IN STAGE III COLON CANCER (PEPITA). A. Hendlisz (1), A. Deleporte (2), J.L. Van Laethem (3), P. Vergauwe (4), M. Van Den Eynde (5), G. Deboever (6), J. Janssens (7), G. Demolin (8), S. Holbrechts (9), M. Clausse (10), J. Vermeij (11), L. D'hondt (12), S. Laurent (13), A. Efir (14), M. Peeters (15), M. Gomez Galdon (1), A. Buggenhout (16), M. Paesmans (2), C. Garcia (2), M. Piccart-Gebhart (2), P. Flamen (2). (1) Institut Jules Bordet, City of Brussels, Belgium ; (2) Institut Jules Bordet, Brussels, Belgium ; (3) Hôpital Erasme, City of Brussels, Belgium ; (4) AZ Groeninge, Kortrijk, Belgium ; (5) Université Catholique de Louvain, Brussels, Belgium ; (6) AZ Damiaan, Oostende, Belgium ; (7) AZ TURNHOUT, Turnhout, Belgium ; (8) Clinique St-Joseph, Liège, Belgium ; (9) CHU Ambroise Paré MONS, Mons, Belgium ; (10) Cliniques Saint Luc, Bouge, Belgium ; (11) ZNA Middelheim, Antwerpen, Belgium ; (12) UCL, Mont-Godinne, Belgium ; (13) UZ Gent, Gent, Belgium ; (14) ULB Brugmann, Brussels, Belgium ; (15) Universitair Ziekenhuis Antwerpen, Antwerpen, Belgium, [16] Erasme University Hospital, City of Brussels, Belgium.

Introduction : Adjuvant chemotherapy improves stage III colon cancer outcome but is not effective for all patients. PePiTA trial's main hypothesis is that the absence of metabolic response of the primary tumor after 1 preoperative chemotherapy course predicts the absence of benefit from adjuvant chemotherapy (at 3-year DFS). This strategy's aim is to spare patients from useless toxicities, improve healthcare resource allocation, and guide translational research. This interim analysis was performed for safety and feasibility of Metabolic Response Assessment (MRA).

Methods : Patients ≥ 18 years, with PS ≤ 1 , diagnosed with colon cancer considered for curative resection are eligible, after signed consent. Baseline PET is repeated after 1 chemotherapy cycle, followed by surgery. PET quality insurance and MRA are performed centrally and the result is blinded for investigators.

Results : From 2010 to 2013, 114 patients – M/F (55%/45%), median age 66 (26-81), ECOG 0/1(92%/8%) – were included in 15 Belgian centers. 11 patients were excluded from analysis : 2 hyperglycemia at baseline PET ; 2 withdrew consent ; 6 PET-revealed stage IV CC ; and 1 second cancer. Preoperative CT was associated with 5% grade 3-4 neutropenia, 1% grade 3 diarrhea, 1% grade 3 hypokaliemia, 1% peritonitis and 1% grade 3 thromboembolic events. Colectomies were performed in all patients after a median of 20 days (interquartile interval 18-21) : 32 right (31%), 69 left (67%), and 2 procedures not detailed. Pathology showed stages 0 (1%), I (13%), II (34%), III (47%), IV (7%), without lymph node downstaging. Postoperative morbidity is 9% (95%CI 5-16%) and includes fistulas (4%), transient ischemic attack (1%), ileus (2%), and evisceration (1%), but no death. Technical or methodological reasons prevented MRA in 19/103 pts. Median SUVmax was 14.4 (4.9-47.8) at baseline, and 10.9 (0-39.3) on day 14. 2 patients presented with complete MR. For the others, median delta SUVmax was -22% (-60 to +31%). MR was observed in 60% of patients, and was absent in 40%, with equal distribution for stages II and III (p = 1.00).

Conclusions : 1 course of chemotherapy is feasible before curative surgery for colon cancer, without inducing excessive toxicity, delay or surgical morbidity. MRA indicated metabolic signs of chemoresistance in 40% of the primary tumors.

- D10 -

PRE-OPERATIVE SEROLOGICAL MARKERS MAY PREDICT POSTOPERATIVE CROHN'S DISEASE RECURRENCE. M. Noben (1), A. De Buck Van Overstraeten (2), S. Lockton (3), G. De Hertogh (2), F. Princen (3), A. Wolthuis (2), G. Van Assche (2), S. Vermeire (2), S. Singh (3), A. D'hoore (2), M. Ferrante (2). (1) Translational Research Center for Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium ; (3) Prometheus Laboratories Inc., San Diego, United States.

Introduction : Preventing postoperative endoscopic (ER) and clinical recurrence (CR) remains a challenging issue in patients with Crohn's disease (CD) undergoing an intestinal resection. Several clinical and histological risk factors have been identified, and may guide selection of appropriate candidates for postoperative prophylactic CD therapy.

Aim : We evaluated if a set of pre-operative serological markers could strengthen the prediction of postoperative ER and CR.

Methods : Our study population consisted of 100 consecutive patients (41 males, 27 active smokers, median age 41.7 years) undergoing an ileal resection with ileocolonic anastomosis for refractory CD, in whom a serum sample was collected \leq 1 week prior to surgery. All patients were followed prospectively and underwent a postoperative endoscopic evaluation at 6 months. The primary endpoint (ER) was defined as a postoperative endoscopic recurrence score of i3 or i4. Secondary endpoints included time to clinical recurrence. Sera were analysed blindly at Prometheus laboratories for the expression of anti-*Saccharomyces cerevisiae* IgA (ASCA A) and IgG antibodies (ASCA G), three different anti-flagellin antibodies (CBir1, Fla2 and FlaX), antibodies to the outer-membrane porin C of *Escherichia coli* (OmpC), and atypical perinuclear antineutrophilic cytoplasmic antibodies (pANCA). The Q3 value of each individual marker in this dataset was defined as the cut-off point. Predictors of both ER and CR in univariate analyses were included in the binary logistic and Cox regression analysis.

Results : Twenty-five patients developed ER at 6 months. Fla2 > 66 EU and active smoking were independently associated with ER (Table 1). During a median follow-up of 23.6 months, 29 patients developed a CR, with Fla2 > 66 EU, pANCA positivity and active smoking as independent risk factors (Table 1). A cumulative risk score was developed by combining 3 risk factors (Fla2 > 66 EU, pANCA positivity, and active smoking). Based on this cumulative risk score, we could observe a significant and gradual increased risk of both ER (Figure 1, linear-by-linear p < 0.001) and CR (Figure 2, LogRank p < 0.001).

Table 1. — Multivariate analysis

	Endoscopic recurrence * (Logistic Regression)	Clinical recurrence * (Cox Regression)
Fla2 > 66 EU	3.0 (1.1-8.7) p = 0.037	2.2 (1.0-4.6) p = 0.041
pANCA positive	2.7 (0.9-8.1) p = 0.083	2.5 (1.2-5.4) p = 0.016
Active smoking	3.1 (1.1-8.8) p = 0.029	2.6 (1.2-5.5) p = 0.011

* Odds ratio (95% confidence interval).

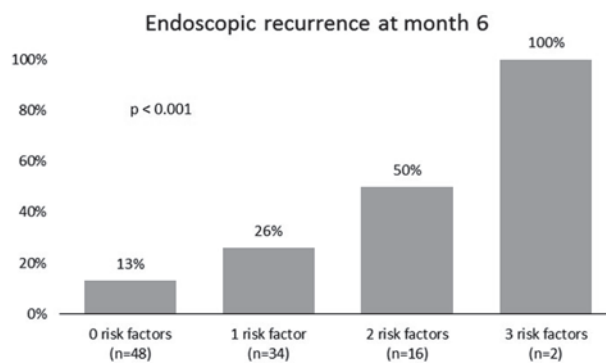


Fig. 1

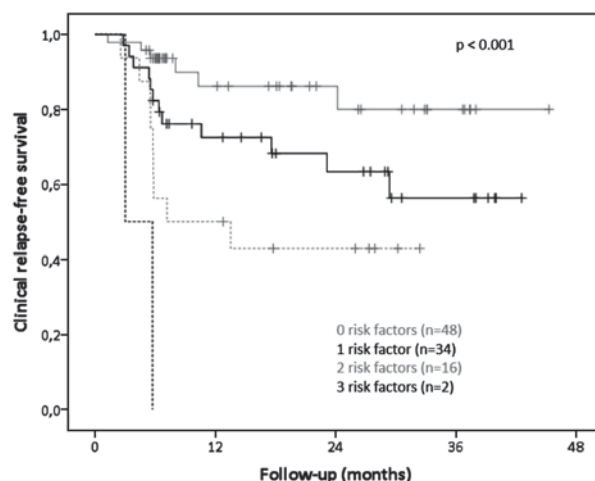


Fig. 2

Conclusions : Pre-operative serological markers, including anti-flagellin Fla2 antibodies, were independently associated with postoperative ER and CR. We identified a risk panel of clinical and serological markers which may guide post-operative prophylactic therapy. Validation of these results in an independent cohort is warranted.

- D11 -

OBETICHOIC ACID, A FARNESOID-X RECEPTOR AGONIST, IMPROVES PORTAL HYPERTENSION IN CIRRHOTIC RATS. L. Verbeke (1), R. Farre (1), J. Trebicka (2), M. Komuta (1), T. Roskams (1), S. Klein (2), I. Vander Elst (1), P. Windmolders (1), T. Vanuytsel (1), F. Nevens (1), W. Laleman (1). (1) KU Leuven, Leuven, Belgium ; (2) University Hospital, Bonn, Germany.

Introduction : The farnesoid-X-receptor (FXR) is a nuclear bile acid receptor involved in bile acid homeostasis, hepatic and intestinal inflammation, liver fibrosis and cardiovascular disease.

Aim : We studied the effect of short-term treatment with obeticholic acid (INT-747), a potent selective FXR agonist, on intrahepatic hemodynamic dysfunction and signaling pathways in different rat models of cirrhotic portal hypertension.

Methods : Thioacetamide (TAA)-intoxicated and bile duct ligated (BDL) rats were used as models. After gavage of 2 doses of 30mg/kg INT-747 or vehicle within 24 hours, in vivo hemodynamics were assessed. Additionally, we evaluated the direct effect of INT-747 on total intrahepatic vascular resistance (IHVR) and intrahepatic vascular tone (endothelial dysfunction and hyperresponsiveness to methoxamine) by means of an in-situ liver perfusion system and on hepatic stellate cell contraction in vitro. FXR-expression and involved intrahepatic vasoactive pathways (e.g. endothelial nitric oxide synthase : eNOS, Rho-kinase, dimethylarginine dimethylaminohydrolase : DDAH) were analyzed by immunohistochemistry, RT-PCR or Western Blot.

Results : In both cirrhotic models, FXR expression was heavily decreased. Immunohistochemically, this coincided with a disappearance of the typical FXR hepatocytic nuclear staining pattern seen in healthy livers. Treatment with INT-747 in TAA and BDL reactivated the FXR downstream signaling pathway and decreased portal pressure without deleterious systemic hypotension (portal pressure decreased from $13.8 \pm 0,6$ mm Hg to $11.6 \pm 0,8$ mm Hg in TAA, $P = 0.045$, $n = 14$ and from $12.3 \pm 0,8$ mm Hg to $10.1 \pm 0,6$ mm Hg in BDL, $P = 0.037$, $n = 15$). This was related to a decrease in total

intrahepatic vascular resistance during liver perfusion (D relative decrease in IHVR after INT-747 treatment in TAA : $-8.06 \pm 0.69\%$, $P < 0.001$, $n = 10$ and in BDL : $-21.8 \pm 2.50\%$, $P < 0.001$, $n = 10$). In the perfused TAA and BDL cirrhotic liver, INT-747 improved endothelial vasorelaxation capacity but not hyperresponsiveness. In both groups, this was associated with an increased eNOS-activity which in TAA related to down-regulation of the Rho-kinase pathway and in BDL to up-regulation of DDAH-2. Furthermore, INT-747 induced a dose-dependent relaxation of cultured rat primary hepatic stellate cells in vitro.

Conclusions : FXR-agonist INT-747 improves portal hypertension in 2 different rat models of cirrhosis by decreasing the IHVR. This hemodynamic effect relates to restoration of endothelial dysfunction by increased intrahepatic eNOS-activity. The mechanisms of eNOS activity increase differ depending on the etiology of cirrhosis.

- D12 -

GASTROSTOMY TECHNIQUES : A SINGLE CENTER PEDIATRIC EXPERIENCE. K. Das (1), M. Miserez (1), T. Claeys (1), J. Daniels (1), M. Stas (2), I. Hoffman (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) University Hospitals Leuven, Belgium, Leuven, Belgium.

Aim : Percutaneous endoscopic gastrostomy (PEG) is widely used for long-term enteral feeding in children. Recently laparoscopic gastrostomy (LG) had gained interest, because of reported lower complication rates and its adaptability to almost any patient. The aim of this study is to compare LG with PEG outcome and complications.

Methods : A retrospective review of all PEG and LG placements in our center between January 2009 and December 2012 was performed. Patient demographics, major comorbidity, operative time, postoperative hospital stay, and postoperative follow-up were recorded. Outcome was assessed using the Clavien-Dindo classification of surgical complications. Statistics were performed using chi square and Mann-Whitney U test. $P \leq .05$ was considered significant.

Results : Of 61 gastrostomies created, 36 were PEG and 25 were LG. Patients in the LG group weighed significantly less (6.6kg vs 12.5kg). Most patients had neurological, tumoral or orofacial comorbidity. In the PEG groups median operative time was shorter (10minutes vs 55minutes, $P < .05$), feedings were started earlier (1day vs 2days, $P < .05$) and postoperative hospital stay was shorter (3days vs 6 days, $P < .05$). There were no intraoperative complications. The majority of adverse events were classified as grade I or II events. Procedure related events scored as grade III or higher were observed in 6 patients in LG and 8 in PEG (24% vs 22%). There was no significant difference in complications or outcome between LG and PEG.

Conclusions : Although patients in the LG group were significantly younger, complication rate and outcome was comparable to PEG. If no other contraindications are present both LG and PEG are equally good options and the decision should be made multidisciplinary and in consultation with the child's parents.

- D13 -

INTRA-ARTERIAL YTRRIUM-90 MICROSPHERES FOR DOWNSTAGING HCC PATIENTS TO TRANSPLANTATION. K. De Man, L. Defreyne, E. Dhondt, E. Delanghe, X. Verhelst, P. Smeets, H. Van Vlierberghe, A. Geerts, R. Troisi, X. Rogiers, B. Lambert. Universiteit Gent, Gent, Belgium.

Introduction and Aim : In our hospital, patients are referred for intra-arterial treatment with Yttrium-90 microspheres if they present with an HCC confined to the liver, but are ineligible for curative treatment options such as liver transplantation, partial hepatectomy or radiofrequent ablation.

Additional eligibility criteria for radioembolisation consist of Child-Pugh score $< B8$, Karnofsky status $\geq 70\%$ and bilirubin < 2 mg/dL. Portal vein thrombosis is not considered a contra-indication.

In the presented study we assessed retrospectively how successful we were in downstaging patients to liver transplantation.

Methods : We retrospectively analyzed all HCC patients who were treated with Yttrium-90 microspheres in our hospital since 2008 and who presented at an age < 70 and with a disease load exceeding the so-called Milan criteria (single tumour ≤ 5 cm or ≤ 3 lesions ≤ 3 cm). We recorded whether the patient was downstaged to a tumour load complying with the Milan criteria, and if so, whether transplantation was carried out.

Results : 35 patients aged < 70 years and with a tumour load exceeding the Milan criteria were evaluable. 29 had Barcelona Clinic Liver Cancer (BCLC) B stage disease and 6 had BCLC C.

18 out of 35 patients were downstaged to the Milan criteria (52%, all BCLC B, except for 1 who was BCLC C), of whom 8 were actually transplanted so far. One patient is on the waiting list, 1 patient showed progression while waiting, 1 patient refused to be listed and 1 patient suffers comorbidity that excludes him from abdominal surgery. Unfortunately, 6 patients died before or during work-out for transplantation : 2 unexplained sudden death, 1 lung embolism, 3

due to liver failure. Overall median survival was 12.9 months. In the group of patients who were downstaged, an overall median survival of 23 months was seen. Patients who underwent transplantation after downstaging had a significant better median overall survival ; a median could not be reached in this group.

17 out 35 patients (49%) did not have a tumour load within the Milan criteria at any point of their follow up and had a significant worse median survival of only 7 months ($p < 0.05$ LogRank).

Conclusions : In our series of BCLC B and C patients aged < 70 who underwent radioembolisation, we obtained a successful downstaging to the Milan criteria in 52% of patients, with an actual transplantation rate of 23%.

- D14 -

LANREOTIDE AUTOGEL 120 mg FOR CHRONIC REFRACTORY DIARRHEA : A MULTICENTER PROSPECTIVE TRIAL. R. Bisschops (1), V. De Ruyter (2), G. Demolin (3), D. Baert (4), T. Moreels (5), P. Pattyn (6), H. Verhelst (7), L. Lepoutre (8), J. Arts (9), P. Ooghe (10), P. Maisonobe (2), J. Tack (1). (1) Gastroenterology, University Hospitals Leuven, Leuven, Belgium ; (2) Ipsen, Merelbeke, Belgium ; (3) CHC, Liège, Belgium ; (4) Maria Middelaars Ziekenhuis, Gent, Belgium ; (5) University of Antwerp, Antwerpen, Belgium ; (6) Ghent University, Ghent, Belgium ; (7) ZOL, Genk, Belgium ; (8) OLV HOSPITAL, Aalst, Belgium ; (9) AZ Sint-Lucas, Brugge, Belgium ; (10) CHU, Charleroi, Belgium.

Introduction : Lanreotide has an inhibitory effect on gastrointestinal secretions and motility. We evaluated the efficacy and safety of the prolonged-release somatostatin analogue lanreotide Autogel 120 mg for the treatment of persistent diarrhea in a multicenter, prospective, open label study.

Methods : The study was performed in 10 centers between July 2009 and August 2012. The study protocol was approved by the Ethics Committee of UZ Leuven, Belgium (ClinicalTrials.gov NCT00891371 ; EudraCT 2009-009356-20). Male or female adult patients with refractory diarrhea (mean of > 3 stools/24 h) for at least 1 month after workup to rule out inflammatory, infectious causes, and rectum disorders, and insufficient /non-response to standard anti-diarrheal were treated for 8 weeks with lanreotide autogel 120 mg deep s.c. every 4 weeks. Patients' stool frequency and consistency (Bristol Stool Form Scale) were recorded in a daily diary. Quality of life (QOL) was assessed using the SF-36 and IBS-QOL questionnaires. Responders were patients with a $\geq 50\%$ decrease or a normalization of the mean number of stools. Adverse events (AEs) were recorded during the entire study.

Results : In total, 36 patients (24 women, 12 men) with chronic refractory diarrhea were studied. The mean age was 55.2 ± 16.4 years. Four weeks after the first injection (Day 28), 44.4% of patients were responders, which increased to 54.3% at Day 56. Response rates were higher in male patients (58.3%) compared to females (37.5%). Overall, the mean number of stools decreased significantly from 5.5 ± 2.3 at baseline to 3.6 ± 2.2 at Day 56 (-30.7%) ($p = 0.0006$). In the responder subgroup at Day 56 this number changed significantly from 5.3 ± 2.4 to 2.2 ± 0.8 ($p < 0.0001$). The median percentage of days per week with less than 3 stools/day increased from 7.1% at baseline to 57.1% at Day 56. At Day 56, the mean change from baseline for the IBS-QOL score was +16.3. Similarly, the SF36-QOL scores improved at Day 56, especially in the domains of social functioning (+14.0), physical role (+11.8) and mental health (+10.1). In the responder subgroup at Day 56, the mean stool consistency score dropped significantly from 6.4 ± 0.7 to 5.2 ± 1.2 ($p = 0.0005$). In total, 93 treatment emergent AEs were reported, of which the majority (79) were mild to moderate and only 9 were serious. The most frequently reported AEs were gastrointestinal disorders (abdominal pain, 25% of patients ; nausea, 11.1% and steatorrhea, 8.3%), injection site reactions (16.7%) and asthenia/fatigue (11.1%).

Conclusions : This study showed improvement of diarrheal symptoms and QOL during therapy with lanreotide autogel 120 mg treatment, which suggest it is effective for the management of persistent refractory diarrhea. Confirmatory studies are needed.

SEVEN SOCIETIES POSTGRADUATE COURSE

Part 1 : Bariatric surgery

- S01 -

What's to expect from current and new procedures in bariatric surgery.
J. Closset, Erasme Hospital, ULB, Brussels, Belgium.

- S02 -

It's more than weight loss, the metabolic side of the story.
M. Lannoo, KUL, Belgium.

- S03 -

The role of the gastroenterologist in postoperative disasters.
T. Moreels, UZA, Belgium.

- S04 -

Gastrointestinal postoperative complaints : is it dumping ?
Y. Van Nieuwenhoven, UGent, Belgium.

- S05 -

NASH and bariatric surgery.
S. Francque, UZA, Belgium.

- S06-

BARIATRIC SURGERY IN ADOLESCENTS. L.W.E. Van Heurn, Maastricht University Medical Centre, The Netherlands.

In the Western world, obesity is an increasing problem, which is associated with a higher mortality and severe morbidity. To reduce the mortality and morbidity of patients with morbid obesity (BMI > 35 kg/m²), bariatric surgery has been popularised over the past decades as a valuable method to lose weight and to decrease the consequences of obesity. Also in youth the prevalence of obesity has substantially increased over time, however bariatric surgery is hardly done and even forbidden in many countries.

It is known that obesity and morbidly obesity in adolescents are associated with higher incidences of the metabolic syndrome, diabetes, cardiovascular abnormalities, obstructive sleep apnoea and steatosis of the liver compared to their peers. Their quality of life is less and their socio-economic prospects reduced with lifelong consequences. Adolescents with obesity have a very high chance to become obese adults.

The generally accepted treatment for morbidly is combined lifestyle interventions including dietary, psychological and physiotherapeutic treatment under close supervision of a dedicated paediatrician. The results of these combined interventions are often disappointing with only small and short-term weight loss. Medical treatment has little effect on weight loss and is hardly used. The consequences, of the more aggressive approach of bariatric surgery are relatively unknown, particularly the long-term outcome and the effect of surgery on growth and development. Adolescents are in an important

phase in their growth and development from child to adult. Many fear that bariatric surgery may jeopardize normal development of the child due to nutritional deficits of vitamins, minerals and other essential nutrients. There are several surgical techniques to obtain weight loss in morbidly obese including gastric banding, a gastric sleeve and a Roux-en-Y gastric bypass. In a systematic review in morbidly obese adolescents, all techniques resulted in a mean weight loss of approximately 50% of additional weight, however, gastric bypasses were more often used in more overweighted patients than gastric banding, and the absolute weight loss was higher in the Roux-en-Y gastric bypass group. Gastric banding has the theoretical advantage of a technique that can be easily reversed and does not include a malabsorption component. The bariatric surgical technique that would fit best in adolescents is therefore unknown. The knowledge of the consequences of bariatric surgery in adolescents is inadequate to advice this method as the golden standard to treat morbidly obese adolescents in whom conservative treatment has not been successful. Additional studies are necessary to assess the benefits and hazards of bariatric surgery if combined lifestyle interventions have failed.

Part 2 : Cystic lesions of the pancreas

- S07 -

Overview & workup.

I. Borbath, UCL and L. Annet, UCL, Belgium.

- S08 -

Multidisciplinary treatment of pseudocyst.

D. Ysebaert, UZA and P. Eisendrath, Erasme Hospital, ULB, Belgium.

- S09 -

IPMN : state of the art.

A. Sauvanet, Université Paris VII & Hôpital Beaujon, Paris, France.

- S10 -

Surgery for premalignant & malignant cystic lesions.

F. Berrevoet, UZ Gent, Belgium.

**XXVith Belgian Week of Gastroenterology
February 13-15, 2014**

ABSTRACTS

A01 — A53	BASL - BLIC - BeSPGHAN
B01 — B18	OG-FWO
P01 — P14	Belgian Pancreatic Club (BPC)
G01 — G13	Belgian Society for Gastrointestinal Endoscopy (BSGIE) and Small Bowel Group
I01 — I31	IBD Research Group (BIRD)
N01 — N05	Research Group of Clinical Nutrition and Metabolism (SBNC)
O01 — O17	Belgian Group for Digestive Oncology (BGDO)
R01 — R17	Pathology Club, Radiology, Nuclear Medicine
C01 — C11	Case Report Session
D01 — D14	Plenary Session
S01 — S10	Seven Societies Postgraduate Course

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