ABSTRACTS

XXVth Belgian Week of Gastroenterology 2013

ABSTRACTS

BASL

- A01 -

LANREOTIDE REDUCES IN A DOSE-DEPENDENT WAY LIVER VOLUME IN PATIENTS WITH POLYCYSTIC LIVER DISEASE. F. Temmerman (1), T. Gevers (2), T. Ho (3), V. De Ruyter (1), R. Vanslembrouck (4), W. Coudyzer (4), W. Laleman (1), D. Cassiman (1), C. Verslype (1), S. Van Der Merwe (1), W. Van Steenbergen (1), J. Van Pelt (5), B. Bammens (1), Y. Pirson (6), J. Drenth (2), F. Nevens (1), F. Nevens (1). (1) UZ Leuven, Leuven, Belgium ; (2) UMC St. Radboud, Nijmegen, Netherlands ; (3) Université Catholique de Louvain, Brussels, Belgium ; (4) Radiology, UZ Leuven, Leuven, Belgium ; (5) University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (6) Université Catholique de Louvain, Brussels, Belgium.

Introduction : The long-acting somatostatin analogue lanreotide (LAN) given 120 mg every 4 weeks reduces liver volume (LV) in patients with polycystic liver diseases (PCLD) after 6 months. In the animal studies the volume reductions on liver and kidney are dose-dependent.
Aim : To investigate whether the effect of LAN is also dose-dependent in human PCLD.
Methods : The 6-month results of the LOCKCYST (placebo *versus* LAN 120 mg) trial and its extension study (LAN 120 mg) and the LOCKCYST II (LAN 90 mg) trial were pooled. LV at baseline and after 6 months were measured by CT-scan and blindly re-analyzed by 2 independent radiologists using different software programs.
Results : The study population consisted of 128 paired observations (112 women ; 95 ADPKD ; median age 49 years (IQR : 45-55). 26 pts were given placebo ; 51 LAN 90 mg /4 wks and 51 LAN 120 mg / 4 wks. Bland-Altman plot showed an excellent agreement in changes in LV by the 2 radiologists and between the 2 techniques of volume measurement. In the placebo group, 3 patients reported the occurrence of mild diarrhea but none of them stopped therapy. Severe side effects (steatorrhea and/or abdominal cramps) occurred respectively in the LAN 90 mg group : 4/55 (7%) and in the LAN 120 mg group : 8/51 (15%). Median delta LV in the placebo, LAN 90 mg and LAN 120 mg groups were resp. : +36 mL [(IQR :(-45) – (+138)] ; -82 mL [(IQR :(-285) – (+92)] ; -123 mL [(IQR : (-312) – (+4)] (Kruskal-Wallis One-Way ANOVA on Ranks ; $P = 0.002$). There was no significant difference in changes of the glomerular filtration rate between the placebo group and both treatment groups (ANOVA on ranks : $P = 0.245$).
Conclusion : LAN 120 mg/4wks given for 6 months induces the greatest reduction in total liver volume. The lower LAN dose is still beneficial compared to placebo with a trend to lesser side effects.

- A02 -

A ROLE FOR ALDH ACTIVITY DURING HEPATIC STELLATE CELL ACTIVATION. L. Dolle, S. Verhulst, I. Mannaerts, E.L. Guimaraes, L.F. Thoen, A. El Taghdouini, L.A. Van Grunsven. Free University (VUB), Brussels, Belgium.

Introduction : Hepatic fibrosis is the major complication of virtually all types of chronic liver damage. Hepatic stellate cells (HSCs) remain the main contributors of the increased liver scar tissue formation. HSCs are the liver pericytes that store 80-90% of total liver retinoid in their lipid droplets. It is generally accepted that when HSCs activate retinyl ester levels progressively decrease, and subsequently, through the activation process the amount of lipid droplets also decreases. Retinyl esters can be degraded by aldehyde dehydrogenase (ALDH) enzymes into retinoic acid. Throughout a healthy liver, the HSC population displays heterogeneity in its location, expression of markers (collagens, desmin, aSMA), size of its lipid droplets and in its capacity for retinoid storage. It is not yet entirely clear why HSCs lose retinol during activation, which enzymes are involved in this process and what role the retinol metabolites precisely play in HSCs upon liver injury.
Aim : Determine whether ALDH enzymes are involved in the HSC activation process by regulating the degradation of retinyl esters.
Methods : We isolated HSCs either from wild type or CCl_4-injured mice by Nycodenz density gradient centrifugation, followed by fluorescence activated cell sorting (FACS) based on vitamin A autofluorescence and/or ALDH activity assay using Aldefluor. Gene expression analysis and immunocytochemistry for HSC markers was carried out on freshly isolated HSCs or on culture activated HSCs.
Results : We show that (i) roughly 70% of the total HSC population in healthy mouse livers has high ALDH activity (HSC-$ALDH^+$) ; (ii) This HSC-$ALDH^+$ population from uninjured livers displays elevated expression of HSC activation markers ; (iii) ALDH activity disappears throughout HSC activation *in vitro* and *in vivo* ; (iv) retinol levels regress during HSC activation *in vivo*, which is linked to ALDH activity ; (v) use of the ALDH inhibitor cyanamide has an inhibitory effect on HSC activation *in vitro* as well as in 2 mouse models of fibrosis (CCl_4 intoxication and common bile duct ligation).
Conclusion : Our results suggest that the HSC population in a healthy liver is heterogeneous based on their ALDH activity and that inhibition of this activity hampers activation of HSCs *in vitro* and *in vivo*.

SIX years OF TREATMENT WITH TENOFOVIR FOR CHRONIC HBV INFECTION IS SAFE AND WELL TOLERATED. P. Marcellin (1), M. Buti (2), E. Gane (3), N. Tsai (4), W. Sievert (5), I. Jacobson (6), G. Germanidis (7), J. Flaherty (8), P. Dinh (9), K. Kitrinos (8), J. Mchutchison (8), N. Afdhal (10), J. Piessevaux (11). (1) Hôpital Beaujon, Clichy, France ; (2) Department Of Hepatology, Hospital Vall D'hebron, Universitat Autònoma De Barcelona, Spain ; (3) Auckland City Hospital, New Zealand ; (4) University Of Hawaii, Honolulu, USA (5) Monash University And Monash Medical Centre, Melbourne, Australia ; (6) Weill Cornell Medical College, New York, USA ; (7) Ahepa University Hospital, Thessaloniki, Greece ; (8) Gilead Sciences, Foster City, USA ; (9) Gilead Sciences, Foster City, USA ; (10) Beth Israel Deaconess Medical Center, Boston, USA ; (11) Gilead Sciences, Brussels.

Background : We previously reported that 5 years of tenofovir DF (TDF) therapy in mostly treatment naïve patients results in sustained virological suppression with no development of resistance and was associated with either the halting or regression of fibrosis in 96% of patients.
Aim : Here we present 6 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) of the spine and hip by DXA were added starting at year 4.
Results : In a total 641 patients who were initially randomized and treated, 585 (93%) entered the TDF extension phase, and at year 6, 466 (73%) remain on study. Efficacy results at year 6 are shown in the table. TDF was well tolerated over the 6 year evaluation period. Less than 2% of patients discontinued TDF due to an adverse event, and ≤ 1.5% experienced a confirmed renal event (≥ 0.5 mg/dL increase in serum creatinine from baseline, phosphorus < 2 mg/dL, or CrCL < 50 mL/min). BMD (T scores) was stable over 2 years of evaluation. No resistance to TDF has been detected through year 6.
Conclusions : In these two trials, TDF remains safe and effective over a 6 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.

	HBeAg-negative Patients (Study 102) N = 375	HBeAg-positive Patients (Study 103) N = 266
HBV DNA < 400 copies/mL [a]	81% (281/345)	62% (157/251)
HBV DNA < 400 copies/mL [b]	99.6% (283/284)	99% (167/169)
ALT normalization [b]	86% (228/265)	78% (127/162)
HBeAg loss [b]	-	50% (82/163)
HBeAg seroconversion [b]	-	37% (61/163)
HBsAg loss [c]	-[d]	11% (n = 24)
HBsAg seroconversion [c]	-[d]	8% (n = 18)

[a] Missing = Failure (LTE-TDF analysis set) ; [b] Missing = Excluded (On treatment analysis set) ; [c] Kaplan-Meier % ; [d] One HBeAg-negative patient experienced HBsAg loss/seroconversion at Year 5.

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
Methods : 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.

- A05 -

THE TIME-DEPENDENT ALTERATIONS OF THE UNFOLDED PROTEIN RESPONSE AND CHEMORESISTANCE IN HCC. Y.P. Vandewynckel (1), I. Colle (2), D. Laukens (1), C. Vanhove (1), S. Janssens (3), B. Lambrecht (3), H. Van Vlierberghe (2). (1) Ghent University, Ghent, Belgium ; (2) Ghent University Hospital, Gent, Belgium ; (3) VIB, Gent, Belgium.

Introduction : Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer worldwide. HCC is a chemo-resistant tumor. Systemic therapy with cytotoxic drugs yields low response rates without proven survival benefit. Medications influencing the chemosensitivity profile are therefore needed. The unfolded protein response (UPR) is implicated in the pathophysiology of several diseases including cancer and it is well known that endoplasmic reticulum (ER) stress could be a cause of the chemoresistance in HCC.
Aim : To investigate these phenomena in HCC, we examined the presence and time-dependent alterations of ER stress and multidrug resistance (MDR) in a mouse model of HCC in order to have an *in vivo* method for the evaluation of UPR manipulation strategies as future cancer therapy.
Methods : The *in vivo* study design was a diethylnitrosamine (DEN)-induced mouse model for HCC. Mice (6 groups of n = 14) where sacrificed after 20, 25 and 30 weeks of DEN or saline administration. The activation pattern of the UPR and the expression of MDR-related efflux pumps were determined by qRT-PCR, Western blot, ELISA and immuno-histochemistry. The histology of the HCC nodules was examined by H/E, Sirius red, F4/80 and reticuline staining. Small animal imaging by dynamic contrast enhanced MRI and choline PET was performed.
Results : After 25 weeks of DEN administration this model showed PET-positive hepatocarcinogenesis occurring in a background of inflammation and fibrosis. The major MDR-related efflux pumps including mdr1, mrp1, mrp4 and brcp were significantly upregulated in the HCC nodules from 25 weeks. The master regulator of the UPR, bip, was significantly induced on 25 and even more on 30 weeks. Downstream targets of the perk branch (phosphorylated eif2a part : atf4, chop, gadd34 and nrf2 part : gclc, gsta1 and -2) were significantly upregulated after 25 weeks and continued to rise until 30 weeks. Unexpectedly, we could not show an increase in xbp1 splicing, spliced xbp1 targets or ire1a phosphorylation in the HCC nodules compared to the normal liver tissue. Only after 30 weeks of DEN the atf6 pathway and the downstream targets showed significant induction.
Conclusion : Here we provide the first evidence of the presence and the time-dependent alterations of MDR-related efflux pumps and a specific activation pattern of the UPR in the HCC nodules of a DEN-induced mouse model. This model can be used to manipulate the fine-tuning of the UPR in hepatocarcinogenesis and related MDR development.

- A06 -

VASCULAR HYPORESPONSIVENESS IN NON-CIRRHOTIC NAFLD-INDUCED PHT COMPARED TO OTHER MODELS OF PHT. S.M. Francque (1), W.J. Kwanten (2), F. Couturier (2), J. Govaerts (2), B.Y. De Winter (2), J.G. De Winterman (2), P.A. Pelckmans (1), P.P. Michielsen (1). (1) Antwerp University Hospital, Antwerpen, Belgium ; (2) University of Antwerp, Antwerpen, Belgium.

Introduction : Steatosis without fibrosis has previously shown to induce a significant increase in portal pressure associated with features of hyperdynamic circulation and vascular hyporesponsiveness to vasoconstrictors. The underlying mechanisms of these alterations are unknown. In classical models of PHT, NO is believed to be a key player in its pathophysiology, as well as prostaglandins. ?
Aim : To study the role of NO- and COX-mediated mechanisms of vascular responsiveness to vasoconstrictors in steatosis and other models of portal hypertension (PHT).
Methods : Male Wister Rats were divided in 4 groups : Sham-operated (n = 10),rats (n = 14) fed a methionin-choline-deficient diet (MCDD) for 4 weeks (steatosis group), CBDL (Common bile duct ligation, n = 12) and PPVL (partial portal vein ligation, n = 10). Vasoconstrictor response to phenylephrine (PE) was studied on abdominal aorta rings, in basal conditions, after NO-synthase inhibition by L-NAME, COX inhibition by piroxicam, or a combination of both in an organ bath set-up. Contractions were noted as a percentage of potassium induced pre-contraction. Results were analysed by Two-Way ANOVA and student-T-test.

Results : Maximum contraction of aortic rings was significantly lower in models of PHT (steatosis, CBDL and PPVL) compared to controls (sham-operated) : 143.89 ± 12.86%, 133.70 ± 7.41% and 137.50 ± 8.73% respectively compared to 181.67 ± 11.86 ($p < 0.0001$). Piroxicam significantly reduced vascular response in the sham-operated and PPVL-group, whereas it had no influence in steatosis and only modestly in CBDL. L-NAME restored responses in PPVL. Also in steatosis L-NAME restored vascular contractility to values comparable to controls with L-NAME : 160.91 ± 6.65% vs. 166.36 ± 7.94% ($p = 0.1732$). In CBDL L-NAME only slightly ameliorated vascular response. L-NAME/piroxicam combination did not significantly alter arterial contraction in any of the models.
Conclusion : This study confirms vascular hyporeactivity in a model of non-cirrhotic NAFLD-associated PHT comparable to what is observed in classical models PHT. In normal conditions vascular reactivity is regulated by both vasodilatory NO and vasoconstrictor COX activity. Hyporesponsiveness in steatosis and CBDL (both models of sinusoidal PHT) is in part caused by absence/reduction of COX-mediated vasoconstriction and by NO overproduction. By contrast, COX-mediated vasoconstriction is maintained in PPVL (a model of prehepatic PHT), in which hyporesponsiveness is solely caused by NO overproduction.

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BELGIAN MULTICENTRE EXPERIENCE WITH INTESTINAL TRANSPLANTATION. L.J. Ceulemans (1), A. De Roover (2), O. Detry (2), R. Troisi (3), X. Rogiers (3), R. Reding (4), J. Lerut (4), D. Ysebaert (5), T. Chapelle (5), D. Monbaliu (1), J. Pirenne (6). (1) Intestinal Transplant Programme, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (2) Abdominal Surgery And Transplantation, CHU Sart Tilman, Liège, Belgium ; (3) General Hepato-Biliary Surgery And Transplantation, Ghent University Hospital, Gent, Belgium ; (4) Abdominal Surgery And Transplantation, Université Catholique de Louvain, City of Brussels, Belgium ; (5) Abdominal Surgery And Transplantation, Antwerp University Hospital, Antwerpen, Belgium ; (6) Intestinal Transplant Programme, University Hospitals Leuven, KU Leuven, Zonhoven, Belgium.

Introduction : Compared to Total Parenteral Nutrition (TPN), Intestinal Transplantation (ITx) is perceived by gastroenterologists as a surgically, medically and immunologically challenging procedure whose results remain inferior to other organ Tx. For this reason, ITx has been relatively rarely applied in Belgium (versus certain other European and North American centers).
Aim : Aim of this multicenter review was therefore to analyse the overall Belgian experience (activity, indications, results) with ITx.
Methods : All Belgian Tx centers took part in this Belgium Liver Intestine Transplant Committee survey. Patient-specific data were entered in the standard ad hoc international ITx registry form. Patient/graft survival was calculated (Kaplan-Meier). Nutritional (TPN) independence and Quality of Life (QoL) (Karnofsky score) were analyzed.
Results : Between 03/1999 and 11/2012, 21 ITx were performed in 20 patients (1 reTx), representing a yearly activity of 1.6 Tx, distributed among 5 centers : KULeuven (12), ULg (5), UZGhent (2), UCLouvain (1), UZA (1). Median age was 38 years (8 months – 56 years). 10 recipients were male and 10 female. 5 were pediatrics (< 18 years) and 15 adults. Indications were anatomical or functional short bowel syndrome (SBS) : intestinal ischemia (5), volvulus (5), Crohn (2), chronic intestinal pseudo-obstruction (2), splanchnic thrombosis (2), Churg-Strauss (1), necrotizing enterocolitis (1), microvillus inclusion (1), intestinal atresia (1) and chronic rejection after first ITx (1). All patients had severe complications of SBS and TPN. 9 patients received an isolated ITx (*plus* kidney Tx in 2 ; *plus* pancreas Tx in 1) ; 10 received a combined liver and ITx ; 2 received a multivisceral Tx. At time of Tx, 11 patients were hospitalized and 10 at home. 20 grafts were procured from deceased donors ; one segmental intestinal graft was procured from a living donor. ABO blood group was identical in 63% and compatible in 37%. Median cold ischemia time was 5 hours 30' (3 hours 17' - 9 hours 31'). All patients received tacrolimus-based immunosuppression. Basiliximab (anti-IL2 receptor antibody) induction was administered in 16 patients. One center transfused donor-specific blood in 11 patients as part of their immunomodulatory protocol. 5-year patient and graft survival is 59% and 55.6%, respectively. 8 patients died : 6 to sepsis, 1 to intracerebral hemorrhage and 1 sudden death remained unexplained. 1 patient developed postTx lymphoma. 2 chronic rejections occured for which one reTx was performed. Of 12 survivors, 11 are nutritionally independent (TPN-free) ; in 10, QoL is excellent (Karnofsky score > 90%).
Conclusion : ITx has come of age in Belgium. A 59% 5-year patient survival is achieved and is similar to results reported by the International ITx registry. ITx is not yet an alternative to TPN in stable patients, but a life-saving option that should be considered early in selected patients with reduced life expectancy due to significant complications from TPN and bowel failure.

- A08 -

FIBROSCAN : THE MOST RELIABLE NON-INVASIVE METHOD TO ASSESS SEVERE FIBROSIS AND CIRRHOSIS IN ALD. M. Fernandez, E. Trepo, T. Gustot, D. Degre, L. Verset, P. Demetter, M. Adler, C. Moreno. Erasme Hospital, Brussels, Belgium.

Introduction : Fibroscan(FS)has proved invaluable in the non-invasive assessment of liver fibrosis in various chronic liver diseases. Surprisingly,no clear consensus with regard to the best cut-offs for the degree of liver fibrosis exists in alcoholic liver diease (ALD), which is common in Western countries.
Aim : The aims of the study were to compare the liver stiffness(LS) by the Fibroscan(FS) and different biochemical markers with histological score of liver fibrosis, and to establish the best LS cut-offs for severe liver fibrosis (F = 3) and cirrhosis (F4). We also evaluated the influence of high-AST on the reliability of our LS cut-offs.
Methods : Our retrospective study included 139 consecutive compensated ALD patients (= 5 drinks a day in the preceding year), who underwent liver biopsy (Percutaneous in 54 or Transjugular liver biopsy in 85 patients).Fibrosis was staged using Metavir classification. Standard liver imagery, FS, FIBROTEST (FT), Fib-4, APRI and FORNS scores and the simple biochemical markers such as AST and ALT were tested in all patients. ? ?Areas under the receiver operating characteristic curves (AUROC) were used to determine the diagnostic accuracy for severe liver fibrosis (F = 3) and cirrhosis (F4).
Results : Characteristics of our ALD population were the following : 68% men, mean age 54 ± 0.86 years, mean porto-systemic gradient 6.8 ± 0.61 mmHg. Distribution of liver fibrosis was as follows : F0 : 17.3%, F1 :6.5%, F2 :23%, F3 :12.2% and F4 :41%. 41 of the 139 patients presented with ASH signs at liver biopsy. Mean AST, ALT, GGT and LS, were 78.5 ± 7.03 UI/l, 68.2 ± 7.63 UI/l, 442.14 ± 53.56 UI/l and 25 ± 2.03kPa, respectively. Failure rate of FS was of 10%. AUROC (95% confidence interval) for the diagnosis of severe fibrosis (F = 3) and cirrhosis (F4) were 0.89 (0.83-0.95 ; $p < 0.0001$) and 0.94 (0.90-0.97 ; $p < 0.0001$) respectively, better than the biochemical scores like FT (0.81 and 0.88), APRI (0.67 and 0.75), FIB-4 (0.70 and 0.72) and FORNS (0.65 and 0.78). The best cut-off values of LS for predicting F = 3 and F4 were respectively 10.5kPa (Se :91% Sp :67% with PPV :75% NPV : 87%) and 15.7 kPa (Se : 90% Sp :87% with PPV :82% NPV :93%). According to these cut-offs, the use of liver biopsy could have been avoided in 80% and 88% of patients. AST presented a significant positive correlation with LS levels (i.e. AST and LS concomitantly increased). However, surprisingly, diagnostic accuracy and cut-offs value were not modified by removing AST > 100UI/l conversely to previous studies. Furthermore, FS correlated significantly ($r = 0.67$; $p < 0.0001$) with the porto-systemic gradient.
Conclusion : Our results suggest that LS is currently the most reliable non-invasive indicator of severe liver fibrosis and cirrhosis in ALD. Hence this method should be recommended in the initial assessment and follow up of liver fibrosis in our ALD patients.

- A09 -

TWO MODELS OF PROGENITOR CELLS SHOW DISCREPANCIES IN PHENOTYPE AND DIFFERENTIATION PRE-DISPOSITION. N. Van Hul (1), R. Espanol Suner (1), C. Sempoux (2), C. Casteleyn (3), F. Lemaigre (4), I. Leclercq (1). (1) Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Woluwe-Saint-Lambert, Belgium ; (2) Cliniques Universitaires St Luc, Woluwe-Saint-Lambert, Belgium ; (3) Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Antwerpen, Belgium ; (4) Liver and Pancreas Development Unit, de Duve Institute, Université Catholique de Louvain, City of Brussels, Belgium.

Introduction : Liver progenitor cells (LPC) are quiescent in the healthy liver, but are activated when massive hepatocytic cell damage occurs and their replication is inhibited or overwhelmed, characteristics of chronic liver injuries.
Aim : The aim of the present work is to characterize and compare the LPC phenotype observed in two widely used models of LPC proliferation, which are considered as being equivalent and therefore used as substitutes or alternatives.
Methods : Adult C57Bl/6j wild type mice received choline-deficient diet supplemented with 0.15% ethionine (CDE) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet for 3 weeks. Histochemical analysis by markers of LPC as CK19, Osteopontin (OPN), SOX9, E-cadherin and -catenin. To follow the fate of LPC, we performed lineage tracing experiments with osteopontin (OPN)-iCreERT2 ;Rosa26RYFP mice. Transgenic mice were exposed to 3 weeks CDE or DDC diet, followed by two weeks of recovery from liver damage by returning to normal chow.
Results : The CDE diet is specifically hepatotoxic while inducing a proliferative response in the liver. These combined events activate the LPC compartment, giving rise to transit amplifying cells. Phenotypically, these cells are small, oval and elongated, a shape promoting migration, and expand from the portal tracts towards the central veins invading the whole liver parenchyma. Myofibroblast activation and extracellular matrix (ECM) deposition precedes this cell expansion, and a laminin sheet sustains those transit amplifying cells. In the DDC model (proto)porphyrin is accumulating in

the hepatobiliary system, manifesting in its progressive deposition hereby creating porphyrin plugs obstructing the bile duct(ule)s. The DDC diet gives rise to the proliferation of ductular-like cells that remain restricted to portal areas. These cells show a biliary-like shape and form bile duct-like structures with patent lumen, delineated by a thick layer of laminin. Cell tracking experiments revealed that LPC were able to generate functional hepatocytes during the recovery period from CDE exposure, but no hepatocytic differentiation was observed when recovering from DDC diet.
Conclusion : Our results delineate remarkable phenotypic discrepancies and dissimilar potential differentiation pre-dispositions exhibited by LPC arising after CDE and DDC diet. While the LPC induced during CDE diet have a more undifferentiated phenotype able to become hepatocytes, the accumulating cells observed in the DDC portal areas resemble more like dysmorphic cholangiocytes participating to the restoration of the bile duct(ule)s. We therefore emphasize the importance of, and target carefulness when, choosing appropriate experimental models.
*The authors equally contributed to this work.

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EFFICACY AND SAFETY OF TENOFOVIR IN CHRONIC HBV INFECTED PATIENTS WITH LAMIVUDINE RESISTANCE. S. Fung (1), P. Kwan (2), M. Fabri (3), A. Horban (4), M. Pelemis (5), P. Husa (6), H. Hann (7), J. Flaherty (8), B. Massetto (9), P. Dinh (10), A. Corsa (9), K. Kitrinos (8), J. Mchutchison (8), E. Gane (11), J. Piessevaux (12). (1) Toronto General Hospital, Toronto, Canada ; (2) University Of British Columbia, Vancouver, Canada ; (3) Clinic For Infectious Diseases, Novi Sad, Serbia ; (4) Medical University Of Warsaw, Warsaw, Poland ; (5) Clinic For Infectious And Tropical Diseases, Belgrade, Serbia ; (6) Faculty Hospital Brno, Brno, Czech Republic ; (7) Thomas Jefferson University, Philadelphia, United States ; (8) Gilead Sciences, Foster City, United States ; (9) Gilead Sciences, Foster City, United States ; (10) Gilead Sciences, Foster City, United States ; (11) Auckland City Hospital, Auckland, New Zealand (Aotearoa) ; (12) Gilead Sciences, City of Brussels, Belgium.

Background : Tenofovir DF (TDF) has demonstrated excellent efficacy and safety through 5 years in treatment-naïve chronic HBV patients in Studies 102/103.
Aim : Although TDF is active in vitro vs. Lamivudin Resistant (LAM-R) HBV, its efficacy has not been established in LAM-R patients in a prospective, randomized trial.
Methods : Phase 3b, double-blind, randomized (1 :1) comparison of TDF and Emtricitabin (FTC)/TDF in chronic HBV patients receiving LAM at time of screening with HBV DNA ≥ 103 copies/mL and documented LAM-R (INNO-LiPA HBV, v2/v3). Patients were stratified at entry by ALT (≥ or < 2 x ULN) and HBeAg status and assessed for efficacy and safety over 96 weeks including bone mineral density (BMD) monitoring by DXA.
Results : 280 patients were randomized and treated ; 133/141 (94%) and 125/139 (90%) in the TDF and FTC/TDF groups, respectively, completed 96 weeks. Baseline (BL) demographics included : mean age 47 years, 75% males, 34% Asians ; HBV genotypes : A 22%, B 14%, C 19%, and D 45%. Mean (SD) baseline HBV DNA was 6.5 (1.9) log10 copies/mL ; 42% had ALT ≤ ULN. Efficacy results (missing = failure) at Week 96 are shown in the table. Both treatments were well tolerated with 1% (3/280) discontinuing for an AE (1-TDF, 2-FTC/TDF). No patients had a confirmed increase in serum creatinine of ≥ 0.5 mg/dL from BL, 1% (2-TDF) had serum phosphorus < 2 mg/dL, and 3% (5-TDF, 4-FTC/TDF) had CrCL < 50 mL/min (pre-treatment CrCL range for these 9 patients : 49-61 mL/min). No clinically relevant bone loss was observed by assessment of spine and hip BMD T and Z scores, and there were no nontraumatic fractures reported. No resistance to TDF was detected through 96 weeks.
Conclusions : A high rate of HBV DNA suppression with no detectable TDF resistance was achieved with TDF in patients with documented LAM-R through 96 weeks. Similar efficacy between the mono- and combination therapy arms supports the use of TDF monotherapy in this population. TDF was safe and well tolerated, with a low rate of renal events and no evidence of clinically relevant bone loss.

	TDF (N = 141)	FTC/TDF (N = 139)
HBV DNA < 400 copies/ml	89% (126/141)	86% (120/139)
Normal ALT	70% (99/141)	70% (97/139)
Normalized ALT [a]	62% (49/79)	63% (52/83)
HBeAg loss [b]	15% (10/65)	13% (9/68)
HBeAg seroconversion [b]	11% (7/65)	10% (7/68)
HBsAg loss	0	0.7% (1/139) [c]

[a] Includes only patients with ALT > ULN at BL ; [b] HBeAg-positive patients only ; [c] No anti-HBs observed.

LIVER CELL PROLIFERATION DETERMINES MELD SCORE IMPROVEMENT IN DECOMPENSATED ALCOHOLIC LIVER DISEASE. N. Lanthier (1), L. Rubbia-Brandt (2), N. Lin-Marq (2), Y. Chalandon (3), J.L. Frossard (1), L. Spahr (1). (1) Gastroenterology and Hepatology, University Hospitals of Geneva, Geneva, Switzerland ; (2) Pathology, University Hospitals of Geneva, Geneva, Switzerland ; (3) Hematology, University Hospitals of Geneva, Geneva, Switzerland.

Introduction : The prognostic significance of liver progenitor cell (LPC) and macrophage expansion in the regeneration of decompensated alcoholic liver disease (ALD) remains ill defined.
Aim : In a well-characterized population of patients with acutely decompensated ALD (Spahr L. *et al*. Hepatology, 2011, A62), we analysed macrophage infiltration, proliferative LPC and differential expression of hepatic genes at baseline in relation to outcome at 3 months follow up.
Methods : Fifty-eight patients (MELD 20) were included. Liver biopsy at inclusion was used for (1) immunohistological analysis of proliferative LPC ($MIB1^+/CK7^+$), proliferative hepatocytes ($MIB1^+/CK7^-$ parenchymal cells), morphometric analysis of macrophage infiltration (CD68) and LPC expansion (CK7), and (2) transcriptome profiling using Affymetrix GeneChip Human arrays. Serum markers of regeneration (cytokines and growth factors) were measured by immunoassays. A $>= 3$ points decrease in MELD at 3 months as compared to baseline defined the improvers. Fifteen abstinent cirrhotics served as controls. CD68 and SPINK3 mRNA expressions were determined in various mice models of liver injury.
Results : At baseline, patients with decompensated ALD presented a significant expansion of $CD68^+$ macrophages and $CK7^+$ cells compared to abstinent cirrhotics. Patients who will improve ($n = 34$) were characterized at baseline by a higher number of $CK7^+/MIB1^+$ cells (1.9 ± 1.5 versus 0.9 ± 0.9 cells/field, $p < 0.01$), $MIB1^+$ hepatocytes (4.1 ± 3.6 versus 1.8 ± 1.4 cells/field, $p < 0.01$), an increased expansion of liver macrophages (4.4% versus 3.3% of surface area, $p < 0.05$) and a higher level of serum HGF ($p < 0.05$), compared to those who will not ($n = 24$). Transcriptome analysis revealed that the first pathways upregulated in improvers were related to cell cycle and a 7-fold increase of liver serine peptidase inhibitor Kazal type I (SPINK1) compared with non-improvers ($p = 0.005$). SPINK1 liver expression positively correlated with CD68 ($r = 0.46$) and cyclinE1 ($r = 0.6$). In mice, a 20-fold increase in liver SPINK3 expression, the homolog of human SPINK1, was evidenced following partial hepatectomy, concurrent with hepatocyte proliferation.
Conclusion : Baseline markers of liver macrophages and liver cell proliferation predict the clinical outcome in decompensated ALD. This study reveals an unexpected implication of SPINK1, an acute phase reactant, in liver regeneration and human ALD.

NON-INFERIORITY OF Q12H TELAPREVIR VERSUS Q8H IN TREATMENT-NAÏVE, HCV GENOTYPE 1-INFECTED PATIENTS. Yves Horsmanns (1), Maria Buti (2), Kosh Agarwal (3), William Sievert (4), Ewa Janczewska (5), Stefan Zeuzem (6), Lisa Nyberg (7), Robert Brown (8), Christophe Hezode (9), Mario Rizzetto (10), Raymundo Parana (11), Sandra De Meyer (12), Donghan Luo (13), James Witek (13). (1) Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium ; (2) Hospital Valle Hebron and Ciberehd Del Institut Carlos III, Barcelona, Spain ; (3) Kings College Hospital, London, Uk ; (4) Monash Medical Centre And Monash University, Melbourne, Australia ; (5) Outpatients Clinic For Hepatology, Myslowice, Poland ; (6) Johann Wolfgang Goethe University Medical Center, Frankfurt Am Main, Germany ; (7) Kaiser Permanente, San Diego, CA, USA ; (8) Columbia University College of Physicians and Surgeons, New York, Ny, USA ; (9) Hôpital Henri Mondor, Créteil, France ; (10) University of Torino, Torino, Italy ; (11) Medical School, Federal University of Bahia, Bahia, Brazil ; (12) Janssen Infectious Diseases Bvba, Beerse, Belgium ; (13) Janssen Research & Development LLC, Titusville, NJ, USA.

Background : The ADVANCE study showed that telaprevir (TVR, T) dosed every 8 hours (q8h) combined with peginterferon alfa-2a (P) and ribavirin (R) had superior efficacy to PR alone, while the C208 study showed similar efficacy with TVR dosed every 12 hours. Here we report the primary endpoint of OPTIMIZE, a Phase III, randomized, open-label, international, non-inferiority study comparing twice daily (bid) versus q8h TVR administration (NCT01241760).
Methods : Treatment-naive patients with genotype 1 (G1) HCV infection were randomized to 750 mg q8h or 1125 mg bid TVR plus P 180 μg/week and R 1000 or 1200 mg/day for 12 weeks, then PR alone for 12 weeks if Week 4 HCV RNA was '< 25 IU/mL, target not detected' or 36 weeks if detectable. Randomization was stratified by fibrosis stage and *IL28B* genotype. The primary endpoint was $SVR12_{planned}$, defined as HCV RNA < 25 IU/mL 12 weeks after the last planned dose of PR. The pre-specified non-inferiority margin was –11%. Secondary objectives included additional efficacy outcomes, safety and tolerability.

Results : 744 patients were randomized and 740 treated. 60% of patients were male, 92% were Caucasian, 14% had cirrhosis, 85% had baseline HCV RNA ≥ 800,000 IU/mL, 57% had G1a, and 29% had IL28B CC. Outcomes were similar between treatment arms [Table]. Non-inferiority was demonstrated for the TVR bid vs q8h regimen : difference 1.5% (95% CI : -4.9%, 12.0%). RVR was 69.4% for TVR bid vs 67.4% for q8h.

Treatment outcome, n/n (%)		T12(q8h)/PR (n = 371)	T12(bid)/PR (n = 369)
SVR12		270/371 (72.8%)	274/369 (74.3%)
SVR12 by fibrosis stage	No, minimal or portal fibrosis Bridging fibrosis or cirrhosis	209/268 (78.0%) 61/103 (59.2%)	213/264 (80.7%) 61/105 (58.1%)
SVR12 by *IL28B* genotype	CC CT TT	92/106 (86.8%) 141/208 (67.8%) 37/57 (64.9%)	97/105 (92.4%) 139/206 (67.5%) 38/58 (65.5%)
On-treatment virologic failure*		36/371 (9.7%)	38/369 (10.3%)
Relapse**		19/293 (6.5%)	23/300 (7.7%)

*Meeting virologic stopping rules or having virologic breakthrough.
**Assessed in patients with HCV RNA < 25 IU/mL at the planned end of treatment.

The adverse event (AE) profile was generally similar between arms. The most frequent AEs by preferred term during the TVR treatment phase were fatigue (47.3%), pruritus (42.7%), anemia (41.6%), nausea (36.5%) and rash (35.3%). AEs that most frequently led to discontinuation of TVR were rash (5.3%), anemia (4.6%), pruritus (2.6%), fatigue (1.2%), and rash, maculopapular (1.1%). Any SAE was reported in 8.5% in the TVR phase.
Conclusions : The efficacy of 1125 mg bid TVR was non-inferior to 750 mg q8h offering simplified dosing to treatment-naïve G1 HCV-infected patients. In this study with a robust representation of cirrhotic patients, safety and tolerability were generally similar between regimens and consistent with the known profile of TVR.

ENDOPLASMIC RETICULUM STRESS INHIBITION REDUCES STEATOSIS BUT NOT GLUCOSE INTOLERANCE IN FOZ MICE. V. Legry (1), B. Lambert (2), L. Poekes (1), G. Farrell (3), I. Leclercq (1). (1) Université Catholique de Louvain, City of Brussels, Belgium ; (2) Université Catholique de Louvain, Brussels, Belgium ; (3) Anu Medical School At The Canberra Hospital, Canberra, Australia.

Introduction : Non-alcoholic fatty liver disease is associated with obesity and insulin resistance, but the mechanisms linking those features remain elusive. Recently, endoplasmic reticulum (ER) stress has been suggested as a link between nutrient overload, increased lipogenesis and insulin resistance. However, results appear controversial depending on the model used.
Aim : We therefore compared ER stress and response to ER stress inhibitor tauro-ursodeoxycholic acid (TUDCA) in 2 models of obesity, insulin resistance and steatosis : the leptin-deficient *ob*/*ob* mice and high fat diet-fed *Alms1*-/- mice (the *foz*/*foz* mice).
Methods : Male *foz*/*foz* mice under high fat diet (for 6 weeks as from weaning) and *ob*/*ob* mice under normal diet (10 weeks of age) received for the last 2 weeks TUDCA (500 mg/kg/day, IP). As TUDCA decreased food intake in both strains of mice, we used pair-fed mice (which received the same amount of food that the TUDCA-treated mice as controls). To evaluate glycaemic homeostasis, we performed a glucose tolerance test after 13 days of treatment and quantified plasma glucose and C-peptide levels to calculate the HOMA insulin resistance index. Liver histology, hepatic lipid content, lipogenesis and ER stress markers were investigated.
Results : In *ob*/*ob* mice, in which lipogenesis is greatly enhanced, ER stress has been clearly described. Conversely, we could not find convincing evidence of ER stress in *foz*/*foz* mice despite moderately increased lipogenesis compared to their wild-type littermates. In *ob*/*ob* mice, TUDCA improved glucose tolerance and insulin sensitivity, compared to the pair-fed control group. Although liver lipid content and steatosis were not significantly reduced, *ob*/*ob* mice had a lower liver weight. Moreover, expression of ER stress response and lipogenesis genes was reduced (decreased expression of GRP78, ATF4 and SREBP1c, FAS, respectively, $p < 0.05$). On the contrary, TUDCA treatment failed to improve glucose tolerance or HOMA-IR level in *foz*/*foz* mice. Also, it did not reduce ER stress markers. However, TUDCA reduced hepatic steatosis on histological findings, hepatic lipid content ($p = 0.05$) and lipogenesis genes expression (SREBP1c, FAS, $p < 0.05$) in *foz*/*foz* mice compared to the pair-fed group.

Conclusion : To sum up these results, alleviating ER stress in a mouse model of simple steatosis (*ob*/*ob*) prevents the development of insulin resistance. On the contrary, ER stress does not seem to be involved in *foz*/*foz* mice, in which NASH will eventually ensue. These results suggest that the link described between steatosis, ER stress and insulin resistance is likely to be aetiology-specific.

Invited Lecture BASL-BLIC Spring Meeting Lecture

- A14 -

THE CONCEPT OF PROGENITOR/STEM CELLS IN LIVER DISEASE IN 2012. L. Van Grunsven / VUB Brussels.

- A15 -

BOCEPREVIR-BASED TRITHERAPY OF HCV-INFECTED BELGIAN LIVER TRANSPLANTED PATIENTS : PRELIMINARY RESULTS. D. Degre (1), I. Colle (2), H. Van Vlierberghe (2), C. Moreno (1). (1) Erasme Hospital, Brussels, Belgium, (2) Ghent University Hospital, Ghent, Belgium.

Introduction : Management of recurrent hepatitis C infection remains a major challenge after liver transplantation. Protease-inhibitor based regimens improve virological response in non-transplanted HCV genotype 1 patients.
Aim : We report here the Belgian experience of Boceprevir-based tritherapy after liver transplantation.
Methods : Safety and efficacy of Boceprevir treatment in combination with PegIFN+ribavarin were investigated in 7 HCV genotype 1 liver transplanted patients (3/7 men with a mean of age 53+/-10.4 years), with recurrent chronic HCV. Two patients had cholestatic hepatitis, 5 had chronic hepatitis with F1 (n = 1), F2 (n = 1), F3 (n = 1) and F4 (n = 2) fibrosis stage according to the METAVIR score.
Results : The median time between LT and HCV treatment initiation was 249 days [47-2411].Lead-in period was not the same in all patients with a median time of 7 weeks [4-26 weeks]. The median follow-up period was 33 [15.86-54.43] weeks. All patients had achieved a steady state of immunosuppressive drugs before the initiation of Boceprevir : 3 patients had tacrolimus and mycophenolate mofetil, 1 had cyclosporine, 1 had cyclosporine, mycophenolate mofetil and corticoid and 1 had everolimus and mycophenolate mofetil. Because of drugs interaction, immunosuppressant treatment was reduced in all but one patient. Cyclosporine and Tacrolimus doses were reduced by 1.9 and 3.77, respectively. Anemia occurred in all patients requiring ribavirin doses reduction in all patients, blood transfusion in 6 patients, erythropoietin administration in 4 patients and boceprevir cessation in one patient after 8 weeks of tritherapy. Median HCV viral load at the beginning of HCV treatment and at the time of boceprevir initiation were respectively 7.54 10^6 [1.07 10^6 – 7 10^7] and 5.85 10^5 [2.59 10^4 -5.5 10^7]IU/ml. After 8 weeks of tritherapy, 5 patients had HCV viral load below 30 IU/ml and remained negative till end of follow-up, 1 patient had 402 IU/ml and relapsed and 1 had stopped treatment because of severe anemia and thrombocytopenia at W4.
Conclusion : These results confirm the feasibility of boceprevir ? based tritherapy in liver transplanted patients. Efficacy results are promising but a close monitoring of immunosuppressive drug levels and of hematological side effects is required.

- A16 -

HIGH FAT INDUCED INSULIN RESISTANCE : THE EMERGING ROLE OF HEPATOKINES. N. Lanthier, V. Lebrun, O. Molendi-Coste, I.A. Leclercq. Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Woluwe-Saint-Lambert, Belgium.

Introduction : A link between serum hepatokine levels (proteins produced by the liver and acting distantly) and insulin resistance in type 2 diabetes has been recently suggested.
Aim : Here, we wanted to explore the liver expression of several hepatokines at the initiation of a high fat diet in mice, as well as their relation with liver macrophage (Kupffer cell) activation.
Methods : Male mice of 5 weeks of age were fed a normal diet (ND) or a high fat diet (HFD) for 3 days, known to induce steatosis and insulin resistance. A preventive Kupffer cell (KC) depletion was obtained by intravenous injection of clodronate loaded liposomes and compared with PBS liposomes. The mRNA expression of hepatokines (selenoprotein-P, fetuin-A, fibroblast growth factor 21, angiopoietin-like protein 3) was evaluated by RT-PCR on liver tissue.
Results : Short term HFD induced steatosis, KC activation and insulin resistance with a significant increased expression of selenoprotein P (1.6 fold, $p < 0.001$), fetuin-A (1.7 fold, $p < 0.01$) and fibroblast growth factor 21 (9 fold, $p < 0.01$).

However, the liver expression of angiopoietin-like protein 3 remains unchanged under short term HFD. Kupffer cell depletion in this setting did not reduce hepatic steatosis but significantly ameliorated insulin sensitivity proved by clamp studies. This amelioration in insulin sensitivity in KC-depleted mice was associated with a significant decrease in fetuin A mRNA expression (0.7 fold, $p < 0.001$) compared to animals with KC. Interestingly, while selectively depleting liver macrophages without affecting adipose tissue macrophage infiltration, intravenous clodronate injection was associated with a significant reduction in epididymal adipose tissue expansion compared to PBS injection (1.1% of body weight versus 1.6% of body weight, $p < 0.001$), suggesting a role of liver-derived products in peripheral tissue alterations associated with obesity.
Conclusion : We demonstrate liver hepatokine overexpression at the initiation of HFD feeding, concurrent with hepatic steatosis and insulin resistance. Targeting KC in this setting improved insulin sensitivity and was associated with a decreased adiposity and a reduced liver fetuin A expression. The impact of this hepatokine on adipose tissue metabolism needs to be confirmed and the search for pathogenic liver-derived factors in obesity associated disorders intensified.

- A17 -

REPLICATION AND SUSCEPTIBILITY TO TELAPREVIR OF T54S VARIANT IS MODULATED BY OTHERS HCV NS3 MUTATION. S. Ortiou (1), S. Bontems (2), J.Y. Servais (1), D. Perez-Bercoff (1), J. Fritz (3), D. Vaira (2), J. Delwaide (4), V. Arendt (5), T. Staub (5), J.C. Schmit (1), C. Devaux (1). (1) Laboratory of Retrovirology, CRP-Santé, Luxembourg, Luxembourg ; (2) Aids Reference Laboratory of Liège, University of Liège, Liège, Belgium ; (3) Luxembourg Centre for Systems Biomedicine, University Of Luxembourg, Luxembourg, Luxembourg ; (4) Department of Gastroenterology and Hepatology, University Hospital of Liège, Liège, Belgium ; (5) National Service of Infectious Diseases, CHL, Luxembourg, Luxembourg.

Introduction : NS3 inhibitors are currently used for clinical treatment of genotype 1 (G1) HCV-infected patients. Although pre-existing resistant variants have been reported they are not necessarily associated with viral failure suggesting a role for other mutations.
Aim : In this study, we have investigated the frequency of naturally occurring NS3 substitutions and resistance mutations as well as their effect on susceptibility to NS3 inhibitors, replication capacity (RC) and infectivity in HCV cell culture.
Methods : NS3 sequencing was performed on 150 G1a and 58 G1b-infected, NS3 inhibitor na ?ve patients from Luxembourg and Liège, Belgium. Mutations were introduced by PCR in Jc1 FLAG2 p7-nsGluc2aUbi-NS3 plasmid. RNA was transfected into Huh7D cells, EC50 in presence of telaprevir (TVR) was determined 72h after electroporation as well as replication capacity. Infectivity assays were further performed with serial dilutions of viral supernatant based on TCID50 (MOI 0.5-0.031).
Results : At least one low/medium level resistance mutation was detected in 9% of G1a (V36M/L, T54S) and 3% of G1b patients (T54S, D168E). In G1a sequences T54S was also associated with V55A and V55I mutants. Few compensatory mutations were detected but not in combination with resistant variants. High amino acid entropy was observed in G1a (10% K68R, N174S/G 40%) and in G1b sequences (36% V48I, 33% Y56F, 20% V132I). The Y56F substitution associated with absence of the turn structure at the catalytic triad amino-acid H57 was found in combination with the T54S mutation. Using Jc1, the T54S mutant showed a 5 -fold reduction in susceptibility to TVR and a similar RC than wt Jc1 but was poorly infectious. Combination with V55A did not change susceptibility to TVR but abolished RC (T54S = 157 ± 72%, T54S+V55A = 3 ± 2%) whereas the combination of V55I mutation had no effect. Although the Y56F variant exhibited a similar susceptibility to TVR, RC and infectivity as wt Jc1, combination with the T54S mutation but not with T54A or R155K significantly decreased EC50 value (T54S = 1162 ± 511nM vs T54S+Y56F = 327 ± 178 nM)..
Conclusion : A significant proportion of mutations conferring resistance to NS3 inhibitors were observed in NS3 inhibitor naive patients. Replication capacity of the T54S mutant was strongly reduced by the V55A mutation whereas its susceptibility to TVR was improved by the Y56F polymorphism. Our data substantiate the role of natural polymorphisms in the modulation of resistance to NS3 inhibitors.

- A18 -

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

Introduction : Peroxisome proliferator-activated receptors (PPARs) have been implicated in the pathogenesis of NASH, merely based on animal models. Data on gene expression in liver tissue of NASH patients are scarce.
Aim : To study PPAR a, b/d and g expression in liver tissue of a large cohort of obese patients assessed for the presence of NAFLD.
Methods : Patients presenting to the obesity clinic underwent a thorough metabolic and hepatic work-up. If NAFLD was suspected, a liver biopsy was performed. Gene expression was studied by mRNA quantification (real time RT-PCR). Liver histology was scored using the Brunt definition and the NASH CRN Scoring System.
Results : 125 patients were consecutively included (mean age 45.0 ± 12.4 y, mean BMI 38.7 ± 6.67 kg/m2). Liver PPARa expression negatively correlated with the presence of NASH according to Brunt *et al* ($p = 0.001$) and with the severity of steatosis ($p = 0.003$), inflammation ($p = 0.001$), the NASH activity score ($p = 0.008$), and fibrosis ($p = 0.003$). PPAR b/d and PPARg expressions did not correlate with any of the histological features. PPARa 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, $p = 0.012$) but not to PNPLA3 polymorphism. Liver PPARg expression did not correlate with parameters of glucose metabolism or serum lipids.
Conclusion : Human liver PPARa expression decrease significantly correlates with NASH severity. It also consistently correlates with visceral adiposity, insulin resistance and adiponectin decrease, all suggesting that PPARa is a potential therapeutic target for NASH treatment. Liver PPARg expression does not seem to correlate with disease severity.

- A19 -

EMBOLIZATION OF LARGE PORTOSYSTEMIC SHUNTS FOR REFRACTORY HEPATIC ENCEPHALOPATHY : A EUROPEAN SURVEY. W. Laleman (1), M. Simon-Talero (2), G. Maleux (3), M. Perez (2), K. Ameloot (1), G. Soriano (4), J. Villalba (4), J.C. Garcia-Pagan (5), M. Barrufet (6), R. Jalan (7), J. Brookes (8), E. Thalassinos (9), A.K. Burroughs (9), J. Cordoba (2), F. Nevens (1). (1) Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (2) Department of Hepatology, Hospital Vall D'hebron, Universitat Autònoma De Barcelona, Barcelona, Spain ; (3) Interventional Radiology, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (4) Hospital De La Santa Creu I Sant Pau And Centro De Investigación Biomédica En Red De Enfermedades Hepáticas Y Digestivas (Ciberehd), Instituto De Salud Carlos Iii, Barcelona, Spain ; (5) Hepatic Hemodynamic Laboratory, Institut D'investigacions Biomèdiques August Pi I Sunyer And Centro De Investigación Biomédica en Red De Enfermedades Hepáticas Y Digestiva, And 8vascular And Interventional Unit, Hospital Clinic, Barcelona, Spain ; (6) Vascular And Interventional Unit, Hospital Clinic, Barcelona.

Introduction : Refractory hepatic encephalopathy (HE) remains a major cause of morbidity in cirrhotic patients. Large spontaneous portosystemic shunts (SPSSs) have been previously suggested to sustain HE in these patients.
Aim : We aimed to retrospectively assess the feasibility, efficacy and safety of patients treated with embolization of large SPSSs for the treatment of chronic therapy-refractory HE in a European multicentric working group and to identify patients that may benefit from this procedure.
Methods : Between July 1998 and January 2012, 37 patients (Child A6-C13, MELD 5-28) with refractory HE were diagnosed with single large SPSSs which were considered eligible for embolization in 6 different European liver units. The data-sets of of these 37 patients whose data were collated into a preset standardized case-report form.
Results : On a short-term basis (i.e. within 100 days after embolization), 22 out of 37 patients (59.4%) were free of HE ($P < 0.001$ vs before embolization) of which 18 (48.6% of patients overall) remained HE-free over a mean period of follow-up of 697 ± 157 days ($P < 0.001$ vs before embolization). Overall, we noted improved autonomy, decreased number of hospitalizations or severity of the worst HE episode after embolization in three quarters of the patients. Logistic regression identified the MELD-score as strongest positive predictive factor of HE recurrence with a cut-off of 11 for patient selection. As to safety, we noted 1 major non-lethal procedure-related complication. There was no significant increase in de novo development or aggravation of preexisting varices, portal hypertensive gastropathy or ascites.
Conclusion : This multicenter European cohort study demonstrated a role for large SPSSs in chronic protracted or recurrent HE and substantiated the effectiveness and safety of embolization of these shunts provided there is sufficient functional liver reserve.

- A20 -

(GT)N REPEAT GENETIC VARIANT IN THE PROMOTER OF HO-1 IS NOT ASSOCIATED TO ALCOHOLIC LIVER DISEASE. A. Lemaire, E. Trepo, R. Ouziel, T. Gustot, C. Moreno, D. Degré, C. Minsart, V. De Wilde, O. Le Moine, J. Devière, M. Abramowicz, A. Le Moine, A. Lemmers. ULB Faculty Of Medicine, Anderlecht, Belgium.

Introduction : Alcoholic hepatitis (AH) is observed in 10% of heavy drinkers and represents the most serious forms of alcoholic liver disease (ALD). Induction of heme oxygenase-1 (HO-1) was shown to prevent liver fibrosis and ethanol induced oxidative stress-related damage in mice models of chronic ethanol feeding. A functional (GT)n repeat variant in HO-1 promoter region is tightly correlated with HO-1 protein expression inducibility (i.e. short (< 26) (GT)n repeat carriers present increased HO-1 expression derived anti-inflammatory and cytoprotective effects).
Aim : We have conducted a genetic association study on HO-1 promoter (GT)n repeat variants in ALD.
Methods : The DNA from 487 biopsy-proven ALD patients (comprising 383 cirrhosis and 193 AH) and 203 healthy controls was harvested for microsatellite analysis using PCR amplification of the promoter region with fluorescently labelled primers followed by fragment length analysis with a laser based automated DNA sequencer. The same cohort was previously validated for another genetic polymorphism study (rs738409 C > G PNPLA3/adiponutrin, demonstrated to be associated with increased risk of ALD and alcoholic cirrhosis).
Results : Analysis of allelic frequency distribution disclosed two peaks at 23 and 30 (GT)n repeats in controls as well as in ALD patients. For genotypic frequencies analysis, only the extreme (short (< 26) and long (> 29)) (GT)n repeat values were studied. The distribution of homozygote long (GT)n profiles (LL) of controls was not different from that of cirrhotic patients and that of patients with AH (OR = 0.88 (0.60-1.29) and OR = 0.77 (0.49-1.21) respectively). The proportion of LL genotype was not significantly higher in patients with alcoholic cirrhosis and AH than in those without AH (OR = 0.76 (0.47-1.24)). Among cirrhotic patients, the length of the (GT)n repeat variant was not correlated to the MELD and Child Pugh scores ($r = -0.07$ $p = 0.23$ and $r = -0.06$ $p = 0.3$ respectively). Among AH patients, the length of the (GT)n repeat variant was not correlated to the Maddrey score ($r = -0.06$ $p = 0.48$).
Conclusion : In a representative European Caucasian cohort of ALD patients, genetic (GT)n repeat variants in the promoter of HO-1 are not associated to the presence of the disease or to its severity.

- A21 -

SARCOPENIA IN SYMPTOMATIC PCLD PATIENTS : A MORE OBJECTIVE PARAMETER FOR LIVER ALLOCATION ? F. Temmerman (1), J. Pirenne (2), R. Vanslembrouck (3), W. Coudyzer (3), D. Monbaliu (2), R. Aerts (2), W. Laleman (1), D. Cassiman (1), C. Verslype (1), S. Van Der Merwe (1), W. Van Steenbergen (1), J. Van Pelt (4), J. Drenth (5), F. Nevens (1). (1) UZ Leuven, Leuven, Belgium ; (2) Abdominal Transplant Surgery, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (3) Radiology, UZ Leuven, Leuven, Belgium ; (4) University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (5) UMC St. Radboud, Nijmegen, Netherlands.

Introduction : Invalidating polycystic liver disease (PCLD) with extreme hepatomegaly can be an indication for liver transplantation (LT).In Eurotransplant, a decreased mid-upper arm circumference (MUAC) is used to give symptomatic pts priority on the waiting list (female : < 23.1 ; men : < 23.8cm). This is based on the assumption that decreased MUAC reflects malnutrition but this has never been validated in PLD.
Aim : To study the prevalence of malnutrition assessed by sarcopenia (depletion of skeletal muscle) in symptomatic. PCLD.
Methods : Liver volume (LV) ; skeletal muscle area (SMA) and subcutaneous adipose tissue (SAT) at vertebra L3 were measured by single CT-scanning. Skeletal muscle area was corrected for stature (SMA-index). Sarcopenia was defined as SMA-index < $38.5 cm^2/h^2$ (female) ; < $52.4 cm^2/h^2$ (men).LV was corrected for body surface area (LV-index).Lean body mass was estimated from skeletal muscle area.
Results : Studygroup (n = 43) :39 women ; mean age of 51y. Sarcopenia was present in 42% ;decreased MUAC in 37%. Pts were divided in 4 groups (Table) : A/decreased MUAC and sarcopenia (n = 8) ; B/decreased MUAC and no sarcopenia (n = 8) ; C/no decreased MUAC with sarcopenia (n = 7) ; D/no decreased MUAC and no sarcopenia. There was no difference regarding age & creatinine clearance. In group A, pts had larger liver volumes than group C ($p < 0.05$). Group C had normal MUAC but more subcutanous fat ($p < 0.05$). The LV-index in group A was higher than in group C and D ($p < 0.05$). In group D, pts had more lean body mass and subcutaneous fat *vs* the other groups ($p < 0.05$). MUAC was well correlated with subcutaneous fat ($r = 0.759$, $P < 0.0001$) but less with lean body mass ($r = 0.393$, $p = 0.009$). Pts were then divided in group LT (n = 20) vs no LT (n = 23).12 pts of group LT had no decreased MUAC, however 25% was sarcopenic.
Conclusion : Sarcopenia is highly prevalent is pts with symptomatic PCLD (42%). MUAC is more strongly correlated with subcutaneous fat rather than lean body mass ; as such a subgroup with sarcopenia despite normal MUAC exists. The SMA and LV indexes are objective parameters to asses malnutrition in PCLD pts. These parameters should also be taken into consideration in the allocation for LT.

- A22 -

IMPACT OF INVASIVE ASPERGILLOSIS ON SHORT-TERM MORTALITY IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS. T. Gustot, E. Maillart, M. Bocci, R. Surin, J. Schreiber, V. Lucidi, D. Degre, V. Donckier, F. Jacobs, C. Moreno. Erasme Hospital, Brussels, Belgium.

Introduction : Alcoholic hepatitis (AH), in its severe form, is a lethal disease in the short-term. Although infections are frequent complications of AH, the incidence of invasive aspergillosis (IA), and its impact on short-term survival remain unknown.
Methods : We retrospectively analyzed 82 patients prospectively followed for biopsy-proven severe AH (modified Discriminant Function (mDF) > 32) from June 2006 to December 2011 with a follow-up of 3 months after biopsy. AH were treated in 58 patients with corticosteroids, 4 with corticosteroids and pentoxiffyline (PTX), 1 with PTX alone and 20 did not received specific treatment. Demographic, bacteriological and therapeutic data were collected. The diagnosis of IA was based on the revised criteria of EORTC/Mycoses Study Group and the AspICU except for host factors.
Results : Fourty cases of IA classified as proven (n = 5), probable (n = 8) or possible (n = 1) were diagnosed (17%) after a median delay of 34 [0-79] days after AH diagnosis. The sites of infection were the lungs (n = 10) and the central nervous system (n = 2) and was disseminated in 2. *Aspergillus fumigatus* was isolated in 10 cases (71% of IA) : 5 in bronchoalveolar lavage (BAL), 4 in bronchial secretions and 1 in a brain biopsy. Diagnosis of other IA was based on radiological signs and galactomannan detection. Patients with IA were younger, had higher total bilirubine, creatinine and Prothrombin Time at day 28 ($p < .01$) and were more frequently admitted in ICU. 12 patients with IA received corticosteroids but 2 did not receive any treatment for AH. The occurrence of IA was similar in non-responders to corticosteroids vs responders as defined by the Lille score. The 3 month-mortality was higher in patients with IA than without IA (93 vs. 50%, $p < .01$). Multivariate logistic regression analysis showed that age = 53 y, a Lille score = 0.45 and the presence of IA were associated with a higher risk of mortality at 3 months.
Conclusion : IA is a frequent complication of corticosteroid-treated severe AH, carrying a high risk of mortality. Systematic screening for IA should be recommended in these patients while further studies are needed to identify high risk population requiring antifungal prophylactic treatments.

Invited Lecture : **Marc Hautekeete Lecture**

- A23 -

LIVER DISEASE ASSOCIATED WITH IBD : WHAT SHOULD THE GASTROENTEROLOGIST KNOW ? K. Muri Boberg. Oslo, Norway.

- A24 -

PREVALENCE OF ADRENAL INSUFFICIENCY IN CIRRHOSIS AND ITS EFFECT ON SURVIVAL. A. Matin, R. Chawlani, A. Kumar, A. Arora. Sir Ganga Ram Hospital, New Delhi, India.

Introduction : Although adrenal insufficiency (AI, hepato-adrenal-syndrome) is well known fact in cirrhotic patients but factors associated with it and its effect on mortality is still not clear.
Aim : We investigated AI in patients with cirrhosis without hemodynamic instability, and studied its influence on short-term mortality.
Methods : In consecutive cirrhotic patients AI was defined by a total serum cortisol (TC) < 18 μg/dl, 60-min after 250 μg synacthan injection and/or when delta-fraction (post-synacthan cortisol–basal-cortisol) was < 9 μg/dl.
Results : 143 patients were included (median age 50 years [range 27-73], males 86.71%). They were divided into two groups according to presence or absence of AI. The median CTP and MELD-scores were 11(range5-15) and 21 (range 6-57). The etiology of cirrhosis was alcohol (53.8%), cryptogenic (26.5%), viral (16.7%), and others (3%). 83 (58%) patients had AI and rest 60 (42%) had normal adrenal function. Serum bilirubin ($p = 0.022$), ALT ($p = 0.043$), AST ($p = 0.038$), ALP ($p = 0.036$), were significantly higher in AI group and sodium ($p = 0.032$), total cholesterol ($p = 0.011$), HDL ($p = 0.001$) and LDL ($p = 0.002$) were significantly lower in AI group. CTP score ($p = 0.938$), MELD score ($p = 0.111$) and basal cortisol ($p = 0.320$) were not different between those with and without AI. On 120-day follow-up, 53 patients died. Thus, the 120-day transplant-free survival was 63%, and this was higher in patients without AI than in patients with AI (78.3% vs 51.8% ; $p = 0.001$). On multi variant analysis presence of AI, TLC and MELD independently predicted 120-day mortality.
Conclusion : AI is present in more than half of cirrhotic patients but does not parallel the severity scores of cirrhosis. Its presence predicts early mortality in these patients, and this prediction is independent of CTP or MELD scores.

- A25 -

GLYCOMICS AS A NEW TOOL FOR PRESERVATION FLUID ANALYSIS IN LIVER TRANSPLANTATION. X. Verhelst (1), B. Blomme (1), A. Geerts (1), I. Colle (1), R. Troisi (1), X. Rogiers (1), N. Callewaert (2), H. Vanvlierberghe (1). (1) Universitair Ziekenhuis Gent, Gent, Belgium ; (2) VIB, Gent, Belgium.

Introduction : N-glycan analysis of serum has proven to be useful in the diagnosis of chronic liver diseases. However, N-glycan analysis of preservation fluid in liver transplantation has not been reported before. Considering that hepatocytes play a major role in protein glycosylation, changes in glycoproteins produced by hepatocytes might reflect alterations in liver graft quality.
Aim : The aim of this pilot study was to explore the feasibility of N-glycan analysis of preservation fluid presuming that this might be an interesting path towards the development of tools for organ quality monitoring.
Methods : In this single center pilot study eleven consecutive adult liver transplant recipients were included between 1th of December 2011 and 1th of April 2012. A sample of preservation fluid (University of Wisconsin solution or histidine-tryptophan-ketoglutarate) was collected after flushing of the right liver vein at the moment of engraftment of the liver and immediately frozen to -20 ?C. N-glycans present in glycoproteins in preservation fluid were desialyated and analyzed by deoxyribonucleic acid sequencer-assisted flurophore-assisted capillary electrophoresis (DSA-FACE) technology.
Results :
1/ Glycoproteins are present in preservation fluid of liver transplant patients after flushing of the right liver vein before engraftment.
2/ N-glycan analysis present in preservation fluid showed the presence of 13 peaks, exactly as we previously observed in human serum. N-glycomic profiles of preservation fluid show a clear concordance between the 11 patients. The most notable observation is a low peak 1 height, representing an agalacto, core-a-1,6-fucosylated biantennary (NGA2F) glycan and a high peak 5 value representing bigalacto, biantennary (NA2) glycan. This pattern demonstrates striking similarities with human serum of healthy volunteers. Both HTK and UW showed similar electropherograms.
Conclusion : N-glycans can be found and analyzed in preservation fluid of liver transplant recipients using DSA-FACE technology. Electropherograms of preservation fluid show an important similarity with electropherograms of serum of healthy human volunteers. These findings for the first time prove the feasibility of this technique in preservation fluid.

- A26 -

HCV SCREENING IN AFRICAN (MALIAN) WOMEN : RELEVANCY OF THE NS3 EPITOPE. N. Bouare (1), D. Vaira (1), A. Gothot (1), J. Delwaide (1), S. Bontems (1), L. Seidel (1), P. Gerard (2), C. Gerard (1). (1) Chu Sart Tilman, Liège, Belgium ; (2) University Of Liege, Liège, Belgium.

Introduction : Samples reacting only with HCV-NS3 epitope must be classified as “indeterminate” according to the manufacturer’s instructions. However, they also may be predictive of seroconversion. Reasonably, in such cases, it is recommended to test a sample of the same patient drawn a few weeks later (INNO-LIA™ HCV Score B30068 v4 2010-12-23 p 1/12).
Aim : Our study aims to evaluate the positive predictive value of the NS3 HCV epitope of the LIA-HCV confirmation method in 2 sub-populations of Malian women.
Methods : Two prospective studies were held in Bamako (Malian capital). They concerned 1000 pregnant women selected between May 26th and Jun 16th 2009 in six reference health centers and 231 women > 50 years old who frequented the general practitioners in two hospitals between October 25 and December 24th 2010. Blood collection and samples preparation/storage were performed in good conditions. HCV screening was performed by using Monolisa Ab/Ag Ultra and Innotest Ab IV. HCV-LIA was used as confirmation test. PCR HCV-RNA analysis and LiPA Genotyping Assay were performed.
Results : Among 17 HCV-LIA positive profiles, NS3, C1 and C2 were clearly predominant (94.1%, 94.1% and 88.2%). There was an obvious association between the intensity of the NS3 HCV band and HCV viraemia and this association was highly significant when the NS3 intensity band was > = 3 ($P < 0.001$) and then between HCV viraemia and the co-existence of HCV C1/NS3 bands when the band intensity was > 2 ($P < 0.01$). One sample with a TR > 5 with both HCV EIA tests exhibited an isolated NS3 band (4+) was concluded as “indeterminate” according to the manufacturers’ recommendations but was however found PCR(+).
Conclusion : These results indicate that intense reactivity on the NS3 epitope of the HCV-LIA is highly predictive of HCV viraemia ; this also support the hypothesis that reactivity of isolated NS3 band intensity > = ? 0.5 may be indicative of HCV seroconversion.

- A27 -

DISTINCT METABOLIC PROFILE AND IMPAIRED HISTOLOGICAL RESPONSE TO WEIGHT LOSS IN ANA+ OBESE PATIENTS. L. Vonghia (1), A. Verrijken (2), L. Van Gaal (2), E. Van Marck (3), 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) Departement of Endocrinology, Metabolism And Clinical Nutrition, University Hospital Antwerp, Antwerpen, Belgium ; (3) Department of Pathology, Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Obesity is often accompanied by several comorbidities including metabolic disturbances, as insuline resistance, and a wide spectrum of liver damage, ranging from NAFLD, NASH and cirrhosis and altered immune function. Autoantibodies as antinuclear (ANA) and anti smooth muscle (ASMA) antibodies can be expressed in various clinical settings including liver diseases.
Aim : To evaluate the clinical significance of the expression of ANA and ASMA in the baseline assessment and follow up of obese patients.
Methods : 522 obese patients were consecutively enrolled, with a one year follow up in 166 patients. Serum tests, including ANA and ASMA, glucose, insulin and c-peptide levelus during oral glucose tolerance test (OGTT), ultrasound and fat measurement at CT scan were performed at baseline and at 12 months follow up. A liver biopsy was performed in 314 patients at baseline and in 86 at follow up.
Conclusion : In our obese population, the positivity to ANA seams to identify a subset of patients with a lower glucose metabolism impairment at baseline and a, probably related, impaired improvement of glucose metabolism, visceral fat and histological features of NAFLD/NASH at follow up. In this population ANA+ seems to suggest a distinct pathogenetic profile.

- A28 -

CHANGES IN ELASTOGRAPHY AS PREDICTOR OF OUTCOME IN PATIENTS WITH COMPENSATED ADVANCED LIVER DISEASE. M. Esmat Gamil, D. Cassiman, W. Laleman, C. Verslype, S. Van Der Merwe, W. Van Steenbergen, J. Van Pelt, F. Nevens. Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

Introduction : Liver fibrosis and its progression to cirrhosis result in significant morbidity and mortality in patients with chronic liver disease. In recent years great interest has been generated in the non-invasive assessment of fibrosis thereby identifying patient populations at risk of progressing to cirrhosis. Liver stiffness measured with transient elastography (TE) has been shown to have a high diagnostic performance to identify patients with cirrhosis. The clinical significance of the wide range of TE values found in patients with cirrhosis is however still unclear. Furthermore, whether TE is of valid for the monitoring of progression in these patients with advanced fibrosis needs to be determined.
Aim : Determine whether repeated TE measurements in patients with chronic liver disease is predictive for clinical outcome.
Methods : This is a prospective study during which patients with an asymptomatic and stable chronic disease were screened. Ninety patients with a TE value = 12 kPa were selected to undergo a second TE measurement after 2 years. A second measurement was possible in 72 patients (80%). Of these 72 patients 65% suffered from a chronic viral hepatitis.
Results : Thirty six patients (50%) did not had cirrhosis at biopsy and the median TE value in these patients was significantly lower vs the patients with cirrhosis : 12.3 kPa [12.1 – 15.6] vs 17.6 kPa [14.1 – 27.6] ($p = 0.001$). The mean interval between the two measurements was 29 ± 10 months with a further follow up of 12 ± 10 months. The median TE value preceeding the event of the 11 patients who had a liver related death or needed a liver transplantation was 27.0 kPa [21.2 – 34.4] vs 14.7 kPa [12.3 – 21.6] for the others ($p = 0.003$). Of the patients who had a second measurement, 9% in the total population and 19% of the patients with a F4 developed the endpoint (evolution to F4 ($n = 3$) or a complication of cirrhosis ($n = 7$)). A delta TE with a cut-off of 13 kPa had the highest prognostic performance (in the F4 patients : sensitivity = 0.71 and specificity 0.86, Youden index = 0.58).
Conclusion : In this study we assessed whether repeated TE measurements in patients with chronic liver disease may be predictive of clinical events during follow-up. We confirmed a wide range of TE values in patients with cirrhosis. In patients with an advanced liver disease TE measurements are sensitive enough to detect changes which are clinically important. In this regards an increase in TE values of > 13 kPa over a period of 2 years is a strong predictor of liver ? related complications.

- A29 -

TRANSCATHETER ARTERIAL EMBOLIZATION WITH DOXORUBICIN ELUTING SAP MICROPSHERES IN TREATMENT OF HCC. J. Dekervel (1), H. Van Malenstein (1), V. Vandecaveye (2), F. Nevens (1), S. Heye (3), W. Laleman (1), J. Vaninbroukx (3), C. Verslype (1), G. Maleux (3). (1) Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (2) Radiology, UZ Leuven, Leuven, Belgium ; (3) Interventional Radiology, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

Introduction : A superior safety profile of TACE using superabsorbent polymer microspheres containing doxorubicin (SAPm-TACE) over conventional TACE (cTACE) has already been established. We present the follow-up data of HCC patients treated with SAPm-TACE in our center.
Methods : We performed a single center, single arm prospective study on all patients undergoing SAPm-TACE between 2005 and 2011. Patient data on demographics, etiology and stage of disease was collected. Treatment response was obtained using standard protocol MRI, four to six weeks after each procedure. Survival rates and serious adverse events (SAE) after treatment were registered.
Results : Between 2005 and 2011, 64 patients underwent one or more SAPm-TACE procedures. The majority of the patients (n = 58) were men, mean age was 65 yrs. Most common underlying liver diseases were (N)ASH (42%) and hepatitis C virus infection (35%). Almost all patients (n = 58) had cirrhosis, which was well compensated in 81% of cases (Child-Pugh A). 66% of patients were classified as having intermediate stage HCC (BCLC class B).
In total 142 SAPm-TACE procedures were conducted in our patient cohort (mean of 2,2 procedures per patient). Standard dose of doxorubicin was 50 mg/m^2. Dose reduction (25 mg/m^2) was applied in case of elevated bilirubin levels or lower performance status.
SAPm-TACE was generally well tolerated. Almost all patients had post-embolisation syndrome (abdominal pain, nausea, fever) in a greater or lesser degree. Serious adverse events were mainly ischemic (n = 4, cholecystitis or pancreatitis) and infectious (n = 3, sepsis or bacteremia).
Disease control (ie. partial remission or stable disease) was seen in 86%, 55% and 37% after one, two and three procedures respectively. One pathologically confirmed complete response (after three procedures) was reported (explant specimen).
Patients were followed up during an average period of 13 months (SD 10). Mean survival after first TACE was 14 months (SD 10). One and two year survival rates were 73% and 44% respectively. No treatment-related mortality was reported. Interestingly, 16 patients underwent orthotopic liver transplantation after one or more SAPm-TACE procedures, which were either performed as a bridge to liver transplantion or to downstage the HCC within the Milan criteria. Two patients died in the post-transplantation period (non-HCC related). Two other patients show recurrent disease (HCC metastasis) today.
Conclusion : SAPm-TACE for patients with HCC can be performed with a good safety profile in patients with intermediate HCC and well compensated liver disease, resulting in substantial response rates and one year overall survival. Moreover, SAPm-TACE seems to be a valid option to manage patients awaiting liver transplantation. A two-arm study is needed to prove a survival benefit of SAPm-TACE over cTACE with doxorubicin and lipiodol.

- A30 -

ROLE OF PROTON PUMP INHIBITORS IN THE OCCURRENCE AND PROGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS. M. De Vos, B. De Vroey, B. Garcia Garcia, C. Roy, F. Kidd, J. Henrion, P. Deltenre. Hôpital de Jolimont, Haine-Saint-Paul, Belgium.

Introduction : Proton pump inhibitors (PPIs) may facilitate intestinal bacterial overgrowth and bacterial translocation in cirrhotic patients. Currently, no robust data exist demonstrating that PPIs increase the risk of spontaneous bacterial peritonitis (SBP), and the question of whether PPIs worsen the prognosis of SBP patients remains unsettled. It has also been suggested that PPI use might be unsuitable for cirrhotic patients.
Aim : To analyze : 1/ the role of PPIs in the occurrence of SBP in cirrhotic patients ; 2/ their impact on the prognosis of SBP patients ; and 3/ the suitability of their use.
Methods : In this retrospective case-control study, PPI use was first assessed in cirrhotic patients consecutively admitted with SBP (group I) and in a control group that included the same number of uninfected cirrhotic patients with ascites (group II). In a second step, the impact of PPIs on SBP was assessed in group I by comparing survival of patients with and without PPIs.
Results : 102 patients were included, 51 in each group. 1/ SBP patients were more frequently treated by PPIs than controls (49 vs. 25%, p = 0.014). 2/ In group I, patients with (n = 25) and without (n = 26) PPIs had similar survival rates at 1 month (64.0 ± 9.6% vs. 59.4 ± 10.0%), 3 months (41.2 ± 10.2% vs. 44.6 ± 10.6%) and 1 year (26.6 ± 9.6% vs.

28.9 ± 10.1%), and similar median age at death (53 vs. 57 years). 3/ The reason for PPI use was inappropriate or undocumented in 34% of group I and II patients.
Conclusion : PPIs were more frequently used in SBP patients than in non-infected cirrhotic patients with ascites, but did not influence the prognosis in SBP. Overuse of PPIs was encountered in one-third of cirrhotic patients and should be avoided.

- A31 -

VIROLOGICAL RESPONSE IN BELGIAN CHC PATIENTS TREATED WITH PEGINTERFERON ALFA/ RIBAVIRIN : A SUB-ANALYSIS. J.P. Mulkay (1), S. Bourgeois (2), L. Lasser (3), C. De Galocsy (4), Y. Horsmans (5), H. Van Vlierberghe (6). (1) Ulb Saint-Pierre, Brussels, Belgium ; (2) Stuivenberg Hospital, Antwerpen, Belgium ; (3) Ulb Brugmann, Brussels, Belgium ; (4) Hôpitaux Iris Sud Bracops, Brussels, Belgium ; (5) Université Catholique De Louvain, Brussels, Belgium ; (6) Ghent University Hospital, Gent, Belgium.

Introduction : PROPHESYS, a large, observational, prospective multinational cohort study of patients treated for chronic hepatitis C, included 7,163 chronic hepatitis C patients and evaluated combination therapy with PegInterferon alfa (Peg-IFNa) plus ribavirin (RBV) for chronic hepatitis C (CHC) in real-world clinical settings.
Aim : Evaluate the rates and predictors of sustained virological response (SVR) in Belgian patients treated with PegInterferon alfa (Peg-IFNa) plus ribavirin (RBV) in - real practice.
Methods : We present data from 384 patients from Belgian centers, hepatitis C virus (HCV) mono-infected, treatment-naïve patients enrolled in PROPHESYS and treated with Peg-IFNa-2a (40KD) or Peg-IFNa-2b (12KD) plus ribavirin. This treatment was prescribed in accordance with Belgian label between June 2007 and March 2011. For Genotype 1 population, baseline predictors of sustained virological response (SVR) were explored by multiple logistic regression (MLR) analysis.
Results : The majority of patients enrolled in PROPHESYS in Belgium were infected by Genotype 1 (40.9%) and Genotype 3 (31.3%) and received Peg-IFNa-2a (95%). The patients were predominantly male (59.1%), Caucasian (76.6%), younger than 45 (53.4%), with a BMI > 25kg/m² (49.7%) ; 22.7% had cirrhosis or transition to cirrhosis. At baseline, 56.2% had HCV-RNA > 800,000IU/ml. Overall 45.3% of all patients in PROPHESYS achieved a rapid virological response (RVR : HCV RNA < 15 IU/ml at week 4) and 49.5% achieved an SVR. The rate of RVR was respectively 26,8% in G1, 69,2% in G2, 70% in G3 and 28,1% in G4.Virological response and relapse rates varied according to genotype : SVR and relapse rate were respectively 43.9% and 30.6% in G1 ; 48.7% and 25.7% in G2, 61.7% and 26.6% in G3, 38.6% and 22.8% in G4. The positive predictive value (PPV) of RVR for SVR was not similar across genotypes (G1 : 59.5%, G2 : 55.6%, G3 : 72.6%, G4 : 75%). In the mLR analysis, baseline factors significantly associated ($p < 0.05$) with SVR in G1 patients were lower HCV RNA and higher platelets count.
Conclusion : Overall 49.5% of patients achieved an SVR with Peg-IFNa and ribavirin consistent with findings from the global PROPHESYS database except for Genotype 2 patients. The rate of SVR in this genotype 2 group should be interpreted with caution because of the low patient numbers. Among patients with the “difficult to cure” G1 infection, RVR was achieved by more than a quarter of patients. Lower HCV RNA and higher platelet count were associated with SVR in Genotype 1 patients.

- A32 -

LIVER STIFFNESS BY SHEAR WAVE ELASTOGRAPHY IS INFLUENCED BY MEAL-RELATED HAEMODYNAMIC MODIFICATIONS. L. Vonghia, W. Verlinden, P. Pelckmans, P. Michielsen, S. Francque. Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Shear Wave Elastography has an emerging role in the non-invasive evaluation of liver fibrosis. Physiological factors can influence liver stiffness (LS) and should be considered when performing LS measurement.
Aim : To evaluate the effect of meal and meal-related haemodynamic variations on LS measurement by Shear Wave Elastography.
Methods : 10 healthy volunteers were enrolled in the study. LS, echo color Doppler analysis of portal vein and hepatic artery, as well as liver and spleen dimensions were evaluated before a meal (T0) and 30 ? (T30), ?60 ?(T60) and 120 ? (T120) postprandially.
Results : LS values increased 30´ postprandially and decareased from 60´ postprandially, returning to baseline values after 2 hours (T0 : 5.2 ± 1.06 kPa, T30´ : 6.3 ± 0.8 kPa, T60´ : 6 ± 1.3 kPa, T120´ 5 ± 1 kPa ; p :0.02). Portal flow showed comparable kinetics (T0 0.52 ± 0.22 L/min, T30 1.08 ± 0.32 L/min, T60 0.77 ± 0.52 L/min, T120 0.48 ± 0.21 L/min ; p : 0.02). Portal mean velocity was significantly increased at T30 (T0 11.3 ± 2.5 cm/sec, T30 16.1 ± 4.7 cm/sec ; p :0.02),

and returned to values comparable to baseline from T60 on (T60′ 14.2 ± 3.6 cm/sec, T120′ 13.5 ± 3.4 cm/sec ; p : > 0.05). Hepatic artery maximal (Vmax) and mean (Vmean) velocity, after a decrease at T30, progressively increased up to T120, where they reached values higher than baseline (Vmax : T0 52.8 ± 36.8 cm/sec, 30′ 45.4 ± 17.4 cm/sec, T60 55.6 ± 23.5 cm/sec, T120 73.7 ± 44.2 cm/sec ; p : 0.04 ; Vmean : T0 36.4 ± 27 cm/sec, T30 28.3 ± 11.7 cm/sec, T60 35.6 ± 16 cm/sec, T120 48.1 ± 29 cm/sec ; p : 0.04). The Resistivity Index (RI) of the hepatic artery increased from T30 after meal (T0 0.62 ± 0.07 cm/sec, T30 0.74 ± 0.08 cm/sec ; p : 0.02, T60 0.73 ± 0.05 cm/sec ; p :0.02) and decreased at T120 (T120 0.69 ± 0.08 cm/sec p > 0.05). Liver and spleen diameters did not show variations.
Conclusion : LS transiently increases postprandially in relation to the meal-induced haemodynamic variations in portal and hepatic arterial flow, and returns to baseline 120' after the meal. LS evaluation should hence be performed fasting or at least 120 ' after a meal.

- A33 -

PREVALENCE AND OUTCOME OF DIASTOLIC DYSFUNCTION IN LIVER TRANSPLANTATION RECIPIENTS. S. Raevens, M. De Pauw, A. Geerts, F. Berrevoet, X. Rogiers, R. Troisi, H. Van Vlierberghe, I. Colle. Ghent University Hospital, Gent, Belgium.

Introduction : Cirrhotic cardiomyopathy (CCMP) denotes a chronic cardiac dysfunction in cirrhotic patients. It is characterized by systolic and diastolic dysfunction (DD), and electromechanical abnormalities, in the absence of any other cardiac disease. Liver transplantation (LTx) is known to have a favorable effect on the existence of CCMP, but the presence of CCMP means in itself a risk factor for undergoing a LTx.
Aim : The aim of the study was to estimate the prevalence of DD among LTx candidates, to compare outcome between patients with and without DD, and to determine if tricuspid regurgitation severity is a predictor of post-transplantation outcome.
Methods : A total of 173 LTx recipients was retrospectively evaluated. Diastolic dysfunction was diagnosed by echocardiography. Difference in outcome between patients with and without DD was evaluated in terms of mortality and occurrence of cardiovascular complications post-transplantation. ?
Results : Diastolic dysfunction was diagnosed in 74 (43%) patients. Patients with DD compared with those without DD had significantly older age (P < 0,0001). Regarding outcome, no statistically significant difference between patients with or without DD could be documented. Moderate to severe tricuspid regurgitation is, in contrast to no or mild tricuspid regurgitation, associated with worse post-transplantation outcome (P = 0,01 on short-term, P = 0,02 on long-term).
Conclusion : In a population of 173 liver transplantation candidates, a prevalence of systolic and diastolic dysfunction of 2% and 43% respectively could be registered. Outcome does not seem to be strongly affected by the presence of DD. Tricuspid regurgitation severity on echocardiography is predictive of patient survival.

- A34 -

CLASS II HDAC INHIBITION HAMPERS HEPATIC STELLATE CELL ACTIVATION BY INDUCTION OF MICRORNA-29. I. Mannaerts (1), N. Eysackers (1), O. Onyema (1), K. Van Beneden (1), A. Mai (2), M. Odenthal (3), L. Van Grunsven (1). (1) Vrije Universiteit Brussel, Jette, Belgium ; (2) Sapienza University, Rome, Italy ; (3) University Hospital of Cologne, Köln, Germany.

Introduction : The conversion of a quiescent vitamin A storing hepatic stellate cell (HSC) to a matrix producing, contractile myofibroblast-like activated HSC is a key event in the onset of liver disease following injury of any aetiology. Previous studies have shown that class I histone deacetylases (HDACs) are involved in the phenotypical changes occurring during stellate cell activation in liver and pancreas.
Aim : In the current study we investigate the role of class II HDACs during HSC activation.
Methods : We characterized the expression of the class II HDACs freshly isolated mouse HSCs. We inhibited HDAC activity by selective pharmacological inhibition with MC1568, and by repressing class II HDAC gene expression using specific siRNAs.
Results : Inhibition of HDAC activity leads to a strong reduction of HSC activation markers a-SMA, lysyl oxidase and collagens as well as an inhibition of cell proliferation. Knock down experiments showed that HDAC4 contributes to HSC activation by regulating lysyl oxidase expression. In addition, we observed a strong up regulation of miR-29, a wellknown anti-fibrotic miR, upon treatment with MC1568. Our in vivo work suggests that a successful inhibition of class II HDACs could be promising for development of future antifibrotic compounds.
Conclusion : In conclusion, the use of MC1568 has enabled us to identify a role for class II HDACs regulating miR-29 during HSC activation.

- A35 -

THE VALUE OF GP73 AS A MARKER FOR DIFFERENTIATING BETWEEN BENIGN AND MALIGNANT LIVER TUMOURS. M. Bröker, J. IJzermans, C. Witjes, R. De Man. Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : The differential diagnosis of a primary focal liver lesion includes benign liver tumours like Hepatocellular adenoma (HCA) and Focal Nodular Hyperplasia (FNH), as well as Hepatocellular Carinoma (HCC). A good differentiation between these primary liver tumours is essential. GP73 is a new promising marker that could be useful in the diagnosis and screening of HCC. However, all studies investigating GP73 included cirrhotic patients and/or healthy persons as control groups only. ? ?
Aim : The aim of this study is to determine the predictive value of GP73 in the differentiation of benign and malignant tumours of the liver.
Methods : A total of 252 patients were included with among them 84 patients with an HCC, 84 patients with an HCA and 84 with a FNH. We examined GP-73-levels in their blood samples with a quantitative ELISA assay. The levels of GP73 in the HCC patients were compared to levels of GP73 in patients with benign liver tumours. The ROC (received operating curve), sensitivity and specificity of GP73 were calculated and compared with alpha-fetoprotein (AFP).
Results : The GP-73 area under ROC was 0.701.The sensitivity was 60% and the specificity of 77% to differentiate HCC-patients from patients with a HCA and FNH. The AFP area under the ROC was 0.912 with a sensitivity of 65% and specificity 96%.
Conclusion : Although literature suggests GP73 is a valuable serum marker and superior to AFP, GP73 does not seem useful for discriminating between malignant and benign liver tumours. Therefore, GP73 should not be used as a diagnostic marker if there is tumor in the liver of unknown origin.

- A36 -

DISCOVERY OF A HYPERFERRITINEMIA : WHICH INVESTIGATIONS ? A GLEM/LOK SURVEY. A. Geubel (1), H. Büscher (2), C. De Galocsy (3), R. Fiasse (1). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) Department of Gastroenterology and Hepatology, Universitary Hospital Antwerp, Antwerpen, Belgium ; (3) CH Joseph Bracops, Brussels, Belgium.

Introduction : In routine clinical practice, hyperferritinemia is a common observation which requires attention due its important diagnostic and therapeutic consequences, especially if this biochemical abnormality reflects the existence of iron overload.
Methods : A questionnaire devoted to the differential diagnosis of hyperferritinemia, to its related diagnostic tests and to the most discriminant parameters of iron overload was established by two experts (A.G. and H.B.) and sent by mail to ? the different GLEMs/LOK**s**. Completed questionnaires which constituted the basis for the present analysis, were returned by 70 respondents during the period April 19th, 2011 to December 20th, 2011.
Results : The majority of gastroenterologists (60.2%) were well aware of the diagnostic value of high transferring saturation to detect iron overload while only 53% did mention Magnetic Resonance Imaging (MRI) as a useful diagnostic tool in this regard, especially in HFE-negative and/or doubtful cases. In the latter, only 14.6% believed in its value for initiating an iron-depleting treatment. Genetic test for HFE was considered as the first diagnostic approach for hemochromatosis by 86.4% of the respondents**,** who largely underestimated its prevalence in the population of patients with hemochromatosis (34.6%). Only 41.5% of the respondents ? were aware of the value of the search for the mutation of transferrin (TfR2-Y250X) and of ferroportin (C326S and C326Y) in the case of HFE-negative iron overload. The prevalence of iron overload in HFE positive subject**s** was underestimated (26% instead of 50%) while the prevalence of complications of iron overload in HFE ?positive cases was overestimated (44% instead of 20%). The majority of respondents (65.7%) were aware of the predominance of Kupffer cells iron overload in the case of ferroportin mutation while almost all (96%) were aware of the fact that non-alcoholic steato-hepatitis (NASH) constitutes ?in our population the major cause of hyperferritinemia not due to hemochromatosis.
Conclusion : The importance of detection of iron overload is part of the competence of our gastroenterologists even if their knowledge about the disease biology and spectrum as well as the potential help for newer diagnostic tools, i.e. genetics and imaging, remain largely perfectible. Due to the high prevalence of iron overload in our population, this observation should constitute a strong encouragement for our scientific societies to include biology and management of iron overload for topics of post-graduate courses.
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INDICATIONS AND RESULTS OF COMBINED LIVER/KIDNEY TRANSPLANTATION : SINGLE CENTER EXPERIENCE. L.J. Ceulemans, D. Monbaliu, R. Aerts, W. Coosemans, I. Jochmans, N. Meurisse, F. Temmerman, W. Laleman, S. Van Der Merwe, J. Van Pelt, W. Van Steenbergen, C. Verslype, T. Roskams, E. Lerut, B. Bammens, B. Sprangers, M. Naesens, K. Claes, B. Meijers, P. Evenepoel, Y. Vanrenterghem, D. Kuypers, F. Nevens, J. Pirenne. Abdominal Transplant Program, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

Introduction : C*reatinine* weighs *heavily* on the Model for End-Stage Liver Disease (MELD) score *(10*((0.957*ln(**Creatinine**))+(0.378*ln(**Bilirubin**))+(1.12*ln(**INR**))*+6.43).
Subsequently, MELD-based prioritization for liver transplantation has substantially increased the numbers of combined Liver/Kidney Tx (CLKTx). Given the scarcity of donor organs, indications/results of CLKTx must be analyzed with scrutiny.
Aim : Review indications/results of CLKTx.
Methods : CLKTx performed between 01/1997-12/2011 were analyzed : donor/recipient characteristics ; etiology of liver/kidney disease ; % patients fulfilling standard indications for both LTx and KTx, or only for LTx or KTx (and receiving *"preventive"* KTx or LTx) ; immunosuppression ; rejection ; 1-/10-year patient/graft survival.
Results : 62 CLKTx were performed (8.6% of LTx). Mean age was 52y(4-69). 30 patients(48.4%) were male ; 32(51.6%) female. 59(95.2%) were adults ; 3(4.8%) pediatrics. Mean MELD score was 21(6-41). 40(64.5%) were dialysis-dependent. Hepatorenal polycystosis was the predominant disease (24 patients(39%)). In the other 38(61%), etiology of liver disease was : postethyl(13), hepatitis B or/and C(9), congenital fibrosis(3), cholestatic (3), reTx(2), drug-induced(2), alpha-1-antitrypsine deficiency(2), cryptogenic(1), Alström(1), hyperoxaluria(1), nodular hyperplasia(1). Hepatocellular carcinoma was associated in 3 ; cholangiocarcinoma in 1. Etiology of kidney disease was : reTx for failing previous KTx(10), IgA-nephropathy(9), drug-induced(4), glomerulonefritis(3), angiosclerosis(3), hepatorenal syndrome(3), solitary kidney polycystosis(2), Alström(1), diabetes(1), hyperoxaluria(1), unknown(1). 37 patients(59.7%) fulfilled standard indications for LTx and KTx (mean eGFR 12.2ml/min(5.1-18.9)). 17 patients(27.4%) with standard liver indication received combined *preventive* KTx because of advanced (not terminal) renal disease (mean eGFR 27.9 mL/min(20.2-41)). 8 patients(12.9%) with standard renal indication (mean eGFR 7.7ml/min(5.1-10.7)) received combined *preventive* LTx because of advanced (not terminal) liver disease. Mean donor age was 38y(3y-66y). Causes of death were : cerebrovascular accidents(29) ; trauma(26) ; others(7). 60 donors(96.8%) were brain dead ; 2(3.2%) were non-heart beating donors. Orthotopic LTx was performed first, followed by heterotopic KTx. Mean liver/kidney cold ischemia times were 7h38'(3h-11h)/13h44'(7h-23h45'). Immunosuppression was tacrolimus-based in 55(88.7%) ; cyclosporine-based in 7(11.3%). 52(83.9%) received mycophenolate mofetil ; 4(6.5%) azathioprine ; 3(4.8%) sirolimus. Induction was used in 26(42%) (basiliximab (24) ; ATG(2)). Early acute rejection occurred in 2 kidneys(3.2%) and 2 livers(3.2%), and simultaneously in kidney/liver in 1(1.6%). All responded to steroids. Delayed kidney graft function developed in 2(3.2%) and primary non function (PNF) in 1(1.6%). 3 patients(4.8%) underwent kidney reTx (PNF/rejection/rejection+Polyomavirus). 2(3.2%) developed chronic allograft nephropathy, of which 1(1.6%) awaits kidney reTx. 2(3.2%) required early liver reTx (artery thrombosis). None developed chronic liver rejection. 8 patients succumbed : infection(7), oropharyngeal carcinoma(1). 1-/10-year (death-uncensored) kidney/liver graft survival are 91.9%/90.3% and 82.7%/86%. 1-/10-year patient survival are 93.5% and 89.2%.
Conclusions : CLKTx is indicated not only in patients with terminal liver and kidney disease but also in selected patients with terminal disease of one organ and irreversible -not yet terminal- disease of the other. In the latter group, CLKTx prevents morbidity/mortality associated to accelerated kidney/liver failure after single organ transplantation. Rejection rates are extremely low. 10-year survival close to 90% is achieved.

VASCULAR CORROSION CASTING IN RESEARCH ON LIVER DISEASES AND TRANSPLANTATION : AN OVERVIEW OF STUDIES. C. Casteleyn (1), C. Van Steenkiste (2), F. Heindryckx (3), S. Coulon (4), N. Van Hul (5), C. Debbaut (6), P. Cornillie (7), D. Monbaliu (8), S. Francque (9), B. Tambuyzer (10), S. Van Cruchten (10), C. Van Ginneken (10). (1) Laboratory of Applied Veterinary Morphology, Department of Veterinary Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Antwerpen, Belgium ; (2) Maria Middelares Ziekenhuis, Gent, Belgium ; (3) Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden ; (4) Ghent University Hospital, Gent, Belgium ; (5) Université Catholique de Louvain, Brussels, Belgium ; (6) Universitair Ziekenhuis Gent, Gent, Belgium ; (7) Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium ; (8) Abdominal Transplant Surgery, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (9) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerpen, Belgium ; (10) Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Antwerpen, Belgium.

Introduction : During the study of liver diseases that alter the hepatic vasculature, visualization of the blood and biliary vessels in experimental animals is often required to determine the effect of a potential treatment. Most often, researchers rely on histological slices, which, unfortunately, do not show the three-dimensional (3D) organization of the vascular trees. In addition, such 3D information is a key for the development of liver perfusion techniques during transport of livers for transplantation.
Aim : The aim of this presentation is to demonstrate some applications of vascular corrosion casting in research on liver diseases and transplantation.
Methods : Vascular corrosion casting is an anatomical technique used to visualize vessel organization. It is based on the injection of a liquid plastic into the vessels of the organ or tissues under investigation, i.c. the liver and splanchnic area. After polymerization of the plastic and maceration of the soft tissues with 25% KOH, a cast of the vessels is obtained. These casts can subsequently be studied by means of stereomicroscopy or scanning electron microscopy (SEM). They can also be scanned in a (micro-)CT followed by computerized 3D rendering.
Results : The value of the vascular corrosion casting technique in hepatology is demonstrated in various studies, including the determination of wall shear stress in a rat model of portal hypertension, the study of anti-angiogenic substances as potential treatments for hepatocellular carcinoma induced in the mouse, the elucidation of the role of anti-angiogenic factors in a mouse model of non-alcoholic steatohepatitis, the assessment of the total intrahepatic resistance and its potential functional and structural determinants in a rat model of non-alcoholic fatty liver disease, the comparison of two mouse models of hepatobiliary disease, the modelling of the impact of living donor liver transplantation on the hepatic hemodynamics using a rat model, and the determination of the perfusion characteristics of the human hepatic microcirculation.
Conclusion : From these studies it can be concluded that vascular corrosion casting has recently revived as a research tool in the field of vascular disorders. This technique is no longer exclusively used in descriptive anatomy, but has made its entry into experimental research. Together with SEM and (micro-)CT combined with 3D reconstruction it allows in-depth examination of the (micro)circulation and hemorheology.

- A39 -

RELATIONSHIP BETWEEN LIFESTYLE/DIETARY HABITS, PHYSICAL EXERCISE AND THE NAFLD SPECTRUM. G. Bronne (1), A. Foucart (1), P. Vereerstraeten (2), P. Demetter (3), L. Verset (3), M. Barea (4), C. Moreno (5), A. Van Gossum (5), J. Devière (5), D. Degré (5), M. Adler (5). (1) Institut Paul Lambin, Departments of (2) Nephrology, (3) Pathology, (4) Gastroenterology, Hepato-Pancreatology and Digestive Oncology, (5) Dietetics. Hôpital Erasme, Brussels, Belgium.

Background and Aims : Dietary composition and physical exercise (PE) are suggested to affect the pathophysiology of Non Alcoholic Fatty Liver Disease (NAFLD). So we sought to study the relationship between these factors and the different histological severity forms of NAFLD.
Patients and Methods : Fifty-six patients (median age 52 yrs, 57% females, median BMI 31.8 kg/m^2) with obesity or weight ≥ 25 kg/m^2 and ≥ 2 criteria of the metabolic syndrome, seen consecutively at the Liver Clinic with a complete work up including demographics, clinical, biochemical, dietary and PE evaluations and liver biopsy were studied. Severity of steatosis, NASH and liver fibrosis was evaluated according to Kleiner and Brunt scores. Dietary assessment was based on food consumed during the preceding week and converted to total calories, macro-and micro-nutrient intake per day. PE was evaluated via the International PE Questionnaire, converted into Metabolic Equivalent of TASK (MET) units per week. Uni- and multivariate analyses were performed in order to define predictive factors related to 3 different pathological entities : severe steatosis (37%), non alcoolic steatohepatitis (NASH : 68%) and significant fibrosis (41%).
Results : Female gender and high levels of ALT (≥ 60 IU) were associated to severe steatosis ; female gender and high levels of polyunsaturated fatty acids to NASH ; low levels of AST and total cholesterol daily intake to significant fibrosis. Intensity of PE, total caloric intake and dietary proteins, total and simple sugars, fibers, lipids, saturated fatty acids, cholesterol, vitamins D, C and E, selenium and caffeine were not different across the two stages of severity within the 3 entities of the NAFLD spectrum.
In conclusion : This pilot study question the role of lifestyle behavior on the physiopathology of severe NAFLD as well as a tailored approach for the dietary manipulations of its subgroups.

OG-FWO

GASTROINTESTINAL REGULATORY MECHANISMS

- B01-

EXPRESSION OF ANOCTAMIN 1, AN ICC MARKER, IN THE INTESTINE OF WILD-TYPE AND MUTANT ZEBRAFISH. L. Uyttebroek (1), I.T. Shepherd (2), G. Hubens (1), J.P. Timmermans (3), L. Van Nassauw (1). (1) Laboratory of Human Anatomy and Embryology, Faculty of Medicine & Health Sciences, University Of Antwerp, Antwerpen, Belgium ; (2) Emory University, Atlanta, United States ; (3) Laboratory Of Cell Biology And Histology, Department of Veterinary Sciences, University of Antwerp, Antwerpen, Belgium.

Introduction : Over the last decade, the zebrafish has emerged as a leading model organism to study human disease conditions. Interstitial cells of Cajal (ICC) are specialized cells that generate electrical slow waves initiating gastrointestinal (GI) motility. ICC are known to be affected in various GI disorders. The Ca2+-activated Cl--channel, anoctamin 1 (Ano1), is recently described as a specific ICC marker in mammals, and suggested to be involved in slow wave generation and ICC proliferation.
Aim : At present, we aimed to study the presence and distribution of Ano1 in the intestine of wild-type and mutant zebrafish. The mutant zebrafish, *lessen,* shows the characteristics of Hirschsprung's disease (HSCR), a congenital disorder characterized by aganglionosis of the distal intestine.
Methods : Using multiple immunofluorescent staining, sections and whole mounts of adult zebrafish intestine were analyzed. Spatio-temporal expression of Ano1 was also examined on isolated intestines of wild-type and mutant embryos (3 to 6 days post fertilization, dpf).
Results : In adult zebrafish, a granular Ano1 immunoreactivity revealed ICC-like cells in two distinct layers forming a 3-dimensional network. A loose layer of bipolar immunoreactive cells, resembling intramuscular ICC were interposed between smooth muscle cells in the circular muscular layer. A layer of multipolar immunopositive cells, resembling myenteric plexus ICC, were intertwined with the myenteric plexus. Both layers were interconnected. Close associations between nerve fibers and ICC-like cells were observed. In 3dpf wild-type embryos, few cells expressing faint granular Ano1 immunoreactivity were observed, scattered throughout the intestinal wall. By 5-6 dpf, a clear 3-dimensional network of cells expressing Ano1 is formed throughout the intestine. Expression of Ano1 in mutant embryos started 1 day later in mutant embryos, and the ICC-like network was less dense, especialy in the distal intestine.
Conclusion : We demonstrated that Ano1, as in mammals, is a selective marker for ICC-like cells in the zebrafish intestine. Ano1-positive ICC-like cells first appear at 3 dpf in the embryonic intestine, indicating that proliferation of ICC-like cells begins at this time point. The expression of Ano1 is delayed in the *lessen* mutant, as observed in human HSCR conditions and mutant mice (*lethal spotted*) showing HSCR characteristics. Furthermore, it is hypothesized that ICC-like cells generate spontaneous contractile activity of the embryonic intestine, because the first appearance of ICC-like cells in the embryonic intestine occurs at the same time that the first spontaneous contractility is observed in the embryonic intestine.

- B02 -

MITOCHONDRIAL ROS IS INVOLVED DURING TNF-A-INDUCED OXIDATIVE STRESS IN INTESTINAL EPITHELIAL CELLS. D. Babu (1), G. Leclercq (2), Q. Remijsen (3), R. Motterlini (4), R.A. Lefebvre (1). (1) Heymans Institute of Pharmacology, Gent, Belgium ; (2) Ghent University, Ghent, Belgium ; (3) Department Of Biomedical Molecular Biology, Gent, Belgium ; (4) Inserm U955, Créteil.

Introduction : Using the mouse intestinal epithelial cell line, MODE-K as an in-vitro model, we previously showed that TNF-alpha/cycloheximide (CHX)-induced apoptosis corresponded with the occurrence of reactive oxygen species (ROS) production, and that resveratrol (75 μM), a polyphenolic antioxidant in red wine, reduced both effects (Babu *et al*., Curr. Pharm. Des., 2012). Activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and mitochondrial oxidative phosphorylation are considered to be the two major endogenous sources of ROS production in cells.
Aim : We therefore investigated the involvement of mitochondrial ROS to TNF-alpha-induced oxidative stress in MODE-K cells and the influence of resveratrol in this model.
Methods : Carboxy-H2DCFDA is a widely used fluorescent probe to measure total intracellular ROS ; DHR123 is a probe that can easily cross cell membranes and react with ROS in mitochondria to generate the positively-charged rhodamine 123 (R123), and thus is a useful probe to measure mitochondrial ROS production. 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 36h and serum starved overnight. Then, the cells were exposed to various concentrations of TNF-alpha/CHX for 6 h ; simultaneous detection of ROS production and cell death has been performed using either carboxy-H2DCFDA or

DHR123 together with Sytox Red in a single experimental setup using flow cytometric analysis. For experiments involving resveratrol, the confluent cells were pretreated with resveratrol for 1 h followed by its co-treatment with TNF-alpha/CHX for 6h.
Results : Treatment of TNF-alpha/CHX increased mean fluorescence intensity (deltaMFI) of both carboxy-H2DCFDA and DHR123-derived fluorescence in a concentration-dependent manner with concomitant increase in cell death as measured with Sytox Red positivity. Pretreatment with resveratrol significantly reduced the TNF-alpha/CHX-induced deltaMFI of both ROS sensitive probes implying that resveratrol reduced both the total ROS and mitochondria-derived ROS ; in parallel resveratrol reduced TNF-alpha/CHX-induced cell death.
Conclusion : Mitochondrial ROS thus seems an important contributor to TNF-alpha-induced oxidative stress in intestinal epithelial cells. The anti-apoptotic effect of resveratrol might largely be due to its inhibitory action on mitochondrial ROS production.

- B03 -

BETA-GLUCAN PARTICLES AS NOVEL ANTIGEN DELIVERY SYSTEMS : TOWARDS ORAL VACCINATION. R. De Smet, S. Verschuere, L. Allais, T. Demoor, B. De Geest, C. Cuvelier. Ghent University, Ghent, Belgium.

Introduction : Several enteric pathogens infect the body following oral uptake and cause life-threatening diseases such as cholera, dysentery and typhoid fever. Oral vaccination is essential to generate protective local immunity against intestinal pathogens. However, antigen delivery to the inductive sites for mucosal immunity (the small intestine Peyer's patches, PP) has proven to be particularly challenging. Recently, there has been a lot of interest in the use of microparticles as antigen delivery systems in the development of more efficient mucosal vaccines.
Aim : We evaluated the potential of beta-glucan microcapsules to deliver antigen transmucosally in the PP regions of the murine small intestine.
Methods : beta-glucan microparticles (2-4 μm) were prepared from *Saccharomyces cerevisiae* and loaded with FITC or Alexa Fluor 488-conjugated bovine serum albumin. Firstly, the appropriateness of beta-glucan particles as antigen delivery systems for oral and intestinal applications was assessed in stability tests. Secondly, beta-glucan particles were administered to male C57BL/6 mice (8-10 weeks old) via intestinal loops at a particle concentration of 100*106/ml. After one hour of incubation, transmucosal particle transport and uptake in the PP was evaluated by flow cytometry, confocal microscopy and transmission electron microscopy.
Results : Fluorimetric analysis of stability tests of the particles demonstrated that the BSA concentration within particles is stable during the first 12 hours of simulated gastric or simulated intestinal fluid treatment. In the intestinal loop experiments we could not observe any particle uptake by means of flow cytometry in the main antigen presenting cell population, the dendritic cells, however, a modest particle uptake was repeatedly detected in the B-cell population. Confocal microscopic images showed localization of beta-glucan particles in the follicle associated epithelium and B-cell internalization. Moreover, transmission electron microscopy demonstrated transcellular transport of yeast particles in M-cells.
Conclusion : In conclusion, stability tests show that antigen concentration of beta-glucan particles remains stable in gastric and intestinal environment. This means that the beta-glucan particles can be administered orally without prior enteric coating and are suitable for mucosal delivery of antigen in the murine gastro-intestinal tract. Our data suggest that M-cells, but not subepithelial dendritic cells, are crucial for the transmucosal transport of beta-glucan particles from the intestinal lumen to the PP and transcytosis of antigen to underlying antigen presenting cells and immune cells.

- B04 -

GHRELIN RECEPTOR MODULATES CD4 T CELL FUNCTION DURING INTESTINAL INFLAMMATION. G. Matteoli, M. Di Giovangiulio, G. Farro, N. Stakenborg, P.J. Gomez-Pinilla, I. Depoortere, G. Boeckxstaens. Translational Research Center for Gastrointestinal Disorders (Targid), Kuleuven, Leuven, Belgium.

Introduction : Recent work suggests that the orexigenic hormone ghrelin have potent anti-inflammatory properties in preclinical model of intestinal inflammation. Ghrelin is secreted predominantly from enteroendocrine cells of the stomach and acts as an appetite-regulating hormone increasing food intake and long-term regulation of body weight. The effects of ghrelin are mediated via the G protein-coupled receptor (GPCR) named as growth hormone secretagogue receptor (GHSR). Interestingly, the expression of this receptor has been shown on innate and adaptive immune cells.
Aim : Taken together, the above suggests that ghrelin may have immunomodulatory properties. However, the mechanism, by which ghrelin suppresses intestinal inflammation, is still unclear. Therefore, we decided to investigate the effect of ghrelin on T cell function during colitis.

Methods : *T cell* transfer chronic *colitis* was induced in the recombination activating gene 1 knockout mice (Rag1-/-) by adoptive transfer of naive CD4 T helper cells (CD4-Ths) from genetically-compatible wild-type (WT) or growth hormone secretagogue *receptor* knockout mice (GHSR-/-). Development of colitis was evaluated every week by assessing body weight loss, diarrhea score and blood in the feces. Eight weeks after T cell transfer, the degree of intestinal inflammation was assessed by analyzing spleen weight, colon length, histology and cytokine expression in the colon.
Results : The lack of the ghrelin receptor on CD4-transferred T cells significantly worsened the course of colitis in Rag1-/- mice. Indeed, Rag1-/- mice reconstituted with GHSR -/- CD4 Ths (GHSR Rag1) showed colitis symptoms (body weight loss, alteration in the feces) significantly earlier than Rag1-/- mice reconstituted with WT CD4 Ths (WT Rag1). In line, GHSR Rag1 mice displayed increased body weight loss (WT Rag1, 14.5 ± 4.0% vs GHSR Rag1, 25.5 ± 3.0%) and higher diarrhea score compared to WT Rag1 (WT Rag1, 2.1 ± 0.2 vs GHSR Rag1, 3.5 ± 0.5). Eight weeks after T cells transfer, GHSR Rag1 mice had significantly increased spleen weight (WT Rag1, 70.5 ± 21.2 mg vs GHSR Rag1, 147.5 ± 42.1 mg) and shortening of the colon (WT Rag1, 8.9 ± 0.4cm vs GHSR Rag1, 7.9 ± 0.5) Histological analysis also showed a higher lesion score in GHSR Rag1 mice compared to WT Rag1 mice (WT Rag1, 7.9 ± 4.0 vs GHSR Rag1, 10.3 ± 4.8). In addition, gene expression of colonic specimen revealed higher levels of IL6 (4.3 ± 1.3 fold), *TNFa* (4.6 ± 1.6 fold), *IFN* (5.6 ± 2.3 fold) and *IL17a* (2.8 ± 0.8 fold) in GHSR Rag1 mice versus WT Rag1 mice.
Conclusion : Our observations strongly suggest that ghrelin may significantly ameliorate experimental chronic colitis by modulating T helper effector cell function in the gut. A better understanding of the underlying regulatory mechanisms of ghrelin will be crucial to further explore the potential therapeutic properties of ghrelin in inflammatory bowel disease.

- B05 -

THE SELECTIVE HISTAMINE H4 ANTAGONIST JNJ7777120 REDUCES POSTINFLAMMATORY VISCERAL HYPERSENSITIVITY. A. Deiteren (1), J. De Man (1), N. Ruyssers (1), T. Moreels (2), P. Pelckmans (2), B. De Winter (1). (1) University of Antwerp, Antwerpen, Belgium ; (2) Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Visceral hypersensitivity is identified as an important factor underlying abdominal pain in irritable bowel syndrome (IBS). The histamine H4 receptor (H4R) has been suggested to be an interesting new target for IBS treatment, however its involvement in visceral hypersensitivity has not been studied to date.
Aim : The aim was to investigate the effects of the selective H4R antagonist JNJ7777120 on visceromotor responses (VMR) in a rat model for post-inflammatory visceral hypersensitivity.
Methods : Colitis was induced in male Sprague-Dawley rats by intrarectal administration of 15 mg trinitrobenzene sulphonic acid (TNBS) in 50% ethanol while controls received a saline instillation. Animals were monitored individually by colonoscopy on day 3 to confirm the presence of colitis and then every 4 days starting from day 10 to monitor convalescence and determine the exact timepoint of endoscopic colonic healing in each animal. Three days after endoscopic resolution of inflammation, visceral sensitivity was assessed by electromyographic registration of VMR to colorectal distension (10-60 mmHg, 20 s, 4 min interval) and expressed as the total area under the curve (AUC). JNJ7777120 (10-35-70-140 mg/kg), a selective H4R antagonist supplied by Janssen Research & Development, or its vehicle was administered 30 min prior to VMR assessment. Compliance was evaluated by applying graded volumes (0-2.5 mL) to the colorectally inserted balloon and recording the corresponding colonic pressures. Afterwards, colonic tissue was assessed by endoscopy, macroscopy, histology and myeloperoxidase activity for remaining signs of inflammation.
Results : Colitis was present 3 days after TNBS instillation and was resolved endoscopically after 15 days (range 10-26 days). After endoscopic healing of colitis, vehicle-treated rats displayed significant post-inflammatory visceral hypersensitivity compared to vehicle-treated controls (total AUC 1953 ± 319 *vs* 671 ± 158 μV ; n = 9 ; $p < 0.05$). Post-inflammatory visceral hypersensitivity was dose-dependently reduced by JNJ7777120 (10-140 mg/kg). The 10 mg/kg dose had no significant effect on VMR (total AUC 1729 ± 152 *vs* 1953 ± 319 μV for vehicle ; n = 5-9 ; ns), whereas 35 mg/kg attenuated visceral hypersensitivity (total AUC 1191 ± 226 *vs* 1953 ± 319 μV for vehicle ; n = 9 ; $p < 0.05$). Finally, both 70 and 140 mg/kg fully reversed the heightened VMR to colorectal distension (total AUC 713 ± 105 μV for 70 mg/kg and 594 ± 152 μV for 140 mg/kg *vs* 1953 ± 319 μV for vehicle ; n = 9 ; $p < 0.05$). The highest dose of 140 mg/kg dose had no effect on VMR in controls. In addition, colonic compliance was similar in post-colitis and control rats and remained unaltered by JNJ7777120 (10-140 mg/kg, n = 5-9/group). Postmortem analysis confirmed subsidence of colonic inflammation.
Conclusion : Visceral hypersensitivity to colorectal distension after resolution of colitis was dose-dependently reduced by the selective H4R antagonist JNJ7777120. Our results indicate that this receptor subtype could indeed be a potential new target for the treatment of visceral hypersensitivity.

Invited Lecture

- B06 -

CALCIUM IMAGING IN THE GUT. G. Henning, Reno, USA.

- B07 -

CA IMAGING AT KHZ RATES DISCRIMINATES DIRECT FROM SYNAPTIC-DRIVEN ACTIVATION OF NERVE VARICOSITIES. M. Martens (1), W. Boesmans (2), P. Vanden Berghe (3). (1) Translational Research Center For Gastrointestinal Disorders (Targid), Lab For Enteric Neuroscience (Lens), KU Leuven, Leuven, Belgium ; (2) Translational Research Center For Gastrointestinal Disorders (Targid), Lab For Enteric Neuroscience (Lens), KU Leuven, Leuven, Belgium ; (3) Translational Research Center For Gastrointestinal Disorders (Targid), Lab For Enteric Neuroscience (Lens), KU Leuven, Leuven, Belgium.

Introduction : Calcium imaging is a powerful tool to record neuronal activity. Recording at kHz frame rates, extends the classical possibilities as individual Ca2+steps associated with action potential spikes can be resolved (Michel *et al*. J. Phys., 2011). In most imaging setups based on CCD technology, fast imaging goes at the expense of spatial resolution.
Aim : Our aim was to record fast calcium events in individual varicosities in the mouse enteric nervous system using a 2kHz CMOS camera with improved spatial resolution (512x512 pixels), to test whether this would allow to differentiate direct from synaptically driven activity.
Methods : Mouse large intestines were dissected, loaded with Fluo4-AM and visualized under an upright microscope. After the recording, tissues were fixed and processed for immunohistochemical staining using antibodies against peripherin and synaptotagmin to identify nerve fibers and vesicle release sites.
Results : We found that the system had sufficient sensitivity and resolution in space and time to resolve stepwise Ca2+ increases in individual varicosities during a 20Hz pulse train (30 varicosities, 7 ganglia, 4 mice). The response frequency was confirmed using Fourier transforms and varicosity localization using high resolution CCD camera recordings and post-hoc immunostaining.
Removal of extracellular Ca2+ (0mM Ca2+, 2mM EDTA) reduced the amplitude by 50% (4 ganglia, 2 mice) and TTX (10-6 M) abolished the responses (4 ganglia, 3 mice), indicating the need for action potential firing. To investigate whether responses were dependent on synaptic transmission we compared both in control and hexamethonium (10-4M) conditions the Ca2+ transients elicited by a 1 pulse stimulus, classically used to elicit fast excitatory postsynaptic potentials. A large fraction of the secondary responses (with delays of 50 ms) disappeared in the presence of hexamethonium, to reappear after washout.
The improved spatial resolution made it also possible to assess signal propagation in individual neuronal fibers. In 2kHz recordings, we were able to continuously monitor the signal as it travelled through ganglia and connecting fibers and calculated propagation speeds of about 80 mm/s (7 ganglia, 4 mice).
Conclusion : Improved sensitivity and spatial resolution further adds to the possibilities of Ca2+ imaging in that 1/ stepwise Ca2+ increases associated with action potentials can be monitored in individual varicosities ; 2/ direct and synaptically driven activity can be distinguished and 3/ signal propagation speed can be measured.

- B08 -

CORTICOTROPHIN-RELEASING FACTOR AND UROCORTINS IN THE HEALTHY AND INFLAMED MOUSE ILEUM. R. Buckinx (1), L.R. Avula (1), K. Alpaerts (1), L. Van Nassauw (2), J.P. Timmermans (1). (1) Laboratory Of Cell Biology And Histology, Department Of Veterinary Sciences, University Of Antwerp, Antwerpen, Belgium ; (2) Laboratory Of Human Anatomy And Embryology, Faculty Of Medicine & Health Sciences, University Of Antwerp, Antwerpen, Belgium.

Introduction : Corticotrophin releasing-factor (CRF) is most widely known for its role in the central nervous system, activating the hypothalamic-pituitary-adrenal axis in the stress response. Besides this central role, direct peripheral effects of CRF signaling affect gastrointestinal (GI) motility and permeability. Moreover, CRF influences inflammatory processes such as cytokine secretion and immune cell activation. However, apart from CRF proper, CRF receptors are also activated by urocortins (UCNs). The protein expression of these CRF-related peptides in the GI tract is to date unknown.
Aim : We have aimed to identify the cellular localization of CRF, UCN1, UCN2 and UCN3 in the healthy and inflamed mouse ileum.

Methods : The expression of CRF-related peptides was evaluated in the ileum of healthy and *Schistosoma mansoni*-infected mice. Using PCR, we have confirmed the presence of mRNA for CRF, UCN1, UCN2 and UCN3 in the ileum. We have further explored the cellular localization of the peptides using immunohistochemical stainings of both cryosections and whole mount preparations. Identification of extrinsic fibers was done using Fast Blue retrograde tracing.
Results : CRF was located in stromal cells close to the crypts of Lieberkuhn and in extrinsic neuronal fibers, but not in intrinsic neurons. UCN3, and to a lesser extent UCN1, were found in myenteric and submucosal neurons whereby the expression of UCN3 tends to be increased in the inflamed ileum. Moreover the tunica muscularis and muscularis mucosae showed clear immunoreactivity for both UCN2 and UCN3.
Conclusion : We demonstrate for the first time the presence of urocortin proteins in the mouse ileal wall, with an important expression in muscle tissue and enteric neurons. This is in contrast to CRF, of which we only identified an extrinsic neuronal expression, next to an immunoreactivity in some stromal cells. Previous research has shown that UCNs have a higher affinity and selectivity for CRF receptors than CRF itself. These observations thus suggest that urocortins, more than CRF proper, are the peripheral signaling molecules acting on CRF receptors in the ileum, and are involved in fine-tuning peripheral CRF-related gastrointestinal effects.

- B09 -

INFLUENCE OF SPRY4 KNOCK-OUT ON THE MAPK/ERK PATHWAY IN THE MOUSE WK641E GIST MODEL. P. Vandenberghe, A. Thys, P. Gromova, P. Hague, J.M. Vanderwinden. Ulb Faculty Of Medicine, Anderlecht, Belgium.

Introduction : We previously identified altered expression of Sprouty homologs (Spry), a family of regulators of the MAPK/Erk pathway, in the mouse WK641E GIST model with the upregulation of Spry4 and downregulation of Spry1 in the Kit-expressing cells, compared to wild-type (WT) controls (Gromova P. *et al*. JCMM, 2009).
Aim : Here we investigated the influence of Spry4 knock-out (KO) in the mouse WK641E GIST model.
Methods : WK641E (Rubin B. *et al*. Cancer Res., 2005) and Spry4KO (Klein O.D. *et al*. Dev. Cell., 2006) mice were mated to produce the desired genotypes. Antrum was dissected out at P14, fixed overnight in paraformaldehyde, cryopreserved in graded sucrose solutions, embedded in OCT and cut on a cryostat. Immunofluorescence was carried out using specific antibodies for Kit, Spry4 and (Thr202-Tyr204) phospho-Erk1/2 (pErk).
Results : In WT antrum, Spry4-ir and pErk-ir were detected in the myenteric ganglia and nerve fibres in the muscle layers, but not in the Kit-ir ICC while in WK641E homozygous antrum, both Spry4-ir and pErk-ir were detected in the hyperplastic Kit-ir cells as well as in myenteric ganglia and nerve fibres, as previously reported (Gromova P. *et al*. JCMM, 2009). In Spry4 KO antrum : Spry4-ir was undetectable and pErk-ir was present in the myenteric ganglia and nerve fibres in the muscle layers, but not in the Kit-ir ICC. Surprisingly, in WK641E homozygous/Spry4KO antrum, pErk-ir was absent in the hyperplastic Kit-ir cells, while strong pErk-ir was observed in the myenteric ganglia and nerve fibres.
Conclusion : While the oncogenic Kit mutation WK641E leads to Erk activation (phosphorylation) despite the upregulation of Spry4 in Kit-expressing cells, Spry4KO appears to induce adaptive changes interfering with WK641E regulatory pathways leading to the abolition of pErk-ir in the Kit-expressing cells. Modulation of expression of other members of the Spry family, namely Spry1 and Spry2, in WK641E homozygous/Spry4KO animals is currently under investigation using qPCR and IF. Quantitative assessment of Kit hyperplasia is also envisioned.

- B10 -

TISSUE CLARIFICATION & LIGHT SHEET MICROSCOPY : IS THIS THE END OF SLICING ? J.M. Vanderwinden, P. Hague. ULB Faculty Of Medicine, Anderlecht, Belgium.

Introduction : Histological diagnostic using tissue sections is a most useful technique. However, two dimensional (2D) sampling provides only limited insight into the volumetric relationships between components. Tissues such as the gut wall, in which structural anisotropy (i.e. the property of being directionally dependent) is a hallmark, would greatly benefit from gathering information in three dimensions. (3D).
Aim : Here we report on our recent experiments with tissue clarification and light sheet microscopy for fluorescence optical sectioning imaging of “thick” 3D structures.
Methods : Various paraformaldehyde-fixed mouse tissues (some expressing fluorescent proteins (FP), some labeled with the nuclear stain TOPRO), zebrafish embryos immunostained with Alexa488 and Cy3 and several insects were embedded in 1% agarose, dehydrated overnight in tetrahydrofuran (THF), cleared in benzyl ether (BE) (Erturk A. *et al*. Nature Medicine, 2011) and imaged using a dedicated light sheet microscope (LaVision BioTec, D) equipped with a

1.26-12.6 macro zoom objective (NA 0.5) and sCMOS camera (2560 x 2160 pixels). A white laser and filter sets (Ex. - Em.) 472/30 - 525/30, 542/27 - 595/40 and 617/73 - 680/30 were used to image green, red and far-red fluorescence, respectively. Imaris (Bitplane, CH) was used for 3D rendering.
Results : THF-BE cleared very efficiently most tissues, except for pigments in mouse skin and spleen. All FPs vanished during the overnight protocol used while autofluorescence (AF), TOPRO, Alexa488 and Cy3 were readily imaged. Volumes up to several millimeters were imaged in a matter of minutes with the light sheet microscope. Nominal maximal resolution was 0.6 micrometer in X-Y and 4 microns along Z axis. Photobleaching and alteration of image quality along the Z axis appeared moderate, while opaque/pigmented structures caused shadowing artefacts. Quantification of fluorescence intensity and image deconvolution were not attempted. AF provided fairly poor contrast in the mouse gut, except for immune cells, e.g. in Peyer's patches. Conversely, AF in insects, TOPRO and immunostaining in zebrafish embryo illustrate the potential of light sheet microscopy for "deep" imaging.
Conclusion : Clearing with THF-BE is effective and readily compatible with various classical fluorophores. Unfortunately, the procedure mandates tedious optimization for FPs (Erturk A. *et al*. Nature Protocols, 2012) - and possibly also for AF. In addition, BE is an hazardous chemical corrosive for most plastic ware and for microscope lenses. Light sheet microscopy, requiring dedicated expensive equipment, is unlikely to gain widespread acceptance. Nevertheless, it offers genuine asset for imaging large and thick samples and deserves further consideration, also for human material, which often exhibit marked AF.

- B11 -

HUC/D PROTEIN IN THE ENTERIC NERVOUS SYSTEM : MORE THAN A NEURONAL MARKER ? A.S. Desmet, C. Cirillo, J. Tack, P. Vanden Berghe. Translational Research Center for Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : The Enteric nervous System (ENS) is referred to as the "brain in the gut" and it consists of two main layers, the myenteric and the submucous plexus. Alterations in gastrointestinal motility associated with defects in the ENS have been described in patients with neurodegenerative diseases and can even be seen in the submucous plexus, accessible via routine intestinal biopsies. HuC/D protein is an RNA-binding protein routinely used to specifically label and quantify neurons ; however HuC/D labeling seems to be ambiguous in both the CNS and the ENS. Until now these observations were somewhat ignored.
Aim : We aimed to evaluate whether changes in HuC/D expression pattern occur in the submucous plexus of patients with intestinal motility disorders and in an *in vitro* animal model of neuronal damage.
Methods : Submucous plexus was obtained from duodenal biopsies taken from 16 control subjects (mean age 52 ± 1) and 19 patients with motility disorders (mean age 51 ± 5) referring to our gastroenterology unit. Myenteric plexus preparations were obtained from mouse intestine and used to mimic neuronal damage *in vitro* (0.1% Benzalkonium chloride application). Human and mouse preparations were then stained with HuC/D to identify neurons and with S100 to mark glial cells.
Results : No differences in the number of neurons/ganglion were observed in patients with motility disorders compared to control subjects (4.8 ± 0.1 vs. 4.4 ± 0.1, $p > 0.05$). However, in patients with motility disorders we found abnormalities in HuC/D expression pattern, in that the HuC/D immunoreactivity was present mainly in the neuronal nuclei while the glial network was distorted. These findings correlated well with the HuC/D labeling in mouse myenteric plexus, showing a more intense immunoreactivity in the nucleus than in the cytosol when neuronal damage was artificially induced.
Conclusion : Our findings support the idea that in diseased neurons, the HuC/D protein resides predominantly in the nucleus rather than in the cytosol, suggesting that the protein may translocate during neuronal damages to the nucleus. The functional meaning of such translocation needs to be further investigated both in humans and in animal models of neurodegeneration.

Invited Lecture
- B12 -

THE USE OF CELL-TYPE SPECIFIC GENETIC MODELS TO UNRAVEL SPECIFIC PATHWAYS IN THE GUT. A. Friebe. Wurzberg, Germany.

- B13 -

ABSENCE OF INTESTINAL INFLAMMATION AND POSTOPERATIVE ILEUS IN A LAPAROSCOPIC MOUSE MODEL. P.J. Gomez Pinilla (1), A. Lissens (1), M.M. Binda (1), M. Di Giovangiulio (1), A. Nemethova (1), G. Farro (2), S.H. Van Bree (3), G. Matteoli (1), J. Deprest (1), G. Boeckxstaens (1). (1) University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (2) Translational Research Center for Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (3) Academic Medical Center, Amsterdam, Netherlands.

Introduction : Postoperative ileus (POI) is characterized by impaired gastrointestinal motility resulting from manipulation-induced intestinal inflammation. The introduction of laparoscopic surgery has dramatically reduced the duration of POI. To what extent this results from a reduction in intestinal inflammation remains unclear.
Aim : In the present study, we compared the degree of intestinal inflammation and gastrointestinal transit following laparoscopic surgery and open abdominal surgery.
Methods : Laparoscopic surgery was performed in mice using a 2-mm endoscope with the abdominal cavity pressure kept at 15 mm Hg via CO2 insufflation. Standardized intestinal manipulation of the small bowel (IM) was applied using a plastic covered trocar (14 GA) and a grasper during laparoscopy (LAP) or after laparotomy (OPEN). 24 Hours after surgery, mice were gavage fed with FITC-Dextran (70KD) and gastrointestinal transit was determined 90 min later. To this end, the geometrical center (GC) was calculated as follows : S (percent of total fluorescent signal in each segment x the segment number)/100. Intestinal inflammation was assessed by the number of myeloperoxidase (MPO) positive cells and the level of cytokine expression. In addition, the duration of POI and the plasma levels of IL-6 and IL-1alpha were compared in patients undergoing open (n = 20) or laparoscopic (n = 26) colectomy. For human, data are presented as median and IQR.
Results : In mice IM following OPEN, but not during LAP induced a significant ($p < 0.05$) delay in gastrointestinal transit (OPEN, 8.5 ± 0.2 versus LAP, 10.3 ± 0.2) compared to laparotomy (10.0 ± 0.3) or laparoscopy (9.4 ± 0.4). The intestinal muscularis externa was infiltrated with MPO positive cells 24 hrs after OPEN but not following LAP (OPEN, 231 ± 30 versus LAP, 24 ± 5 positive cells).
In addition, pro-inflammatory cytokine expression was only up-regulated in the intestinal muscularis externa 24 hrs after IM in OPEN surgery (IL-6 ; 40.4 ± 7.2 x10-5, IL-1a 46.6 ± 12.9 x10-5 and IL-1alfa 34.3 ± 6.2 x10-5 respect to housekeeper gene (rlp32)) while no significant increase in genes expression was detected in IM during LAP (IL-6 ; 15.8 ± 2.6 x10-5, IL-1a 7.9 ± 1.7 x10-5 and IL-1alfa 4.1 ± 0.8 x10-5 respect to housekeeper gene (rlp32). In line, patients undergoing colectomy have significantly higher plasma levels of IL-6 and IL-1alfa in open colectomy compared to laparoscopic colectomy (IL-6 ; open : 228 pgr/ml (66-620), laparoscopic : 62 pgr/ml (23-93), IL-1alfa open : 114 fgr/ml (0-303), laparoscopic : 0 fgr/ml (0-64)).
In addition, the time until passing stool and tolerance of solid food was significantly increased after laparotomy compared to the laparoscopic approach (open : 96 hours (52-163), laparoscopic : 72 hours (44-97)).
Conclusion : Our data confirm that inflammation of the muscularis externa is underlying the delayed gastrointestinal transit observed after open surgery. Most importantly, we demonstrate that under laparoscopic conditions there is absence (mice) or reduction (human) of this inflammatory response, explaining the faster recovery following laparoscopy compared to open surgery.

- B14 -

ACUTE ANXIETY AND CHRONIC CO-MORBID ANXIETY IMPAIR GASTRIC ACCOMMODATION IN FUNCTIONAL DYSPEPSIA. N. Weltens (1), H.G. Ly (1), R. Vos (2), L. Holvoet (1), L. Van Oudenhove (1), J. Tack (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium.

Introduction : Functional Dyspepsia (FD) symptoms have been associated with both impaired gastric accommodation and psychiatric co-morbidity, especially anxiety & depression. Traditionally, gastric accommodation is quantified as the postprandial gastric volume increase averaged over one hour, thereby ignoring putative complex kinetics of the exact time course of this response. Further, experimentally induced acute anxiety has been shown to impair accommodation in healthy subjects. However, the putative interplay between psychopathology and gastric motor function in FD remains understudied.
Aim : To analyze in detail the time course of the postprandial gastric volume response (i.e. accommodation) in FD and to investigate whether this process is influenced by psychopathological factors.
Methods : 259 outpatients recently diagnosed with FD (Rome II criteria) underwent a standard gastric barostat investigation with liquid meal to assess accommodation. Generalized anxiety disorder (GAD), state anxiety & depression were measured using validated self-report questionnaires. Mixed models were used to analyze the time course of the postprandial gastric volume response. Psychological variables were added to the model as continuous (state anxiety) or

dichotomous (GAD & depression, based on established cutoffs) covariates and their potential interaction with linear, quadratic, third-, and fourth-order effects of time were assessed (only significant interactions were retained in the models).
Results : The postprandial gastric volume response could be modeled by a combination of significant linear, quadratic, third-, and fourth-order effects of time ($p < .001$). The linear effect represents an initial meal-induced gastric relaxation, followed by a slower increase to a maximum and return to baseline, indicated by the quadratic, third-, and fourth-order effects, respectively. A significant main effect of state anxiety (beta = -1.7, $p = .012$) was found, indicating that this covariate impairs accommodation irrespective of time after meal (i.e. shifting the whole curve downwards). For GAD, significant time-by-GAD (beta = 10.3, $p = .0015$), $time^2$-by-GAD (beta = -0.4, $p = .0036$) and $time^3$-by-GAD (beta = .005, $p = .0016$) interaction effects indicate that this covariate is associated with a significantly slower initial postprandial gastric volume increase and return to baseline. No significant effects of depression were found.
Conclusion : To the best of our knowledge, we are the first to report that the postprandial gastric volume response in FD can be modelled by a combination of linear, quadratic, third-, and fourth-order effects of time. Furthermore, we show that gastric accommodation is impaired by both acute (state) anxiety and chronic co-morbid (generalized) anxiety in FD. These findings may help elucidate the complex interaction between psychopathology and gastric motor dysfunction in FD.

- B15 -

THE NON-DIABETIC BB-RAT : A SPONTANEOUS MODEL FOR IMPAIRED GASTRIC ACCOMMODATION.
C. Vanormelingen, R. Farré, T. Vanuytsel, T. Masaoka, S. Salim Rasoel, J. Toth, T. Thijs, H. Vanheel, L. Van Oudenhove, I. Depoortere, P. Vanden Berghe, J. Tack. Translational Research Center For Gastrointestinal Disorders (Targid), Kuleuven, Leuven, Belgium.

Introduction : Nitric oxide (NO) is an important mediator of gastric accommodation to a meal. Intestinal inflammation leads to loss of nitrergic myenteric neurons and disturbed motor function, but spontaneous animal models to study the relationship between these changes are missing. The Biobreeding (BB) rat consists of a diabetes-resistant (BBDR), control strain, and a diabetes-prone (BBDP) strain. In our facility 50% of the BBDP rats develop diabetes after the age of 90 days. BBDP rats develop ganglionic inflammation, loss of nNOS expression and nitrergic motor control in the small intestine, independently of hyperglycemia.
Aim : To evaluate the neuromuscular neurotransmission and the presence of inflammation in the gastric fundus of different BB rat strains.
Methods : Gastric fundus muscle strips of rats 70 and 220 days old (control, non-diabetic (BBDP) and diabetic (BBDP-H) (N = 6)), were suspended along their circular axis. Responses to electrical field stimulation (EFS ; 8V, 35 ms and 1-16 Hz) under NANC conditions were evaluated, as well as the impact of NO synthase inhibitor L-NAME ($3 \times 10\text{-}4$) and the P2Y1 receptor antagonist MRS2179 (10-5), separately or combined. Total relaxation during stimulation was evaluated as area under the curve (AUC), and corrected for cross-sectional area and weight. Nitrergic and P2Y1 mediated components were evaluated by the relaxation under L-NAME and MRS2179. Statistics for strip experiments were done using mixed model analysis. Expression of nNOS was quantified by Western blot relative to PGP9.5. Myeloperoxidase (MPO)-activity was determined for segments of mucosa and muscularis propria and inflammation was also evaluated histologically.
Results : Relaxation under NANC conditions was reduced at all frequencies in BBDP and BBDP-H rats of 220 days when compared to control rats (eg at 1 Hz, 29 ± 2.5 and 30 ± 3 vs. 55 ± 1 g/mm2/s ; $p < 0.01$). In all animals, muscle relaxation was inhibited by L-NAME and MRS2179. The nitrergic component was significantly smaller in BBDP (70 and 220 days) and BBDP-H (220 days) rats compared to controls. Significant loss of nNOS proteins was seen in BBDP rats of 220 days but not at 70 days. The P2Y1 component was significantly impaired in BBDP-H rats of 220 days. MPO activity was increased in the fundic mucosa and muscularis propria of BBDP (70 and 220 days) and BBDP-H (220 days) rats compared to controls, and this was confirmed by a significant increase in polymorphonuclear cells (PMN) on histology.
Conclusion : BBDP rats showed altered fundic muscle function, which is at least partially explained by loss of nitrergic function in the myenteric plexus, and may be related to local inflammation. These fundic changes develop independently from diabetes. The non-diabetic BBDP rat may provide a spontaneous model for post-inflammatory impaired gastric accommodation.

- B16 -

BAY 58-2667 DOES NOT IMPROVE DELAYED GASTRIC EMPTYING IN APO-SGC MICE. S. Cosyns (1), J.P. Stasch (2), P. Brouckaert (3), R.A. Lefebvre (1). (1) Heymans Institute of Pharmacology, Gent, Belgium ; (2) Institute of Cardiovascular Research Bayer Healthcare, Wuppertal, Germany ; (3) Department of Biomedical Molecular Biology, Gent, Belgium.

Introduction : In apo-sGC mice (his105phe mutation in sGCbeta1) both sGCa1beta1 and a2beta1 are heme-deficient and can no longer be activated by NO ; these mice can thus be considered as a model for oxidized/heme-free sGC. Gastrointestinal consequences of lacking NO-sensitive sGC are most pronounced at the level of the stomach ; apo-sGC mice show an enlarged stomach, hypertrophy of the smooth muscle layers of fundus and pylorus and delayed gastric emptying. Vascular relaxation by the NO- and heme-independent activator BAY 58-2667 is increased when sGC is oxidized and loses its heme group. The gastric fundus of apo-sGC mice is relaxed more efficiently by BAY 58-2667 than that of WT mice. We now tested *in vivo* whether BAY 58-2667 could restore the delayed gastric emptying in apo-sGC mice.
Methods : In 16h fasting WT and apo-sGC mice, BAY 58-2667 or its solvent were administered intraperitoneally (IP ; 300 μg/kg) or intravenously (IV ; 300, 100 or 30 μg/kg) and 15 min (IP injections) or 5 min (IV injections) later 250 mL of a liquid phenol red meal was gavaged. Fifteen min after gavage, mice were sacrificed by cervical dislocation and the stomach and small bowel were excised. Gastric emptying was calculated as the amount of phenol red that left the stomach as % of the total amount of phenol red recovered. *In vitro*, the influence of BAY 58-2667 (10 μM) was studied on pyloric rings of WT and apo-sGC mice.
Results : Gastric emptying was significantly delayed in apo-sGC mice compared to WT mice ($P < 0.001$ for gastric emptying after IP administration of solvent in apo-sGC [16 ± 5% gastric emptying] versus WT mice [63 ± 3% gastric emptying] ; $P < 0.01$ for gastric emptying after IV administration of solvent in apo-sGC versus WT mice). IP administration of BAY 58-2667 (300 μg/kg) did not improve the delayed gastric emptying in apo-sGC mice. IV injection of BAY 58-2667 (300 μg/kg and 100 μg/kg) delayed gastric emptying significantly in WT mice ($P < 0.05$), but had no influence on gastric emptying in apo-sGC mice. IV injection of a lower dose of BAY 58-2667 (30 μg/kg) did not have this inhibitory effect on gastric emptying in the WT mice, but it was also not able to improve the delayed gastric emptying in apo-sGC mice. *In vitro*, BAY 58-2667 induced relaxation in WT pyloric rings (12 ± 4% of the spontaneous tone present in the pyloric rings at a load of 0.25 g, $n = 7$), but did not relax apo-sGC pyloric rings ($n = 7$).
Conclusion : The NO- and heme-independent sGC activator BAY 58-2667 does not improve delayed gastric emptying in apo-sGC mice. This might be related to its inability to relax the pylorus of apo-sGC mice.

- B17 -

ENDOCRINE AND SMOOTH MUSCLE RESPONSES OF THE BITTER AGONISTS, DENATONIUM BENZOATE, IN MOUSE STOMACH. B. Avau, T. Thijs, J. Laermans, J. Tack, I. Depoortere. Translational Research Center for Gastrointestinal Disorders (Targid), KUleuven, Leuven, Belgium.

Introduction : Bitter taste receptors, T2R, and their associated G-proteins, gustducin and transducin, and the downstream signaling cation channel, TRPM-5, have been identified in the gut. Their presence suggests a functional role as nutrient sensors in gastrointestinal responses to ingested bitter compounds. *In vivo* studies from our group have shown that intragastric administration of bitter agonists affects ghrelin release, food intake and gastric emptying (Janssen *et al*. PNAS, 2011, 108 : 2094).
Aim : The aim of this study was to elucidate the mechanisms behind the observed effects of the bitter agonist, denatonium benzoate (DB), on ghrelin release and smooth muscle contractility.
Methods : The effect of DB on the release of ghrelin was studied by radioimmunoassay in the ghrelinoma cell line, MGN3-1. The response of fundic smooth muscle strips from wildtype, a-gustducin-/- and TRPM5-/- mice to cumulative concentrations of DB was measured isometrically in the absence or presence of pharmacological blockers. The expression of the bitter taste receptor for DB, T2R108, was demonstrated by real-time PCR.
Results : Stimulating ghrelinoma cells with DB resulted in a dose-dependent release of octanoyl ghrelin (EC50 : 84 μM), with a maximal increase (73 ± 18%) at 500 μM. At higher concentrations, the ghrelin release started to decline. Preincubation of the cells with the bitter taste receptor blocker, probenecid, inhibited ghrelin release with 76% ($P < 0.05$). The expression of T2R108 mRNA was confirmed in fundic smooth muscle and mucosa. The contractile response to DB in fundic smooth muscle strips was bell-shaped. DB (10-5-10-4M) caused a TTX-insensitive contraction (13 ± 1% of ACh), followed by a pronounced relaxation (88 ± 1% of 10 μM nitroglycerine) at higher concentrations. The contraction was enhanced (32 ± 1% of ACh) by treatment with the Ca2+-activated K+ channel blocker, charybdotoxin, but not by iberiotoxin, apamin or TRAM-34. The DB-induced contraction under charibdotoxin was reduced by the PLCbeta-blocker, U73122, and the PKC inhibitor, GF109203X. Thapsigargin, which depleted intracellular Ca2+-stores, the IP3-

receptor blocker, 2-APB, the L-type Ca2+ channel blocker, nifedipine, abolished DB-induced contractions. The response to DB in muscle strips from a-gustducin-/- mice was reduced with 47%, but was not affected in TRPM5-/- mice. The relaxation at higher concentrations could only be blocked by the PKC inhibitor, calphostin C.
Conclusion : The bitter agonist, DB, induces ghrelin release from ghrelinoma cells via bitter taste receptor signaling. In smooth muscle strips, DB activates the G-protein, gustducin, resulting in Ca2+-release from intracellular stores and influx via L-type Ca2+-channels. The resulting contraction is partially masked by the DB-induced activation of Ca2+-activated K+-channels. The relaxation at higher concentrations is mediated via a PKC-dependent mechanism. Our studies indicate a functional role for bitter taste receptors in gastric endocrine and smooth muscle cells.

- B18 -

INTRAGASTRIC ADMINISTRATION OF DENATONIUM BENZOATE DECREASES HUNGER AND THE OCCURENCE OF PHASE III. E. Deloose, M. Corsetti, L. Van Oudenhove, I. Depoortere, J. Tack. Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : In mice, intragastric administration of a mixture of bitter agonists, including denatonium benzoate (DB), induced a prolonged suppression of food intake (Janssen, *PNAS* 2011). We recently reported a close correlation between gastric phase 3 (ph3) of the migrating motor complex (MMC) and hunger peaks (Deloose, *DDW* 2012).
Aim : The aim of the present study was to investigate the effect of DB on the occurrence of ph3 and hunger ratings in man.
Methods : After an overnight fast, 11 healthy volunteers (6 men ; 26 ± 8 years ; 24 ± 2kg/m^2) underwent a 36-channel antroduodenal high resolution manometry twice, with at least one week interval. Fasting motility was recorded for a full MMC cycle (2 ph3). 20 min after the occurrence of the 2nd ph3, DB (1μmol/kg) or placebo was administered intragastrically in a double-blind randomized fashion through a nasogastric tube, to bypass the tongue. Motility recording continued until the next ph3 or 3 hrs. Subjects scored hunger every 5 min on a 10 cm visual analogue scale (VAS).A baseline hunger score was calculated by averaging VAS ratings during 10 min before administration of DB or placebo. This baseline score was subtracted from the hunger rating at each time point until 60 min after administration. The resulting delta hunger VAS scores were entered as the dependent variable in a linear mixed model analysis with time and administered agent (DB vs placebo) as continuous and categorical independent variables, respectively. The effect of interest is the interaction between time and agent, representing the difference in linear slope of the delta hunger scores between DB and placebo. Proportions were analyzed using Chi-square. Data are mean ± SEM.
Results : Baseline hunger scores did not differ between placebo and DB (58 ± 9 vs 47 ± 8 mm VAS ; $p = 0.7$). The slopes of delta hunger differed significantly between placebo and DB (time-by-agent interaction effect $p = .0003$). While mean hunger ratings remained stable after administration of placebo (slope of delta hunger 0.03 ± 0.03 ; $p = .2$), a progressive significant decrease in hunger occurred after DB (slope of delta hunger -0.11 ± 0.03 ; $p = .0012$).The interval between baseline ph3 (98 ± 19 vs 85 ± 14 min ; $p = .8$) as well as their origin (58% vs 57% gastric ; $p = .9$) did not differ significantly between the two treatment days. During the 60 min interval after placebo, a ph3 occurred in 36% of the volunteers compared to only 11% after DB ($p < .0001$). 75% of the ph3 were gastric after placebo compared to 0% after DB ($p < .0001$). DB was generally well tolerated, with only 1 subject reporting abdominal cramps on the night after the experiment.
Conclusion : Administration of DB decreases hunger ratings and inhibits the occurrence of ph3 with a gastric origin. These data suggest potential involvement of GI bitter taste receptors in the control of hunger and interdigestive motility in man.

- B19 -

LIRAGLUTIDE IMPROVES PARAMETERS OF LATE DUMPING SYNDROME DURING OGTT IN PATIENTS WITHOUT GASTRECTOMY. T. Vanuytsel (1), R. Bisschops (2), L. Holvoet (1), P. Caenepeel (2), J. Arts (2), S. Kindt (2), G. Boeckxstaens (1), M. Lannoo (2), E. Deloose (1), C. Andrews (1), J. Tack (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium, (2) University Hospitals Leuven, Leuven, Belgium.

Introduction : Dumping syndrome is a common complication of esophageal and gastric surgery. Therapeutic options in patients with symptoms resistant to dietary measures are limited. Rapid delivery of the hyperosmolar, carbohydrate-rich contents from the stomach into the duodenum, and their spread through the small bowel, are the key mechanism for induction of early and late dumping symptoms. Long-acting glucagon-like peptide 1 (GLP-1) analogues are used in the treatment of type 2 diabetes mellitus. GLP-1 also slows down gastric emptying, is involved in the ileal brake and inhibits

intestinal motility. These direct gastrointestinal effects of GLP-1 analogues may prove beneficial in the treatment of dumping syndrome, especially in patients with a preserved antropyloric region.
Aim : To conduct a pilot mechanistic study assessing the therapeutic potential of the long-acting GLP-1 analogue liraglutide in dumping syndrome.
Methods : Twelve patients (9 female, 50 ± 3 ys) with dumping syndrome, evidenced by oral glucose tolerance test (OGTT), a score of 10 or more on the dumping syndrome severity score (DSSS) (Arts, *CGH* 2009) and insufficient response to dietary measures, were studied in this exploratory study. An OGTT was performed before and after a 2-day treatment with subcutaneous liraglutide 0.6 mg once daily. Glycemia, hematocrit, heart rate and blood pressure were measured at 30 min intervals after the ingestion of 75g glucose for 180 min. A subgroup analysis was performed for the 6 patients (3 female, 51 ± 4 ys) with an intact stomach. The OGTT parameters before and after liraglutide treatment were compared using a mixed model analysis with time and treatment as within-subject factors. Nadir glycemia was compared by a paired t-test. Proportions were compared by McNemar's test.
Results : Liraglutide had no significant effect on the glycemia in the mixed patient population (nadir 67 ± 4 vs. 60 ± 5 mg/dl ; $p = 0.35$), but increased the nadir glycemia in patients without gastrectomy (71 ± 3 vs. 55 ± 6 mg/dl ; $p = 0.02$). Before and after liraglutide, the OGTT was pathological based on glycemia criteria (< 60 mg/dl between 120 and 180 min) in 7/12 vs. 2/12 patients in the mixed population ($p = 0.10$) and 5/6 vs. 0/6 in the patients without gastrectomy ($p < 0.05$) respectively. Changes in hematocrit were not affected by liraglutide. The proportion of patients with more than 10% increase in heartrate at 30 minutes was lower after liraglutide in both the entire population (9/12 vs. 3/12 ; $p = 0.01$) and in those without gastrectomy (4/6 vs. 1/6 ; $p = 0.08$).
Conclusion : Liraglutide, a long-acting GLP-1 analogue, improved pathophysiological correlates of late dumping symptoms in patients without gastrectomy and had minor effects on early dumping parameters. These results provide ground for further large-scale clinical evaluation of liraglutide in dumping syndrome patients without gastrectomy.

- B20 -

ROME III FUNCTIONAL DYSPEPSIA SUBDIVISION IN PDS AND EPS :RECOGNIZING POSTPRANDIAL SYMPTOMS REDUCES. F. Carbone (1), L. Holvoet (2), C. Andrews (1), J. Tack (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium.

Introduction : The Rome III consensus proposed to subdivide functional dyspepsia (FD) into 2 groups : meal-related dyspepsia or postprandial distress syndrome (PDS), and meal-unrelated dyspepsia or epigastric pain syndrome (EPS). However, in clinical practice, overlap between both has been reported to be as high as 50%, thereby hampering clinical applicability. Although EPS is referred to as meal-unrelated dyspepsia, relationship of symptoms to meal ingestion in this category is not formally addressed in the Rome III criteria.
Aim : The ***aim*** of our study was to investigate whether taking into account the relationship of epigastric pain and nausea to meal ingestion may help to improve separation between EPS and PDS.
Methods : Consecutive ambulatory tertiary-care patients with epigastric symptoms filled out Rome III gastro-duodenal questionnaires with supplementary questions. Those fulfilling Rome III FD criteria and a negative endoscopy were identified and subdivided into "pure" PDS patients (i.e. meeting criteria for PDS without EPS symptoms), "pure" EPS (i.e. meeting criteria for EPS without PDS symptoms), and overlapping PDS-EPS (i.e. symptoms of both PDS and EPS). Proportions of patients with symptoms were compared using Fisher's exact test.
Results : Out of 598 patients, 80 (age 46 ± 1.7 years ; 65% female) fulfilled Rome III FD diagnostic criteria, and could be subdivided into pure PDS (29%, age 48 ± 2.8 years, 73% female), pure EPS (17%, age 46 ± 4.9 years, 62% female) and overlapping PDS-EPS (55%, age 44 ± 2.3 years, 62% female). Symptom occurrence ratings were highest in the PDS-EPS patients, but these included a high proportion of meal-related symptoms, including postprandial fullness (94%) and early satiation (80%), but also postprandial epigastric pain, postprandial nausea and belching, whose relationship to meals was not specified in the questionnaire. Compared to pure EPS patients, the overlap PDS-EPS patients were characterized by a higher occurrence of postprandial epigastric pain (70% vs. 31%, $p < 0.0001$), while the occurrence of epigastric pain in between meals did not differ (45% vs. 46%). In addition, the overlap PDS-EPS patients reported a higher occurrence of postprandial nausea (34% vs. 0%, $p < 0.0001$), and belching (56% vs. 23%, $p < 0.0001$). When postprandial epigastric pain and postprandial nausea were considered as PDS symptoms, the "adapted" subdivision identified 52% pure PDS, 17% pure EPS and only 31% overlapping PDS-EPS patients.
Conclusion : EPS and PDS symptoms frequently coexist in FD patients, with postprandial symptoms substantially contributing to the overlap. A more rigorous linking of postprandially occurring symptoms to PDS, regardless of their qualitative nature, may improve the separation between PDS and EPS. Further evaluation in a larger patient population is warranted.

EFFECT OF THE H1-RECEPTOR ANTAGONIST EBASTIN ON VISCERAL PERCEPTION AND CLINICAL SYMPTOMS IN IBS. S. Van Wanrooij, M. Wouters, L. Van Oudenhove, S. Vermeire, P. Rutgeerts, G. Boeckxstaens. Translational Research Center for Gastrointestinal Disorders (Targid), Kuleuven, Leuven, Belgium.

Introduction : Previously we reported that the non-specific mast cell stabilizer and histamine 1 receptor (H1R) blocker ketotifen improves VH and IBS symptoms. As no reduction in release of mast cell mediators was observed, we hypothesized that most likely H1R blockade was underlying the beneficial effect of ketotifen. To evaluate our hypothesis, we performed a double blind randomized controlled trial evaluating the effects of the H1R-antagonist ebastin in IBS patients.
Methods : IBS patients with positive Rome III criteria were randomized to receive ebastin or placebo treatment during 12 weeks, and were stratified by visceral sensitivity (normo- vs hypersensitive). Sensitivity to rectal distensions was assessed before and after treatment. A patient unable to tolerate a distension of 21 mmHg above minimal distension pressure (MDP) was considered hypersensitive. IBS symptoms and health related quality of life were scored before, during and after treatment using the following questionnaires : SGA of relief and abdominal pain, GSRS and IBSQOL. Primary endpoint was a reduction in VAS score during +21mmHg distensions of at least 15%. Secondary endpoints were global symptom relief, abdominal symptoms, bowel habits and quality of life. Clinical outcomes were evaluated in a linear mixed model, with treatment effects as a dependent and time as a fixed variable.
Trial registration number : NCT01144832
Results : 55 IBS patients (62% female, 35 ± 12) were randomly assigned to ebastin (N = 28) or placebo (N = 27). VH to rectal distension was present in 45% of patients. Symptom scores evoked by rectal distention (+3, +9 and +21 mmHg above MDP) were not significantly influenced by ebastin. During the 12 weeks there was a significant linear reduction of abdominal pain under ebastin (slope = -1.35, $p < 0.0001$), but not during placebo (slope -0.29, $p = 0.4$). This effect was significant over placebo ($p = 0.03$). At the end of the 12 weeks treatment, significantly more patients treated with ebastin had at least considerable relief of symptoms (46% vs 12%, $p = 0.01$, chi2-test), and reported lower abdominal pain VAS scores (ebastin 38 ± 5, placebo 62 ± 4) compared to the placebo group. Other abdominal symptoms, scored using the GSRS questionnaire, were not altered during ebastin treatment. QoL was significantly improved on all IBS-QOL subscales in the ebastin group compared to baseline and placebo. Finally, no difference in response to ebastin was observed between the hypersensitive and normosensitive subgroups.
Conclusion : Although we failed to show a significant effect on visceral perception assessed by rectal distension, ebastin resulted in a significant improvement in global symptom relief, abdominal pain, and QoL compared to placebo. These data indicate a poor (or even lack of) relationship between barostat findings and clinical response, but most importantly demonstrate that H1R blockade may represent an effective treatment for IBS.

ANTIDIARRHOEAL AGENTS AND PARACELLULAR PERMEABILITY OF E COLI-INFECTED CACO-GOBLET INTESTINAL MODEL. B. De Servi (1), M. Meloni (2). (1) N/A Vitroscreen Srl, Milan, Italy ; (2) N/A Vitroscreen Srl, Milan, Italy.

Introduction : Diarrhoea continues to be an important cause of morbidity and mortality worldwide, in spite of the advances in health technology, improved management, and increased use of oral rehydration solutions in recent decades, persisting as a major cause of death in children under five years of age. It has been demonstrated that gelatine tannate protects intestinal cells from damage induced by *Escherichia coli* whilst also preventing *E coli* adhesion in Caco-Goblet® intestinal epithelium model. The present study aims to evaluate this dual-inhibitory effect upon *E coli* for a new product combining gelatine tannate with a mixture of probiotics (TSC Duo) as compared to other antidiarrhoeal agents already available in the market.
Aim : We assessed the efficacy of TSC Duo (5 mg/mL) in counteracting the increase in paracellular permeability induced by *E coli* to diosmectite (5 mg/mL), a probiotic mixture (12.5 mg/mL), and *Saccharomyces boulardii* (12.5 mg/mL) using an *in vitro* intestinal epithelial model (Caco-Goblet) pre-infected with *E coli*.
Methods : The Caco-2 monolayer is a relevant, well-established model that recreates *in vitro* the intestinal mucosa deprived of mucous cells ; the modified model Caco-Goblet, including mucus-secreting goblet cells (HT 29-MTX), represents a more predictive model to study paracellular permeability and product interaction with mucus. After pre-inoculation with *E coli* at the concentration of 1e+08 CFU/mL for 30 minutes, we tested the effect of the above antidiarrhoeal agents applied for either 1 h or 24 h onto to the Caco-Goblet cells. Cell permeability was measured as percent change in transepithelial electrical resistance (TEER) as well as passage of Lucifer Yellow (LY).
Results : TSC Duo was able to counteract the negative effect of E coli on TEER (reduction) by as much as 123.08% and 149.54% of recovery after 1 h or 24 h of treatment, respectively ; the probiotic mixture was effective at inducing a TEER recovery of 107.17% at 1 h but only 17.92% at 24h. These values were significantly lower for diosmectite and

S boulardii (1 h : 68.81% and 16.05%, respectively ; 24 h : -4.50% and 11.42%, respectively). Similarly, the passage of LY was significantly reduced following treatment with TSC duo for 1 h and 24 h (0.41 ± 0.00 and 1.34 ± 0.34, respectively) as compared to diosmectite (1.90 ± 0.32 and 2.30 ± 0.14, respectively), probiotic mixture alone (0.54 ± 0.02 and 6.44 ± 0.42, respectively) and *S boulardii* (1.70 ± 0.04 and 4.16 ± 1.20, respectively).

Conclusion : The most significant recovery of barrier integrity and fence properties as shown by TEER and LY results, respectively, for gelatine tannate combined with probiotics could be explained by its positive interaction with the mucous proteins in the epithelial surface. These findings obtained on a biologically relevant and well-established *in vitro* model are of utmost importance, contributing to the increasing body of evidence that gelatine tannate, either alone or combined with probiotics, protects intestinal cells from *E coli* infection by inhibiting the adhesion and internalisation of bacteria, preventing the increase of tight junction permeability and modulating cytokine gene expression.

CASE REPORTS

- C01 -

GIANT FILIFORM POLYPOSIS REVEALED BY INTESTINAL OBSTRUCTION IN A PATIENT WITH NORMAL COLON. G. Mavrogenis (1), P. Ngendahayo (2), P. Kisoka (1), M.L. Nicholas (1), E. Kovacs (1), Y. Hoebeke (1), P. Warzee (1). (1) Notre Dame, Charleroi, Belgium ; (2) IRSPG, Gosselies, Belgium.

A 31-year-old patient with no previous history of inflammatory bowel disease (IBD) was addressed for further evaluation of recurrent episodes of intestinal obstruction. An abdominal CT-scan revealed wall thickening of the ascending colon and dilated small bowel loops. Colonoscopy disclosed a large polypoid mass with finger-like projections in the right colon. Progression of the endoscope beyond this point was not possible. Biopsies were negative for malignancy. Prompted by the suspicious endoscopic appearance of the lesion and the signs of intestinal obstruction, a right-sided hemicolectomy was performed. The resected specimen included a mass with innumerable clustered polyps with frequent branches almost completely obliterating the lumen of the colon. Microscopic examination was consistent with giant filiform polyposis. The non-polypoid mucosa of the terminal ileum and right colon was normal. Filiform polyposis (FP) of the colon is a rare entity, usually encountered in the colon of patients with IBD. However, single cases of FP have been reported in patients with Histiocytosis X, intestinal tuberculosis or ischemic colitis. FP is morphologically characterized by multiple slender worm-like projections consisting of submucosal cores lined with normal mucosa. The polyps can range in size from 1.5-3 cm in length and up to 0.5 cm in diameter. When these polyps adhere to each other, they form large tumor-like masses. A Pubmed research revealed less than twenty cases of FP without history of IBD. Among them, in only three cases the lesion was circumferential, measuring 4 to 15 cm in largest diameter. FP alone is not an indication for surgical resection, but complications such as acute massive haemorrhage, intussusception or intestinal obstruction may necessitate surgical intervention.

- C02 -

REVERSAL OF PORPHYRIA CUTANEA TARDA AFTER HCV TREATMENT. L. Vonghia (1), V. Van Marck (2), A. Bervoets (3), J. Lambert (3), P. Pelckmans (1), P. Michielsen (1), S. Francque (1). (1) Department Of Gastroenterology And Hepatology, Antwerp University Hospital, Antwerpen, Belgium ; (2) Department of Pathology, Antwerp Universitary Hospital, Antwerpen, Belgium ; (3) Department Of Dermatology, University Hospital Antwerp, Antwerpen, Belgium.

A 57-year-old man presented at our department for disturbed liver tests : ALT 530 U/L (nv : 7-56U/L), AST 263 U/L (nv : 5-40 U/L), Alkaline Phosphatase 190 U/L (nv :36-95 U/L), GammaGT 465 U/L (nv : 13-45 U/L). Moreover, he presented skin lesions (erosions on hands, ears and nose), hypertrichosis, skin photosensitivity and weight loss (BMI at presentation 19.8). The medical history was positive for alcohol abuse, cannabis use and nicotine abuse. He was then investigated for infectious diseases : HCV-Ab positive and HCV-RNA 5.68 × 10E6 IU/ml/ (6,75log IU/ml), Genotype 1a, Human Immunodeficiency virus (HIV)-Ab negative, HAV-Ab IgG positive and IgM negative, HbsAb positive, HbcAb, HbeAb, HbcAg, HBsAg, HbeAg negative. Futher serological investigations showed positive ANA nuclear (1/80 fine-speckled) and negative ANA cytoplasmic positive (1/80 granular), Anti-ds DNA, anti ENA, SSA, SSB, Sm/rnp, SCl70, Jo1, CENP-B, AMA and Anti-skin antibodies. Iron and ferritine levels were within normal limits (respectively 77 microg/dl ; nv : 49-181 microg/dl and 315 ng/ml ; 30-400 ng/ml) and iron saturation was low (19% ; nv : 22-55%). The liver disease assessment was completed by an abdominal ultasound that showed a slight hyperechogenic liver parenchyma, and a subsequent liver biopsy showing an unspecific chronic active hepatitis with lobular inflammation and periportal fibrosis with some porto-portal septa (METAVIR A2F2). Moreover, a 24 hour-urine collection showed elavated porphyrin levels (3694.9 microg/24 h ; NV 50-200 microg/24 h). The biopsy performed on skin erosions on the wrist evidenced the following : a wide ulceration covered by a crust, a slight epidermal hyperplasia, dilated dermal bloodvessels with PAS positive wall, absence of significant dermal inflammatory infiltrate. The immunofluorescence showed positivity for IgM and C3 and negativity for IgA, IgG and C1q. These histological findings, together with clinical manifestations, were indicative of Porphyria Cutanea Tarda. A treatment with Peginterferon and Ribavirin for 48 weeks was started and was successful. In fact, the patient had a rapid viral response followed by a sustained virological response with a reversal of liver enzymes to normal (ALT 13 UI/l, AST 16 UI/L). At 12 weeks of treatment porphyrin levels at 24 hour-urine collection returned within normal values (168,4 microg/24h ; NV 50-200 microg/24 h) and the skin lesions improved. Porphyria Cutanea Tarda results from reduced activity of uroporphyrinogen decarboxylase (UROD). It is mainly an acquired disease (type 1, 75% of cases), related to iron defects, HCV, HIV, alcohol, drug use, estrogen and smoking. However it can also be due to inherited UROD partial defects (type 2, 20%) or to other inherited defects (type3, < 1%). The clinical presentation is characterized by blisters, bullae, increased fragility, scarring and hyper- and hypopigmentation affecting sun-exposed areas of the body, hirsutism and photosensitivity. Sunlight

avoidance, phlebotomy or iron chelation, chloroquine/hydroxychloroquine, HCV treatment are the therapeutical option in this setting.

- C03 -

COMBINED KIDNEY/INTESTINAL TRANSPLANT FOR ENTERIC HYPEROXALURIA SECONDARY TO SHORT BOWEL SYNDROME. L.J. Ceulemans (1), Y. Nijs (1), F. Nuytens (1), K. Claes (2), B. Bammens (2), M. Naesens (2), P. Evenepoel (2), D. Kuypers (2), D. Monbaliu (1), J. Pirenne (1). (1) Abdominal Transplant Surgery, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (2) Nephrology And Renal Transplantation, University Hospitals Leuven, KULeuven, Leuven, Belgium.

Introduction : Intestinal transplantation (ITx) is indicated for irreversible bowel failure and complications of total parenteral nutrition (TPN). ITx has been performed as part of multivisceral grafts including the liver or other splanchnic organs and sporadically the kidney. In contrast, only one case report of combined KTx and ITx (CKITx) -not as part of a multivisceral graft- can be found in a patient with an indication for a second KTx and suffering from encapsulating peritoneal sclerosis. We report two patients with renal failure due to enteric hyperoxaluria secondary to short bowel syndrome (SBS) in whom we performed CKITx to prevent recurrence of hyperoxaluria.
Case reports :
Case 1 : A 41-year-old female, TPN-dependent after complete small bowel resection due to volvulus, developed end-stage renal disease (ESRD) secondary to enteric hyperoxaluria. An entire small bowel and kidney were transplanted (7-year-old donor). The postoperative course was uneventful. She developed an acute rejection 46 months postTx due to non-compliance, but fully recovered. Currently -67 months postTx- she is TPN-/dialysis-free.
Case 2 : A 56-year-old female, suffering from Crohn, became TPN-dependent after multiple bowel resections. Simultaneously, she developed secondary hyperoxaluria leading to ESRD. An entire small bowel and kidney were transplanted (9-year-old donor). 9 months post-Tx, she developed hydronephrosis due to ureteral calculi, reversible by placement of a double-J-stent. Recurrence of enteric hyperoxaluria was excluded showing normal oxalate urinary excretion (20 mg/24 h ; normal :10-41 mg/24 h) and normal oxalate/creatinine ratio (27mg/g ; normal : < 32 mg/g). Currently, 11 months post-Tx, she is TPN-/dialysis-free.
Discussion : In primary hyperoxaluria (caused by *excessive endogenous production* of oxalate in the liver) liver Tx is usually recommended in addition to KTx to prevent disease recurrence. We report 2 patients with secondary hyperoxaluria (as a consequence of *excessive absorption* of oxalate) in whom we performed an additional ITx and prevented hyperoxaluria recurrence. No other case of CKITx for this indication has been reported.
It may be that -in similar situations- preference was given to isolated KTx to avoid additional morbidity and mortality, associated with ITx. It is also possible that CKITx has been performed but not reported. The Intestinal Transplant Registry (ITR) indicates that of 534 isolated ITx (2005-2012), 19 (3.5%) were combined with KTx (Personal communication/ITR). CKITx may have also been performed in patients primarily referred for ITx and suffering from simultaneous renal failure. Indeed, post-Tx renal failure increases the risk of mortality by a factor > 4 and therefore, *pre-emptive* KTx will decrease this risk.
A putative advantage of CKITx is that the kidney function can be used as a surrogate for bowel rejection (as in combined kidney/pancreas transplantation).
Conclusion : CKITx is a valuable treatment option in KTx candidates with enteric hyperoxaluria secondary to SBS and furthermore, *pre-emptively*, in those with advanced but not yet terminal renal failure.

- C04 -

A SUBDURAL HEMATOMA AS A COMPLICATION OF GASTRIC CANCER. I. Vos, S. Altintas, M. Peeters, G. Moorkens. Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Disseminated intravascular coagulation (DIC) is a systemic process producing both thrombosis and hemorrhage. It is caused by a number of disorders : the most common of which are sepsis, trauma and malignancy. Malignancy is the third most frequent cause of DIC, accounting for approximately 7% of the clinically relevant cases.
Case report : A 32-year-old man, recently diagnosed with metastatic gastric carcinoma, was referred to our hospital for chemotherapy with TPF (docetaxel, cisplatin and fluorouracil) and zoledronate. A few hours after hospitalization he mentioned symptoms of blurry vision, strength loss in both hands, sensory disturbances in both cheecks, a feeling of pressure in head and derealisation. Clinical neurological examination was completely normal. CT scan of the brain showed no abnormalities. The lab results showed abnormal coagulation tests (INR 1.47, D-dimers 20.0 μg/ml and low platelets count of $54 \times 10^{E}9$/L) and elevated liver enzymes (AST 56 U/L, ALT 130 U/L, g-GT 575 U/L, AP 3149 U/L).

The working hypothesis was a diffuse intravascular coagulation (DIC). Unless the thrombopenia and general condition, we decided to give the chemotherapy. The day after chemotherapy the neurologic complaints nearly disappeared, except for his headache.
Two weeks later the patient mentioned headache at the right fronto-parietal region and still blurry vision. EEG showed generalised slowing of the basic rythm. MRI of the brain revealed a large subdural hematoma at the right cerebral hemisphere with important mass effect and midline shift. A surgical drainage was performed with a good result. Ten days later he presented at the emergency room with pain in both legs, a severe headache and nausea. CT scan was performed which showed a recurrent subdural hematoma in the right cerebral hemisphere with midline shift.
Conclusion : We report a particular case of a young man with a subdural hematoma due to a DIC as paraneoplastic phenomenon. The treatment of this entity is therapy for the underlying malignant disease.

- C05 -

CASE REPORT : BOERHAAVE SYNDROME SUCCESSFULLY TREATED WITH AN OTSC® MACROCLIP. A. Lemmers, P. Eisendrath, S. Cappeliez, A. Brasseur, J.L. Vincent, O. Le Moine, J. Deviere. ULB Faculty of Medicine, Anderlecht, Belgium.

A 50 year old man without previous medical history other than GERD presented to the emergency department for sudden epigastric pain radiating to the back following vomiting. Blood C-reactive protein concentration was increased to 15 mg/dL. A thoraco-abdominal CT showed a small pneumoperitoneum and a large pneumomediastinum, suggesting a Boerhaave syndrome. Antibiotics and high doses PPI were started and the patient kept fasting in the ICU. In the absence of any hemodynamic instability, a gastroscopy under general anesthesia was planned for the next day. This revealed a 7mm large defect by retroflexed view on the lesser gastric curve just under the Z line. Water soluble contrast injection identified a leak into the mediastinum. Due to its intragastric position, we decided not to treat the leak by stent, but rather to close it with an OTSC® macroclip (OVESCO) (size 12 mm, sharp teeths type). The silicone cap with the loaded clip was placed at the tip of the gastroscope, the edges of the hole were taken sequentially within the twin grasper, tracted to invaginate them in the cap before releasing the clip. The leak closure was confirmed by water soluble contrast injection. A nasogastric tube was inserted and fasting was pursued until a water soluble contrast study 48 hours later showed no residual leak. He was discharged from ICU on day 4 with oral antibiotics for 5 additional days. The abdominal pain and the inflammatory syndrome resolved. At 6 weeks, the patient was asymptomatic and control gastroscopy revealed satisfactory healing of the mucosa at the defect site and spontaneous migration of the clip. Biopsies of the oesophagus and cardia showed normal tissue. Available pictures : initial CT (pneumomediastinum) ; therapeutic endoscopy (endo+ Rx) ; control water soluble contrast ingestion ; control endoscopy.

- C06 -

RITUXIMAB INDUCED COLITIS : A CASE REPORT. M. Claeys, R. D'hondt, K. Hertveldt, M. Cool, G. Deboever, G. Lambrecht. AZ Damiaan, Oostende, Belgium.

Introduction : We present a case of severe colitis following maintenance therapy with rituximab in a patient with a follicular NHL. Rituximab is a chimeric monoclonal antibody directed against the CD-20 antigen of B-lymfocytes. Treatment is usually well tolerated but severe adverse effects have been reported.
Case report : A 47 year old man was diagnosed with a grade I follicular NHL stage IIIa in june 2010. He received combination chemotherapy with CHOP (6 cycles) and rituximab (8 cycles) which resulted in complete remission. After harvesting stem cells two monthly maintenance therapy with rituximab (375 mg/m^2) was initiated. After his 8th cycle of maintenance therapy he presented with abdominal pain, watery diarrhea, dyschezia, fever and voiding problems. Treatment with oral ciprofloxacin by his general physician was unsuccessful. Abdominal CT revealed a slight thickening of the colonic wall and on colonoscopy widespread inflammation of the large intestine with punched out lesions was visualized. C. difficile toxine analysis was positive but treatment with oral metronidazole failed to improve his symptoms. Histopathology was not suggestive for ulcerative colitis nor Crohn's disease and analysis for both CMV and HSV was negative. Congo red stain was normal and no acid-fast bacteria were reported on a Ziehl-Neelsen stain. Additional CD3, CD20 and CD 68 immunohistochemical staining showed a complete absence of CD20 B-cells. The diagnosis of rituximab induced colitis was established. Rituximab therapy was discontinued and the patient was given high dose steroids. His symptoms gradually improved, inflammatory parameters declined and colonoscopy revealed a favorable evolution. To this date no relapse has been reported.
Discussion : We report a case of severe and extensive rituximab induced colitis. The patient received 15 cycles of rituximab in total. To our knowledge only four other cases of rituximab induced colitis were reported in literature.

Administration of rituximab results in depletion of systemic as well as intestinal B-cell populations and it has been associated not only with deterioration of existing inflammatory bowel disease but also with new onset colitis. The role of B-cells in the pathofysiology of inflammatory bowel disease is still in debate. Some mice models have suggested that mesenteric B-cells aggravate intestinal inflammation. Other models conversely suggest a beneficial contribution of mucosal B-cells by effects on the clearance of apoptotic cells or to B-cell-dependent regulation of CD4 T-cell activity. In other auto-immune diseases as well (e.g. autoimmune encephalomyelitis) the modulatory role of B-cells is being increasingly recognized.
Conclusion : Our experience supports previous observations that the use of rituximab, however safe in the majority of patients, may cause serious side effects. It can not only be detrimental in patients with existing inflammatory bowel disease but may also induce new onset colitis.

- C07 -

EXTRAHEPATIC BILIARY OBSTRUCTION NOT ALWAYS RESULTS FROM STONES AND BILIARY STRICTURES. W. Van Steenbergen (1), D. Vanbeckevoort (2), G. Maleux (3). (1) Hepatology, UZ Leuven, Leuven, Belgium, (2) Radiology, UZ Leuven, Leuven, Belgium, (3) Interventional Radiology, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

Introduction : Extrahepatic bile duct obstruction is mostly related to biliary stone disease or to malignant or benign biliary strictures. However, the clinician should be aware that other, rare causes may lead to biliary colics and cholestasis. We here report on two patients who both presented with a rare and unexpected cause of bile duct obstruction.
Case 1 : In this 27-yr old patient, a diagnosis of lupus nephritis was made in October 2012 after he had presented with renal insufficiency and severe hypertension. In November, he presented with biliary colics and jaundice ; lab results showed cholestasis with a total bilirubin of 7 mg/dl. Sonography revealed thickening of the gallbladder wall with the presence of “sludge” but without bile duct dilatation. Cholestasis was complicated by cholangitis and ERCP was performed. A diagnosis of hemobilia was made with evacuation of blood from the papilla and with blood clots in the bile ducts. Sphincterotomy and extraction of blood clots was performed which led to regression of symptoms and of cholestatic tests. MRI demonstrated a bloodfilled gallbladder and excluded the presence of a hepatic artery aneurysm. A diagnosis of hemorrhagic cholecystitis complicated with hemobilia was made and a cholecystectomy was planned. When a patient with SLE presents with biliary colic and cholestasis, a differential diagnosis of hemobilia should be included. Importantly, this complication may result either from hemorrhagic cholecystitis or from hepatic artery aneurysm, and final therapy with either cholecystectomy or aneurysm embolization should be directed to one of these possible etiologies.
Case 2 : This 88-yr old patient underwent cholecystectomy in May 2011 ; this procedure was complicated by section of the common hepatic duct for which primary suture was performed. One month later she presented with abnormal liver tests. CT demonstrated dilatation of the intrahepatic bile ducts and a large biloma in the right liver lobe that was drained percutaneously. She was referred for ERCP. This examination revealed a large and irregularly delineated mass in the common hepatic duct, and mild dilatation of the intrahepatic ducts. Initially a diagnosis of a large bile duct stone or of a bile duct tumor was made. However, after re-evaluation of the CT that had been performed in the other hospital, a diagnosis was made of a large pseudo-aneurysm of the hepatic artery that had led to extrinsic compression of the common hepatic duct. Angiographic embolization of the aneurysm was performed and the bile duct was stented.
Conclusion : Hepatic artery aneurysm has to be recognized as a rare cause of biliary obstruction either by rupture with hemobilia or by direct compression of the bile duct. This diagnosis has to be taken into account in patients with vasculitis and with a possible trauma to the bile duct.

FOUR PATIENTS WITH AMANITA PHALLOIDES POISONING. S. Vanooteghem (1), J. Arts (1), S. Decock (1), P. Pieraerts (2), W. Meersseman (3), C. Verslype (3), P. Van Hootegem (1). (1) AZ Sint-Lucas, Brugge, Belgium ; (2) General Practicioner, Zedelgem, Belgium ; (3) UZ Leuven, Leuven, Belgium.

Introduction : Mushroom poisoning is a rare problem. We report four concomitant cases of amatoxin intoxication, the most deadly cause of mushroom poisoning.
Case : Four women, living together in a convent, were admitted because of nausea, vomiting and diarrhea. Symptoms started approximately 10 hours after eating wild mushrooms, self-picked in the forest. Laboratory data, 24 hours after ingestion, showed normal liver enzymes in 2 patients and normal INR and bilirubin in all 4 patients. Because of suspected amatoxin intoxication, a therapy with intravenous fluid, N-acetylcysteine and silibinin was started. 36 hours after intoxication, complaints of vomiting and diarrhoea improved in all 4 patients. Blood analysis however showed a dramatic increase of the liver enzymes in 3 of 4 patients (ALT-range 48 hours after ingestion : 558-1762 U/L), and an elevation of bilirubin and INR in all 4 patients. Two patients were transferred to a transplant centre 48 hours after mushroom poisoning because they developed stage 2 hepatic encephalopathy. With maximal supportive therapy, all patients gradually improved from day 3. They were discharged from the hospital between 6 to 10 days after admission.
Discussion : Our patients showed the typical clinical syndrome of an amatoxin intoxication. This syndrome can be divided into 3 phases. The gastrointestinal phase (starts 6-40 hours after consumption) is characterized by vomiting and diarrhoea and lasts 12-24 hours. In amatoxin intoxication, symptoms develop typically more than 6 hours after ingestion, while other toxic mushrooms cause symptoms earlier, after 0.5-3 hours. Blood analysis at this initial stage shows normal liver and kidney function. A careful history is very important, to avoid the risk that patients are discharged too early with a diagnosis of a common gastroenteritis. The second phase is characterised by an apparent recovery 36-48 hours after ingestion, while biochemistry shows a progressive increase of transaminases. In the third phase (2-6 days after ingestion), patients can develop hepatic failure, often complicated by renal failure. The therapy of amatoxin intoxication consists of supportive care and medical therapy with silibinin and N-acetylcysteine. Therapy with activated charcoal can be beneficial, if started early after the intoxication. We did not treat our patients with activated charcoal because of vomiting and presentation of the patients 24 hours after ingestion. Patients who develop liver failure should be transferred to a transplant centre. (S. Thiessen, C Verslype, A. Meulemans *et al*., Tijdschr. Geneesk., 2012, 68, 934-942) (L. Santi, C. Maggioli, M. Mastroroberto, M. Tufoni, L. Napoli, P. Caraceni, Int. J. Hepatol., 2012, e-Pub ahead of print) (C.N. Broussard, A. Aggarwal, S.R. Lacey *et al*., Am. J. Gastroenterol., 2001, 96, 3195-3198).
Conclusion : Amatoxin intoxication is a rare cause of liver failure. When suspected, careful monitoring of the liver function and treatment with silibinin and N-acetylcysteine are mandatory.

PLENARY SESSION

- D01-

COMPREHENSIVE GERIATRIC ASSESSMENT SCREENING TOOL IN ELDERLY PATIENTS WITH GASTRO-INTESTINAL CANCER. M. De Man, S. Costers, K. Hendrickx, P. Dobbels, M. Boriau. OLV Hospital, Aalst.

Introduction : Elderly patients constitute an important part of the cancer patient population. Although there is consensus that age and general condition should be taken into account in treatment strategy it remains unclear how this should be applied in daily practice.
Aim : The aim of the study is to evaluate the use of a screening comprehensive geriatric assessment (CGA) tool in patients with gastrointestinal cancer.
Methods : Patients with gastrointestinal cancer of seventy years or older were included after written informed consent. Data on diagnosis, treatment, age, performance status (PS), nutritional status, Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL) and comorbidities were collected.
As proposed in a screening global geriatric assessment (Ludovico Balducci, Martin Extermann : Oncologist 2000 ; 5 ; 224-237) patients were classified in three groups : group A : fit patients who are functionally independent and have no serious comorbidity, group B : intermediate patients who have one or more IADL's and/or have one or two comorbid conditions and group C : frail patients who have one or more ADL's and/or more than two comorbid conditions . Each group is compared in age, PS and applied treatment.
Results : Between 08/04/2010 and 15/12/2011 65 patients were included.14 patients were considered fit (group A), 30 patients intermediate (group B) and 21 patients frail (group C).
Between the patient groups there is no statistical difference in age : mean age (min-max) group A : 74,4 (71-93) , group B : 77,6 (70-87), group C : 76,5 (70-85) yrs. Presence of metastatic disease was : group A : 5 (35%), group B : 7 (23%) and group C : 4 (19%) pts. Patients in group A and B had almost all a good performance status (PS 0-1 group A 13 (93%), group B 29 (93%) pts). Although frail patients of group C more often had a poor performance status still 22 (66%) pts in group C had a PS of 0-1.
Most of the patients in the different CGA groups were treated without apparent difference between the groups. Group A surgery (S) 11(79%), chemotherapy (CT) 8 (58%) radiotherapy (RT) 0 pts. Group B : S 14 (47%), CT 8 (27%) and RT 1 (3%) pts. group C : S 15 (71%), CT 7 (33%) and RT 2 (10%) pts.
Conclusion :
(1) PS and age, although important, are not sufficient to identify the frail elderly patient.
(2) Patients in different CGA groups receive active treatment without apparent difference.
(3) Screening CGA is necessary before treatment discussion of elderly patients in multidisciplinary team. Patients defined as intermediate or frail should receive increased medical attention or a geriatric intervention.

- D02 -

DISTRIBUTION OF BODY FAT CONTRIBUTES TO THE PREDICTION OF COMPLICATIONS AFTER COLORECTAL SURGERY ? D. Leonard (1), F. Penninckx (2), D. Brandt (1), C. Bugli (1), N. Abbes Orabi (3), R. Chamlou (4), C. Coimbra (5), A. D'hoore (2), R. Droissart (4), J. Etienne (6), C. Jehaes (7), B. Majerus (8), F. Mboti (9), B. Monami (7), F. Pierard (10), J.P. Saey (3), L. Stainier (8), N. Tinton (11), J. Van De Stadt (9), Y. Van Molhem (12), A. Kartheuser (1). (1) UCL Saint-Luc ; (2) UZ Leuven ; (3) CHR St Joseph, Mons ; (4) Clinique Saint-Jean, Brussels ; (5) CHU Sart Tilman, Liège ; (6) Clinique Sainte-Elisabeth, Namur ; (7) Clinique St-Joseph, Liège ; (8) Clinique St. Pierre, Ottignies ; (9) ULB Erasme ; (10) Clinique Saint-Luc, Namur ; (11) Hopital St Joseph, Gilly ; (12) OLV, Aalst.

Introduction : Obesity measured by BMI has not been found to be an independent risk factor for post-operative mortality and morbidity in several previous studies whereas WC and W/HR have been shown to be better risk predictors than BMI in the field of cardiovascular disease.
Aim : To determine whether body fat distribution, measured by Waist Circumference (WC) and the Waist/Hip Ratio (W/HR), contributes to a better prediction of mortality and morbidity after colorectal surgery compared to Body Mass Index (BMI) or Body Surface Area (BSA).
Methods : A prospective multi-centric international study was performed in patients undergoing elective colorectal surgery. BMI, BSA, W/HR were derived from body weight, height, waist and hip circumferences measured preoperatively. Odds ratios for mortality, wound abscess, conversion, intra- and post-operative surgical complication, medical complication, anastomotic leak and evisceration were calculated using logistic regression. P values presented are two-tailed, and p values < 0.05 were considered to indicate statistical significance.

Results : 1349 patients (mean age : 64.8y +/- 13.2 ; 754 males, sex ratio M/F : 1.3) from 38 centres in 11 countries, who underwent elective colorectal surgery were included. There were 761 (56.4%) laparoscopic procedures (conversion rate : 12.7%). Median BMI was 25.6 [13.7-50.0], BSA 1.85 [1.28-2.62], WC 96cm [53-181] and W/HR 0.96 [0.49-2.42]. Intra-operative adverse events occurred in 204 (15.1%) patients, medical complications in 178 (13.2%), surgical complications in 240 (17.8%) [78 (6.3%) anastomotic leak ; 55 (4.1%) wound abscess]. Mortality was 0.7%. With increasing BMI, logistic regression analysis showed an increased risk of wound abscess only (OR = 52.0, $p < 0.05$). An increased risk of wound abscess, conversion, intra- and post-operative surgical complications was observed with increasing WC (OR = 34.7, $p < 0.05$; OR = 10.2, $p < 0.05$; OR = 6.7, $p < 0.05$; OR = 4.5, $p < 0.05$). Increasing W/HR significantly increased the risk of conversion, intra- and post-operative complications, medical complications, anastomotic leak and death (OR = 15.7, $p < 0.05$; OR = 11.0, $p < 0.05$; OR = 7.7, $p < 0.05$; OR = 13.2, $p < 0.05$; OR = 13.7, $p < 0.05$; OR = 653.1, $p < 0.05$). These risks did not increase with increasing BSA.
Conclusion : Our study shows that W/HR is highly predictive of adverse events after elective colorectal surgery and supports its use in routine clinical practice.

- D03 -

OBESITY, METABOLIC SYNDROME AND NASH : WHAT IS THE LINK WITH PRIMARY CILIUM DYSFUNCTION ? L. Poekes (1), V. Legry (1), V. Lebrun (1), O. Schakman (1), G.C. Farrell (2), Y. Horsmans (1), I. Leclercq (1). (1) UCL St Luc ; (2) Canberra Hospital, Australia.

Introduction : Fatty Aussie Mice (*foz/foz*), carrying a mutation in the Alms1 gene encoding a protein of the primary cilium, are proposed as a new model of NASH. Indeed, upon a high fat diet (HFD) they become obese, insulin resistant and develop a progressive NASH. Their unique metabolic phenotype has been linked to hyperphagia resulting from abnormal ciliary function in the central nervous system. ?
Aim : The aim of our study is to verify the dependence of the phenotype on over-feeding and to better understand the link between primary cilium dysfunction, metabolic syndrome and NASH.
Methods : We studied male *foz/foz* (Alms1-/-) and wild-type (WT) littermates fed a normal diet (ND) or a HFD for 4 weeks and analyzed metabolic parameters, tissue inflammation, basal metabolism, activity level and gut flora. We next performed a pair-feeding experiment in which *foz/foz* mice had access to the strict same amount of HFD consumed by WT the day before to unravel the respective roles of hyperphagia and intrinsic consequences of Alms1 mutation and cilium dysfunction.
Results : Compared to WT mice, *foz/foz* mice fed a HFD, ate more (14.1 ± 0.9 vs 18.2 ± 2.5 kcal/d, $p < 0.001$), became more obese (26.8 ± 1.9 vs 42.5 ± 8.9 g, $p = 0.01$), insulin resistant and glucose intolerant ($p = 0.008$). They developed steatosis, adipose tissue and liver inflammation and will in the long term develop NASH, contrarily to WT mice in which liver alteration will remain slight. Compared to *foz/foz* mice fed *ad libitum*, pair-fed mice had a reduced body weight gain ($+85.8\% \pm 32.8$vs $+54.3\% \pm 5.4$, $p = 0.09$) and glucose intolerance. However, compared to WT mice receiving the exact same amount of food, *foz/foz* mice gained more weight ($+54.3\% \pm 5.4$vs$29.7\% \pm 10.6$, $p < 0.001$) and were more glucose intolerant. To explain altered metabolic phenotype in *foz/foz* mice, we evaluated basal metabolism and activity level. Upon ND, WT and *foz/foz* mice had similar oxygen consumption, body temperature and activity level and pattern around the nychtemera. Switch to a HFD reduced drastically the activity level in both genotypes ($p < 0.01$), irrespectively of food intake. HFD induced a similar reduction in the weight of the ceacum (a surrogate for gut flora), and a similar increased intestinal permeability in vivo. Despite matched HFD intake, *foz/foz* mice developed increased adipose tissue inflammation and decreased hepatic and muscular insulin signaling compared to WT mice.
Conclusion : Our results suggest that, beside causing hyperphagia,Alms1 deficiency and ciliary dysfunction alter inflammatory response and insulin sensitivity, and that such alterations participate to the metabolic and hepatic phenotype leading to NASH. The understanding of the mechanisms at play may uncover new potential therapeutic targets.

- D04 -

THE EFFECT OF WEIGHT LOSS ON NONALCOHOLIC FATTY LIVER DISEASE IN AN OVERWEIGHT AND OBESE POPULATION. A. Verrijken, S. Francque, I. Mertens, M. Ruppert, G. Hubens, E. Van Marck, P. Michielsen, L. Van Gaal. UZ Antwerp.

Introduction : Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide and is frequently associated with obesity and insulin resistance. Currently, there is no approved therapy. Weight loss is recommended for overweight and obese patients with NAFLD, with data on histological improvement mostly restricted to bariatric surgery series.
Aim : We aimed at studying the effect of weight loss on metabolic and histological parameters of NAFLD in an overweight and obese population.
Methods : Patients presenting for a problem of overweight or obesity underwent a metabolic and liver assessment. If NAFLD was suspected, a liver biopsy was proposed. Patients were invited to participate in a weight reducing program (hypocaloric diet in combination with physical activity or bariatric surgery). Patients were re-evaluated after 12 months of treatment, including liver biopsy. All biopsies were scored according to the NASH Clinical Research Network Scoring System.
Results : 120 patients (70.8% female) were prospectively included (mean age 46.4 ± 1.08 years) ; 49.2% were treated with lifestyle intervention and 50.8% underwent bariatric surgery. In 56 (46.7%) patients a second liver biopsy was performed (58.9% were treated with lifestyle intervention). After 12 months of treatment mean BMI fell from 39.0 ± 0.51 kg/m^2 to 31.5 ± 0.43 kg/m^2 ($P < 0.001$) and visceral fat dropped from 214 ± 7.31 cm^2 to 136 ± 8.46 cm^2 ($P < 0.001$). A significant improvement was observed in the metabolic profile with reduction in blood pressure, lipid profile and insulin resistance (HOMA-IR). Serum alanine aminotransferase ($P < 0.001$), aspartate aminotransferase ($P < 0.001$), gamma glutamyltransferase ($P < 0.001$), and alkaline phosphatase ($P = 0.037$) significantly decreased, suggesting NAFLD improvement. Significant histological improvement was noted in the NASH Activity Score (from 3.96 ± 0.30 to 1.48 ± 0.29, $P < 0.001$) and its individual components ; steatosis (from 1.55 ± 0.14 to 0.52 ± 0.11, $P < 0.001$), lobular inflammation (from 1.21 ± 0.12 to 0.45 ± 0.10, $P < 0.001$) and ballooning (from 1.20 ± 0.10 to 0.52 ± 0.11, $P < 0.001$) and this was also significant in both intervention groups separately. However, mean decreases were higher in the surgery group. Fibrosis stage also showed significant improvement in patients who underwent bariatric surgery (from 1.09 ± 0.24 to 0.52 ± 0.25, $P = 0.046$).
Conclusion : Weight loss by lifestyle intervention can achieve a significant, histologically documented, improvement of NAFLD in overweight and obese patients. Bariatric surgery results in even more pronounced improvement, including fibrosis regression.
This work is part of the project “Hepatic and adipose tissue and functions in the metabolic syndrome” (HEPADIP) European Commission 6th Framework Program (Contract LSHM-CT-2005-018734).

- D05 -

RADIOFREQUENCY ABLATION IN THE ESOPHAGUS : RESULTS OF A PROSPECTIVE MULTICENTER BELGIAN REGISTRY. R. Bisschops (1), P. Deprez (2), H. Willekens (1), D. De Looze (3), E. Macken (4), F. Mana (5), H. Orlent (6), J.J. Carausu (1), G. Coremans (1). (1) UZ Leuven ; (2) UCL St Luc ; (3) UZ Ghent ; (4) UZ Antwerp ; (5) UZ Brussel, Brussels, Belgium ; (6) AZ Sint-Jan Brugge-Oostende.

Introduction : Radiofrequency ablation (RFA) has been incorporated in the treatment algorithms for early neoplasia in Barrett (BE) and squamous dysplasia. It can be used as a primary treatment for flat low-grade dysplasia (LGD) and high-grade dysplasia (HGD) or in combination with endoscopic resection (ER) of visible lesions to obtain complete remission of intestinal metaplasia (IM) and dysplasia (D). To date, data outside of clinical trials on this technique are very limited.
Aim : This multicenter registry aims at monitoring all RFA procedures performed in Belgium with a specific focus on oncological and procedural safety.
Methods : All RFA procedures are prospectively monitored for : indication, treatment before RFA, aim of the treatment, short/ long term complications/symptoms, and prospective long-term pathological outcomes (complete remission (CR) for IM and D) through standardized case report files. Data are collected by the study coordinating centre (UZ Leuven). All participating physicians received formal training through the European RFA academia.
Results : Currently, 6 out of 9 centres performing RFA have entered the RFA registry. Between 2/2008 and 11/2012, 312 RFA procedures (124 circumferential,188 focal) were performed in 151 patients (age 65 yr ; 87% male). Indications for RFA were : primary RFA in BE HGD (54), BE LGD (12), IM BE (1), squamous HGD (2) and BE cancer (1) ; add-on RFA after ER was performed in 73 (48%) of patients. ER showed 5 LGD, 21 HGD, 46 T1a, and 1 T1b cancer. Pre-RFA histology confirmed 5 IM, 22 LGD, 72 HGD, 2 indefinite for dysplasia, 2 squamous HGD, and 2 adenocarcinoma. Of the latter, 1 patient was treated outside the standard clinical protocol and a second showed no residual lesion after RFA.

Early complications occurred in 13.9% of patients and 6.7% of procedures : 14 small mucosal lacerations (5 after sizing only), 6 minor bleedings with spontaneous haemostasis, and 1 pneumonia. Delayed serious complications occurred in 15.2% of patients and 7.3% of procedures : 11 stenosis (+1 perforation after dilation), 5 severe bleedings requiring re-admission, 2 poor healing and 6 hospitalizations due to severe pain complaints.
Additionally, 113 patients treated with intent to obtain CR-IM and CR-D reached the end of RFA treatment. 2 patients died of metastatic cancer (1 disease related) and 4 (2.6%) needed surgical referral for a BE related cancer occurring in between RFA sessions (2 T1b, 1 T2 lesion, 1 poor healing with T1a). In 1 patient no CR- IM was pursued. CR-D and CR-IM was obtained in 107/113 and 106/113 respectively. After a median follow-up of 1.22 years, CR-IM, CR-D, and CR-HGD are 70%, 81%, and 87% in an ITT analysis and 75%, 87%, and 92% per protocol. Median time to recurrence of IM and D was 262 and 209 days respectively. Recurrence was managed endoscopically.
Conclusion : Our registry data confirms that RFA is an efficient and safe treatment option for dysplastic BE, whether or not in study protocols, outweighing the risks of surgery. However, physicians should be aware of pop-up lesions. Significant complications can occur in up to 15% of patients and IM reoccurs in 25% of patients. Therefore, RFA cannot be recommended in asymptomatic low-risk patients with non-dysplastic BE.

State of the art lecture

- D06 -

HIGHLIGHTS OF LAST 25 YEARS IN GASTROENTEROLOGY. P. Deprez /Ucl St Luc, Brussels.

- D07 -

ENDOSCOPIC SUBMUCOSAL DISSECTION VS ENDOSCOPIC MUCOSAL RESECTION FOR LARGE. P. Deprez, E. Kim, H. Ivekovik , L. Shaza, R. Yeung, A. Jouret-Mourin,H. Piessevaux. UCL St-Luc.

Introduction : Endoscopic mucosal resection (EMR) and submucosal dissection (ESD) are techniques which allow for removal of large (> 2cm) superficial neoplasms of the gastrointestinal tract. Whereas the use of EMR in the treatment of duodenal lesions is well documented, the data on the performance of ESD in duodenum are scarce.
Aim : We present results on the performance of the ESD compared to EMR for large superficial duodenal neoplasms in our cohort of patients.
Methods : We cross-examined our database of endoscopic procedures, between 1/2006 and 1/2012 to indentify patients with ESD/EMR done in duodenum. Patients with superficial, duodenal neoplasms, were eligible for the study. Patients with ampullatory lesions or familial adenomatous polyposis were excluded from the study. Morphology of duodenal lesions, procedure-related data presence of postprocedural adverse events and clinical outcomes were analysed. Results are expressed as medians and IQR, and compared with Student's t-test, Pearson's chi-squared test or Fisher's exact test for proportions.
Results : 158 patients with the ESD/EMR procedure done in duodenum were reviewed. A total of 51 patients were eligible for the study (ESD group 19 pts, EMR group 32 pts). No significant differences between two groups were observed in terms of age (ESD 64 [60-70] vs. EMR 65 [60-68] years, p = 0,94), sex (men 7 vs. 13, p = 0,78), lesion size (20 [15-29] vs. 20 [14-25] mm, p = 0,40), location (D1-D3, p = 0,89), presence of advanced lesions (high grade dysplasia, adenocarcinoma T1m1, carcinoid, p = 0,69), procedure time (95 [78-144] vs. 100 [73-120] min, p = 0,26), size of specimen (16 [13-21] vs. 14 [12-20] mm, p = 0,28) and en bloc resection (3 vs. 2 specimens, p = 0,27). Number of intraprocedural events (perforation/haemorrhage) was higher in the ESD group (9/1 vs 3/1, OR = 7,77, CI95% : 1,65-39,81, p = 0,003), in contrast to the presence of adverse postprocedural events (delayed bleeding/incomplete resection/recurrence : 1/1/0 vs 4/8/2, OR 0,093 (95%CI 0,004-0,823), p = 0,01). No significant differences were found in terms of length of hospital stay (p = 0,06), requirements for blood transfusion (p = 0,27) and surgery (p = 0,58).
Conclusion : ESD in duodenum is a time-consuming and technically challenging procedure. Compared with EMR, lower rates of postprocedural adverse effects observed in our study, are counteracted by higher rate of intraprocedural perforation and haemorrhage. Proper expertise in management of these situations is a prerequisite for safe endoscopic treatment of duodenal lesions.

- D08 -

POSITIVE AND NEGATIVE MOOD MODULATE ESOPHAGEAL PAIN PERCEPTION IN HEALTH. N. Weltens (1), N. Schaub (2), L. Van Oudenhove (1), H.G. Ly (1), Q. Aziz (2), J. Tack (1), S. Coen (3). (1) KULeuven ; (2) Wingate Institute, Queen Mary University Of London, UK ; (3) King's College London, UK.

Introduction : It is generally accepted that mood plays an important role in the altered perception of visceral sensations in functional gastrointestinal disorders (FGID). However, compared to somatic pain, evidence supporting this hypothesis in the context of visceral pain is sparse and mostly originates from brain imaging studies demonstrating an effect of negative mood on neural but not behavioral (i.e. pain ratings) responses to visceral stimuli. Moreover, evidence for an influence of positive mood on visceral pain perception is completely lacking to date.
Aim : To investigate the effect of positive and negative mood on esophageal pain perception.
Methods : 68 healthy volunteers (HV) (36 men ; mean age 26.7 years) participated in the study. Positive, negative, and neutral mood were induced using a combination of Velten mood induction statements, validated emotional pictures and music. Esophageal balloon distensions at individually determined pain toleration level were used throughout the study to evoke visceral pain. Volunteers attended one visit during which negative, positive, and neutral mood was induced over 3 separate experimental runs, in a counterbalanced order. 10 painful stimuli were pseudo-randomly delivered during each run. Participants rated mood and arousal at the beginning and end of the run, and pain intensity and unpleasantness after each stimulus. Data were analyzed using a repeated measures ANOVA and post hoc Bonferroni's multiple comparison test.
Results : Ratings of mood were significantly lower following negative emotion induction and significantly higher following positive emotion induction, compared to neutral induction ($p < .0001$). Participants were significantly more aroused following both negative and positive mood induction than following neutral induction ($p < .0001$). These results confirm the efficacy of the emotion induction procedure. Mean valence and arousal at the start of each run were not significantly different, indicating that the observed results were not due to order effects. A significant effect of emotion on pain ratings was found, with significantly higher and lower pain ratings during negative and positive mood, respectively, compared to neutral ($p < .0001$ for pain intensity and unpleasantness). Interestingly, despite these significant differences at group level, inter-individual variability of pain modulation was large.
Conclusion : We are the first to report that both negative and positive mood modulate esophageal pain perception in HV. Moreover, we observed large inter-individual differences in the effect of emotion on visceral pain perception. This might represent a susceptibility factor or "endophenotype" for the development of visceral pain disorders (i.e. FGID). Further research is needed to elucidate the mechanisms that underlie this variability.

- D09 -

PSYCHOLOGICAL STRESS INCREASES INTESTINAL PERMEABILITY IN MEN VIA CRH-MEDIATED MAST CELL ACTIVATION. T. Vanuytsel, S. Van Wanrooy, E. Houben, S. Salim Rasoel, J. Toth, L. Holvoet, H. Vanheel, C. Vanormelingen, S. Verschueren, R. Farre, L. Van Oudenhove, P. Van Den Berghe, G. Boeckxstaens, K. Verbeke, J. Tack. KULeuven.

Introduction : Increased intestinal permeability and psychological stress have been implicated in the pathogenesis of irritable bowel syndrome and other chronic gastrointestinal disorders. Animal models suggest that stress increases small intestinal permeability (SIP) via CRH-mediated activation of mast cells. However, evidence that stress exerts the same effect on SIP in humans is lacking.
Aim : To investigate the effect of acute psychological stress on SIP in humans.
Methods : 33 healthy volunteers (15 men, 21 ± 0.2 yr), were enrolled in a first study in which SIP was determined in 4 conditions : 1) control ; 2) after ingestion of indomethacin (75 mg 16h and 50 mg 6h before the test) ; 3) during and after a 30 min anticipatory stress protocol, involving 20 auditory countdown sequences, half of these followed by a painful electrical stimulus in a randomized fashion ; 4) during and after a public speech (PS) in an exam situation. In vivo SIP was determined by measuring the urinary lactulose-mannitol ratio (LMR) during the first two hours after drinking a 150mL solution containing 5g lactulose and 2g mannitol. Samples were analyzed by HPLC-ELSD. In a second study, SIP was tested in 13 volunteers (4 men, 22 ± 0.4 yr) in 5 conditions : 1) control ; 2) after treatment with oral cromoglycate 200 mg qid for 2 weeks ; 3) after intravenous injection of 100μg CRH ; 4) after injection of CRH with cromoglycate pretreatment ; 5) during and after PS with cromoglycate pretreatment. Stress-related symptoms were scored by the STAI-state questionnaire. Data were analyzed by a mixed model analysis with 'condition' as a within-subject factor with Bonferroni post-hoc test.
Results : Anticipatory stress and PS both increased the total STAI-state score (46.6 ± 2.3 and 53.2 ± 2.0 vs. 30.3 ± 0.7 ; both $p < 0.0001$). Salivary cortisol was elevated during PS only (18.8 ± 1.4 vs. 9.9 ± 0.8ng/ml ; $p < 0.0001$). Indomethacin, serving as positive control, significantly increased the LMR (0.060 ± 0.007 vs. 0.025 ± 0.004 ; $p < 0.0001$). The PS condition (0.044 ± 0.007 ; $p < 0.01$), but not the anticipatory stress protocol (0.036 ± 0.005 ; $p = 0.76$) increased the SIP.

There was a significant correlation between salivary cortisol and LMR within the PS condition ($r2 = 0.32$; $p = 0.01$). Moreover, including cortisol as an independent variable in the model renders the effect of condition non-significant, while the effect of cortisol becomes highly significant ($p = 0.0001$), indicating that the effect of stress on permeability is mediated via a component of the HPA-axis. Intravenous administration of CRH significantly increased the LMR (0.038 ± 0.005 vs. 0.028 ± 0.003 ; $p = 0.04$) which was inhibited by the mast cell stabilizer cromoglycate (0.025 ± 0.003 ; $p = 0.99$). Finally, SIP was unaltered by PS in case of cromoglycate pretreatment (0.025 ± 0.002 ; $p = 0.84$).
Conclusion : Acute psychological stress alters small intestinal permeability by a pathway involving CRH and mast cells. These findings may provide further insight in the pathogenesis of stress-related chronic gastrointestinal disorders and symptoms.

- D10 -

COLON TRANSIT TIME IN CHILDREN AND YOUNG ADULTS WITH SPINA BIFIDA. S. Vande Velde (1), S. Van Biervliet (1), M. Van Winckel (1), V. Meerschaut (1), N. Herregods (1), L. Pratte (2). (1) UZ Ghent ; (2) UGhent.

Introduction : Spina Bifida (SB) patients frequently present constipation and incontinence.
Aim : Analyze colon transit time (CTT) in children and young adults with SB in relation to neuronal lesion, mobility, bowel habits and continence in comparaison to age-matched healthy controls.
Methods : Study performed at the Spina Bifida Reference Center of the Ghent University Hospital. All patients age 6-18 yr, not using antegrade continence enemas are asked to participate. Care as usual (including laxatives and retrograde enemas) is continued during the study but retrograde enemas (RCE) are stopped 48h prior to the X-Ray. 49 SB patients meet inclusion criteria, 35 participated. Data from the medical file and prospective questionnaires regarding constipation and incontinence were collected. The SB patients are constipated if = 2 of the Rome III criteria for paediatric functional constipation are fulfilled. The SB patients are incontinent if involuntary faecal loss is > once a month. The control group are 21 healthy age-matched children, not suffering from constipation or incontinence according to the Rome III criteria. Total and segmental CTT is measured using the 6-day method. Non parametric tests are used and multivariate analysis is performed. There is ethical approval (EC UZG 2010/348).
Results : The questionnaires confirm persisting constipation despite treatment in 13/35 SB patients. Seven patients are spontaneously continent and 10 are pseudo-continent. SB patients have a significant ($P = 0.006$) longer total CTT compared to controls (median CTT 100,8h vs. 43,2h). Of the SB population 13 patients have a normal total CTT. No clinical parameter (lesion level, mobility or mental ability) is associated with the CTT as evaluated by multivariate analysis. Constipated SB patients have a significantly longer total CTT than non-constipated patients ($P = 0.001$) (CTT 122,4h vs. 61,2h).
There is a significant difference of CTT in continence status ($P = 0.014$), spontaneous continent patients have a normal CTT (CTT 33,6h) and other patients have an elongated CTT. An abnormal CTT predicts the necessity of treatment to achieve continence ($p < 0.05$). RCE influences not CTT as there is no significant difference between the RCE users and the other SB patients. The total CTT ($P = 0.027$), right ($P = 0.001$) and left CTT ($P = 0.003$) is significantly different from the control population. No difference in rectosigmoidal CTT is found between patients and controls.
Conclusion : CTT in patients with SB is significantly prolonged indicating a neurogenic bowel. SB patients with a normal CTT are more likely to achieve spontaneous continence. Better knowledge of the CTT will tailor the future treatment of SB patients to achieve faecal pseudo-continence.

- D11 -

TREATMENT OF HCV GENOTYPE 1 PATIENTS WITH F3/ F4 : THE INTERNATIONAL TELAPREVIR EARLY ACCESS PROGRAM. C. Moreno (1), M. Colombo (2), I. Fernández (3), D. Abdurakhmanov (4), P.A. Ferreira (5), S. Strasser (6), P. Urbanek (7), A. Streinu-Cercel (8), A. Verheyen (9), W. Iraqi (10), R. Demasi (11), A. Hill (12), J.M. Läuffer (13), I. Lonjon-Domanec (10), H. Wedemeyer (14). (1) ULB Erasme ; (2) IRCCS Milano, Italy ; (3) Hospital Universitario 12 De Octubre, Madrid, Spain ; (4) E. M. Tareev Clinic, Moscow, Russia ; (5) University of São Paulo, Brazil ; (6) Royal Prince Alfred Hospital, Sydney, Australia ; (7) Charles University, Prague, Czech Republic ; (8) University Bucuresti And Institute For Infectious Disease Ibi, Bucharest, Romania ; (9) Janssen Pharmaceutica Belgium ; (10) Janssen Pharmaceuticals, France ; (11) Tibotec, USA ; (12) Metavirology, London, UK ; (13) Janssen-Cilag, Switzerland ; (14) Medizinische Hochschule Hannover, Germany.

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

Aim : To provide early access to telaprevir for subjects with genotype 1 chronic hepatitis C with advanced hepatic fibrosis or compensated cirrhosis who are either treatment naïve or treatment experienced and to gather information on the safety and tolerability of telaprevir treatment in combination with Peg-INF-alfa and RBV.
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/mm^3 were required at entry. This interim (ITT) analysis included 16 week data from the first 609 patients.
Results : Mean age was 54 years and mean weight 79kg ; 67% were Male and 98% Caucasian, 66% had HCV RNA levels ≥ 800,000 IU/mL, 45%/55% had severe fibrosis/ cirrhosis, 28% had genotype 1a. Overall, 20% were treatment naïve, 28% prior relapsers, 47% prior non-responders and 5% had prior viral breakthrough. Up to week 16, 59% of patients developed grade 1-4 anemia (Hb < 11 g/dL or > 2.5 g/dL reduction), with 31% severe cases (Hb < 9 g/dL or > 4.5 g/dL reduction) ; 171 patients (28%) dose reduced ribavirin ; 148 (24%) received EPO ; 31 (5%) were transfused and 19 (3%) discontinued treatment for anemia. By week 16, 42% of patients developed grade 1-3 rash, including 4% severe cases (Grade 3) and one Stevens-Johnson syndrome (resolved) ; 30 patients (5%) discontinued treatment for rash. 85 patients (14%) developed serious adverse events (SAEs). Three cirrhotic patients died during the PR phase due to hepatic failure or ischemic colitis with subsequent multi-organ failure. HCV RNA responses at weeks 4 and 12 (ITT analysis) are shown below :

Time on treatment	Week 4		Week 12	
HCV RNA suppression	< 25 IU/mL	Not detected	< 25 IU/mL	Not detected
Naïve (n = 124)	86%	59%	88%	85%
Relapser (n = 171)	80%	63%	87%	85%
Partial responder (n = 94)	80%	52%	85%	77%
Null responder (n = 176)	68%	41%	76%	68%
Viral breakthrough (n = 28)	82%	68%	89%	86%
Overall (n = 609)	77%	54%	83%	79%

Conclusions : In this telaprevir early access program – clinical trial for patients with severe fibrosis or compensated cirrhosis, 79% of patients had undetectable HCV RNA by week 12 (ITT). SAEs occurred in 14%, discontinuation due to anemia/rash was similar to phase III registration trials.

- D12 -

A LACK OF ROSEBURIA HOMINIS DEFINES DYSBIOSIS IN PATIENTS WITH ULCERATIVE COLITIS. K. Machiels (1), M. Joossens (2), J. Sabino (1), V. De Preter (1), I. Arijs (1), V. Eeckhaut (3), V. Ballet (1), K. Claes (1), K. Verbeke (1), M. Ferrante (1), J. Verhaegen (1), P. Rutgeerts (1), S. Vermeire (1). (1) KULeuven ; (2) VUB ; (3) UGhent.

Introduction : Bacteria play an important role in the onset and perpetuation of the intestinal inflammation in inflammatory bowel disease (IBD) and intestinal dysbiosis has been described. Unlike in Crohn's disease (CD), where dysbiosis has been better characterized, in UC, only small cohorts have been studied and showed conflicting data.
Aim : Our aims were to evaluate in a large cohort if the microbial signature described in CD is also present in UC and if we could characterize predominant dysbiosis in UC. To assess the functional impact of dysbiosis we quantified metabolites of the bacterial species driving dysbiosis.
Methods : Bacterial DNA was extracted from 214 fecal samples belonging to 127 UC patients and 87 age and sex matched controls. The predominant microbiota was analyzed using denaturing gradient gel electrophoresis (DGGE) analysis. Identified bands were purified, after which they were excised and sequenced on an ABI Prism 3130 Genetic Analyser. Results were confirmed using real-time PCR. Metabolites were measured using GC-MS.
Results : The microbial signature previously described in CD was not present in UC based on the DGGE analyses. More specifically, no differences between groups were detected for *Ruminococcus gnavus* and *Bifidobacterium adolescentis*. Although *Faecalibacterium prausnitzii* was reduced in UC patients compared to control subjects, the predominant difference was not significant (Uncorrected p = 0.053). When comparing DGGE banding patterns of UC patients with control subjects, 2 band-classes were significantly different after stringent correction for 49 band-classes. One was identified as *Bifidobacterium longum*, and was more present in UC patients versus controls (pcorr = 0.01). The other was identified as *Roseburia hominis* and was reduced in UC patients compared to control subjects (pcorr = 0.03). Short chain

Fatty acids (SCFA) were in general reduced in UC patients, but no direct correlation between these SCFAs and the identified bacteria was found.
Conclusion : The composition of the fecal microbiota of UC patients differs from that of healthy individuals : we found a predominant increase in *Bifidobacterium longum*, known as an important lactic and acetic producer, and a reduction in *Roseburia hominis and Faecalibacterium prausnitzii,* both well-known butyrate producing bacteria of the Firmicutes phylum. These results underscore the importance of dysbiosis in IBD but suggest that different bacterial species play a role in the pathogenesis of UC and CD. The mechanism through which these species contribute to inflammation cannot solely be related to their produced metabolites and requires further investigation.

- D13 -

PRIMARY RESPONSE TO INFLIXIMAB IN CROHN'S DISEASE IS ASSOCIATED WITH THE TNFRSF1A GENE POLYMORPHISM. T. Billiet (1), I. Cleynen (1), V. Ballet (2), M. Ferrante (1), P. Rutgeerts (1), S. Vermeire (1). (1) KULeuven ; (2) UZ Leuven.

Background : The introduction of anti-TNF alpha antibodies has had a major impact on the treatment of patients with chronic inflammatory diseases. Remarkably the response to infliximab differs depending on the underlying disease. Whereas inflammatory bowel disease (IBD), rheumatoid arthritis and psoriasis all show good efficacy, there is no efficacy or even worsening of disease observed in multiple sclerosis patients. The *TNFRSF1A* gene has been implicated in susceptibility to multiple sclerosis but not to Crohn's disease (CD), rheumatoid arthritis or psoriasis. Both genetic and functional evidence suggested that rs1800693 is the causal variant in this gene. This variant leads to expression of a novel soluble form of TNFR1 which blocks TNF and therefore mimics the effect of anti-TNF agents. We hypothesized that the G risk allele of rs1800693 is associated with primary nonresponse to infliximab in IBD patients.
Methods : A single-center cohort of 863 IBD patients (616 CD and 247 ulcerative colitis (UC)) were evaluated for primary clinical response to infliximab at weeks 4-10 following initiation of infliximab and were genotyped for rs1800693. Patients who had no clinical/CRP benefit after two or three infusions were considered as primary non-responders. Statistical analyses were conducted using PLINK.
Results :

	CD				UC			
	Alleles	Genotypes			Alleles	Genotypes		
	G	AA	AG	GG	G	AA	AG	GG
Responders	38%	211 (38%)	269 (48%)	78 (14%)	43%	55 (32%)	88 (50%)	32 (18%)
Non-responders	47%	19 (33%)	23 (40%)	16 (27%)	42%	23 (32%)	38 (53%)	11 (15%)

In our population of 885 healthy controls the G allele frequency (44%) was similar to the frequency observed in the control population in the multiple sclerosis study (40%). A statistically significant association between the G risk-allele and primary nonresponse to infliximab was found in the CD cohort ($p = 0.022$) : 27% of CD patients with GG genotype showed non-response compared to 14% in the responders ($p = 6.09e-03$, OR = 2.34 [1.26-4.37]). There was a gene dosage effect as the risk for non-response increased from 8% in heterozygous carriers of the risk allele to 17% in homozygous mutant patients ($P = 0.05$). However, in the (much smaller) UC cohort, this association could not be confirmed ($P = 0.53$ for the recessive model, OR = 0.69 [0.33-1.45]).
Discussion : We found that the *TNFRSF1A* rs1800693 GG genotype was associated with a 2.3 fold increased chance for primary non-response to infliximab in CD patients. The absence of effect in UC can be explained by low sample size, or could indicate different mechanisms driving response in CD or UC. The exact mechanisms how this variant leads to more resistance to anti-TNF agents in CD is unclear. Possibly, the anti-TNF effects exerted by the new soluble form of TNFR1 in the GG homozygous patients result in less TNFalpha-driven inflammation. It will be important to study mucosal expression profiles in these patients.

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

Invited Lecture
- G01-

HOW TO IMPROVE THE ADENOMA DETECTION RATE ? R. Bisschops. KULeuven.

The prerequisite to improve polyp detection is increased quality of endoscopy in general. The quality of colonoscopy is determined by bowel preparation and a good basic technique with sufficient insufflation and inspection time during withdrawal. The quality of bowel preparation, assessed by different available and validated scales (e.g. Boston Bowel preparation scale) has been shown to determine polyp detection rate, caecal intubation rate and difficulty of colonoscopy. Other prerequisites for a well conducted examination are sufficient withdrawal time during which the endoscopist "searches the folds" for small lesions, using sufficient insufflation and different positions. Additionally antispasmodics can help if colonic contractions impede visibility. Patient discomfort can be improved using good sedation and carbon dioxide insufflation. All the above mentioned measures become however obsolete if the bowel preparation is inadequate. Good bowel preparation is nowadays obtained by either high volume PEG solutions or low volume hyperosmotic agents. Recent ESGE guidelines strongle recommend a split dosage 4 liter PEG solution. However due to the larger volumes this is sometimes not so well tolerated. An alternative in patients without cardiac or renal failure and without ascites is a combination of picosulphate and magnesium citrate.
In general the use of new imaging devices for the detection of more polyps in an average risk population is very marginal according to recent literature. A meta-analysis compared the yield and miss rates of high-definition (HD) colonoscopy with NBI versus HD-white light endoscopy (WLE) in a total of 3059 participants. There was no difference between HD-NBI and HD-WLE for the detection of adenomas, patients with polyps, patients with adenomas, detection of adenomas < 10 mm and flat adenomas. Classical chromo-endoscopy does improve polyp detection but due to its practical limitation of the use of dyes and spray catheter it has never been implemented, nor is it clear if it is cost-efficient. It is also not clear if the additional polyp detection is clinically important. Most studies show an increased detection of diminutive polyps, however there are no data indicating that this affects for instance interval cancers. Nevertheless, the use of HD endoscopy systems, is probably preferable and needs to be taken into consideration when systems have to be replaced in an endoscopy unit.
In two specific situation high definition endoscopy and advanced imaging is formally indicated. In patients with longstanding IBD related colitis, chromo-endoscopy with methylene blue 0.1% or indigo carmine 0.4% has now become the gold standard for surveillance of colitis related neoplasia. It has been shown to be superior to the random biopsy protocol in several trials and in a meta-analysis. It is indicated in patients diagnosed with pancolitis after 8 years of disease-onset and in patients with left sided colitis after 10 years. Prerequisites for screening are besides a good bowel preparation, a quiescent colitis to allow for detection of subtle lesions that should be targeted for biopsy. Chromo-endoscopy is often regarded as time-consuming and messy, due to the staining agents that have to be sprayed through a catheter. Recent data suggest however that in the future, this will very likely be replaced by virtual chromo-endoscopy. In a recent randomized controlled trial, no significant difference could be found in neoplasia detection between HD-NBI or HD chromo-endoscopy. However since this was not a non-inferiority trial, this needs to be confirmed in future studies before chromo-endoscopy as the new gold standard can be abandoned.
In patients with Lynch syndrome, several studies, although small in sample size, have consistently demonstrated an increased polyp and adenoma detection with chromo-endoscopy or virtual chromo-endoscopy (NBI and i-scan). In patients with Lynch syndrome, increased polyp detection of smaller polyps is probably clinically relevant, because malignant transformation can already be present in small polyps.
In conclusion, the most important factor to increase polyp detection is the quality of bowel preparation and the colonoscopy itself. The additional value of advanced imaging for colonoscopy in an average risk population is not clear, and it is not formally indicated in routine colonoscopy. However, in addition to the general prerequisites, high definition colonoscopy with advanced imaging is necessary for surveillance of patients with longstanding colitis and HNPCC patients.

- G02 -

LOWER DIAGNOSTIC YIELD OF CAPSULE ENDOSCOPY SINCE REIMBURSEMENT POLICY IN BELGIUM.
J. Van Hauwe, L. Crape, C. Jacobs, P. Hindryckx, M. De Vos, D. De Looze. Ghent University Hospital, Gent, Belgium.

Introduction : Since the first of July 2008, capsule endoscopy (CE) is partially reimbursed for patients with obscure or overt gastrointestinal bleeding.
Aim : To evaluate the impact of reimbursement of CE on the referral pattern and diagnostic yield of CE.
Methods : We retrospectively selected data from patients who underwent CE for occult or overt bleeding at the University Hospital of Gent between July 2002 and October 2012, with a total of 408 patients. Following data were analyzed : number of CEs, indications, transfusion-dependency, lowest haemoglobin (Hb) level before CE, diagnostic yield and most relevant finding. A comparison was made of these data before and after reimbursement.
Results : 408 patients underwent CE : 173 before (group 1) and 235 after July 1st, 2008 (group 2). Indications were occult bleeding with anaemia in 60.5% and overt bleeding in 39.5%. The mean age was 64 years. Transfusion was needed in 73.5%. Overall, relevant findings were found in 44.4%. Comparison between the 2 groups showed no significant difference in age ($P = 0.793$) nor gender ($P = 0.255$). The diagnostic yield differed significantly, respectively 56.1% in Group 1 and 35.7% in group 2 ($P < 0.001$). The distribution of relevant findings on CE was comparable in both groups : 38% active bleeding, 30% angiodysplasia, 26.5% erosions, 8% tumors, 14.4% other findings.
There was a significantly higher transfusion need ($P = 0.002$) and a lower mean Hb level ($P = 0.013$) in group 1. Overall, patients with overt bleeding, transfusion need and lower Hb levels were more likely to have a relevant CE finding ($P = 0,034$, $P < 0,001$ and $P < 0,001$ respectively).
Conclusion : In time, there is a decreasing diagnostic yield of CE in patients with obscure GI bleeding, which started with the advent of reimbursement of this tool. This is caused by earlier referral at a stage of less severe anemia. Further exploration of the results is necessary to find out if earlier use of capsule endoscopy leads to a more cost-effective management of patients with obscure bleeding. Since tumours were diagnosed in 8% of the patients, CE remains a crucial step in the diagnostic strategy of obscure GI bleeding.

- G03 -

DOUBLE BALLOON ENTEROSCOPY IS AN ADEQUATE THERAPEUTIC TOOL TO ADDRESS ANGIODYSPLASIA-RELATED ANEMIA.
M. Janssens (1), X. Verhelst (2), P. Hindryckx (1), D. De Looze (1), M. De Vos (1), H. Peeters (1). (1) Ghent University Hospital, Gent, Belgium ; (2) Ghent University Hospital, Gent, Belgium.

Introduction : Double balloon enteroscopy (DBE) allows the endoscopic exploration of the small bowel and is now routinely performed in large endoscopy units. Apart of being a diagnostic tool, it can also be used as a therapeutic tool, e.g. for coagulation of angiodysplastic lesions.
Aim : We investigated the indications for which patients underwent a DBE, the therapeutic use of DBE and evaluated the long-term outcome of patients after therapeutic DBE.
Methods : We conducted a retrospective cohort study in a large Belgian tertiary referral center. A total of 135 patients underwent a DBE at our institution from 2007 until march 2011. Clinical data were retrieved from the patient files. Referring centers and primary care physicians were interviewed for clinical outcome using a questionnaire and were asked for recent hemoglobin and hematocrit levels. At least one value was collected after DBE. All data were analyzed with SPSS 19.
Results : Of all patients 68.9% underwent a DBE for suspected gastro-intestinal bleeding, 5.2% for suspicion of Crohns disease, 25.9% for other reasons (such as chronic diarrhea, suspicion of tumour, Peutz-Jegher…). A complete follow up was collected in 83 of the 135 patients. Mean follow up period was 28 months (range 1-42 months). In those 83 patients a total of 107 DBE procedures were performed. 62 of these 83 had a suspicion of GI bleeding.
In 51.6% (32 of 62) of these patients the diagnosis of angiodysplasia was made, 8.1% (5 of 62) had erosions and 24.2% (15 of 62) were normal. 16.1% (10 of 62) had other diagnoses such as tumours, Crohns disease….
In 53.2% (33 of 62) argon plasma coagulation (APC) was performed, in 6.5% (4 of 62) of the patients biopsies were taken, in 1.6% (1 of 62) polyps were removed and in 38.7% (24 of 62) no therapeutic action was performed. In the group of patients with angiodysplasia (32 of 62), 90.6% (29 of 32) were treated with APC. Interestingly, the mean Hb level of the patients with angiodysplasia rose from 9.38 (SD 1.99) before DBE to 11.34 (SD 2.09) after DBE. Mean hematocrit values rose from 21.17% (SD 5.71) before to 35.36% (SD 5.77) after DBE. 90.6% (29 of 32) of patients with angiodysplasia had anemia before DBE compared to 65.6% (21 of 32) after DBE ($p = 0.042$).
The need for transfusion diminished from 68.8% (22 of 32) before DBE to 37.5% (12 of 32) after DBE ($p = 0.033$).
Conclusion : This study shows that DBE significantly reduces the rate of anemia and the need for blood transfusion in patients with angiodysplasia. Intervention with DBE also improved the levels of hemoglobin and hematocrit values on the long-term in those patients. Therefore DBE is an adequate therapeutic tool to address angiodysplasia-related anemia.

- G04 -

FAECAL TRANSPLANTATION FOR RECURRENT CLOSTRIDIUM DIFFICILE INFECTIONS : FLEMISH EXPERIENCE. T. Holvoet (1), S. Naegels (2), D. Marichal (3), A. Mast (3), E. Cesmeli (3), G. Ghillebert (4), D. De Looze (1). (1) Ghent University, Ghent, Belgium ; (2) Zna Middelheim, Antwerpen, Belgium ; (3) Az Sint Lucas, Gent, Belgium ; (4) Heilig Hart Ziekenhuis, Roeselare, Belgium.

Introduction : Infections with Clostridium difficile are an important health problem with frequent, difficult to treat relapses. In patients with recurrent infections, studies show there is an imbalance in the colonic flora (dysbiosis) that may be responsible for the persistence of the infection. Faecal transplantation (FMT) is a simple technique that is infrequently used and treats recurrent C. difficile infections by restoring the microbiotic balance.
Aim : In this study we wanted to examine the Flemish experience regarding FMT for treating recurrent infections with Clostridium difficile.
Methods : Multicenter retrospective study to examine the Flemish experience with FMT in treating recurrent Clostridium infections. Participating centers were surveyed by means of a questionnaire.
Results : Four centers in Flanders treated a total of 8 patients (3 male, median age 71 years) with FMT for recurrent C. difficile infections (median number of recurrences = 4). Five transplantations were performed via colonoscopy (74%), one via enema and in two patients transplantation occurred by jejunal delivery. In all cases but one, donors were related to the patients. All subjects were cured after FMT with total resolution of the diarrhoea and negative fecal cultures. In a mean follow up time of 22 months, no relapses were reported nor were there any infectious complications. Two patients died of unrelated causes.
Conclusion : FMT is a valuable option for treating recurring infections with Clostridium difficile. Flemish experience is limited, but the global cure rate of 100% merits a more prominent place of FMT in the treatment of intractable Clostridium infections.

- G05 -

REACTIONS TO THE ENDOSCOPIC DISCOVERY OF SMALL (< 1 CM) COLONIC ADENOMAS. BELGIAN GLEM/LOK SURVEY. M. Van Outryve (1), C. De Galocsy (2), H. Büscher (3), J.L. Coenegrachts (4), R. Fiasse (5). (1) Universitair Ziekenhuis Antwerpen, Antwerpen, Belgium ; (2) Hôpitaux Iris Sud Bracops, Brussels, Belgium ; (3) AZ St Jozef, Malle, Belgium ; (4) Virga Jesse Hospital, Hasselt, Belgium ; (5) Université Catholique de Louvain, Brussels, Belgium.

Introduction : Colonoscopy with polypectomy reduces lifetime risk of colorectal cancer. Small (< 1 cm) and especially diminutive (< 6mm) colonic polyps however have a low risk of progression to cancer.
Aim : What about the detection rate and endoscopic characterisation of small adenomas, and the necessity of removal and pathological examination of small colonic polyps.
Methods : A questionnaire concerning prevalence/diagnosis, therapy and follow-up of small colonic adenomas was distributed to the Belgian GLEM/LOK groups in 2011.
Results : We received 77 answers, 73 were complete. Only 14% of the gastroenterologists (GE) always used high magnification colonoscopy. Small polyps were discovered in mostly 20-50% of the patients. Polyps were measured in 2/3 of the cases, but their morphology according to the Paris classification (1) was described in only 24%. Polyps of any size were removed by 80% of the GE ; the remaining GE only resected polyps of at least 3mm diameter. 58% of diminutive polyps were removed by biopsy forceps, the other ones by polypectomy snare. Even polyps without adenomatous characteristics on NBI, chromoendoscopy or other techniques were resected by 84% of the GE. Nearly all GE followed American (2) or other guidelines in screening and surveillance of colorectal adenomas and cancer. Only 28% of the responding GE already participated in a quality assessment of colonoscopy.
Conclusion : Most participating Belgian GE have excellent detection rates of small colonic polyps and adenomas, and adhere to international guidelines concerning their removal and follow-up. There is still some reluctance to participate to GLEM/LOK surveys or quality assessments of colonoscopy. The endoscopic differentiation of small polyps (adenoma or not) will improve with the increasing use of NBI, chromoendoscopy with magnification and others. In a near future, small non adenomatous polyps will probably be resected and discarded without pathology, or even left in place. This will save costs and manpower.
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Invited Lecture

- G06 -

WHAT TO DO WITH DIMINUTIVE POLYPS : RESECT AND/OR DISCARD ? J. East. Oxford, UK.

Improvement in detection through better operator technique and higher resolution instruments has led to an avalanche of diminutive lesions being sent to pathologists for assessment. These tiny polyp are thought to have relatively low risk of developing into malignant lesion ; however they are important in defining surveillance intervals. With the advent of easily available optical biopsy techniques such as narrowed spectrum endoscopy (NBI, FICE, iSCAN) or confocal endomicroscopy which provide a high level of diagnostic accuracy in expert hands, the concept that endoscopists might make an optical diagnosis for a diminutive polyp, resect the polyp, but not send the tissue for pathological assessment has been proposed. This is the so called "DISCARD" strategy (1, 2). More controversially if a diagnosis of a diminutive hyperplastic polyp is made in the rectosigmoid, that lesion might be left in situ. This represents a paradigm shift in endoscopic approach to polyps but potentially would result in very significant cost savings and a more efficient patient pathway. The ASGE has set out standards to be met for technologies and operators if such a strategy were to be undertaken in a PIVI statement (3).

In order to convince pathologists, patients, and those paying for healthcare that this strategy is safe and effective, a number of aspects need to be considered : What is the risk of advanced neoplasia or carcinoma in diminutive polyps ? What level of accuracy is required ? Can this be delivered by community based endoscopists ? Could computer aided detection help ? What training is needed ? How will endoscopists be accredited ? How will endoscopists be reimbursed ? What digital documentation is required ? What are the medicolegal implications ? Will National and European societies support such a strategy ? And will patients accept DISCARD ?

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- G07-

ERCP BY LAPAROSCOPIC TRANSGASTRIC ACCESS IN PATIENTS WITH GASTRIC BYPASS WITH BILIARY DISEASE. A. Badaoui (1), G. Dapri (2), C. D'haese (3), A. Rosiere (1), T. De Ronde (1), P. Deprez (4), J. Himpens (3). (1) UCL, Mont-Godinne, Belgium ; (2) ULB Saint-Pierre, Brussels, Belgium ; (3) AZ St Blasius, Dendermonde, Belgium ; (4) UCL St-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Preexisting biliary disease in morbidly obese patients can lead to biliary complications because of rapid weight loss after Roux-en-Y gastric bypass (RYGBP). The conventional endoscopic access to the biliary tract is limited principally for anatomic reasons. ERCP with balloon assisted enteroscopy has been reported to be feasible but has several limitations as a time-consuming procedure, with failures to identify the anastomosis site and the lack of adapted accessories. ERCP by laparoscopic transgastric access (ERCP-LTA) represents an alternative.

Methods : We report all patients with gastric bypass who experienced biliary complications (mainly choledocolithiasis) and were managed by ERCP-LTA. After creating a laparoscopic pneumoperitoneum, Four trocars were placed, and an incision was made in the anterior and distal part of the bypassed stomach close to the pylorus. A side-viewing endoscope (TJF 160R, outer diameter of 11,3mm, Olympus Corp, Tokyo, Japan) was introduced through the incision after removing the supraumbilical trocar, or through a 18-mm trocar on the left-upper quadrant and after creating a purse-string suture on the greater curvature of the gastric remnant. ERCP with sphincterotomy was performed under fluoroscopic guidance. Gallstones and/or biliary sludge were retrieved from the common bile duct with an inflated balloon catheter. Carbon dioxide gas was used for endoscopic insufflation. After removal of the scope, gastrostomy incision was closed. Finally, if necessary, laparoscopic cholecystectomy was performed. Antibiotics were given for 7 days.

Results : A total of 10 patients underwent ERCP-LTA between May 2008 and Nov 2012 (9 women and 1 man with a mean age of 48 y, range 26-66). Biliary stones or sludge extraction after sphincterotomy could be achieved in 9 patients. In the 10th patient sphincterotomy was performed for papillary stenosis. A cholecystectomy was performed during the same laparoscopy in 4 patients. The mean duration of the procedure was 66 min (35-98). No complications occurred, liver tests quickly normalized in all patients who were discharged on post-operative day 4 (3-5). Laparoscopic access was converted to a minilaparotomy in 1 patient with multiple previous surgeries due to bypass complications.

Conclusion : Laparoscopic transgastric ERCP can provide an effective biliary access after RYGBP. This technique seems feasible, safe, and reliable for the management of common bile duct stones and and may represent the new standard with a minimally invasive approach. Moreover, laparoscopic access to the biliary tract has the advantage of allowing ERCP and cholecystectomy to be performed consecutively in a single procedure, but requires a close collaboration between endoscopists and surgeons.

- G08 -

COMBINED LAPAROSCOPY-ASSISTED ERCP AND CHOLECYSTECTOMY AFTER BARIATRIC ROUX-EN-Y GASTRIC BYPASS. C. Snauwaert, P. Laukens, F. Goudsmedt, B. Dillemans. AZ Sint-Jan Brugge-Oostende, Brugge, Belgium.

Introduction : Endoscopic access to the biliary tree in Roux-en-Y gastric bypass (RNYGB) patients with biliopancreatic disorders is technically challenging. However, in certain conditions such as choledocholithiasis, endoscopic sphincterotomy with stone extraction (and cholecystectomy) should be performed to prevent recurrent biliary complications.
In our experience, the disadvantages of the balloon enteroscopy-assisted ERCP technique post-RNYGB are its very long procedural time and its inadequate instruments, leading to insufficient success rates, approaching approximately 60% in most of the literature. Therefore, we aim to perform laparoscopy-assisted ERCP as initial approach in this population.
To date, concomitant cholecystectomy at the time of RNYGB is not recommended given the relatively low rate (about 8 percent in large studies) of postoperative symptomatic gallstone disease.
Hence, it is to be expected that a fair amount of post-RNYGB patients with symptomatic gallstone disease will need both endoscopic biliary intervention and cholecystectomy. In these cases, combining laparoscopy-assisted ERCP and cholecystectomy may be a valuable strategy.
Aim : The goal of the present study is to assess the feasibility of combined laparoscopy-assisted ERCP and cholecystectomy after RNYGB.
Methods : We analysed retrospectively data of post-RNYGB patients who underwent a laparoscopy-assisted ERCP in our centre from November 2010 to November 2012. Informed consent was obtained from all patients according to usual clinical practice. Patients were identified using an electronic database that captures all endoscopic procedures performed at our hospital. Clinical data including age, gender, pre-operative diagnostic results, indication for procedure and final pathologic findings were recorded in the electronic patient file.
Results : Eight post-RNYGB patients (one male, seven females) were identified (mean age 58.7 years, range 48-80 y). Two patients had history of open surgery, six patients were operated laparoscopically. None of the patients had prior cholecystectomy. Indications included ascending cholangitis (n = 1), radiologically proven choledocholithiasis (n = 3), recurrent biliary pancreatitis (n = 3) and combination of a typical pain syndrome, cholelithiasis, abnormal serum aminotransferases and a dilated common bile duct on ultrasound (n = 1). All patients underwent cholecystectomy prior to the ERCP. All procedures were performed laparoscopicallywithout conversion to laparotomy.
After creating a gastrotomy on the greater curvature near the antrum, the gastric remnant was lifted up to the abdominal wall and ERCP was performed through a trocar placed into the gastrotomy. ERCP findings included normal cholangiogram(n = 1), choledocholithiasis (n = 6) and biliary sludge (n = 1). All patients underwent successful biliary cannulation and sphincterotomy (+/- balloon extraction). No needle-knife pre-cut sphincterotomy was necessary to achieve biliary access. There were no postoperative complications related to the ERCP.
Pathologic examination of all cholecystectomy specimens revealed chronic cholecystitis with cholesterolosis and/or cholelithiasis.
Mean hospital stay was 2.2 days (range 2-3d).
Conclusion : In post-RNYGB patients with symptomatic gallstone disease, combining laparoscopy-assisted ERCP and cholecystectomy is a feasible approach, without extra complications in our series.

- G09 -

ENDOSCOPIC ULTRASONOGRAPHY-GUIDED DRAINAGE IN THE MANAGEMENT OF MALIGNANT BILIARY OBSTRUCTION. A. Badaoui (1), C. Gillain (2), T. Aouattah (3), J.F. De Wispelaere (1), T. De Ronde (1), P. Deprez (4). (1) UCL, Mont-Godinne, Belgium ; (2) Grand Hôpital de Charleroi, Hôpital Saint-Joseph, Gilly, Belgium ; (3) Clinique St. Pierre, Ottignies, Belgium ; (4) UCL-St-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : EUS-guided biliary drainage (EUSGBD) has been proposed as an alternative technique to percutaneous transhepatic drainage (PTBD), for the management of patients with malignant biliary obstruction in whom ERCP fails or cannot be achieved. We report our experience of EUSGBD performed either as the sole drainage or associated wit ERCP or percutaneous right biliary drainage.

Methods : The files of all patients with malignant biliary obstruction managed by EUSGBD were retrospectively reviewed, for the cause of obstruction, the reason for performing EUSGBD, the type of access, and the morphological and clinical outcomes. The procedure was performed with linear-array echoendoscopes (Hitachi/EG 3830UT, Pentax or Aloka Alpha 7/Olympus) under fluoroscopy. The left biliary ducts or the common bile duct (CBD) were punctured with a 19-g needle (Cook Medical or Boston Scientific) under EUS-guidance. Before inserting a 0,035 inch guidewire (Boston Scientific) into the biliary ducts, cholangiography was performed, to assess the route of drainage and the connection to the right hepatic ducts. If necessary, the tract was enlarged with a 6Fr cystostome (Endoflex) before insertion of covered metallic stent (Boston Scientific or Taewong Medical) between the left biliary ducts and the gastric wall or between the CBD and the duodenal wall. No dilatation of the tract was performed. Intravenous antibiotics were administered during 1-7 days.
Results : Nineteen patients underwent EUSGBD between Aug 2004 and Nov 2012 (11 W and 8 M, aged 65, 14-85 y). Causes of biliary obstruction were pancreatic adenocarcinoma (7), colorectal cancer with liver or hilar involvement (3), hilar cholangiocarcinoma (3), IPMN (2), metastatic rhabdomyosarcoma in a child (1), hepatocarcinoma (1), ampulloma (1), and gallbladder cancer (1). Rendez-vous, transduodenal and transgastric routes were used in 3, 3 and 13 pts respectively. Combined ERCP/PTBD and EUSGBD was performed in 6 pts, due to persistant cholangitis after incomplete drainage. Mean duration of procedure was 40 (35-45) minutes. The post-procedure course was favourable with rapid normalization of hyperbilirubinemia. Three hepatico-gastrostomies were complicated with asymptomatic pneumoperitoneum. Surgery was performed in 1 patient after EUS-GBD and chemotherapy could be either initiated or resumed after jaundice resolution in 15 patients. The mean follow-up was 5 months (1-10).
Conclusion : EUS-GBD represents a feasible alternative in patients with malignant biliary obstruction and unsuccessful ERCP. Prospective trials are however awaited to compare the respective safety and efficacy of EUSGBD and PTBD.

- G10 -

OUTCOME OF EMBOLIZATION OF POST-SPHINCTEROTOMY BLEEDING REFRACTORY TO ENDOSCOPIC THERAPY. G. Maleux (1), J. Bielen (2), A. Laenen (3), S. Heye (1), J. Vaninbroukx (1), W. Laleman (4), P. Verhamme (2), A. Wilmer (2), W. Van Steenbergen (2). (1) Interventional Radiology, University Hospitals Leuven, KU Leuven, Leuven, Belgium ; (2) UZ Leuven, Leuven, Belgium ; (3) KU Leuven, Leuven, Belgium ; (4) Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

Introduction : Bleeding related to sphincterotomy is a common complication which can be treated by medical and endoscopic management in the large majority of cases. However, severe and persistent post-sphincterotomy bleeding is rare and, if refractory to medical and endoscopic treatment, embolisation may be a valuable alternative treatment option. Data on technical and clinical outcome of percutaneous embolotherapy are unknown.
Aim : The aim of this study was to retrospectively analyse the technical and clinical outcomes of embolotherapy for post-sphincterotomy bleeding refractory to medical and endoscopic therapy. Additionally, factors potentially influencing the 30-day mortality were analysed.
Methods : From November 1998 till November 2012, 34 patients (16M, 18F ; mean age 71years) underwent percutaneous embolotherapy for post-sphincterotomy bleeding refractory to medical and endoscopic management. Demographic, laboratory, angiographic and clinical follow-up data were collected. Additionally, several clinical and biochemical factors potentially influencing the 30-day survival were analysed.
Results : Indication for initial sphincterotomy was benign (n = 28) or malignant (n = 6) disease. In 13 patients (38%) a precut was performed ; in the remaining 21 patients (62%) complete sphincterotomy was performed. Eleven patients were still on antithrombotic medication at the time of sphincterotomy : aspirin (n = 7) and heparin (n = 4).
A mean delay of 3.6 days (standard deviation 4.4 days ; median 1.0 day ; min. 0 days and max. 16 days) was noted between sphincterotomy and clinical evidence of massive bleeding. Angiographic evaluation revealed contrast extravasation (n = 31), pseudoaneurysm (n = 2) and a combination of both (n = 1).
In 27 patients (79.4%) one bleeding point was identified on angiography ; in the remaining 7 patients (20.6%) 2 bleeding points were identified. Embolization was succesful in 33 out of 34 patients (97%). Recurrent bleeding occurred in 3 patients (9%) and 30-day mortality was 20.6% (n = 7). Factors significantly influencing 30-day mortality were : INR (P = 0.008) and aPTT (P = 0.012). Overall survival at five years was 68.7%.
Conclusion : Angiographic embolization is very effective in stopping a post-sphincterotomy bleeding refractory to medical and endoscopic therapy. Rebleeding rate is low (9%) but 30-day mortality remains significant (20%). Hemostatic disorders seem to significantly influence the 30-day survival. Long-term survival is satisfactory.

Invited Lecture
- G11 -

LARGE COLONIC POLYPS : PIECEMEAL EMR OR ESD OR... SURGICAL RESECTION ? H. Piessevaux. UCL St-Luc.

- G12 -

"BEAR-CLAW" OR OVER-THE-SCOPE CLIP SYSTEM (OTSC)-A BREKTHROUGH,SURGERY SPARING, ENDOSCOPIC DEVICE. J. Toshniwal (1), L. Fry (2), K. Vormbrock (2), M. Zabielski (2), K. Mönkemüller (2). (1) Sir Ganga Ram Hospital, New Delhi, India ; (2) Marienhospital, Bottrop, Germany.

Introduction : The "bear-claw" or over-the-scope clip system, OTSC (Ovesco Endoscopy, Tübingen, Germany) is a new clipping device developed for closure of large luminal gastrointestinal (GI) defects.
Aim : To evaluate the clinical outcomes of patients treated with the OTSC.
Methods : Prospective, single-arm, pilot study conducted in a regional hospital with tertiary care endoscopy facility. This study involved 11 clip applications in 10 patients(median age 77.4 years [range 55-90 years], 6 woman) with GI defects from fistulas and anastomotic dehiscence ; and peptic ulcer and posterior wall duodenal ulcer bleeding. Closure of leaks was confrimed clinically and by using contrast agent under fluoroscopy.
Results : Two bleeding gastric ulcer, four bleeding posterior wall duodenal ulcers, three fistulas, one anastomotic dehiscence were treated using the OTSC-system.
In addition a self-expanding metal stent was anchored securely in place with an OTSC-system. The diameter of leaks ranged between 12 and 20 mm. A complete sealing of leaks was achieved in all patients. Also, hemostasis was achieved in all patients. Hemostasis could not be achieved in them with normal conventional methods (through the scope clip, injection adrenaline). There were no clip failures or complications. However, during introduction of the loaded clip on the tip of the endoscope, the hood tended to migrate over the scope, i.e. retracting, thus diminishing the exposed hood. This leads to diminished tissue suction and closure. Thus we modified the technique by tightly taping the hood on the tip of the scope. This trick may explain why all our cases were successful.
Conclusion : The OTSC system is a useful device in a variety of clinical scenarios including the management of in larger GI leaks, GI bleeding and stent anchoring ; even in very old and frail patients, who are very high risk candidates for surgical intervention and are associated with high mortality and morbidity.

- G13 -

CLINICAL MANIFESTATIONS ASSOCIATED WITH ENTAMOEBA COLI : IMPACT OF HIV AND METRONIDAZOLE. O. Lheureux, T. Sersté, V. Muls, M. Buset, S. De Wit, O. Vandenberg, A. Dediste. ULB Saint-Pierre, Brussels, Belgium.

Introduction : Entamoeba coli (EC) is not a known pathogen in human and its causal relationship with diarrhea is generally doubted.
Aim : The aim of this study was to assess the relation between diarrhea and the presence of EC detected the stool. The role of a treatment test with metronidazole and the place of the immunological status (Human Immunodeficiency Virus, HIV) in the clinical manifestations associated with EC have also been precised.
Methods : All consecutive patients in whom EC was detected in stool analysis (UMC Saint Pierre, Brussels, Belgium) between January 2007 and December 2011 were retrospectively included. The underlying cause which led to perform the stool analysis (either diarrhea, flatulence, or hypereosinophilia) was collected. Stool specimens were first examined for cysts or parasites by light microscopy after wet mount preparation, trichrome staining and formal-ethyl acetate concentration. Bacterial stool cultures were then secondly obtained for conventional phenotypic identification. Patients were divided groups according to their immunological status (HIV). The causal relationship between diarrhea and EC was qualified as highly suspicious when no well known pathogen was identified in stool specimen and when diarrhea stopped immediately after a treatment test with metronidazole.
Results : A total of 145 stool samplings were collected from 145 patients. The prevalence of the HIV was 29/145 (20.0%). The underlying causes to perform the stool examinations were as follows : 87 diarrheas (60.0%), 31 flatulences (21.4%), 27 hypereosinophilias (18.6%). In patients with diarrhea, there was no known pathogen(s) in patients with diarrhea in 79/87 (= 90.8%) cases. EC was associated with another *non-pathogenic* microorganism (mainly Blastocystis hominis) in 51/79 (= 64.6%) patients.
The proportion of patients who improved diarrhea with metronidazole was compared with patients who spontaneously improved their symptoms : the difference was significant (43/49 = 87.8% with metronidazole versus 19/30 = 63.3%

without metronidazole, p = 0.003). When EC was found alone (not associated with any micro-organism), there was no statistical improvement of diarrhea with metronidazole (11/13 = 84.6% with metronidazole versus 11/15 = 73.3% ; p = 0.06). Concerning immunity and clinical manifestations, the frequency of diarrhea in the presence of EC in the stool analysis was higher in HIV patients than in non-HIV patients (64/116 = 55.2% in non-HIV versus 23/29 = 79.2% ; p = 0.021).
Conclusion : In conclusion, EC is frequently associated with diarrhea and other non-pathogenic microorganisms. Metronidazole seems to improve diarrhea, particularly when other non-pathogenic microorganism are found in the stool. In HIV patients the frequency of diarrhea in the presence of EC is significantly higher.

- G14 -

STENTING IN UNEXTRACTABLE LARGE COMMON BILE DUCT STONES IN ELDERLY AND HIGH RISK PATIENTS. A. Krishnan, R. Ramakrishnan, J. Venkataraman. Stanley Medical College, Chennai, India.

Introduction : Endoscopic sphincterotomy (ES) and stone extraction is the treatment of choice for bile duct stones. Therefore, if ES and conventional stone extraction fail, further treatment is mandatory. Insertion of a biliary endoprosthesis is an effective option. Different endoscopic modalities are available for the extraction of common bile duct stones. However, there is no clear consensus on the better therapeutic approach.
Aim : The aim is to analyze the effectiveness of “interim” plastic biliary stent deployment in difficult stones in elderly and high risk patients
Methods : Patients who had co-morbid illness and elderly patients who are not fit for surgery were included. Endoscopic plastic biliary stenting was performed in 65 patients with large and/or multiple common bile duct stones or those difficult to extract with conventional endoscopic therapy. Liver function test before and after stenting also recorded. Bile duct drainage and endoscopic placement of 7 Fr plastic biliary stents were established in all patients. The diameters of the CBD stones were measured on the radiographs before and after stenting.
Results : In this 22 patients has multiple CBD stones (> 3) and 46 patients had large stones (> 2cms). Stone retrieval was possible, after a median of 24 days (19-38 days). All patients had reductions in the stone number and/or stone size. In 18 patients there was spontaneous clearance of the stones from the CBD. The median number and size of stones per patient was significantly reduced after biliary stenting compared with before 5 (3) vs 2.0 (1.0) [P < 0.0001] and 2.8 (1.5) to 2.0 (1.0) [P < 0.001] respectively. Liver function test also showed a significant after stenting (p < 0.001). All the stones were black and amorphous in consistency.
Conclusion : Plastic biliary stenting is safe and effective in the management of difficult stones in elderly and high risk patients. It may fragment common bile duct stones and decrease stone sizes. Unlike the reports for cholesterol stones, shorter period of deployment is sufficient for pigment stones, because these are either black or mixed and are amorphous, unlike the hard cholesterol stones reported for hard cholesterol stones.

- G15 -

TRANSORAL FUNDOPLICATION FOR GASTROESOPHAGEAL REFLUX : A LONG TERM SINGLE-CENTER CLINICAL STUDY. S. D’amico, O. Plomteux, J. Weerts, F. Fontaine, B. Bastens. St Joseph Hospital, Liège, Belgium.

Introduction : Transoral incisionless fundoplication with EsophyX® has been used for several years to treat patients with chronic gastroesophageal reflux (GERD). Its long term effectiveness can now be analysed. We evaluated clinical outcomes using GERD health-related quality of life (HRQL) questionnaire, upper gastrointestinal endoscopy and medication use.
Aim : The primary effectiveness endpoint was patient quality of life assessed by GERD-HRQL questionnaire. Secondary effectiveness endpoints were PPI usage and reflux oesophagitis grade.
Methods : 24 patients with chronic GERD inadequately controlled by proton pump inhibitors (PPIs) medication underwent EsophyX® from 2007 until 2011. 3 were lost of follow-up. 13 patients at 8 months and 15 patients at 35 months completed the follow-up.
Results : At 8 months median follow-up, median GERD-HRQL scores were significantly reduced (p < 0,001) from 20 (6-34) to 2 (0-28) compared to the baseline on PPI in 84,6% (11/13) of patients. 11 patients were off daily PPI after the procedure. Among 11 patients with oesophagitis, significant improvement occured in 9 patients (81,8%). At 35 months median follow-up, 66,6% (10/15) of patients were clinically improved with a median GERD-HRQL significantly reduced from 17 (2-24) to 6 (0-23). 11 patients (73,3%) could discontinue their everyday use of PPI.
Conclusion : Our results show that transoral incisionless fundoplication is effective to improve quality of life, to reduce symptoms and to decrease the daily use of PPI of patients with chronic gastroesophageal reflux at 35 months follow-up.

- G16 -

SUCCESS RATE AND SAFETY OF COLONOSCOPY IN PATIENTS WITH LIVER CIRRHOSIS. E. Macken (1), T. Steinhauser (2), S. Francque (2), P. Michielsen (2), P. Pelckmans (1), T. Moreels (1). (1) Antwerp University Hospital, Antwerpen, Belgium ; (2) Universitair Ziekenhuis Antwerpen, Antwerpen, Belgium.

Introduction : Colonoscopy is a mandatory examination in patients with liver cirrhosis before liver transplantation in order to detect extrahepatic malignancy. Although caecal intubation rate is an important quality parameter, is it not known if this goal is harder to achieve in the presence of liver cirrhosis with or without ascites.
Methods : Caecal intubation rate and complication rate in 93 patients with known liver cirrhosis were retrospectively compared with a control group of 1269 patients in 2009. Age, sex, Child-Pugh classification, presence of ascites, type of sedation and cleanliness of the colon were recorded.
Results : 93 patients with liver cirrhosis were included. Male-female ration was 58 :32 with a mean age of 58 years. The majority of patients were Child-Pugh B. Caecal intubation rate was significantly lower in patients with liver cirrhosis than in patients without liver cirrhosis (83% vs 94%, $p < 0.0001$). Caecal intubation rate did not differ significantly between different sexes, ages sedation or cleanliness of the bowel in the cirrhosis group. However, caecal intubation rate was lower in patients with ascites in the cirrhosis group than in patients without ascites, although these numbers did not reach a satistically significant difference (91% vs 76%, $p > 0.05$).
Complication rate was significantly higher in the cirrhosis group (2.2% vs 0.4%, $p < 0.05$). Mortality in the cirrhosis group was 1.1% (vs 0% in the control group). One death was due to perforation after the procedure, one patient needed urgent surgery due to an incarcerated umbilical hernia. There were no cases of bleeding or spontaneous bacterial peritonitis.
Conclusion : This retrospective analysis showed that caecal intubation rate is significantly lower in cirrhotic patients. The presence of ascites could probably play a role, although the numbers did not reach statistically significance. Also, complications occur significantly more often in cirrhosis patients and mortality is significantly higher. Care should be taken in this patient population when performing colonoscopy.

- G17 -

CHARACTERIZATION OF NEUTRALIZING MONOCLONAL ANTIBODIES AGAINST CLOSTRIDIUM DIFFICILE TOXINS A AND B. D. Staelens (1), M. Van De Wouwer (2), E. Brouwers (2), S. Caluwaerts (3), B. Lacy (4), P. Rottiers (3), P. Vanhoenacker (3), N. Geukens (5), P. Declerck (2), S. Vermeire (1), P. Rutgeerts (1), G. Van Assche (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (2) Laboratory for Pharmaceutical Biology, Leuven, Belgium ; (3) Actogenix Nv, Gent, Belgium ; (4) Vanderbilt University Medical Center, Nashville, United States ; (5) Pharmabs, Leuven, Belgium.

Introduction : *Clostridium difficile* associated disease (CDAD) is increasing in incidence and severity with significant morbidity and mortality. Antibiotic treatment is not invariably successful with a relapse rate around 30%. Only toxin A and/or B (TcdA, TcdB) producing strains are proven to be pathogenic.
Aim : We aim to develop a novel treatment modality based on targeted mucosal delivery of anti- *Clostridium difficile* toxin-antigen binding fragments.
Methods : SJL/J mice were immunized with *Clostridium difficile* toxoid by a standard protocol for hybridoma generation. Monoclonal antibody (MA) production was evaluated on coated TcdA and TcdB. Antigen binding fragments (Fabs) were generated using a standard protocol based on papain treatment. Selected MAs were evaluated for their *in vitro* neutralization capacities using human fibroblasts (IMR-90) and TcdA or TcdB at 570 pM or 56 pM, respectively. Affinities were determined on a Biacore 3000. Relative epitope-mapping was based on pair-wise combination of MAs in sandwich ELISAs. Western blot with different domains of TcdA and TcdB and selected MAs as primary antibody were used for initial epitope mapping. *In vivo* neutralization capacities of the MAs were evaluated by murine ileal loops in C57BL/6 mice (65 nM TcdA, 4 hours incubation, 2 cm loops either with or without 72 - 113 μM MA). Gene expression was analyzed by standard RNA extraction and RealTime-PCR.
Results : Out of 99 monoclonal antibodies that were reactive against TcdA and/or TcdB, 4 neutralized TcdA *in vitro* (average EC50 : 15 nM), 6 neutralized TcdB *in vitro* (average EC50 : 3.02 nM) and 1 antibody neutralized both toxins (EC50-TcdA : 13 nM, EC50-TcdB : 140 nM) *in vitro*. Biacore affinity studies on 4 TcdA neutralizing MAs demonstrated high affinity for TcdA and moreover, very slow dissociation rates. Relative epitope mapping resulted in the selection of 6 neutralizing MAs representing 3 distinct epitopes at each toxin. Western blot experiments indicate that the three selected MAs against TcdA are binding at the receptor-binding domain, while the selected MAs against TcdB bind at the glucosyltransferase domain. *In vivo* neutralization activity was confirmed for three selected MAs by a decrease in mRNA levels of *IL6*, *CXCL1* and *TNFalpha* after injection in murine ileal loops of TcdA and the MA, compared to injection

with TcdA alone. Fabs derived from the best neutralizing MAs showed *in vitro* neutralization of TcdA or TcdB without significant loss in affinity.
Conclusion : This study identifies several monoclonal antibodies with high affinity and bioactivity *in vitro* as well as *in vivo*. We will further seek *in vivo* confirmation in the well-established Syrian gold hamster model of CDAD with local delivery of Fab fragments of the selected MAs using the ActoGeniX *Lactococcus*-based mucosal delivery system.

- G18 -

PROCTOLOGICAL PROBLEMS IN RELATION TO CHEMOTHERAPY. T. Lagaert, B. Van Duyhuys, K. Gorleer, B. Strubbe, I. Bruggeman, P. Hindryckx, D. De Maeseneer, S. Van Belle, I. Moors, T. Kerre, K. Geboes, S. Laurent, D. De Looze. Ghent University Hospital, Gent, Belgium.

Introduction : In daily practice anal problems in patients under chemotherapy are often seen, cause significant morbidity and are difficult to treat.
Aim : Literature about this problem is scarce, even unexisting. Therefore, we want to make a survey of proctological problems in patients under chemotherapy, and evaluate factors that promote the development of anal disease.
Methods : From March, 15th until November 30th, 2012 all patients spontaneously reporting anal complaints at the different departments of oncology and currently under chemotherapy, are selected for this study. Informed consent is obtained from all patients. The following data are systematically collected : performance status (grade 0 is normal-grade 4 is severe), medical history, current oncological disease and chemotherapy, chemotherapy-related toxicity (grade 0-4), proctological complaints, diagnosis and outcome.
Results : Twenty-three people, 14 women and 9 men, with a mean age of 50 years (range 20-80) are collected. The main presenting symptom is anal pain (n = 21) and in 2 patients anal blood loss. Proctological diagnoses were anal fissure (n = 12), external hemorrhoidal thrombosis (n = 3), anal abscess (n = 2), anal ulceration (n = 2), internal hemorrhoidal bleeding (n = 2), no diagnosis (n = 2). Mean WHO performance status was 1,72 (range 1-4), mean toxicity scores for respectively oral mucositis, nausea-vomiting, diarrhea and constipation are 0,76-0,88-0,94 and 1,50 (ranges 0-4). Patients were under chemotherapy for breast cancer (n = 8), AML (n = 3), renal cell carcinoma (n = 2), rectal carcinoma (n = 2), ALL (n = 2), MDS (n = 2), sarcoma (n = 1), testis carcinoma (n = 1), aplastic anemia (n = 1) and non-Hodgkin lymphoma (n = 1).
Conclusion : Anal fissure is the most frequent encountered proctological problem in patients under chemotherapy. Constipation was the most commonly seen toxicity of chemotherapy, while oral mucositis was rarely seen in this patient cohorte. Treatment of anal problems is most often conservative, but preventive measures should be directed towards prevention of constipation

- G19 -

TOLEROGENIC PROTOCOL WITH LOW IMMUNOSUPPRESSION FOR INTESTINAL TRANSPLANTATION : 90% 10-YEAR SURVIVAL. L.J. Ceulemans, D. Monbaliu, G. Van Helleputte, M. Hiele, G. Van Assche, S. Vermeire, G. De Hertogh, F. Nevens, T. De Rijdt, M. Waer, J. Pirenne. Intestinal Transplant Program, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

Introduction : Results of intestinal transplantation (ITx) remain inferior compared to other solid organ Tx because of higher rates of rejection and need for more profound immunosuppression (IS) with its associated side-effects. This is due to the intrinsic immunogenicity of the bowel, exacerbated at time of transplantation by inflammation/ischemia-reperfusion injury (IRI), endotoxin translocation and their enhancing effect on alloimmunity.
Aim : We hypothesized that reduction of periTx IRI and endotoxin translocation in conjunction with recipient immunomodulation (in the form of donor-specific blood transfusion (DSBT)), would promote acceptance of intestinal grafts while reducing the need for IS.
Methods : An immunomodulatory protocol consisting in i) maneuvers destined to reduce IRI and endotoxin translocation (hemodynamic stable donors, selective bowel decontamination (Nilstat, Tobramycine, Amphotericine B and Colistine sulphate), glutamine administration in donors and receptors, short ischemic times and more recently administration of anti-TNF alpha) ; ii) perioperative administration of DSBT (200-400cc whole blood undepleted of circulating leukocytes) was used in 10 consecutive deceased ITx recipients 5 isolated ITx ; 5 combined liver and ITx, median age : 38.5 years (2 years 8 months - 57 years). Indications were anatomical or functional short bowel syndrome : volvulus (4), intestinal ischemia (3), Crohn (2) and chronic intestinal pseudo-obstruction (1). IS was tacrolimus-based. Early postTx trough levels were kept < 15 ng/ml and gradually tapered individually to 5-8 ng/ml (isolated ITx) or < 5 ng/ml (combined liver and ITx). As induction therapy, basiliximab was used in 8 and ATG in 2. Low dose steroids (no bolus) and

azathioprine were used and adjusted individually. The analyzed end-points were : incidence of early (< 3 months) and late (> 3 months) Acute Rejection (AR) and Chronic rejection (CR), nutritional independence (TPN-freedom), patient and graft survival.
Results : Early (reversible) AR was seen in one Crohn patient (10%). Late AR was seen in 2 patients (20%) at 4 months and 46 months postTx. In the former, AR was reversed but the patient succumbed to aspergillus sepsis ; in the latter, AR was due to therapy withdrawal/non-compliance, but was reversible with treatment and the patient is doing well. No CR was documented so far. The longest survivor succumbed 11 years and 10 months postTx due to acute diffuse NSAID-induced ulcera in the transplanted bowel. At last follow-up (median : 1780.5 days (254-3698)), the 8 survivors are TPN-free. The 10-year patient and graft survival is 90%.
Conclusion : The immunomodulatory protocol described herein seems to promote intestinal graft acceptance and nutritional independence under low IS, resulting in low incidence of IS-side effects (infection, cancer, drug toxicity) and in improved long-term survival.

- G20 -

CLIP AND ENDOLOOP DEFECT CLOSURE DURING ENDOSCOPIC EMR OR ESD RESECTION OF NONAMPULLARY. L. Shaza (1), H. Ivekovic (1), R. Yeung (1), A. Jouret-Mourin (2), H. Piessevaux (2), P. Deprez (2). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) Cliniques Universitaires St Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Endoscopic removal (ER) of non ampullary duodenal adenomas although effective and safe for small lesions is associated with higher risk for bleeding due to the extensive blood supply in the second duodenum where most adenomas occur. Delayed bleeding after duodenal ER is a feared complication that can be reduced by careful per procedure haemostasis and may be reduced by closure of the mucosal defect.
Aim : We present results on the performance of the defect closure with clips and endoloops in our cohort of patients.
Methods : We cross-examined our prospectively maintained database of endoscopic procedures, between 6/2005 and 3/2012 to identify patients with ESD/EMR performed in the duodenum either for sporadic or familial adenomatous polyposis, excluding ampullary adenomas. Morphology of duodenal lesions, procedure-related data such as type of closure, presence of post-procedural adverse events were analysed. Results are expressed as medians and range, and compared with Pearson's chi-squared test.
Results : 72 patients (62y [33-83], 35 M and 37 F) were included with a total of 83 resected adenomas by EMR technique in 68 pts and ESD in 15 pts (11 hybrid procedures combining EMR and ESD due to poor lifting after recurrence or previous failed attempts of removal).
Conclusion : In this large series of EMR-ESD removal of duodenal adenomas, delayed bleeding occurred in 19% and was significantly reduced by prophylactic closure of the mucosal defect with clips and endoloops (7%). Delayed perforation rate was low and was further decreased by prophylactic clipping, that was however not feasible after resection of the very large adenomas (> 3cm) at highest risk for perforation.

- G21 -

HIV-HELICOBACTER PYLORI CO-INFECTION : ANTIBIOTIC RESISTANCE, PREVALENCE, IMPORTANCE. M. Nkuize (1), P. Itoudi (1), S. Dewit (1), D. Touma (1), V. Muls (1), Y. Miendje Deyi (2), R. Ntounda (1), T. Serste (1), M. Buset (1). (1) ULB Saint-Pierre, Brussels, Belgium ; (2) ULB Brugmann, Brussels, Belgium.

Introduction : Human immunodeficiency virus (HIV) and *Helicobacter pylori (HP*) infections are world while burden.
Aim : To evaluate anti-*HP* drug resistance and first line treatment response among treatment-naive HIV-HP co-infected patients.
Methods : This is a longitudinal observational study since 1st January 2007 until 31 December 2011. Consecutive patients, *HP* treatment naive, with a gastric biopsy positive for *HP* infection (Histology and culture) were selected.
Parameters studied at the date of endoscopy were related to : demographics (age, gender, ethnicity, body mass index (BMI)), HIV and immunity (status, CDC stage, CD4 counts, antiretroviral treatment (ARV) *HP* (antibiotic sensitivity, type of treatment), and antibiotics used twelve months prior including amoxicillin, clarithromycin, tetracycline, quinolones, tetracycline, and metronidazole.
Data were compared between groups A (*HP*+-HIV-) and B (*HP*+-HIV+) patients.
Results : We found 353 patients : 260 in group A vs. 93 in group B.
Patients from group B were at CDC stage A (54%), heterosexual (64.6%), Black African (51%) or Caucasian (44%), and under ARV treatment (61%), and had a mean CD4 counts of 504.

Compared to group A, patients from group B were often males ($p = 0.01$), used less frequently antibiotics ($p < 0.0001$), had a lower BMI ($p < 0.0001$), and yielded more *HP* strains resistant to fluoroquinolones ($p = 0.005$) and metronidazole ($p = 0.01$). Those resistances were significantly frequent in females, Black Africans, and heterosexuals. A triple therapy was more prescribed at first line, and the response rate was 12/56 (78.6%) and 206/242 (85%) in groups B and A respectively. There were no significant differences for other parameters.

Conclusion : A high prevalence of HP strains resistant to fluoroquinolones and metronidazole were found in HIV-*H. Pylori* co-infected patients, notably in women, Black Africans, and heterosexuals, than among HIV uninfected patients. This could have an impact on the choice and the type of anti-*HP* treatment.

The response rate to triple therapy against *H. pylori* might be lower in HIV infected patients.

Invited Lecture

- I01 -

ROLE OF LYMPHANGIOGENESIS IN PHYSIOPATHOLOGY OF IBD. J.F. Rahier, UCL, Mont-Godinne.

Abnormalities in lymphatic vasculature have been noted in the original descriptions of CD. Nowadays, the lymphatic system is re-emerging as a critical player in inflammatory and immune processes. Recent studies report lymphangitis, lymphangiogenesis, bacterial infiltration and lymph node infection, immune cell trafficking, and fat-wrapping in CD suggesting altered lymph drainage and implicating the lymphatic system as a player in IBD.
Both forms of IBD show lymphatic remodeling with intestinal vessels proliferating in each layer of the inflamed small and large bowel. This remodeling is also found in non-inflamed sections of IBD suggesting that lymphangiogenesis can be present prior to the appearance of mucosal features of inflammation. Whether lymphangiogenesis in IBD is pathological or protective is an open question. A close association between inflammation, granulomas, tertiary lymphoid follicles, and the lymphatic vasculature is noted in CD together with lymphangiectasia and lymphocytic perilymphangitis. Beside, unexpected distribution of lymphatic vessels is observed in both forms of colonic IBD. Lymphatic vessels are seen throughout the inflamed colonic mucosa and reach the upper third of the lamina propria, challenging established dogma regarding the absence of lymphatic vessels in colonic mucosa.This may impact luminal antigen sampling and uptake, as well as migration of antigen-presenting cells.
The role of lymphatic vessels in the pathophysiology of IBD remains uncertain. Lymphatic trafficking between the primary sites of inflammation and local draining lymph nodes certainly plays an important role in limiting progression of inflammation. Studies of decoy receptors bring evidence of the lymphatic system as an active player in immune function during IBD. Indeed, D6-deficient mice are more susceptible to DSS colitis and fail to resolve colitis with higher circulating levels of chemokines. Experimental evidence strongly supports the idea that lymph stasis from lymphatic vessels promotes fat accumulation. Disrupted or malfunctioning lymphatic drainage leads to massive edema, increased numbers of fibroblasts, adipocytes, and keratinocytes in the skin and subcutaneous tissue and a mild inflammation. No data are available on the clearance capacities of lymphatic vessels in IBD. Some of the lymphatics might be immature or non-functional, as described in tumour-associated lymphangiogenesis. Macroscopic remodeling of mesenteric and serosal lymphatic vessels occurs during CD. Fat-wrapping which is a feature of Crohn's disease patients undergoing ileal resection and which correlates with the extent of transmural inflammation may be another consequence of lymphatic contractile dysfunction or disruption in CD.
Improved knowledge and appreciation of the roles that the lymphatic system plays in immune cell trafficking, infection, fat transport and edema resolution is necessary to better understand the pathogenesis of chronic inflammatory conditions such as CD and may provide the basis for new therapeutic strategies.

- I02 -

THE THERAPEUTIC POTENTIAL OF HELMINTH PROTEINS IN A CHRONIC COLITIS TRANSFER MODEL. M. Heylen (1), N. Ruyssers (1), J. De Man (1), P. Pelckmans (2), B. De Winter (1), T. Moreels (2). (1) University of Antwerp, Antwerpen, Belgium ; (2) Antwerp University Hospital, Antwerpen, Belgium.

Introduction : The hygiene hypothesis is based on a possible relation between the higher incidence of inflammatory bowel disease in the Western world and a decreased exposure to parasitic helminth infections.
Aim : Our aim was to determine whether treatment with *Schistosoma mansoni* worm adult proteins (SWAP) can improve the course of colitis in a murine chronic colitis transfer model and which immunological pathways are involved.
Methods : Colitis was induced in immunodeficient SCID mice by the adoptive transfer of naive (CD4+CD25-CD62L+) T cells, isolated from BALB/c mice ; controls were injected with PBS (CONTROL) (Heylen *et al*., Inflamm. Bowel Dis., in press). Mice were treated twice with vehicle (COLITIS) or SWAP (25 g/week ; i.p.) at weeks 4 and 5 after adoptive transfer (COLITIS+SWAP) (n = 6 in each group). Every 2 weeks, changes in body weight were recorded and colonoscopy was performed. After sacrifice at week 6, post-mortem colonic macroscopic, microscopic and myeloperoxidase (MPO) assessment were performed. Mesenteric lymph nodes (MLN) were collected and prepared for flow cytometric characterization of CD4+ T cells.
Results : CONTROL mice gained weight during the experiment. COLITIS mice lost 13.7+/-3.1% of their initial body weight, while COLITIS+SWAP mice lost 4.7+/-4.1%. In COLITIS mice the colonoscopic score significantly increased over time from 2.8+/-0.7 at week 2 to 6.8 ?0.8 at week 6, whereas in COLITIS+SWAP mice the maximum score of 4.7+/-0.8 was reached at week 4 and significantly decreased at week 6 (3.3+/-0.6). The macroscopic score was significantly higher in COLITIS (10.0+/-0.7) versus COLITIS+SWAP mice (5.8+/-1.3). The microscopic score and MPO

activity of COLITIS+SWAP mice (7.3+/-1.0 and 4.0+/-1.1 units MPO/g tissue) tended to decrease compared to COLITIS mice (9.0+/-0.5 and 6.3+/-1.0 units MPO/g tissue). None of the above mentioned inflammatory parameters increased in CONTROLS. Flow cytometric analysis of the MLN showed comparable amounts of CD4+ cells in the COLITIS and COLITIS+SWAP mice (74.4+/-2.5% and 54.7+/-10.9% respectively), which were significantly increased compared to CONTROLS (2.6+/-0.7%). The amount of regulatory T cells (Treg) characterized by CD4+CD25+FOXP3+ were similar in COLITIS (2.2+/-0.9%) and COLITIS+SWAP mice (2.1+/-0.4%), and tended to increase compared to CONTROLS (0.0+/-0.0%). COLITIS and COLITIS+SWAP mice showed increased levels of IL-17A producing CD4+ cells (9.2+/-2.1% and 6.2+/-2.4% respectively) compared to CONTROLS (1.0+/-0.4%). No differences in IFN-gamma, IL-4 and IL-10 producing CD4+ cells were detected.
Conclusion : Treatment with *Schistosoma mansoni* proteins improves the colonoscopic and macroscopic inflammation signs in the course of colitis, induced by the adoptive transfer of naive T cells in SCID mice. Immunologically, we demonstrate the Th17 and Treg basis for this adoptive transfer model, however the underlying immunological mechansims by which helminth proteins induce protection remain to be elucidated.

- I03 -

DIFFERENTIALLY EXPRESSED MICRORNAS REGULATE EXPRESSION OF GENES WITH CLINICAL INTEREST IN ACTIVE UC. J. Van Der Goten (1), L. Van Lommel (2), W. Vanhove (1), V. De Preter (1), M. Ferrante (1), F. Schuit (2), P. Rutgeerts (1), S. Vermeire (1), I. Arijs (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 : Ulcerative colitis (UC) is associated with colonic differential expression of genes involved in immune response (e.g. *IL8*, *IL1*), barrier integrity (e.g. cadherins, mucins) and tissue remodeling (e.g. matrix metalloproteinases). MicroRNAs (miRNAs) control gene expression post-transcriptionally by hybridizing to target mRNAs and thereby repressing translation. Recently, miRNAs have emerged as key regulators of various immune-related diseases.
Aim : We investigated if miRNA expression in UC mucosa is altered and correlated our findings with mucosal gene expression.
Methods : Colonic mucosal biopsies were obtained during endoscopy from 17 UC (10 active and 7 inactive) patients, and 10 normal controls. Total RNA, incl. small RNA, was extracted and used to analyze the miRNA expression via Affymetrix GeneChip miRNA 2.0 arrays. To assess gene expression, total RNA isolated from biopsies was analyzed via Affymetrix GeneChip Human Gene 1.0ST arrays. Data was analyzed with Bioconductor software. Predicted target genes were identified using the miRWalk software tool. Microarray data were validated by qRT-PCR analysis.
Results : We identified 51 (24 up- and 27 downregulated) miRNAs and 1543 (976 up- and 567 downregulated) gene probe sets that were significantly different (false discovery rate (FDR) < 5% and > 2-fold change) in active UC *vs*. controls. These genes were mainly involved in immune-related functions (cellular movement, immune cell trafficking). In contrast, in inactive UC *vs*. controls, no significant miRNA expression differences were found, while 155 gene probe sets (73 up- and 82 downregulated) were significantly differentially expressed. Next, we identified potential target genes using *in silico* analysis of the significantly dysregulated miRNAs and genes in active UC *vs*. controls. We focused on target genes of clinical interest in UC with an inverse correlation of expression with the respective miRNA. Out of 333 miRNA-target gene pairs, a highly significant inverse correlation was observed between the expression of hsa-miR-200c-3p and *IL8*, a UC susceptibility gene and inflammatory marker (Spearman rho = -0.92 ; FDR < 0.001), and between hsa-miR-200c-3p and *CDH11*, a gene related to intestinal epithelial barrier function (Spearman rho = -0.88 ; FDR < 0.001). Colonic expression of hsa-miR-200c-3p was 2.4-fold lower in active UC *vs*. controls (FDR = 0.001), while expression of its target genes *CDH11* and *IL8* was respectively 3.2-fold (FDR = 1.9E-06) and 7.8-fold (FDR = 8.7E-06) increased in active UC *vs*. controls. qRT-PCR confirmed array data of hsa-miR-200c-3p, *IL8* and *CDH11*.
Conclusion : Altered expression of miRNAs plays an important role in the expression of immune- and barrier-related genes in inflamed UC mucosa. Integrated analysis of miRNA and gene expression profiles revealed potential targets, such as hsa-miR-200c-3p, for use of miRNA mimics as therapeutics.

- I04 -

SIMULTANEOUSLY RESTORATION OF FOXP3+TREG, TYPE 1-LIKE TREG AND B CELLS BY ANTI-TNF THERAPY FOR IBD. 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 (+) T cells in patients (pts) with Crohn's disease (CD), ulcerative colitis (UC), rheumatoid arthritis (RA), psoriasis and Behçet's disease. Co-expression of CD45RA and Foxp3 distinguishes resting and active Treg (rTreg and aTreg) from Foxp3 (+) effector T cells (Teff). IFX also up-regulates blood total memory and pre-switch 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. IL-10/IFNg producing Tr1-like cells (Tr1L) have been characterized in human blood. Genetically modified B cells induce Tr1L in vivo. Recently resting B cells have a role in expanding Foxp3+Treg.
Aim : To investigate the kinetics of these cells in pts with IBD during IFX therapy.
Methods : Blood was taken from healthy controls (HC, N = 37) and pts with IBD (70 CD, 39 UC) before & during therapy (5 mg/kg IV 0-2-6 and q8 wks). The 3 subsets of Foxp3 T cells, Tr1L and B cells were assessed by flow cytometry after staining for CD4, CD45RA, Foxp3, CD25, CD127 and CD19. Assessment of symptoms, endoscopic healing and histological improvement was used to distinguish responders (RS) from non-responders (NRS) at 4 to 12 weeks after start of therapy. Serum CRP was collected to monitor biological response.
Results :
1. Pts with active IBD before therapy had low circulating **rTreg**(0.43+/-0.080, $p < 0.001$), **aTreg**(0.62+/-0.12, $p < 0.001$), **Foxp3Teff**(2.38+/-0.27, $p = 0.002$), **Tr1L**(4.79+/-0.68, $p < 0.001$) and **B cells** (0.17+/-0.02, $p = 0.002$) (N = 25), compared with HC(1.47+/-0.16),(2.40+/-0.17),(3.75+/-0.34),(16.82+/-1.7) and (0.27+/-0.02) (N = 37)(10e6/L blood mean+/-SEM for Tr1L, 10e9/L for others).
2. Compared with baseline before therapy, changes in these cells after IFX treatment were observed : **rTreg**(RS : 1.57+/-0.21, $p < 0.001$; NRS : 1.14+/-0.24, $p < 0.001$), **aTreg**(RS : 2.70+/-0.26, $p < 0.001$; NRS : 1.48+/-0.33,$p = 0.0057$), **Foxp3+Teff** (RS : 3.19+/-0.24, $p = 0.09$; NRS : 3.02+/-0.41,$p = 0.25$),**Tr1L**(RS : 26.09+/-2.21, $p < 0.001$; NRS : 8.92+/-1.00, $p = 0.013$) and **B cells**(RS : 0.25+/-0.03, $p = 0.035$;NRS :0.14+/-0.01, $p = 0.31$) (N = 59 in RS,15 in NRS).
3. Significant differences between RS and NRS were seen only for **aTreg**, **Tr1L** and **B cells** ($p = 0.0067$, < 0.001, < 0.001), but not in **rTreg** and **Foxp3Teff** ($p = 0.32, 0.72$).
4. **CRP** negatively correlated with **rTreg**, **aTreg**, **Tr1L** (as %of CD4T cells) and **B cells** (absolute number) ($p = 0.0011$, $r = -0.32$),($p < 0.001$, $r = -0.40$),($p < 0.001$, $r = -0.39$) and ($p = 0.044$, $r = -0.21$).
5. **B cells** positively correlated with **rTreg**, **aTreg** and **Tr1L** (absolute number)($p = 0.002$, $r = 0.31$), ($p < 0.001$, $r = 0.49$) and ($p < 0.001$, $r = 0.37$).
Conclusion : Circulating Foxp3T cells,Tr1L and B cells are decreased in active IBD and an increase in these cells (except for Foxp3Teff) correlates with biological response and/or with the clinical response to IFX . The positive correlation between B cells and Foxp3Treg subsets or Tr1L suggests that there might be a cross talk between B cells and Tregs.

Invited Lecture

- I05 -

MECHANISM OF LYMPHOCYTE HOMING REGULATION. A. Hart, St-Mark's Hospital, London, UK.

- I06 -

METALLOTHIONEIN, AN EMERGING DANGER SIGNAL DURING EXPERIMENTAL COLITIS. L. Devisscher (1), P. Hindryckx (1), K. Olievier (1), H. Peeters (1), M. Lynes (2), C. Cuvelier (1), M. De Vos (1), D. Laukens (1). (1) Ghent University, Ghent, Belgium ; (2) Cedars-Sinai Medical Center, Los Angeles, United States.

Introduction : Danger signals have been postulated as regulators of gut mucosal immunity. During intestinal inflammation, the epithelium is compromised and signals, alerting adjacent cells of tissue damage, are released. Hence, we were interested in metallothioneins (MTs), small proteins which have been identified at inflammation sites.
Aim : We explored triggers releasing MTs from colon epithelial cells and identified their role as extracellular danger signal during experimental colitis.

Methods : HT29 cells were subjected to the following treatments : 200ng/ml LPS, 200 microM H2O2, hypoxia (~1% oxygen), 100ng/ml TNF-alpha + 300ng/ml IFN-gamma to induce apoptosis, and repeated freeze/thaw cycles to mimic necrosis. Supernatant was analysed for MT levels using western blot and for lactate dehydrogenase activity (LDH). A Boyden transwell migration assay with blood leukocytes was applied to evaluate the chemotactic potential of extracellular MT and the capacity of monoclonal anti-MT antibody (100g/ml UC1MT) to abolish this. The role of MT as chemo-attractant was further explored using dextran sulphate sodium (DSS)-induced colitis in MT knockout (MT-/-), transgenic (MT+/+) and wild type mice (WT). The therapeutical use of monoclonal therapy was tested in DSS- and 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis. Inflammatory cell infiltrate was evaluated in all experiments together with standard inflammation markers.
Results : Necrosis and TNF-induced apoptosis resulted in detectable MT levels in supernatant of HT29 cells, which was not the case for LPS, H2O2 nor hypoxia treatment. LDH activity was not increased after stimulation with TNF, ruling out an uncontrolled release of MT from TNF-treated cells. Increased leukocyte migration towards this MT-containing supernatant was detected, whereas the addition of UC1MT was able to overcome this chemo-attraction ($p < 0.05$). Significantly less neutrophil infiltration was observed in MT-/- mice compared to MT+/+ and WT mice in DSS colitis ($p < 0.05$). Complementary, i.p. UC1MT treatment reduced the number of F4/80-positive macrophages in DSS- and TNBS-induced colitis ($p < 0.05$). Less inflammatory infiltrate was associated with reduced histological inflammation in all three colitis experiments.
Conclusion : We characterized metallothionein as danger signal released from HT29 cells after necrotic and TNF-induced apoptotic cell death. Inhibiting MT function by monoclonal therapy reduces leukocyte infiltration and represents a novel therapy dampening experimental colitis.

- I07 -

GENE EXPRESSION PROFILING UNDERLINES THE IMPORTANCE OF INFLAMMATORY LOAD IN THE COURSE OF ILEAL CD. J. Van Der Goten (1), I. Arijs (1), K. Machiels (1), L. Van Lommel (2), G. De Hertogh (1), M. Ferrante (1), F. Schuit (2), S. Vermeire (1), P. Rutgeerts (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 : Crohn's disease (CD) is characterized by intestinal inflammation progressing to stricturing and/or penetrating complications in most patients. Therefore, many patients need resective surgery. Biologic treatment seems more effective in the early phase of CD.
Aim : This study compared the ileal gene expression profiles of patients with newly diagnosed CD, post-operative recurrent (POR) CD or late CD and healthy controls to see whether gene expression differences could explain the changing disease behavior over time.
Methods : Ileal biopsies were obtained from inflamed mucosa from 15 patients with < 2 year diagnosis of CD (= early CD), 19 patients with POR CD (Rutgeerts score i3 or i4) < 1 year after ileo-colonic resection, 14 patients with > 2 year diagnosis or > 2 year after ileo-colonic resection (= late CD) and 12 controls. Total RNA extracted from biopsies was used to analyze gene expression via Affymetrix GeneChip Human Gene 1.0 ST arrays. Data was analyzed with Bioconductor software.
Results : Unsupervised clustering identified 2 clusters corresponding to controls and CD patients. With comparative analyses, only small gene expression differences were identified between the different CD groups, while many differences were observed with controls. Comparison of early *vs*. late CD identified only 1 significantly different (false discovery rate < 5% and > 2-fold change) gene probe set (*NPC1L1*). In POR *vs*. late CD, the expression of 4 gene probe sets (*MUC5B*, *S100P*, *C12orf75*, *C12orf36*) was significantly decreased. In early *vs*. POR CD, we identified 28 significantly differentially expressed gene probe sets (11 up- and 17 downregulated), incl. 2 CD susceptibility genes (*FAIM3* up-, *ABCG5* downregulated), 1 antimicrobial peptide (AMP) (*C5* downregulated) and 1 cell-adhesion molecule (CAM) (*CCL19* upregulated). As compared to controls, we identified in early, POR and late CD respectively 580, 355 and 601 significantly differentially expressed gene probe sets mainly involved in inflammatory pathways. A great overlap of 253 gene probe sets was found between these comparisons. Next, we included *IL8* expression as a confounder to exclude the effect of the inflammatory load. After comparison with controls, only a small number of gene probe sets remained significantly different : 23 gene probe sets in early CD (incl. *ITLN1*, *LCN2*, barrier genes *MUC1*, *MUC4*), 96 gene probe sets in POR CD (incl. *ITLN1* ; barrier genes *MUC1*, *CLDN18*, *CDHR1*, *LIN7A* ; AMPs *LCN2*, *C5*, *NOS2* ; CAM *CCL28*) and 13 in late CD vs. controls. This indicates that the majority of significant gene probe sets in comparisons with controls are related to the inflammatory load during the disease course.
Conclusion : Our data demonstrate that there are no different pathogenic mechanisms for early and late disease, highlighting the relativity of time in the natural evolution of CD. Further, we underline the importance of the severity of the inflammatory process during the disease course and the necessity to early interrupt the inflammatory cascade.

- I08 -

DIFFERENTIAL EXPRESSION OF PROLYL HYDROXYLASES 1 AND 3 BETWEEN IBD AND NON-IBD PATIENTS. S. Van Welden, D. Laukens, M. De Vos, P. Hindryckx. Ghent University Hospital, Gent, Belgium.

Introduction : Prolyl hydroxylase domain-containing proteins are oxygen sensing enzymes that hydroxylate under normoxic conditions the hypoxia-inducible factor 1 alpha subunit (HIF-1a). During hypoxia they are inhibited which ultimately leads to the expression of cell survival genes by stabilization of HIF-1a (i.e. hypoxic adaptive response). The PHDs are increasingly being explored because of their role in this hypoxic adaptive response. It has been shown that hydroxylase activity can affect the inflammatory response and it has recently been suggested that pan-hydroxylase inhibitors like dimethyl-oxalylglycine (DMOG), may represent a new therapeutic tool for the treatment of IBD, comprising Crohn's disease (CD) and ulcerative colitis (UC).
Aim : In this study we explored the colonic mucosal expression of the different prolyl hydroxylase-isoforms (PHD1, 2 and 3) in order to identify the key isoform(s) involved in the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC).
Methods : Biopsies were taken in macroscopically inflamed and non-inflamed areas of UC and CD patients. Samples of healthy controls and inflamed regions of patients with infectious colitis were included as controls. Next, the expression of PHD1, 2 and 3 was analysed using qRT-PCR in a medication naïve cohort (N = 79) and results were validated in a second cohort, which also included patients that received immunosuppressive agents (N = 89).
Results : Gene expression analysis revealed a significant increase of PHD1 in inflamed colonic biopsies of both UC ($P < 0.0001$) and CD patients ($P < 0.05$) when compared to healthy controls. A same trend was observed in patients with infectious colitis ($P = 0.063$). Interestingly, only inflamed colonic samples of UC patients showed a significant up-regulation of PHD3 mRNA levels ($P < 0.0001$) compared to healthy controls. This was not seen in patients with active CD, nor in patients with infectious colitis. All results were confirmed in a second cohort of patients and were not influenced by the use of immunosuppressive agents.
Conclusion : We have identified an altered expression pattern of PHD1 and 3 in patients with IBD. PHD1 appears to be the most appealing target for therapeutic intervention in the treatment of colitis. In addition, increased colonic PHD3 expression may have potential as a new diagnostic marker for UC.

- I09 -

ENDOPLASMIC RETICULUM AMINOPEPTIDASE 1 IS A PROMISING CANDIDATE GENE FOR INFLAMMATORY BOWEL DISEASE. D. Laukens, H. Peeters, P. Hindryckx, M. De Vos. Ghent University, Ghent, Belgium.

Introduction : Genome scan data underscore the importance of genetic susceptibility to inflammatory bowel disease (IBD), and the search for functional gene defects is ongoing. Next to the classical coding mutations, polymorphisms which influence gene expression may have an important impact on gene function.
Aim : We aimed to identify differentially expressed genes in familial IBD that are influenced by the genetic background.
Methods : Duplicate genome-wide peripheral blood mononuclear cell (PBMC) expression profiles of 7 families with IBD, containing 15 IBD patients and 32 controls, were generated using Agilent technology. A combination of permutation t-test analysis and heritability score measures was employed to identify differentially expressed genes that are most likely influenced by genetic background. Results were confirmed by quantitative real-time PCR (qRT-PCR) in the same samples and in inflamed colonic biopsies from patients with Crohn's disease (CD, N = 21) and ulcerative colitis (UC, N = 11) and in healthy controls (N = 21). Finally, genotype-expression correlation was investigated in 100 lymphoblastoid cell lines (LCLs) of members of Centre d'étude du Polymorphisme Humain (CEPH) families.
Results : Based on full gene expression profiles, samples from family members and duplicate samples clustered together. Seven genes were differentially expressed in PBMCs from IBD patients compared to healthy controls and showed positive heritability. Following confirmation by qRT-PCR, genotype-expression analysis of these genes was performed in LCLs of 100 individuals from CEPH families. Of these genes, the expression of *endoplasmic reticulum aminopeptidase 1* (*ERAP1*), a gene that was significantly down-regulated in PBMCs of IBD patients ($P = 0.007$), had high heritability score ($h = 1.37$). In addition, ERAP1 mRNA levels were reduced in inflamed colonic samples of patients with UC and CD compared to healthy colonic tissue ($P < 0.001$ and $P < 0.01$ respectively). Finally, various SNPs surrounding the *ERAP1* gene, including rs1363907 that was recently associated with IBD, were highly correlated with its expression in LCLs.
Conclusion : Through analyzing gene expression in familial samples, we found that gene expression was highly influenced by genetic background. Reduced expression of ERAP1 was seen in mononuclear cells and biopsies from IBD patients, and SNPs surrounding this gene were highly correlated with its expression. Therefore, ERAP1, a peptidase that is involved in processing proteins for antigen presentation on MHC class I, is a promising candidate gene for IBD.

Invited Lecture
- I10 -

CHOLINERGIC MODULATION OF INTESTINAL INFLAMMATION. G. Boekxstaens. KULeuven.

It is well accepted that the immune system has a major impact on neural function, contributing to increased pain perception and motility abnormalities in a variety of functional and inflammatory disorders. The communication between the immune system and the gut innervation is however bidirectional, i.e. also the autonomic nervous system has a major impact on the immune system. In addition to its contribution in stress-induced immune activation/dysregulation in functional bowel disorders, the autonomic innervation of the gut is increasingly recognized as an important player in immune homeostasis dampening both the mucosal and muscular immune system. Through the vagus nerve, the brain is informed on gut inflammation and subsequently modulates the immune response in order to prevent excessive inflammation and subsequent tissue damage. Mainly through the enteric nervous system, the vagus nerve interacts with the immune system not only dampening ongoing inflammation, but is also contributing to maintenance of oral tolerance, a key mechanism of immune homeostasis in the gut. The potential mechanisms and receptors involved will be discussed and the evidence indicating the therapeutic effect of vagus nerve stimulation will be summarized. Finally, the current data supporting its relevance to human disease and its therapeutic potential will be discussed.
Insight in the mechanisms underlying these crucial properties may indeed lead to better understanding of immune-mediated diseases of the gut and to improved anti-inflammatory therapies.

Invited Lecture
- I11 -

WHAT IS THE REMAINING PLACE OF CONVENTIONAL IMMUNOSUPPRESSIVE TREATMENT IN IBD ? P. Irving. Guy's & Thomas's Hospital, London, UK.

- I12 -

FAMILIAL AGGREGATION IN THE RESPONSE TO ANTI-TNF IN INFLAMMATORY BOWEL DISEASE PATIENTS. T. Billiet (1), I. Cleynen (1), V. Ballet (2), K. Van Steen (3), M. Ferrante (1), P. Rutgeerts (1), S. Vermeire (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (2) UZ Leuven, Leuven, Belgium ; (3) Montefiore Institute, Liège, Belgium.

Introduction : The introduction of anti-TNF antibodies has greatly improved outcomes for patients with inflammatory bowel disease (IBD). In clinical practice response to anti-TNF is seen in > 80% of patients, although 10-15% are non-responders. The latter is believed to be at least partially explained by genetic factors. Through the immunochip project 163 susceptibility loci for IBD have been confirmed and efforts are underway to link these factors to response or adverse events to drugs.
Aim : At current, nothing is known on response to anti-TNF within families. We therefore investigated if there is a familial aggregation of response to adalimumab (ADA) or infliximab (IFX).
Methods : From our database we identified 74 patients belonging to 34 families that received IFX, ADA or both. This comprised of 28 first degree (parent, child and sibling) and 19 second or higher degree pairs. We studied if the primary response to anti-TNF shows concordance among family members. Clinical response was assessed at week 4 or 10 after a single infusion or induction schedule, respectively. Patients who had clinical improvement with an obvious decrease of the disease were considered as responders. Those who had no benefit after two or three infusions were considered as primary non-responders. We also analyzed the disease location and behavior for each pair.
Results : Overall, 63 (85%) patients showed response and 11 (15%) non-response. No significant difference was observed in response rate between IFX or ADA treated patients. In 87% of pairs we found concordance for response (79%) or non-response (8%). If we considered first degree pairs only, the concordance rate increased to 93% compared to 79% in second or higher degree pairs. Among the 22 first degree pairs with response concordance, 15 or 68% also shared disease location.
Conclusion : A high rate of familial aggregation for response to anti-TNF was observed in this cohort of IBD patients. To our knowledge this has never been investigated in any inflammatory disease before. The fact that the concordance was even higher in first degree relative pairs as compared to second and further degree pairs strengthens the hypothesis that response to anti-TNF therapy could in part be driven by genetic factors. In this respect, the immunochip project could be used to identify underlying genetic factors responsible for response to anti-TNF. This is an ongoing project of the IIBD-GC.

- I13 -

DEPTH OF REMISSION IN CD PATIENTS SEEN IN A REFERRAL CENTER : ASSOCIATED FACTORS & IMPACT ON OUTCOME. M. Poncin (1), C. Reenaers (2), C. Van Kemseke (2), J. Belaiche (2), E. Louis (2). (1) University of Liege, Liège, Belgium ; (2) Centre Hospitalier Universitaire de Liège, Liège, Belgium.

Introduction : A deep remission of Crohn's disease (CD) including clinical but also biological remission and intestinal healing may be associated with better outcome. Our goals were to assess the prevalence of deep remission in routine practice, to evaluate the correlation between deep remission and clinical or demographic characteristics as well as its impact on disease outcome.
Aim : In this retrospective study we aimed at assessing the prevalence of deep remission in routine practice in a referral centre. Secondary objectives were to evaluate the correlation between deep remission and disease outcome, including relapses, hospitalizations and surgeries, and to try to find biological, clinical or demographic factors associated with it.
Methods : We performed a retrospective study in a referral center including patients seen at the outpatient clinic between April 2009 and April 2010. Retrospective follow-up was analysed till October 2011. Selected pateints were in clinical remission defined by a HBI = 4 and had a measurement of CRP. Biological remission was defined by a CRP < 5 mg/l. Tissue remission was defined by the absence of ulcer at endoscopy and/or absence of signs of acute inflammation at MRI. Different stages of remission (clinical only vs biological and clinical vs tissue and clinical) were compared.
Association with demographic, clinical and laboratory markers was studied by univariate and multivariate analyses and rates of relapses, hospitalizations and surgeries over follow-up were compared using Kaplan Meier method and the logrank test.
Results : Out of 815 CD patients in our database, 263 had an appropriate bio-clinical assessment during the studied year. 147 of them (56%) were at least in clinical remission and were further studied. Of those, 102 (69%) were in biological remission. Among 58 patients having also had morphological evaluation, 37 (66%) were in tissue remission. All of these (37/37) were also in biological remission. Biological remission was associated in multivariate analysis with older age ($p = 0.02$), higher hemoglobin ($p < 0.0001$) and lower BMI ($p = 0.01$). Tissue remission was associated in multivariate analysis with older age ($p = 0.03$), lower platelets count ($p = 0.008$), absence of previous surgery ($p = 0.03$), and the use of immunosuppressant ($p = 0.009$). Median follow up after retrospective assessment was 20 months. Time to relapse was significantly longer in patients with biological remission as compared to patients without biological remission (median time to relapse = 19 months vs > 30 months ; $p = 0.008$) and in patients with tissue remission as compared to patients without tissue remission (median time to relapse = 22 months vs > 30 months ; $p = 0.008$).
Conclusions : In this retrospective study in a referral centre, among the patients in clinical remission seen as outpatients, two thirds were in deep remission (either biological and/or tissue remission). Deep remission was associated with a better outcome than clinical remission without biological or tissue remission.

- I14 -

ASSESSMENT OF SERUM CALPROTECTIN AS A POTENTIAL BIOMARKER FOR CROHN'S DISEASE. M.A. Meuwis (1), G. Vernier-Massouille (2), J.C. Grimaud (3), Y. Bouhnik (4), D. Laharie (5), E. Piver (6), L. Siedel (7), E. Louis (8). (1) CHU Sart-Tilman, Liège, Belgium ; (2) Centre Hospitalier Régional Universitaire De Lille, Hôpital Claude Huriez, Lille, France ; (3) Hôpital Nord, Marseille, France ; (4) Inserm Bichat-Beaujon, Paris, France ; (5) University Hospital Pellegrin, Bordeaux, France ; (6) Hôpital Trousseau, Tours, France ; (7) School of Public Health, University of Liege, Liège, Belgium ; (8) Centre Hospitalier Universitaire de Liège, Liège, Belgium.

Introduction : In Crohn's Disease (CD), the correlation between clinical assessment and disease activity at the tissue level is only weak. Hence the prediction of tissue healing, disease relapse and disease progression remains a big challenge. While fecal calprotectin is one of the most informative markers, serum calprotectin has been very little studied in CD.
Aim : We aim at evaluating serum calprotectin in relation to prediction of relapse and mucosal healing for patients showing active disease and patients in remission using the STORI trial cohort.
Methods : We used the STORI trial cohort ($n = 115$) and a cohort of healthy controls ($n = 40$) to determine serum calprotectin level by ELISA. CD patients were studied either at baseline being in clinical remission before infliximab treatment withdrawal ($n = 63$) or, for some of them, at the time of relapse after infliximab withdrawal ($n = 52$). Data were analyzed through correlation analysis, Kaplan Meier curve and cox models, using also available Crohn's Disease Activity Index (CDAI), Crohn's Disease Endoscopic Index of Severity (CDEIS), fecal calprotectin and high sensitivity C-reactive protein quantitation levels (hsCRP). Variables were considered as continuous or discrete ones using either median, P25 or previously defined cut-off values.

Results : Serum calprotectin median values (range) were 8353 (410-49938) ng/mL, 19584 (2886-125000) ng/mL and 1318 (215.8-3770) ng/mL for patients with inactive disease, active disease and healthy controls respectively ($p < 0.0001$ between active and inactive disease and also between these two groups and healthy controls). Serum calprotectin correlated significantly with hsCRP ($r = 0.4092$, $p < 0.0001$) and CDAI ($r = 0.4442$, $p < 0.0001$), but not with CDEIS, on the contrary to fecal calprotectin ($r = 0.6458$, 0.5515, 0.2577 with $p < 0.0001$, $p < 0.0001$, $p = 0.019$ respectively). In univariate analysis, it was not able to predict relapse or mucosal healing. However, in multivariate analysis, used as a discrete variable at a threshold of 5675 ng/ml (P25) it appeared to be complementary to hsCRP (> 5 mg/l) and fecal calprotectin (> 250 microg/g) to predict relapse ($P = 0.0173$, 0.0024 and 0.0002 ; HR : 3.191 3.561 and 4.120, respectively). For mucosal healing (CDEIS < 3), the only selected variable was fecal calprotectin. Serum calprotectin had no added value.
Conclusion : As a biomarker for CD, serum calprotectin does not seem to have added value over hsCRP and seems to be less informative than fecal calprotectin. However it may be complementary to fecal calprotectin and hsCRP for the prediction of relapse.

- I15 -

SEVERE CLASSIC GALACTOSEMIA MIMICKING NEONATAL HEMOCHROMATOSIS - A JEKYLL AND HYDE CASE. I. Scheers (1), S. Nobrega (1), F. Smets (1), C. Sempoux (2), X. Stephenne (1), E. Sokal (1). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) Université Catholique de Louvain, Brussels, Belgium.

Introduction : Neonatal hemochromatosis (NH) is a severe liver disease characterized by intra and extrahepatic iron overload. Gestational alloimmune liver damage has been proposed as the etiology of NH. The proposed mechanism involves the binding of a specific maternal alloantibody to an unspecified fetal hepatocytic protein with consequent activation of the complement cascade and formation of the membrane attack complex (MAC). Several diseases have been associated with a NH phenotype, including perinatal infections and genetic or metabolic diseases.
Aim : With this case report, we aim to demonstrate that acute liver failure from various etiologies may be accompanied by liver siderosis, elevated ferritin levels and positive MAC liver immunostaining. The exclusion of treatable conditions with, in this case, subsequent early avoidance of galactose should be a priority for clinicians.
Methods : We report the case of three siblings displaying acute liver failure (ALF) due to proven congenital galactosemia.
Results : Case 1 was born small for gestational age after 35 weeks of gestation. On the third day of life she developed cytolysis, coagulopathy, hyperammonemia and elevated serum ferritin. She secondary presented multiple organ failure. Liver histology showed impressive loss of hepatocytes, acinar formation, pericellular fibrosis, severe biliary stasis and accumulation of iron mainly in Kupffer cells. Positive liver immunostaining for membrane attack complex (MAC) led to the diagnosis of neonatal hemochromatosis (NH). Salivary gland biopsy was not contributive and abdominal MRI was not performed. For the next pregnancy, the mother received prophylactic intravenous immunoglobulin to prevent possible recurrence of NH. Case 2 was a full term healthy boy. On the third day of life he developed severe acute liver failure (ALF) with coagulopathy and elevated serum ferritin. He was initially treated with exchange transfusion and immunoglobulins, but failed to improve and was referred for liver transplantation. The boy recovered after the implementation of a galactose free diet. Genetic and enzymatic studies confirmed homozygocy for classic galactosemia (CG). The same mutations were found retrospectively in stored liver tissue from fatal case 1. Because NH could not be absolutely excluded, the mother was again treated with immunoglobulins for a third pregancy. Case 3, a healthy girl, was born at term. CG was genetically and enzymatically confirmed and she was fed with a soy milk formula. On the seventh day of live, following an accidental ingestion of a lactose containing milk formula, the girl developed a rapid life threatening liver failure which progressively recovered after liver support treatment and lactose exclusion.
Conclusion : These 3 familial cases of classic galactosemia illustrate that ALF can occur after a single dose of lactose containing milk. In any child with ALF, galactose should be excluded from diet until the diagnosis of classic galactosemia can be ruled out. Moreover, severe metabolic conditions such as galactosemia can be associated with elevated ferritin levels in the serum, iron deposits in the liver and positive MAC immunostaining ; which is thus not specific of NH. We therefore advocate the need for more specific positive diagnostic markers of NH.

Invited Lecture
- I16 -

BONE MARROW TRANSPLANTATION : BACK TO INFANCY. S. Vermeire, KULeuven.

Invited Lecture
- I17 -

IBD PHENOTYPES AND IMMUNODEFICIENCIES IN INFANCY. F. Ruemmele, Paris, France.

- I18 -

CLINICAL COMPARISON OF 8 DIFFERENT CALPROTECTIN IMMUNOASSAYS FOR THE DIAGNOSIS AND FOLLOW-UP OF IBD. D. Labaere, A. Smismans, G. Van Olmen, P. Christiaens, G. D'haens, V. Moons, P.J. Cuyle, J. Frans, P. Bossuyt. Imelda Hospital, Bonheiden, Belgium.

Introduction : Fecal calprotectin (FC) may be of value as non-invasive marker for diagnosis and follow-up of inflammatory bowel disease (IBD). Instead of the time-consuming ELISA, newer fast and/or automated tests have been developed. However, few data are available on their comparability.
Aim : We prospectively evaluated the performance of 8 different calprotectin assays for the diagnosis of IBD and for monitoring disease activity in ulcerative colitis (UC) and Crohn's disease (CD).
Methods : 33 patients with suspected IBD and 31 IBD patients in follow-up provided a stool sample for calprotectin measurement. We determined FC by means of 3 point-of-care tests (POCT) (B ?hlmann Quantum Blue, Eurospital Calfast, and Biotest Certest), 4 ELISA's (Bühlmann, Eurospital, Calpro and Calprolab) and 1 fully automated immunoassay (Phadia). ? Results were compared with endoscopic and histological findings.
Results : After adjusting the optimal cut-off by ROC-curve analysis, sensitivity and specificity for diagnosis of IBD were comparable for all 8 different assays, and ranged from 82 to 83% and from 84 to 89%, respectively. Clinical discordances were limited to 6.7% and only appeared in the non-IBD group. The discriminatory power to distinguish IBD from non-IBD appeared highest for the Bühlman Quantum Blue, Phadia and the Calprolab assay, with ratios of median IBD/non-IBD calprotectin values of respectively 14, 12 and 10. For monitoring of disease activity, the semi-quantitative Certest was omitted because no acceptable combination of sensitivity and specificity could be reached at its fixed cut-off. ? For the other assays, mild UC (Mayo score 1) could be differentiated from remission with a sensitivity of 71-100%. In moderate to severe UC (Mayo 2-3), sensitivity was 100% for all assays. Specificity ranged from 67 to 86% in both subgroups.
In CD patients, only disease activity with a SES-CD (Simple Endoscopic Score for Crohn's Disease) above 7 could be differentiated from remission, with a sensitivity of 83-86%, and a specificity of 75% for all tests.
Disagreement between the different assays occurred in 18% of the CD and UC patients, but only appeared in cases of remission or mild inflammation.
Although all methods correlated significantly, the slopes and/or intercepts differed significantly.
Conclusion : All calprotectin assays showed acceptable and comparable clinical performance for diagnosis of IBD. For follow-up of known IBD, performance of the different assays was moderate to good, except for mild Crohn's disease, which is difficult to differentiate from remission. The ELISA's can be safely replaced by their corresponding quantitative POCT assays. Based on the inter-test disagreement, one cannot compare trial results or multiple results from one patient, if they are obtained with a different assay. Further efforts are urgently needed to harmonize calprotectin assays.

- I19 -

ELEVATED SERUM IMMUNOGLOBULIN A AND G IN CHILDREN WITH FOOD ALLERGY POST LIVER TRANSPLANTATION. R. De Bruyne, M. Dullaers, S. Van Biervliet, S. Vande Velde, P. Gevaert, M. Van Winckel. Ghent University Hospital, Gent, Belgium.

Introduction : Post transplant food allergy (PTFA) is found in 28% of our pediatric liver transplant (LT) patients. The pathogenesis remains incompletely understood but IgE seems implicated in the immunopathology. Hypergammaglobulinemia of one or more immunoglobulin (Ig) isotypes is often seen after pediatric LT mainly in the context of EBV infection and may serve as early indicator of post transplant lymphoproliferative disease.

Aim : Since no data are available on serum Ig levels other than IgE in PTFA, we compared serum Ig levels between pediatric LT patients (n = 49) with (n = 15) and without (n = 34) PTFA, renal transplant (RT) patients (n = 26), FA children (n = 20), children with chronic parenchymal liver disease (n = 31) and healthy controls (n = 83). None of the RT patients has FA. LT and RT patients were treated with a similar immunosuppression protocol.
Methods : Serum Ig levels were collected from patient records. Data were analysed in SPSS.
Z-scores were calculated for each Ig value to correct for age-dependent reference ranges. Kruskall-Wallis test was used to compare serum Ig levels between groups. Subsequently, Mann-Whitney U test was used to compare subgroups mutually. Because of multiple testing a p-value < 0,005 was considered statistically significant.
Results : Serum Ig levels in chronic liver disease patients were not statistically different from healthy controls. No significant difference in distribution of IgM was found across groups. As expected, IgE was higher in FA compared to controls ($p < 0,0001$), LT ($p < 0,0001$), RT ($p < 0,0001$) patients and in PTFA versus non FA LT patients ($p < 0,0001$). IgG was elevated in LT compared to controls ($p = 0,003$) and RT patients ($p = 0,001$) and in PTFA versus non FA LT patients ($p < 0,0001$). IgA was raised in both FA and LT patients compared to controls ($p = 0,001$;$p < 0,0001$) and RT patients ($p = 0,001$;$p < 0,0001$). IgA was also significantly higher in PTFA compared to non FA LT patients ($p < 0,0001$). No significant difference in EBV viral load was found between these two groups. 1 patient in the PTFA group was diagnosed with PTLD and treated successfully with rituximab.
Conclusion : We first report that besides serum IgE, also serum IgA and IgG are increased in PTFA. This might be the result of a disturbed mucosal barrier and inadequate secretory immunity. In view of the potential role of secretory IgA in tolerance to food, these findings might be of relevance for the further unravelling of the pathogenesis of PTFA.

- I20 -

CRYPTOGENIC MULTIFOCAL ULCERATING STENOSING ENTERITIS (CMUSE) : A PICTORIAL CASE SERIES. H. De Schepper, T. Moreels. Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Ulceration of the small bowel poses a rather limited but difficult differential diagnosis. The most common causes are Crohn's disease, NSAID-associated enteritis, lymphoma, CMV and tuberculous enteritis. An important and relatively novel differential diagnosis is CMUSE or cryptogenic multifocal ulcerative stenosing enteritis.
Aim : Review of clinical, endoscopic and radiological findings in patients with CMUSE.
Methods : Five patients referred for balloon-assisted enteroscopy for various reasons showed endoscopic features of CMUSE. These findings and, when available, medical imaging were reviewed.
Results : Patient 1 was treated surgically and endoscopically for suspected jejunal Crohn's disease before she was referred to us for endoscopic dilatation of a new stenosis. Antegrade enteroscopy showed multiple solitary and short ulcerations distorting the jejunal lumen. Biopsies were nonspecific. Patient 2 suspected for Crohn's disease was also referred for endoscopic balloon dilatation of a small intestinal stricture. MR enterography suggested active stenosing Crohn's disease in the ileum with wall thickening restricted to the short strictures. Retrograde enteroscopy showed 7 consecutive very short ulcerative stenoses (1-2 cm length) with prestenotic dilatation. Patient 3 was referred under suspicion of ileal Crohn's disease. Retrograde enteroscopy only showed a short circular stenosing ulcer with normal surrounding ileal mucosa. Biopsies were nonspecific. Patient 4 was admitted with anemia and melena, but with a normal gastro- and coloscopy and absent inflammatory parameters. MR enterography showed no small intestinal pathology. A video capsule examination showed two small ulcerative stenoses in the ileum, with prestenotic retention of the capsule and need for surgical resection of the short affected segment. Pathology of the resection specimen was not specific for Crohn's disease. Patient was started on corticosteroids with good response. Patient 5 was suspected for therapy-resistant ileal Crohn's disease since many years and with intractable abdominal pain. CT enterography was normal. Enteroscopy showed multiple short ulcerative stenoses in the ileum. Patient was empirically treated with infliximab, with excellent clinical and enteroscopic response.
Conclusion : In patients with short and ulcerative enteral stenoses without signs of systemic inflammation, CMUSE is an important but often neglected differential diagnosis. The pathophysiology and relationship to Crohn's disease are the subject of ongoing debate and controversy, but the specific endoscopic characteristics and the lack of clear abnormalities on CT or MRI enterography do suggest CMUSE is a distinct albeit rare chronic inflammatory bowel disease.

SEROLOGICAL AND GENETIC MARKERS AS PREDICTORS OF COMPLICATED DISEASE IN CROHN'S DISEASE. E. Hoefkens (1), M. Ferrante (2), F. Princen (3), V. Ballet (2), I. Cleynen (1), P. Rutgeerts (2), S. Vermeire (2). (1) University of Leuven, Leuven, Belgium ; (2) University Hospitals Leuven, Leuven, Belgium ; (3) Prometheus Laboratories Inc., San Diego, United States.

Introduction : Early prediction around the time of diagnosis of disease progression in Crohn's disease (CD) would help to individualize management and prioritize initiation of biological therapy. Some clinical parameters have been reported but these lack sensitivity and/or specificity. The use of serologic and genetic markers have been proposed to improve accuracy. We studied baseline clinical parameters, together with a serology panel and *NOD2* genotypes in a prospectively recruited and followed cohort of new CD patients.
Aim : We hypothesized that molecular markers could improve the accuracy for predicting complicated disease behaviour and abdominal resections.
Methods : Out of 78 newly-diagnosed CD patients diagnosed at our centre since 2007, 69 patients reached a follow-up of at least 6 months (median FU was 26 months (IQR 15-46)) and were included in this study. Serum and DNA was taken at diagnosis and analyzed for microbial peptides : anti-*Saccharomyces cerevisiae* (ASCA), anti-bacterial flagellins (CBir1, Fla2 and FLAX) and anti-outer membrane porin of *E. Coli* (OmpC) (Prometheus Labs) and were genotyped for *NOD2*-variants (p.Arg702Trp, p.Gly908Arg and p.Leu1007fsX1008). Associations between clinical parameters (age at diagnosis < 40years, ileal location, need for steroids, deep ulcers on endoscopy, smoking), immune responses to microbial peptides (both the presence and the quartile sum score), *NOD2*, and disease progression were evaluated.
Results : During follow-up, 10 patients progressed from B1 (inflammatory disease behaviour) to B2 (stenosing) or B3 (penetrating) behavior after a median of 8 months (5-20) and 13 needed abdominal resection after a median of 11 months (5-37). Kaplan-meier survival analysis indicated the presence of ASCA IgG as the only risk factor for progression of disease behaviour (LogRank p = 0.054). A high serology load was a risk factor (LogRank p = 0.023) for the need for abdominal resection. Individual risk factors for abdominal resection were a positive value for ASCA IgA (LogRank p = 0.015), CBir1 (p = 0.012), B2/B3 behaviour at diagnosis (p < 0.001) and a mutation in one of the 3 snps (p = 0.024). In Cox regression multivariate analysis, ASCA IgA [Odds ratio (OR) 6.6 (95%CI+/- 1.1-39.4), p = 0.039] and B2/B3 behaviour at diagnosis [OR 7.8 (95%CI+/- 1.7-36.8), p = 0.009] remained significant. The proportion of patients needing abdominal surgery significantly increased with increasing quartile sum score for antibodies (linear-by-linear association p = 0.01) and none of the 19 patients in the lowest serology quartile sum score needed abdominal surgery.
Conclusion : In this prospective follow up of newly-diagnosed CD patients, we demonstrate that the presence and magnitude of immune responses to different microbial antigens and the presence of mutations in *NOD2* are associated with a higher risk for surgery.

THERAPEUTIC STRATEGY AND PATIENT OUTCOME DURING THE FIRST 2 YEARS OF PEDIATRIC CROHN'S DISEASE. G. Veereman (1), J. Malachie John (2), E. De Greef (1), F. Smets (3), S. Van Biervliet (4), K. Van Steen (2). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) Systems And Modeling Unit, Montefiore Institute Ulg, Bioinformatics and Modeling, Giga-R ULG, Liège, Belgium ; (3) UCL St-Luc, City of Brussels, Belgium ; (4) UZ Gent, Gent, Belgium.

Aim : BELCRO was initiated in May 2008 through a collaboration of the IBD working group of the Belgian Society for Pediatric Gastroenterology, Hepatology and Nutrition (BESPGHAN) and the Belgian IBD Research and Development Group (BIRD). The aim of the registry is to describe a cohort of pediatric CD patients recruited over a 2 y period and to prospectively follow these patients for 5 y. We here report therapeutic strategy and outcome after the first 2 years of disease.
Methods : Data from the BELCRO database were evaluated at inclusion (M0) in after 6 (M6), 12 (M12) and 24 (M24) months. All analyses were performed using SAS 9.3 (SAS Institute, Cary NC) and hypothesis were tested at 5% level of significance. Mixed models via Procedure Mixed were used to analyze the effect of treatment hypotheses on growth related variables allowing, for patient-specific random effects. Wilcoxon signed ranked test was used to compare individuals at M0 and M24.
Results : Data from 98 newly diagnosed patients were available at M0, 6 were lost to follow up at M24.
The population at diagnosis was described previously (1). Disease severity score decreased over time : for inactive/mild/moderate to severe respectively at M0 4.26%, 24.47%, 71.28% ; at M6 53.73%, 38.81%, 7.46% ; at M12 60.00%, 35.71%, 4.29% at M24 77.14%, 21.43%, 1.43%.
The evolution of therapeutic strategy is presented at M0 and M24 : **mono** 5ASA :7.14 and 7.61% ; immunomodulatory : 1.02 and 19.57% ; exclusive enteral : 1.02 and 1.09% ; steroids : 19.39 and 0% ; mono biologicals : 0 and 10.87% ;

combinations with 5ASA : 30.61 and 22.83% ; with immunomodulatory : 38.78 and 52.17% ; with enteral supplements 12.24 and 6.52% ; with steroids : 37.76 and 16.30% ; with biologicals 1.02 and 39.13%.
Treatment category and growth showed significant associations only for improvement of z score for height and conversely for decreasing z score for BMI on mono immunomodulatory treatment between M6 and M24 ($p = 0.0221$ and $p = 0.0057$).
Individual comparisons at M0 and M24 show that patients with z score for height < -2 at M0 do not improve their z score at M24. Patients on mono immunomodulatory treatment improve their z scores for height at M24 ($p = 0.0353$).
Disease severity at M0 influences therapeutic strategy at M24 only for the prescription of immunomodulatory treatment which is 'mono' in patients with mild disease at M0 ($p = 0.0384$) but' in combination' for patients with moderate to severe disease at M0 ($p = 0.0398$). Disease location L3 is inversely related to the prescription of mono immunomodulatory therapy ($p = 0.027$). Patients with moderate to severe disease at M0 are more likely to be on biologicals in combination at M24 ($p = 0.0176$). Disease severity at M0 is correlated with a lower z score for BMI ($p = 0.0116$). BMI z score improves significantly at M24 ($p = < 0.0001$).
Conclusion : Disease course seems well controlled the BELCRO cohort as demonstrated by decreasing severity scores. Current therapeutic strategy appears satisfactory at M24, decisions are influenced by disease severity at presentation. Patients with growth retardation need more attention.
Reference :
1. Profile of pediatric Crohn's disease in Belgium. De Greef E. *et al*. JCC in press.

Invited Lecture

- I23 -

MANAGEMENT OF ACUTE SEVERE UC. M. De Vos. UZ Ghent.

- I24 -

OUTBREAK OF ECHOVIRUS 11 FULMINANT NEONATAL HEPATITIS IN BELGIUM DURING SPRING 2012.
C. Panagiotaraki (1), D. Van Der Linden (1), S. Clement De Clety (2), L. Houtekie (2), B. Kabamba (2), E. Sokal (2), F. Smets (2). (1) Université Catholique de Louvain, Cliniques Universitaires St Luc, Woluwe-Saint-Lambert, Belgium ; (2) Université Catholique de Louvain, Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Nonpolio enterovirus infections are common during summer and fall. Among neonates clinical presentation varies from asymptomatic viral shedding and non-specific febrile illness to sepsis-like syndrome and severe liver, cardiac or cerebral diseases. Echovirus 11 is the most frequent cause of serious neonatal morbidity and mortality, often presented as fulminant hepatitis, infection of the central nervous system, or both. Newborn EV infections may be acquired after birth or vertically, more likely at the time of delivery through contact with maternal blood, fecal material, vaginal or cervical secretions. Mortality rates are greater for infections that appear during the first week of life, probably through vertical transmission, in comparison to later infections.
Methods : We report four cases of echovirus 11 neonatal infections that occurred between April and June 2012.
Results : The four children were admitted during the first week of life, day 4 to 6, with sepsis-like syndrome. All the cases were biologically characterized by marked transaminase elevation (GOT > > GPT), hemolytic anemia, thrombocytopenia, and severe coagulopathy. Diagnosis was confirmed by positive polymerase chain reaction on blood, stool or cerebro-spinal fluid samples. Three neonates were born by vaginal delivery. Among them one child deceased after one month because of hemodynamic instability due to a major capillary leak syndrome, and was the only one having been treated with corticoids (day 2 to day 10). All three received supportive treatment associated to intravenous immunoglobulins (IVIG) within 7 days of admission (1 to 2 g/kg). The fourth case was born by caesarian delivery and was also treated with early IVIG administration. Contact history was only documented in this last patient (diarrhea in her siblings one week before her birth). Three children presented an inversion of portal blood flow at liver doppler, which is a marker of portal hypertension commonly reported in fulminant hepatitis. Whether this will be associated to long-term sequellae remains to be determined.
Conclusion : This study shows that outbreak of enterovirus is still associated with severe infection and fulminant hepatitis in newborns. Clinical presentation is aspecific although early diagnosis and rapid treatment is mandatory to avoid fatal evolution. There was rationale to administer IVIG, and positive outcome in 3 patients out of 4 might have been favoured by this treatment.

- I25 -

IDENTIFICATION OF FACTORS CAUSING FATIGUE AND IMPAIRED PHYSICAL CONDITION IN PAEDIATRIC IBD. L. Beckers (1), Y. Van Driessche (1), J. Ramet (2), E. Van De Vijver (2). (1) University Of Antwerp, Antwerpen, Belgium ; (2) Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Crohn's disease (CD) and Colitis Ulcerosa (UC) are chronic inflammatory bowel diseases (IBD). The disease is characterized by times of rest (remission) and times of exacerbation (active disease). IBD is a chronic disease which has considerable psychological and social consequences. Even children with inactive disease have significantly more complaints of fatigue and a reduced quality of life compared with their healthy peers.
Aim : To examine the literature and identify the determinants of fatigue and impaired physical condition.
Methods : We searched the electronic databases Pubmed and Embase. The search in the Cochrane Library and Clinical Evidence didn't yield useful articles. Case reports were excluded. 60 articles were retained and were evaluated using the Cochrane checklists. Finally, 16 articles were used for this systematic review.
Results : Patients in remission had a prevalence of fatigue of 44% to 64%. Factors that played a role in influencing fatigue were anaemia, IBS, depression, anxiety, therapy and the personality of the patient. Only few articles are available on fatigue in paediatric IBD. We also found some contradictions in the results of the reviewed articles.
Conclusion : Fatigue is common among patients with IBD in remission. Different factors have an influence on fatigue in adult patients but little is known on fatigue in children. More research needs to be done in order to determine the factors of fatigue in paediatric IBD, and their effect in daily life.

- I26 -

NO EVIDENCE FOR A PROVOKING ROLE OF NUTRITIONAL FACTORS IN DISTAL INTESTINAL OBSTRUCTION SYNDROME. D. Declercq, S. Van Biervliet, E. Robberecht. Ghent University, Ghent, Belgium.

Introduction : The etiology of distal intestinal obstruction syndrome (DIOS) remains unclear. Food and pancreatic enzyme replacement therapy (PERT) intake are often blamed for its occurrence.
Aim : The aim of this study was to evaluate the nutritional and PERT intake of cystic fibrosis (CF) patients at a first DIOS attack.
Methods : All CF patients complete annually a 3-day-intake diary of their caloric, protein, fat, dietary fiber, liquid and PERT intake. Patients diagnosed with a first DIOS attack (n = 12) retrospectively filled in an intake diary of the 3 days preceding the DIOS episode supervised by an expert dietitian. Results were compared to those of one year before and also of 36 CF controls matched for age, sex, genotype and disease severity. All were pancreatic insufficient.
Results : A first DIOS episode was diagnosed in 12 CF patients. ? The median age was 18 years [4,4 - 33,4 years] and 8 were male. Only the absolute median fat intake (114 g [75 ; 154 g]) and pancreatic enzyme intake (63,8 × 104 IU lipase [3 × 105 ; 8,8 × 105 IU lipase]) at the time of the DIOS attack were significantly higher ($p < 0,05$) than a year earlier 96 g [68 ; 139 g] and 48,8 IU lipase [22,5 × 104 ; 9 × 105], respectively. This significant difference disappears when enzyme intake is expressed as units lipase/gram fat (5635 IU lipase/gram fat versus 5112 lipase/gram fat). This was however also found in the controls. We suspect this could result from the dietary recommendations to increase fat intake and concomitant enzyme intake. No other significant dietary differences were found.
Conclusion : CF patients who experienced a first DIOS attack showed no significant difference in intake when compared to one year earlier or versus controls. ? This study does not sustain a potential role of nutritional factors or PERT in the first DIOS episode.

- I27 -

COULD ADJUVANT THERAPY WITH VSL3 BE EFFECTIVE PEDIATRIC CROHN'S DISEASE TYPE L1 ? AN OPEN PILOT REPORT. S. De Smet (1), T. Devreker (2), E. De Greef (2), B. Hausser (3), Y. Vandenplas (3), G. Veereman (4). (1) School For Dieticians, Kh, Leuven, Belgium ; (2) UZ, City Of Brussels, Belgium ; (3) UZ, City of Brussels, Belgium ; (4) University Hospital Brussel (VUB), Brussels, Belgium.

Introduction : The benefit of probiotics in Crohn's disease remains largely hypothetical.
Aim : The aim of this prospective, open therapeutic trail was to observe the effect of probiotics in children diagnosed with Crohn's disease who suffer from terminal ileitis (L1) despite ongoing conventional treatment.
Methods : Over eight months (8/2011-3/2012) 12 patients with confirmed CD, type L1 and persistent ongoing active inflammation in the terminal ileum (documented with MRI and/or endoscopy) were selected. The group consisted of

8 boys and 4 girls, mean age 14.5 yrs (sd 3.3). The ongoing treatment was ineffective in resolving inflammation in the terminal ileum. Six out of 12 patients used Imuran, 3 Infliximab, 3 Modulen, 2 Humira, 1 methotrexate and 1 Pentasa. Seven patients were on monotherapy and 5 on combination. A 2 months treatment with VSL3 was proposed to the patients and their parents, in addition to their ongoing treatment. Free samples of VSL3 were obtained from the company but there was no further support. All patients received 18 boxes VSL3 in order to and take 3 sachets each day during 2 months. Before and after the 2 months therapy with probiotics a clinical assessment, laboratory analysis (CBC, CRP, ESR and albumin) and sonography were obtained. Patients were informed about the storage of probiotics, which must always be refrigerated and a logbook was kept.

Results : Two patients did not finish the 2 months treatment course and dropped out, one because of severe relapse necessitating surgery and one because of intolerance (nausea) to the product. Of the 10 patients that completed the 2 months treatment, based on clinical evaluation, laboratory results and sonography : 1 relapsed, 5 improved and 4 reached clinical remission. Thus in this small pilot therapeutic trial, based on overall clinical judgment 9/12 patients (75%) improved and 4 of them (33%) reached remission. No serious adverse events were noted. The patients who relapsed demonstrated progression of inflammation, with obstruction in one case.

Conclusion : This observation needs to be interpreted with caution but justifies further exploration of the mechanisms that down regulate inflammation and fibrosis in the terminal ileum of CD patients.

- I28 -

ASSESSMENT OF RISK OF BLEEDING FROM ESOPHAGEAL VARICES DURING MANAGEMENT OF BILIARY ATRESIA IN CHILD. X. Stephenne, C. Wanty, T. Helleputte, F. Smets, E. Sokal. Université Catholique de Louvain, Brussels, Belgium.

Introduction : The management of esophageal varices (EV) in children suffering from biliary atresia (BA) remains controversial. Recent studies in children proposed initiating a prophylactic treatment in patients with severe (Grade III) EV and/or EV associated with red color signs.

Aim : Our study was aimed at assessing the risk of bleeding from EV in a series of BA patients, identifying risk factors for bleeding in order to develop a predictive model of bleeding.

Methods : This was a retrospective study including 83 eligible BA patients. Clinical, ultrasonographic, endoscopic, and laboratory parameters were studied from the beginning of medical management up to the occurrence of upper gastrointestinal bleeding.

In patients not presenting any bleeding, data were analyzed until liver transplantation, endoscopic treatment of EV was performed or last follow up.

Risk factors were investigated using univariate and multivariate statistical analyses.

Methodology was originally based on machine learning and “ensemble” feature selection methods.

We therefore builded a large number of models (10,000) using randomly constituted sub-cohorts of patients.

This ensemble approach is highly robust and generally offers good predictive performance.

Results : Out of 80 available endoscopic data patients, 73 (91%) developed EV, of which 31% were Grade I, 37% were Grade II, and 23% were Grade III.

Red color signs were observed in 27% of patients and hypertensive gastritis in 62%.

There were a total of 7% gastro-esophageal varices and 2% isolated gastric varices. ? Seventeen out of 83 patients (20%) presented gastrointestinal bleeding, with a median age of 9.5 months (6-50 months). All bleeding was treated endoscopically using variceal ligation in five patients and sclerotherapy in 12 patients of less than 7 months of age.

In univariate and multivariate analyses, high-grade EV, red color signs on endoscopic examination, and low fibrinogen levels, at first endoscopy, were identified as risk factors for bleeding.

When tested in more than 10,000 different models, these three variables appeared to play the most significant role in predicting bleeding.

Conclusion : Our study confirmed that grade III EV and red color signs are risk factors for bleeding in patients followed up for BA. We identified low fibrinogen levels as an additional risk factor.

The relevance of these three factors to predict bleeding from EV requires validation in a prospective study. These prospective studies must also include analysis of fibrinogen supplementation impact on variceal bleeding risk.

PEDIATRIC CROHN'S DISEASE : A DIFFERENT APPROACH BETWEEN PEDIATRIC AND ADULT GASTRO-ENTEROLOGISTS ? E. Degreef (1), B. Maus (2), I. Hoffman (3), F. Smets (4), S. Vanbiervliet (5), M. Scaillon (6), B. Hauser (1), I. Paquot (7), P. Alliet (8), W. Arts (9), O. Dewit (4), H. Peeters (10), F. Baert (11), G. Dhaens (12), J.F. Rahier (13), I. Etienne (14), O. Bauraind (15), A. Vangossum (16), S. Vermeire (3), F. Fontaine (17), V. Muls (18), E. Louis (19), F. Van De Mierop (20), J. Coche (15), J. Mahachie (2), K. Vansteen (2), G. Veeremanwauters (1). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) Montefiore Institute, Liège, Belgium ; (3) University Hospitals Leuven, Campus Gasthuisberg, Leuven, Belgium ; (4) Université Catholique de Louvain, Brussels, Belgium ; (5) Universiteit Gent, Gent, Belgium ; (6) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (7) CHC Clinique De L'esperance, Liège, Belgium ; (8) Virga Jesse Hospital, Hasselt, Belgium ; (9) Zol, Genk, Belgium ; (10) Ghent University Hospital, Ghent, Belgium ; (11) H. Hart Hospital, Roseselare, Belgium ; (12) Imelda Hospital, Bonheiden.

Introduction : Pediatric gastroenterologists treat Crohn's disease patients up to 15-18 years of age and then transfer them to adult care. However, there is no restriction for adult colleagues to diagnose and treat pediatric patients.
Aim : In the current survey, we investigated differences in presentation, use of diagnostic procedures and initial treatment for pediatric Crohn's disease patients under the care of pediatric versus adult gastroenterologists.
Methods : This comparison was made in the cohort of BELCRO patients diagnosed by a pediatric or adult gastroenterologist.
Results : In the BELCRO cohort, 71% of patients were diagnosed by a pediatric gastroenterologist of whom 58% in a tertiary care centre compared to 37% of the 29% of patients in adult care. Even though patients diagnosed by adult physicians are significantly older, 22% were below the age of 12 y. No difference in presenting symptoms (abdominal pain, diarrhoea, growth failure) or disease severity at diagnosis was found between both groups. Disease classification according to Montreal (1) and the recently adapted Paris (2) classification was similar. Pediatric gastroenterologists performed as many upper endoscopies at diagnosis before and after publication of the Porto criteria (75%) (3), whereas adult physicians performed significantly less upper endoscopies. At diagnosis, adult physicians initially prescribed more monotherapy with 5-ASA and were less inclined to use combination therapy with steroids, immunomodulators, antibiotics or enteral nutrition compared to pediatric colleagues.
Conclusion : Further follow up will indicate whether differences between pediatric and adult practitioners affect long term disease behaviour and outcome. ?
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ETHNIC DIFFERENCES IN CROHN'S DISEASE CHARACTERISTICS. S. Bouhadan, P. Pelckmans, T. Moreels. Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Crohn's disease (CD) phenotype varies among ethnic groups. Immigrants may encounter specific problems when diagnosed with inflammatory bowel diseases (IBD).
Aim : The aim of this study was to define the clinical phenotype of CD in first and second degree Moroccan immigrants in comparison to Caucasian immigrants in Antwerp.
Methods : All first and second degree immigrants were selected from our Antwerp University Hospital patient cohort with IBD. Phenotype differences were assessed between Moroccan and Caucasian immigrants. We looked at age at diagnosis, sex, disease location and behavior (Montreal classification), extraintestinal manifestations, need for anti-TNF treatment and surgery and smoking habits. Chi-square statistics were used for analysis.
Results : 49 immigrant IBD patients were witheld, 32 (65%) with CD and 17 (35%) with ulcerative colitis (UC).+/-56% of the CD patients were Moroccan, and 44% were Caucasian immigrants ($p = 0.03$), with an inverse male/female ratio : 44/56% vs 64/36%. ?Mean age at diagnosis was comparable in Moroccan (26 ± 9 years) and Caucasian (30+/-15 years) immigrants. The majority of immigrant patients had ileocolonic disease (53% L3 Montreal classification) but Moroccan patients tended to have more penetrating (B3) disease : 39% vs 7% ($p = 0.076$), more CD-related surgery : 44% vs 29%

($p > 0.05$), more need for anti-TNF treatment : 44% vs 29% ($p > 0.05$) and more extraintestinal manifestations : 39% vs 21% ($p > 0.05$). There were no differences in smoking habits. ?
Conclusion : These single-center results suggest that first and second degree Moroccan immigrants with IBD suffer predominantly from CD with a more severe phenotype characterized by penetrating disease leading to higher rates of CD-related surgery and need for anti-TNF treatment. In addition, extraintestinal manifestations tend to be more prevalent in the Moroccan immigrant population. Further study is needed to explain why Moroccan immigrants seem to have more severe CD.

- I31 -

NGAL-MMP-9 AS A SURROGATE SERUM MARKER FOR MUCOSAL HEALING IN ULCERATIVE COLITIS. M. De Bruyn (1), I. Arijs (2), W.J. Wollants (2), K. Machiels (2), K. Van Steen (3), M. Ferrante (2), P. Rutgeerts (2), S. Vermeire (2), G. Opdenakker (1). (1) Rega Institute, Leuven, Belgium ; (2) Translational Research Center For Gastrointestinal Disorders (Targid), Kuleuven, Leuven, Belgium ; (3) Montefiore Institute, Liège, Belgium.

Introduction : The current standard for assessing intestinal inflammation and mucosal healing after therapy in inflammatory bowel diseases (IBD) is endoscopy. However, frequent assessments are costly and uncomfortable for the patient. Although C-reactive protein (CRP) is widely used as a marker of inflammation, half of the patients with active ulcerative colitis (UC) do not have elevated CRP levels.
Aim : The aim of this study was to investigate neutrophil gelatinase B-associated lipocalin - matrix metalloproteinase-9 (NGAL-MMP-9) as a surrogate serum marker for mucosal healing after treatment with infliximab (IFX) in UC patients.
Methods : NGAL-MMP-9 serum levels were determined with zymography analysis and sandwich ELISA before and 4-6 weeks after first IFX infusion from 66 active, IFX-na ?ve UC patients (median age at first IFX (years) : 40.9, male/female (%) : 60.6/39.3) as well as from 40 healthy controls (HC, median age (years) : 30.4, male/female (%) : 62.5/37.5). The response to IFX was defined as complete mucosal healing (endoscopic Mayo score 0 or 1) at control endoscopy. Data were analyzed with SPSS statistics 20 using non-parametric tests and p-values of < 0.05 were considered significant.
Results : From the 66 patients with active UC, 28 patients were classified as responder and 38 as non-responder. At baseline, median [interquartile range, IQR] NGAL-MMP-9 serum levels were found to be significantly higher in UC patients versus HC (42.4 [22.8 -79.9] vs 103.8 [56.9-180.4] ng/ml ; $p < 0.0001$). After IFX therapy, median [IQR] NGAL-MMP-9 serum levels significantly decreased in UC responders (116.3 [58.6-204.0] to 32.0 [22.4-64.2] ng/ml ; $p < 0.0001$), whereby only 3 patients (10%) had unchanged serum levels of NGAL-MMP-9 after therapy. For UC non-responders, median [IQR] NGAL-MMP-9 serum levels also significantly decreased after treatment with IFX (94.7 [53.2-139.8] to 54.1 [35.9-103.9] ng/ml ; $p = 0.0469$), however, this decrease was significantly less pronounced in comparison with UC responders ($p = 0.0074$). A strong correlation was found between results obtained from zymography analysis and sandwich ELISA ($r = 0.835$, $p < 0.0001$). NGAL-MMP-9 serum levels correlated with the amount of neutrophils ($r = 0.430$, $p < 0.0001$) and endoscopic Mayo scores ($r = 0.317$, $p < 0.0001$). Moreover, we found that NGAL-MMP-9 serum levels correlate better with endoscopic Mayo scores than CRP levels ($r = 0.299$, $p < 0.0001$). Receiver operating characteristic (ROC) analysis defined a NGAL-MMP-9 serum level cut-off value of 97.7 ng/ml corresponding to mucosal healing (AUC = 0.75, 43.27% sensitivity, 92.86% specificity).
Conclusion : In the search for surrogate markers assessing mucosal healing in UC, NGAL-MMP-9 performs better than CRP. We therefore propagate that the use of NGAL-MMP-9 serum levels can be implemented in clinical practice, thereby potentially overriding the need for endoscopy.

- I32 -

INHIBITION OF TH17 BUT NOT OF TREGS AFFECTS EXTRACELLULAR MATRIX DEPOSITION IN CHRONIC DSS COLITIS. C. Breynaert (1), C. Perrier (1), J. Cremer (1), L. Coorevits (2), M. Ferrante (1), S. Vermeire (1), P. Rutgeerts (1), C. Uyttenhove (3), J. Van Snick (3), J. Ceuppens (2), G. Van Assche (1). (1) Translational Research Center for Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (2) Experimental Immunology, KULeuven, Leuven, Belgium ; (3) Ludwig Institute For Cancer Research, City Of Brussels, Belgium.

Introduction : Studies in experimental colitis have greatly advanced our knowledge of the immune processes driving mucosal inflammation in inflammatory bowel diseases, but have generally failed to unravel the pathophysiology of connective tissue changes related to more chronic colitis.
Aim : To investigate the effect of downregulation of Tregs and Th17 cells on fibrosis in a murine colitis model, characterized histologically by segmental, transmural colitis with persistent lymphoid aggregates and collagen deposition.

Methods : 3 cycles dextran sodium sulphate (DSS) chronic relapsing colitis was induced in female 6 week-old C57BL/6 mice. DSS for 7 days followed by a recovery period of 14 days with normal drinking water was defined as one cycle of DSS. Weekly intraperitoneal injection of 500 μg rat IgG1anti-CD25 (n = 9), mouse IgG1 anti-IL17A (n = 8), mouse IgG1 anti-IL17F (n = 8) or corresponding isotype IgG (n = 9 and n = 8) was started after cycle 1. After euthanasia, the distal colon was harvested for histology. Blood and mesenteric lymph nodes (MLN) were studied using flow cytometry. Collagen deposition was quantified with MSB staining and hydroxyprolin assay.
Results : Although no significant differences in relative weight or disease activity index were observed, spleen weight ($p = 0.002$), colon weight ($p = 0.024$) and colon weight/length ratio ($p = 0.030$) were significantly higher in the anti-CD25 group. Colon length and macroscopic damage scores were not different. Despite enhanced inflammation, collagen deposition in mucosa and submucosa was not affected. Anti-IL17A/F administration did not influence weight curves, colon or spleen weight or macroscopic damage scores, but colon length tended to be shorter in the anti-IL17A group vs isotype. No significant differences were observed in expression of CD4+Foxp3+ Tregs in blood and MLN after administration of anti-CD25 nor in expression of CD4+IL17A+/F+ cells in MLN after administration of anti-IL17A/F. Collagen deposition in mucosa and submucosa tended to be higher in the anti-IL17A group.This was confirmed using a hydroxyprolin assay ($p = 0.027$ vs isotype). No differences in thickness of the muscularis propria were observed in all conditions.
Conclusion : Although administration of aCD25 results in more colonic and systemic inflammation, collagen deposition is not altered, suggesting that Tregs are not crucial in the induction of fibrosis. In contrast, inhibition of IL17A unexpectedly increases collagen deposition in more chronic DSS colitis, without reducing colonic or systemic inflammation, suggesting that IL17 limits collagen deposition.

- I33 -

DIFFERENTIAL EFFECT OF FECAL WATER ON BUTYRATE OXIDATION IN HT-29 CELLS AND HUMAN COLONIC BIOPSIES. L. Boesmans, V. De Preter, K. Windey, G. Vandermeulen, P. Rutgeerts, S. Vermeire, K. Verbeke. Translational Research Center For Gastrointestinal Disorders (Targid), Kuleuven, Leuven, Belgium.

Introduction : Butyrate, a colonic metabolite of anaerobic bacterial fermentation of carbohydrates, is considered as the major energy source for the colonic mucosa. A reduced butyrate metabolism has been reported in ulcerative colitis (UC). We hypothesize that compounds present in the colonic lumen of these UC patients impair the butyrate oxidation in colonocytes.
Aim : In this preliminary study, we evaluated the effect of fecal water (FW) from UC patients versus healthy controls on butyrate oxidation in human colonic biopsies as a representation of the colonic environment. Furthermore the effect of FW on butyrate oxidation was assessed in a human colon adenocarcinoma cell line (HT-29) to investigate whether these cells can be used as a representative model.
Methods : FW was obtained from stool samples from 8 healthy controls and 8 UC patients during active disease and again after achieving remission. To evaluate the effect of FW on butyrate oxidation, colonic mucosal biopsies, obtained from healthy subjects, and HT-29 cells were incubated with FW of UC patients and healthy controls. The butyrate oxidation rate was determined by incubating the biopsies and HT-29 cells with 14C-labelled Na-butyrate, measuring the released 14CO2 by liquid scintillation counting and correcting for protein content. Data are presented as median (IQR) and were compared by Kruskal-Wallis (K-W) and Mann-Whitney-*U* (M-W) tests with Bonferroni correction, with statistical significance set at $p < 0,05$.
Results : Addition of FW of healthy controls or UC patients did not significantly influence the butyrate oxidation in mucosal biopsies (K-W $p = 0,595$). However, incubating HT-29 cells with FW from UC patients with active disease and in remission, significantly increased the butyrate oxidation to 141 (131-166) % (M-W $p = 0,002$) and 142 (132-180) % (M-W $p = 0,043$) respectively. The FW of healthy subjects did not influence the butyrate oxidation in HT-29 cells. The disease activity of the UC patients did not significantly influence the butyrate oxidation, neither in biopsies nor in cells.
Conclusion : We conclude that the butyrate oxidation in HT-29 cells is significantly increased after incubation with FW from UC patients, while there is no significant difference in oxidation rate detected in colonic mucosal biopsies. Therefore the HT-29 cell line seems not a representative model to study the effect of colonic luminal compounds on butyrate oxidation.

INFLAMMATION-INDUCED DOWN-REGULATION OF BUTYRATE OXIDATION CAN BE COUNTERACTED BY BUTYRATE. L. Boesmans, V. De Preter, K. Verbeke. Translational Research Center for Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : Butyrate is produced in the large intestine by anaerobic bacterial fermentation of carbohydrates. Butyrate has well-documented anticarcinogenic and anti-inflammatory effects. Furthermore, butyrate serves as the major energy source for colonocytes via an intracellular beta-oxidation pathway. A reduced butyrate oxidation has been reported in the colonic mucosa of ulcerative colitis (UC) patients. This metabolic defect has been suggested to be the consequence of the inflammation rather than the cause.
Aim : In the present *in vitro* study, we mimicked inflammation in colonic cells and investigated whether butyrate was able to abolish the inflammation-induced decrease in butyrate oxidation.
Methods : Human colon adenocarcinoma cells (HT-29) were incubated for 24 h with either 50 ng/ml TNF-alpha (TNF), 100 ng/ml IFN-gamma (IFN) or 50 ng/ml TNF + 100 ng/ml IFN with or without 4 mM butyrate. Non-incubated cells and cells incubated with 4 mM butyrate served as control conditions. Afterwards, the incubation medium was discarded, the number of living cells was counted and butyrate oxidation was measured by incubating the cells with 14C-labelled butyrate, followed by beta-liquid scintillation counting of the released 14CO2. Data were compared by Mann-Whitney-*U* tests with a statistical significance set at $p < 0,05$.
Results : Incubation with butyrate alone or in combination with TNF, IFN or TNF + IFN resulted in a significantly reduced number of live cells as compared to respectively the control condition ($p < 0,01$) or incubation with the inflammatory stimulus alone (all $p < 0,05$). The butyrate oxidation rate was not influenced by a 24-h incubation with butyrate alone, suggesting that prior incubation with butyrate does not induce the butyrate oxidation pathway, nor with TNF. In contrast, incubation with IFN and the combination TNF + IFN resulted in a significant decrease in butyrate oxidation to respectively 38,3% and 2,7% of the control (both $p < 0,01$). However, simultaneous incubation of butyrate with these inflammatory stimuli at least partially prevented the drop in butyrate oxidation to respectively 93,4% and 37,1% of the control (both $p < 0,05$).
Conclusion : The reduction in number of live cells after incubation with butyrate is in line with previous *in vitro* experiments on tumour cell lines, in which butyrate induced apoptosis and inhibited proliferation. The inflammation-induced down-regulation of butyrate oxidation after incubation with IFN or TNF + IFN could be prevented by the simultaneous incubation with a physiologically relevant concentration of butyrate (4 mM). Increasing the luminal butyrate concentrations in UC patients might have therapeutic potential.

RESEARCH GROUP OF CLINICAL NUTRITION AND METABOLISM (SBNC)

Invited Lecture
- N01 -

NEW GUIDELINES FOR NUTRITION IN ICU. J.C. PREISER. Hôpital Erasme, City Of Brussels, Belgium.

Several aspects of nutrition therapy in critically ill are rapidly moving, in relation with the recently released results of large-scale trials, who evaluated the issues of optimal caloric and protein intake, micronutrient supplementation and pharmaconutrition. In order to facilitate the translation of these findings in daily clinical practice, a group of 38 Belgian, French and Swiss experts was nominated by French-speaking societies of intensive care and nutrition to suggest guidelines, following an analysis of the available literature using the GRADE methodology. Seventy recommendations and statements addressed the following areas : prevalence and consequences of undernutrition, evaluation of energy expenditure and protein needs, assessment of nutritional status, overall strategy of nutrition

Invited Lecture
- N03 -

THE BODY MASS INDEX AND ITS INFLUENCE ON ENDOSCOPY BEFORE GASTRIC BYPASS. P. Itoudi, T. Sersté, R. Ntounda, V. Rolland, B. Cadière, G. Cadière, M. Van Gossum. ULB Saint-Pierre, Brussels, Belgium.

Introduction : Oesogastroduodenoscopy (OGD) prior to bariatric surgery has been recommended to exclude endoscopic injuries and to investigate the presence of Helicobacter pylori (H.pylori).
Aim : The purpose of the study was to determine the relation between the body mass index (BMI) in one hand and the prevalence of H. pylori, the symptomatology, the endoscopic injuries and the effectiveness of the eradication treatment on the other hand.
Methods : Retrospective and observational study on 761 patients who underwent a gastric bypass between January 2008 and December 2011 in CHU Saint Pierre and were ranked in 4 groups following their BMI (group I : 35 to 39 kg/m² ; group II : 40 to 44 kg/m² ; group III : 45 to 49kg/m² and group IV more than 50kg/m². Every patient had had an OGD with biopsies (histology and H. pylori culture). In case of positive H. pylori, an eradication treatment was instaured and it's efficiency was evaluated by an urea breath test.
Results : The distribution of the 761 patients (561 women, 200 men) was as followed : group I : 24% ; group II :53% ; group III : 14% and group IV : 8%. The median age was 39 +/- 2 years (extremes :18-72). There were 563 caucasians (72%), 110 magrhebians (14%) and 88 subsaharians (12%). H. pylori was positive in 25% on histology and 20% with the culture. Thirty-three pourcent of the patients had symptoms (reflux and/or dyspepsia). Hundred and eighty-nine patients suffered an eradication treatment : 92% needed a one line treatment, 6% a two line and 1% a three line treatment. Two patients remained positive unlike the treatment. There was at least one endoscopic injury in 447 patients (59%) : 157 aspecific gratropathies, 158 oesophagitis, 17 gastric ulcers, 4 duodenal ulcers, 6 Barrett oesophagus, one oesophagial candidose and 3 gastrics polyps. The prevalence of H. pylori according groups group was respectively 18%(group I), 26% (group II), 31% (group III) and 27% (group IV).
There was no statistical difference between groups ($p = 0.056$). The prevalence of symptoms following the group was respectively 36%, 31%, 31% and 32% without significance. No statistical difference was found between groups according the effectiveness of the eradication treatment (respectively 94%, 91%, 94%, 94% ; $p = 0.76$). No statistical difference was found between the groups regarding the of endoscopic injuries.
Conclusion : BMI doesn't have any significant influence on the prevalence of H. pylori, symptoms, eradication, treatment effectiveness and endoscopic injury. BMI should not have any influence on the therapeutic strategy regarding the eradication of H. pylori.

- N04 -

NUTRITIONAL ASSESSMENT IN CANCER OUTPATIENTS PERFORMED IN AN ONCOLOGICAL HOSPITAL. J. Cantarero Gamarra , N. Hallot, Y. Lalami, D. De Valeriola , B. Fernez, M. Moreau, M. Csergö, E. Toussaint. Institut Jules Bordet, Brussels, Belgium.

Introduction : Screening policies for nutritional risk are largely applied for cancer patients during hospitalisation. Nevertheless, albeit an increasing number of oncological treatments administered on an outpatient basis, nutritional screening is scarcely applied in this setting.

Aim : The aim of this study was to describe a population of cancer patients, treated in an outpatient setting (one-day centre), regarding nutritional risk and prevalence of undernutrition.
Methods : All adult patients scheduled for chemotherapy in the one-day clinic of our oncological centre were included after informed consent. Nutritional risk and prevalence of undernutrition were assessed by :
1. The PG-Subjective Global Assessment (PG-SGA),
2. The presence and degree of previous weight loss,
3. the Malnutrition Screening Tool (MST),
4. The Nutritional Risk Screening 2002 (NRS2002) and the Malnutrition Screening Tool for Cancer Patients (MSTC).
Results : Two hundred fifty patients were assessed, with a mean age of 59 (29-87). Mean body-mass index (BMI) was 25.2 kg/m2 (13.5-51) ; 44% were overweight (BMI > 25). The majority of patients had solid tumours (n = 230) and specifically, breast cancer (n = 124). 60% of the patients with solid tumours had metastases. Concerning chemotherapy, regimens included neo-adjuvant (16.5%), adjuvant (21%) and palliative (55%). Regarding weight loss during the previous 6-month period, 24% presented with unintended weight loss during the previous month and 47% during the previous 6 months. According to the PG-SGA, the prevalence of undernutrition was 22.8% (n = 57), including 15 patients with severe undernutrition (6%). Concerning nutritional risk assessment, the NRS 2002 identified 19.6%, the MST 25.6% and the MSTC 35% of the patient population as being at risk of undernutrition. Regarding patients with solid tumours (n = 230), 14.6% of patients undergoing neo-adjuvant, 21.1% neo-adjuvant and 32.4% palliative treatment were undernourished. It is noteworthy that the mean BMI of undernourished patients was 23.5 kg/m2 (15.6-51.5). Furthermore, in patients with a normal BMI, or a BMI > 25, undernutrition was detected, respectively, in 22% and 17.3%. Finally, 12.6% of patients with a normal performance status (PS :0) were undernourished.
Conclusion : In a population of cancer patients treated in an outpatient setting, 22.8% were undernourished, 6% severely undernourished and up to 35% were considered at nutritional risk, despite the fact that the majority of patients were treated for breast cancer, an assumingly low nutritional risk disease. Furthermore, the prevalence of undernutrition was significant even in patients undergoing neo-adjuvant therapy, in overweight patients (BMI > 30) and patients with a normal performance status, whereas the above are also considered as low nutritional risk situations. Therefore, nutritional assessment for cancer patients can be useful on an outpatient basis.

Invited Lecture

- N05 -

DIFFERENTIATING SARCOPENIA FROM CACHEXIA AND UNDERNUTRITION. S. Schneider. Chu, Nice, France.

Precise definitions matter in Medicine, and Nutrition follows the rules. Undernutrition, often improperly called malnutrition, represents the consequences at tissue levels of an imbalance between protein-energy intake and expenditure. Sarcopenia is not amyotrophy ; it is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death. Cachexia is not emaciation ; it is a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. Recent definitions allow us to describe more precisely sarcopenia and cachexia in their respective target populations (elderly and chronic diseases respectively). However, with common determinants, these three conditions often overlap in patients, and these definitions based on mechanisms and outcomes will undoubtedly need to be updated.

- N06 -

REFEEDING SYNDROME AND VITAMIN D LEVELS :PROSPECTIVE STUDY IN PATIENTS RECEIVING PARENTERAL NUTRITION. M. Arvanitakis (1), P. Itoudi-Bignoumba (1), A. Ballarin (1), V. Lievin (2), A. Van Gossum (1). (1) Erasme Hospital, Brussels, Belgium ; (2) Erasme University Hospital, City Of Brussels, Belgium.

Introduction : The refeeding syndrome (RS) is defined by all adverse events occurring during nutritional rehabilitation of malnourished patients and is characterized by a decrease in serum phosphate of > 25%. Although this condition is well known, prospective data is scarce. Furthermore, vitamin D could influence phosphatemia, because of its role in phosphate-calcium metabolism.
Aim : The aim of this study was to assess incidence of RS in patients receiving parenteral nutrition (PN) and identify predictive factors, including the eventual role of vitamin D status.

Methods : This was a prospective study including all patients in whom PN was started during a period of 6 months. Demographic data as well as nutritional parameters (% of previous weight loss, body-mass index (BMI), prealbumin, phosphatemia and vitamin D levels) were recorded. Patients were divided in 3 groups, according to the risk of presenting RS following the NICE classification : A. No risk factors (low), B. 1 moderate risk factor (moderate) and C. = 2 moderate risk factors or = 1 severe risk factor (high). All patients had blood samples taken before PN infusion (T0), as well as 2 (T1) and 4 (T3) days later. Occurrence of RS was defined as a decrease of = 25% of phosphatemia following instauration of PN.
Results : 71 patients were included (35 men), with a median age of 57 (20-90). Median BMI was 24 kg/m2 (14-46). Previous weight loss (= 5%) was recorded in 56 patients (78.9%), and 31 (43.7%) had lost = 10% of initial body weight. At least one risk factor for RS was identified in 34 patients (47.9%). Median prealbumin level was 12.5 mg/dl (5-34) and 25 patients (35.7%) had a level of = 10 mg/dl. Median phosphatemia at T0 was 0.99 mmol/l (0.35-1.6). Regarding vitamin D, 55 (71.5%) patients had = 20 ng/ml and 33 (46.5%) had = 10 ng/ml. PN bags included 8 gr of nitrogen and 12 mmol of phosphate (n = 22, 31%) or 12 gr of nitrogen and 19 mmol of phosphate (n = 49, 69%). RS was observed in 30 (42.3%) patients. Regarding predictive factors, older age seemed to be associated with the occurrence of RS (52 vs 61, p = 0.018). Interestingly, T0 phosphatemia was higher in patients presenting with RS (1.06 vs 0.86 mmol/l, p = 0.002). Factors of undernutrition (BMI < 18.5 and prealbumin < 10 mg/dl), as well as vitamin D status, did not seem to be associated with RS. Finally, 32.4% of patients in group A (low risk) developed RS.
Conclusion : RS is frequent in patients in whom PN is started and can occur even in patients with an assumingly low risk, according to the NICE classification. Vitamin D levels do not seem to influence occurrence of RS in this initial study.

- N07 -

ROLE OF ANOREXIA IN THE DEVELOPMENT OF CANCER CACHEXIA. A. Loumaye, M. De Barsy, J.P. Thissen. Cliniques Universitaires St-Luc, City Of Brussels, Belgium.

Introduction : Cachexia is a complex metabolic syndrome associated with underlying illness and characterized by loss of skeletal muscle. In contrast to simple malnutrition, cachexia does not result only from reduced food intake. However, when present, anorexia may represent an opportunity for intervention.
Aim : To study the role of anorexia in cancer cachexia, we investigated prospectively patients referred to the Cancer Center of our Hospital for a colorectal or a lung cancer either at the diagnosis or at relapse. This study is a part a larger protocol aiming to define the role of circulating hormones/cytokines in cancer cachexia.
Methods : All patients have been prospectively recruited at the St-Luc Academic Hospital after written consent. Evaluation included dietary assessment (SNAQ questionnaire), body composition measurements (bioimpedance and anthropometry), quality of life questionnaires (ECOG and QLQC30) and blood sampling.
Results : Seventy-seven patients were recruited (29 lung and 48 colorectal cancer patients). Patients were included at the time of diagnosis (n = 65) or at relapse (n = 12). The prevalence of cachexia, as defined by a 5% involuntary weight loss in less than 6 months, according to K Fearon reached 48%. The presence of cachexia was unrelated to the cancer site, the presence of a relapse, the presence of metastasis and the previous treatments. As expected, cachexia was associated with a muscle mass loss (-14% ; $P < 0.005$) but also a fat mass loss (-29%, $P < 0.008$) as assessed by bioimpedance. Cachexia was associated with reduced physical function (ECOG and QLQC30 ; respectively $P < 0.0001$ and $P < 0.05$), reduced quality of life ($P < 0.004$) and increased symptoms (QLQC30) ($P < 0.001$). Anorexia, defined by a SNAQ score below 14, was much more common in cachectic than in non-cachectic patients (46% vs 13% ; $P < 0.002$). Furthermore, the SNAQ score was negatively correlated to the weight loss ($R = -0.546$; $P < 0.001$), but unrelated to the cancer site or the presence of metastasis. Finally, anorectic patients were also characterized by a lower skeletal muscle mass (-18% vs non-anorectic patients ; $P < 0.005$).
Conclusion : Cachexia is highly prevalent in patients with lung and colorectal cancer even at the time of diagnosis. The frequent coexistence of anorexia and cachexia suggests that decreased food intake may play a more important role than expected in the development of cachexia, even at the time of diagnosis. These observations suggest therefore that dietary intervention may slow down the progression of cachexia in cancer patients.

Invited Lecture - J.C. Preiser
- N08 -

- N09 -

CORRELATION BETWEEN BMI, OBESITY AND GASTRO-ESOPHAGEAL REFLUX DISEASE. A. Krishnan, J. Venkataraman. Stanley Medical College, Chennai, India.

Introduction : Obesity and Gastroesophageal reflux disease (GERD) are increasingly important health problems. Increase in body mass index has been shown to be associated with the increase in the prevalence of GERD symptoms, esophageal mucosal injury, and GERD complications
Aim : To evaluate the association between Body Mass Index and Gastroesophageal Reflux Disease and to determine the correlation Obesity and GERD
Methods : We carried out a prospective cross sectional study to determine the prevalence and analysis of BMI waist - hip ratio in 106 patients with symptomatic GERD. Patients who had dysmotility, those with history of abdominal surgery and pregnant women were excluded from this study. Classic symptoms of GERD and history of other co morbid illness especially related to obesity were recorded after direct questions. Complete histories regarding diet habits, smoking, alcohol consumption, tobacco chewing, use of NSAIDs, oral contraceptives were recorded. Weight, height, waist, hip circumference, waist circumference and abdominal girth were measured and waist hip ratios, BMI were also calculated. Patients were then assigned to four categories according to their BMI.
Results : There were 45 males and 61females, there were 40 females vs 14 males among GERD cases with BMI > 23. Of the 45 males with GERD, 16 (35.6%) had waist circumference > 87 cm, where as 39 out of 61 females had waist circumference > 82. 31 males GERD patients who were of < 23 BMI were significantly more than 14 those with BMI in overweight or obese category : P value 0.008. However, 40 female GERD patients who were overweight or obese exceeded 14 those with BMI < 23 P value 0.02. 29 male GERD patients with waist circumference < 87cms were significantly more than 16 those with higher waist circumference : P value 0.03. 39 females out OF the number of 16 Males in the high waist circumference group but the difference was not statistically significant. 29 males were more than 22 females in the normal waist circumference group : P value 0.05. The number of male GERD patients who were with in reference range for waist hip ratio (25) were significantly more than those with higher waist – hip ratios (20) : P value 0.0001. However 51 female GERD patients with higher waist – hip ratios exceeded 10 those with in reference range : p value 0.0006. Upper GI endoscopy was done in 99 patients. NERD was more prevalent than ERD in both males and females p value : 0.03. There were more female with NERD than ERD p value 0.07. Males were more likely to have ERD compared to females [p value 0.003].
Conclusion : Overweight and obesity are strong independent risk factor of GERD symptoms. Prevalence of obesity among patients with GERD was more based on waist circumference and waist - hip ratio than BMI. The link between obesity and GERD is strong in women

- N10 -

DIFFERENTIAL EFFECT OF WBE AND FOS ON FERMENTATION PATTERNS AND FECAL WATER CYTOTOXICITY IN HEALTHY. K. Windey (1), I. François (2), W. Broekaert (2), J. Delcour (3), J. Herman (4), T. Louat (4), V. De Preter (1), K. Verbeke (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (2) Fugeia NV, Leuven, Belgium ; (3) Centre For Food And Microbial Technology, Leuven, Belgium ; (4) Interdepartemental Valorisation Platform, KU Leuven, Leuven, Belgium.

Introduction : Prebiotic intake has been shown to effect fecal metabolite patterns. However, the relevance of these changes to gut health has not been thoroughly investigated.
Aim : In this study, the effect of 2 prebiotics, fructo-oligosaccharides (FOS) and wheat bran extract (WBE) containing arabinoxylan-oligosaccharides, on colonic metabolism and parameters of gut health, genotoxicity and cytotoxicity, was evaluated.
Methods : A randomized, double-blind, placebo-controlled, cross-over study was conducted in 20 healthy subjects and consisted of a 2-week run-in period and 3 intervention periods of 2 weeks each time separated by a 2-week wash-out period. During the first week of each intervention period subjects received a low dose (15 g/d) of either FOS, WBE or placebo, while the dose was increased to 30g/d during the second week . At the end of the run-in period, each intervention and wash out period a fecal sample was collected for the analysis of the metabolite profile and for production of fecal water. Fecal metabolite patterns were analysed using GC-MS. Fecal water genotoxicity was determined using the Comet Assay in HT-29 cells and expressed as tail length (μm). Fecal water cytotoxicity was assessed using the WST-1

assay and expressed as fold dilution at which 50% of the cells survived (FD50). Data were analyzed using a linear mixed model with intervention and sequence as fixed effects. In addition, cluster analysis was applied to analyze metabolite patterns according to the intervention, genotoxicity and cytotoxicity.
Results : Cluster analysis of the metabolite patterns showed separation, although incomplete, of the samples collected after intake of FOS from those after WBE and all samples collected during the run-in, placebo and wash-out periods. Samples collected during FOS and WBE intake were associated with the presence of esters and cycloalkanes and - alkenes, while sulfides and branched chain fatty acids (BCFA) were more abundant in the samples collected during run-in, placebo and wash-out periods. FOS and WBE did not affect fecal water genotoxicity as compared to placebo. However, WBE significantly reduced fecal water cytotoxicity in comparison to FOS and placebo (-8.97 ± 14.79 vs. -0.64 ± 11.19 and -0.14 ± 15.96 ; $p = 0.043$). Cluster analysis of metabolite profiles in fecal samples according to cytotoxicity revealed a separation between the high toxicity samples and the low toxicity samples. This separation was mainly due to the presence of esters, cycloalkanes and -alkenes in the low toxicity samples and ketones and BCFA in the high toxicity samples.
Conclusion : Intake of FOS and WBE differentially affects fermentation in the colon. Only intake of WBE reduces fecal water cytotoxicity. This reduction is associated with a higher prevalence of esters and cycloalkanes and - alkenes.

- N11 -

INCORPORATION OF COLONIC DERIVED SHORT CHAIN FATTY ACIDS IN CHOLESTEROL : STUDY IN HEALTHY HUMANS.
E. Boets (1), E. Houben (1), K. Windey (1), V. De Preter (1), S. Gomand (2), G. Van Den Mooter (3), J.A. Delcour (4), K. Verbeke (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), Leuven Food Science And Nutrition Research Centre (Lforce), Ku Leuven, Leuven, Belgium ; (2) Laboratory Of Food Chemistry And Biochemistry, Leuven Food Science And Nutrition Research Centre (Lforce), Ku Leuven, Leuven, Belgium ; (3) Laboratory For Pharmacotechnology And Biopharmacy, Ku Leuven, Leuven, Belgium ; (4) Laboratory Of Food Chemistry And Biochemistry, Leuven Food Science And Nutrition Research Centre (Lforce), Ku Leuven, Leuven, Belgium.

Introduction : Consumption of dietary fibre has been reported to lower plasma cholesterol concentrations. This effect is attributed to the production of short chain fatty acids (SCFA) during the colonic fermentation of dietary fibre. However, the effects of SCFA on cholesterol metabolism are equivocal. Acetate is considered as a substrate for cholesterol synthesis whereas propionate might have a dual role : (a) it reduces cholesterol synthesis by inhibiting key enzymes in the cholesterol pathway and (b) it is used as a substrate for cholesterol synthesis.
Aim : The aim of this study was to quantify *in vivo* the utilisation of colonic derived acetate and propionate for cholesterol synthesis in healthy subjects using a stable isotope technique.
Methods : Twelve healthy volunteers (7F/5M ; 26 ± 6y) performed each 2 test days. On each test day either ^{13}C-labelled acetate (400 mg) or ^{13}C-labelled propionate (340 mg) was administered into the proximal colon using a pH-dependent colon delivery capsule (CDC). Together with this CDC the volunteers consumed a standard breakfast labelled with inulin-^{14}C-carboxylic acid. Upon arrival in the colon, inulin-^{14}C-carboxylic acid and ^{13}C- SCFA are metabolised to $^{14}CO_2$ and $^{13}CO_2$, respectively, and are exhaled in breath. Breath samples were collected during the day for analysis of $^{13}CO_2$ and $^{14}CO_2$. Blood samples were collected at regular time points for 12 h. ^{13}C-cholesterol enrichment in plasma was measured using gas chromatography combustion isotope ratio mass spectrometry. Total cholesterol concentrations were measured by standard laboratory techniques. Both measurements were used to calculate the concentration of ^{13}C-cholesterol in plasma and the total recovery (the area under the curve (AUC) of the concentration-time curve). Based on a one-compartment model the fraction of administered ^{13}C-acetate and ^{13}C-propionate incorporated in cholesterol was calculated. Results are expressed as medians and interquartile ranges.
Results : The rise of 13CO2 in breath upon opening of the CDC coincided with an increase in 14CO2, indicating that the CDC released its content in the colon. Increased 13C-cholesterol plasma concentrations were detected in all subjects after 13C-acetate and in 10 subjects after 13C-propionate. The total recovery (AUC) of 13C-cholesterol 12 h after consumption of the CDC was 0.026 [0.014-0.050] mmol.h/l for 13C-acetate and 0.002 [0.001-0.006] mmol.h/l for 13C-propionate. The fraction recovered in cholesterol was 0.088 [0.058-0.137] % of the administered 13C-acetate and 0.005 [0.002-0.009] % of the administered 13C-propionate.
Conclusion : Our results indicated that a small proportion of the colonic produced acetate is used as substrate for cholesterol synthesis. In contrast, propionate incorporation in cholesterol was negligible.

IN VIVO EVALUATION OF BACTERIAL CROSS-FEEDING IN THE COLON USING STABLE ISOTOPE TECHNIQUES. E. Boets (1), E. Houben (1), K. Windey (1), V. De Preter (1), F. Moens (2), S. Gomand (3), G. Van Den Mooter (4), L. De Vuyst (5), J.A. Delcour (6), K. Verbeke (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), Leuven Food Science and Nutrition Research Centre (Lforce), KU Leuven, Leuven, Belgium ; (2) Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Ixelles, Belgium ; (3) Laboratory of Food Chemistry and Biochemistry, Leuven Food Science and Nutrition Research Centre (Lforce), KU Leuven, Leuven, Belgium ; (4) Laboratory for Pharmacotechnology and Biopharmacy, KU Leuven, Leuven, Belgium ; (5) Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Ixelles, Belgium ; (6) Laboratory of Food Chemistry and Biochemistry, Leuven Food Science and Nutrition Research Centre (Lforce), KU Leuven, Leuven, Belgium.

Introduction : Short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate, are produced in the colon by anaerobic bacterial fermentation of undigested dietary carbohydrates. Previous *in vitro* incubation studies of fecal slurries with fermentable carbohydrates demonstrated conversion of acetate into butyrate, i.e. so-called cross-feeding.
Aim : The aim of this study was to demonstrate *in vivo* cross-feeding in the human colon.
Methods : In this preliminary cross-over study, twelve healthy volunteers (7F/5M ; 26+/- 6 y) each performed 3 test days. On each test day, 13C-labelled SCFA (either 400 mg 13C-acetate or 340 mg 13C-propionate or 990 mg 13C-butyrate) was administered into the proximal colon using a pH-dependent colon delivery capsule. Blood samples were taken every hour during the first 4 hours and afterwards every 20 minutes during 8 hours.13C-acetate, 13C-propionate, 13C-butyrate, 13C-pentanoate and 13C-hexanoate enrichments in plasma were measured using gas chromatography combustion isotope ratio mass spectrometry.
Results : After introduction of 13C-labelled acetate into the colon, 13C-enrichment of acetate and butyrate increased in the plasma with 0,467 [0,339-0,746] and 0,275 [0,182-0,574] atom percent excess (APE), respectively. After administration of an uncoated 13C-acetate capsule, only 13C-acetate appeared in plasma but no 13C-butyrate, implicating that the conversion of 13C-acetate into other SCFAs is due to bacterial metabolism and not to human metabolism. Known acetate-converting species include *Roseburia, Eubacterium* and *Faecalibacterium prausnitzii*. Administration of 13C-propionate resulted only in increased 13C-propionate (APE : 1,552 [1,250-2,292]). Similarly, 13C-butyrate consumption induced only an increase in 13C-butyrate (APE : 3,587 [2,706-4,969]), indicating that there was no propionate or butyrate conversion into acetate, propionate, butyrate, pentanoate or hexanoate.
Conclusion : The method used in the present study allowed to evaluate plasma SCFA profiles obtained after administration of different 13C-labelled SCFAs in healthy subjects. Our results indicated that cross-feeding occurs *in vivo* in the complex and dynamic microbial ecosystem of the human colon.

BELGIAN GROUP FOR DIGESTIVE ONCOLOGY (BGDO)

- O01-

DEBULKING AND INTRAPERITONEAL CHEMOPERFUSION FOR PERITONEAL METASTASIS FROM COLORECTAL ORIGIN. W. Ceelen, K. Geboes, S. Laurent, P. Pattyn. Universitair Ziekenhuis Gent, Gent, Belgium.

Introduction : A subset of colorectal cancer (CRC) patients presents with synchronous or metachronous metastasis confined to the peritoneal surfaces. These patients may benefit from cytoreductive surgery with hyperthermic intraperitoneal chemoperfusion (HIPEC).
Aim : We reviewed the morbidity and survival associated with this multimodal approach.
Methods : Colorectal cancer patients with potentially resectable peritoneal metastases were evaluated. Patients with low grade appendiceal tumours including the pseudomyxoma peritonei (PMP) syndrome were excluded from the analysis. Staging consisted of CT and PET-CT ; laparoscopy was rarely performed. Neoadjuvant therapy was prescribed in patients with extensive disease. All patients underwent cytoreduction including peritonectomy procedures as indicated. When optimal resection was achieved, HIPEC was performed with the open abdomen technique and using oxaliplatin 460 mg/m_2 during 30 min at 40-41°C. All consecutive patients were included in the analysis. Overall survival was estimated with the Kaplan Meier method, and multivariate modeling of overall survival was performed with Cox proportional hazards analysis. Statistical significance was assumed whenever the probability of a type I error was < 5%.
Results : In a 7 year period, 123 patients, age 59 (SD :12) and 50% male were treated. In 25 patients (20%), peritoneal metastases were synchronous with the primary tumour. Neoadjuvant chemotherapy was prescribed to 53% of patients. The mean duration of the combined procedure was 8.9 (SD : 2.6) hours. Postoperative mortality was 1.6%, and major complications (requiring prolonged stay and/or reintervention) occurred in 34%. Median hospital stay was 17 days (range 8-169). After a median follow up of 16 months, median overall survival (OS) was 28 months, with a three year actuarial survival of 40.4%. In a multivariate model, the following variables were independently associated with OS : completeness of cytoreduction, tumour differentiation, and response to neoadjuvant chemotherapy.
Conclusion : A multimodal approach consisting of neoadjuvant chemotherapy, cytoreductive surgery, and HIPEC results in a meaningful survival benefit in patients with peritoneal metastases from CRC. In a referral center, the procedure can be performed with low operative mortality and acceptable morbidity.

- O02 -

HEALTH-RELATED QUALITY OF LIFE AFTER ESOPHAGECTOMY : IMPORTANCE OF PREOPERATIVE PHYSICAL FUNCTIONING. P. Nafteux, T. Lerut, J. Moons, J. Durnez, W. Coosemans, H. Decaluwe, G. Decker, H. Van Veer, D. Van Raemdonck, P. De Leyn. University Hospitals Leuven, Leuven, Belgium.

Aim : To evaluate baseline Health-Related Quality of Life (HRQL) factors that influence length of stay (LOS) after esophagectomy for cancer of the esophagus and GEJ.
Methods : 455 Patients operated on with curative intent between January 2005 and December 2009 were analyzed. HRQL-scores were obtained by EORTC QLQ-C30 and OES-18 questionnaires at baseline (= day before surgery) and three monthly post surgery for the first year. Multivariable analyses, using a binary logistic regression model were performed.
Results : There were 372 males and 83 females, with a mean age of 63.1 years. Hospital mortality was 3.7% (17 pts). Overall survival at 12 and 60 months was 84.4% and 50.2%. Disease-free survival was 44.5% at 5 years.
Independent prognosticators for a longer LOS (> 10 days) were : medical (HR 6.2 [3.62-10.56] ; $p < 0.0001$) and surgical (HR 2.79 [1.70-4.59] ; $p < 0.0001$) morbidity, admittance to ICU (HR 33.82 [4.55-251.21] ; $p = 0.001$) and a preoperative perception of poor physical functioning (HR 1.89 [1.14-3.14] ; $p = 0.014$). The perception of physical function, (the only HRQL related factor withheld preoperatively) was postoperatively influenced by following independent prognosticators : admittance to ICU (HR 5.19 [1.94-13.89] ; $p = 0.001$), recurrence (HR 2.81 [1.94-13.89] ; $p = 0.001$) and pre-operative poor physical functioning (HR 5.94 [3.26-10.82] ; $p < 0.0001$). LOS was not withheld in this multivariate analysis ($p = 0.409$).
Conclusion : Besides medical /surgical morbidity and admission to ICU, poor physical function preoperatively is another independent predictor for prolonged LOS. Postoperative HRQL was determined by poor pre-operative physical function, as well as by admission to ICU and recurrence. It can be postulated that besides high quality surgery improvement of perception of preoperative physical functioning might have a beneficial effect on LOS and on the perception of physical function one year post surgery.

- O03 -

EFFECT OF NEOADJUVANT RADIATION DOSE ON OUTCOME IN LOCALLY ADVANCED OESOPHAGEAL CANCER. E. Van Daele (1), C. Brackenier (1), W. Ceelen (2), T. Boterberg (1), K. Geboes (2), S. Laurent (2), P. Pattyn (2). (1) UZ Gent, Gent, Belgium ; (2) Universitair Ziekenhuis Gent, Gent, Belgium.

Introduction : Neoadjuvant multimodality treatment confers a survival benefit in locally advanced oesophageal cancer patients. The optimal dose of radiotherapy (RT) remains undefined.
Aim : To analyse the effect of RT dose on surgical outcome and survival in patients treated with multimodality treatment followed by surgery in locally advanced oesophageal cancer.
Methods : This was a retrospective comparative study based on a prospectively collected database. Patients with clinical stage III oesophageal cancer were treated with a combination of RT, cisplatin 80 mg/m2, and 5-FU 800 mg/m2. Radiation dose was 36 Gy (group 1) or 45-50 Gy (group 2), depending on the referring physician. Ivor Lewis oesophagectomy was performed after a 6-8 weeks period. Surgical and pathological outcome was compared using Fisher exact test. Overall survival (OS) and disease free survival (DFS) were calculated using the Kaplan Meier method, and the effect of RT dose on survival was tested using univariate (log rank test) analysis.
Results : A total of 134 patients were evaluated : 108 received 36 Gy, and 26 received 45-50 Gy. Mean age at surgery was 61 ± 9.43 years, and 84% were male. Median length of postoperative hospital stay was 17 days. Overall postoperative 30 day or in hospital mortality was 7.5%, and anastomotic leakage occurred in 4.1% ; neither was influenced by RT dose. Pathological complete response (pCR) was observed in 15.5% (group 1) and 31% (group 2), P = 0.09. No differences were observed in downstaging of either the T stage or the N stage. Overall five year OS and DFS were 35.1% and 38.3% respectively. In univariate analysis, a higher RT dose was associated with a significantly better DFS (P = 0.01), but not OS (P = 0.6).
Conclusion : In patients with locally advanced oesophageal cancer treated with neoadjuvant chemoradiation, a higher RT dose does not affect surgical outcome, enhances pCR rate and improves DFS without affecting OS.

- O04 -

CAN EXTRACAPSULAR LYMPH NODE INVOLVEMENT BE A TOOL TO FINE-TUNE PN1 FOR ADENOCARCINOMA IN UICC TNM 7. P. Nafteux, T. Lerut, J. Moons, W. Coosemans, H. Decaluwe, G. Decker, H. Van Veer, D. Van Raemdonck, P. De Leyn. University Hospitals Leuven, Leuven, Belgium.

Introduction : The current(7th) International Union Against Cancer (UICC) pN staging system is based on the number of positive lymph nodes but does not take into consideration characteristics of the metastatic lymph nodes itself in particular the presence of extracapsular lymph node involvement.
Aim : The aim of the current study was to examine the prognostic value of extracapsular (EC-LNI) and intracapsular (IC-LNI) lymph node involvement in esophageal cancer.
Methods : From 2000-2010, 499 adenocarcinoma patients with primary R0-resectionwere retrieved from our prospective database. The number of resected lymph nodes, number of positive lymph nodes and number of EC-LNI / IC-LNI were determined. Extracapsular spread was defined as infiltration of cancer cells beyond the capsule of the positive lymph node.
Results : Two hundred and eighteen (43%) patients had positive lymph nodes. Cancer-specific 5 year survival in lymph node positive patients was significantly ($p < 0.0001$) worse compared to lymph node negative patients, being 86.7% versus 25% respectively. In 128 (58.7%) cases EC-LNI was detected. EC-LNI showed significantly worse cancer-specific 5-year survival compared to IC-LNI (n = 90), 15.7% versus 37.9% ($p < 0.0001$). In the pN1-category (n = 67, EC-LNI n = 19, IC-LNI n = 48) (1 or 2 positive LN's - UICC stages IIB and IIIA) this was 20.4% versus 50.7% ; $p = 0.045$). Cancer specific survival in pN1 EC-LNI is comparable to current stage IIIB ($p = 0.88$) whereas pN1 IC-LNI are comparable to current stage IIA ($p = 0.33$). In higher pN categories, this effect of EC-LNI versus IC-LNI was no longer noticed.
Conclusion : EC-LNI is associated with worse survival compared to IC-LNI. In current UICC stages IIB/IIIA, EC-LNI patients show survival rates that are more closely associated with stage IIIB, whereas IC- LNI patients have a survival more similar to stage IIA. When reclassified as such, homogeneity of the TNM model increases. In the future edition of the TNM staging system for esophageal cancer, EC-LNI versus IC-LNI should be considered.

- O05 -

CHARACTERISATION OF GENOME-WIDE COPY NUMBER ABERRATIONS IN COLON CANCER. E. Mampaey (1), N. Van Roy (1), K. De Ruyck (2), L. Ferdinande (1), W. Ceelen (1), Y. Van Nieuwenhove (1), P. Pattyn (1), K. Geboes (1), S. Laurent (1). (1) Ghent University Hospital, Ghent, Belgium ; (2) Ghent University, Ghent, Belgium.

Introduction : Despite improved screening programs and modern treatments, colorectal cancer (CRC) still remains the third most common cancer in men and women and the second leading cause of cancer-related death. The development of CRC is characterised by several genetic and epigenetic alterations. There is still a high rate of recurrence and metastases with a remaining need for identification of molecular biomarkers in order to predict the evolution of the disease.
Aim : Our aim was to characterise copy number alterations in different subgroups of colon cancer patients.
Methods : Genetic alterations were analysed using Array Comparative Genomic Hybridisation (array-CGH) in 100 resection tissues of colon tumours. The tumour DNA was isolated with the Qiagen DNeasy Blood and Tissue kit. Megapool reference DNA was used as normal control. Tumour DNA was labelled with the fluorescent dye Cy3 and the normal control was labelled with Cy5. After labelling the DNA, it was hybridised to a SurePrintG3 Human CGH Microarray, 4 × 180 K. The scanned images were analysed with an in-house developed software program, arrayCGHbase.
Results : In the studied patient population, 3-year survival and recurrence of disease were correlated with the stage of colon cancer but not with tumour localisation.
Preliminary results showed a significant correlation ($p < 0{,}001$) between the number of genetic alterations and overall survival of the patients ($n = 100$). The most prevalent copy number alterations in CRC as described in literature, were confirmed in our samples, for instance gain of chromosomes 7, 13 and 20 and losses of chromosomes 4 and 18. Moreover, we found alterations residing in a number of new interesting chromosome regions. Future analyses will have to reveal the clinical relevance in colon. cancer.
Conclusion : The number of copy number alterations is associated with the overall survival of colon cancer patients. The new findings will be further investigated in depth. The link between KRAS and MSI status and the copy number alterations in the tumours will also be investigated.

- O06 -

THE LYNCH SYNDROME AND HYPERPLASTIC POLYPOSIS SYNDROME : A COMMON MOLECULAR BASIS ? R. Derycke (1), S. Tejpar (2), T. De Ravel De L'argentière (3), S. Decock (4), P. Van Hootegem (4), J. Arts (4). (1) University of Leuven ; (2) University Hospitals Leuven ; (3) UZ Leuven ; (4) AZ Sint-Lucas, Brugge.

Introduction : The presentation of a family diagnosed with HNPCC, hereditary nonpolyposis colorectal cancer, due to a mutation in *hMLH1* gene and the simultaneous diagnose of HPS, hyperplastic polyposis syndrome, in the non carrier son of the proband, led to reflection on this association. Both conditions are known as having an increased colorectal cancer (CRC) risk. HNPCC is a disorder with colorectal and other cancers, characterized by a deficiency in the mismatch repair (MMR) pathway. Mutations occur in the *hMSH2, hMSH6, hMSH3, hMLH1, hMLH3*, *hPMS2* and *hPMS1* genes. The phenotype of HPS is the presence of multiple, large and/or proximal hyperplastic polyps, in which there are no known germ-line predisposition. Candidate genes for HPS are *OGG1, MBD4, MYH*, *MTH1, DNMT* and *EPHB2*. Combined histologic and molecular details are strongly suggestive of a sequential relationship between serrated polyps and CRC. These findings support the evidence of a serrated neoplasia pathway.
Aim : The aim of the study was to find a possible explanation for the simultaneous presentation of HNPCC and HPS and to review critically the literature including the genetic basis of HNPCC and HPS, the known molecular characteristics of HPS-CRC or HNPCC-CRC. The common ground between HNPCC and HPS will be discussed.
Methods : This article is a literature review that aims to associate the current knowledge of the molecular and genetic basis of the HNPCC and HPS.
Results : The association between HNPCC and HPS has been described in different families (Walsh *et al*., 2009), but there's also evidence for the presentations of the two syndromes in one person (Jarrar *et al*., 2009). The simultaneous presentation of HNPCC and HPS in one patient supports the hypothesis of a fusion pathway, that combines mechanisms associated with base excision repair defects (HNPCC) and serrated neoplasia.
It is suggested that these patients are the clinical presentations of the coexistence of the mutator pathway and the methylator pathway.
The convergence of the two pathways may even lead to stronger phenotypes and it is interesting to see if the variable penetrance of the HNPCC phenotype between kindreds and within individuals of a kindred can be explained by the co-occurrence of serrated pathway predisposition.
Molecularly, microsatellite instability is often found in serrated polyps of sporadic origin, indicating a potential role in the genesis of these lesions. The microsatellite instability in serrated polyps is most often the consequence of hypermethylation. Analysis of the base excision repair (BER) genes as candidate genes for HPS has not revealed pathogenic

germline mutations. Candidate genes found in rare cases are *DNMT, OGG1, MTH1, MYH, MBD4* and *EPHB2* (Drini et al, 2011 ; Morak *et al*., 2010 ; Chow *et al*., 2006 ; Kokko *et al*., 2006).
Conclusion : The literature suggests common inheritance of mutations leading the BER and serrated polyps may coexist in some HNPCC kindreds, possible contributing to stronger HNPCC phenotypes. These kindreds may also present with BER mutation negative individuals with multiple serrated polyps. The observation of these mixed kindreds and the molecular similarities between HNPCC related and serrated polyp tumors, both having base excision repair defects, may suggest common drivers. Better family screening, with documentation of the types of polyps and tumors in carriers and non carriers, may allow double diagnosing, and should contribute to our understanding of the association between HNPCC and HPS in the future.

Invited lecture
- O07 -

WHAT IS THE RELEVANCE OF METABOLIC RESPONSE ? M. Peeters. Antwerp.

Invited lecture
- O08 -

NEW TRACERS IN NUCLEAR MEDICINE : WHICH ONES WILL BE RELEVANT IN GI CANCER ? Deroose. Leuven.

Invited lecture
- O09 -

MRI IN RESPONSE PREDICTION. M. Bali. Erasme Brussels.

Invited lecture
- O10 -

COLORECTAL CANCER. H. Prenen. UZ Leuven.

Invited lecture
- O11 -

HEPATOBILIARY AND PANCREATIC CANCER. J.-L. Van Laethem. Brussels.

Invited lecture
- O12-

UPPER GASTROINTESTINAL CANCER : HIGHLIGHTS DURING THE PAST YEAR AND FUTURE PERSPECTIVES. A. Hendlisz, Institut Jules Bordet, Brussels.

Abstract : The upper gastrointestinal cancer field in recent months has been marked both by several resounding failures in pharmaceutical industry-driven studies, but also some important academic achievements. Both phenomena point to interesting perspectives for the future management of patients.
Esophageal cancer : While the two most frequent histological subtypes of esophageal cancer, squamous cell carcinoma (SCC) and adenocarcinoma (ADC), share no etiological features and few biological aspects, most past and ongoing trials have studied the two subtypes together. We hope that this will disappear in future trials.

Nevertheless, arandomized Dutch trial (1) of this type comparing surgery alone versus preoperative carboplatin-taxol based radiochemotherapy (RTCT) reported interesting results. In the mixed histology population (75% ADC, 25%SCC), the trimodality approach was associated with a significantly better resection rate (92% versus 69%, $p < 0.001$) and median overall survival (OS) (49.4 versus 24.0 months, hazard ratio [HR] 0.657 ; 95% confidence interval [CI] 0.495 to 0.871, $p = 0.003$) than surgery alone. This pivotal study has thereby introduced a new standard for RTCT, now a quasi-mandatory step before any curative surgery for esophageal cancer.

Improvements in radiotherapeutic techniques (2) and in minimally invasive surgery (3) will probably help further evolve the therapeutic modalities for localized forms of this cancer. FDG-PET/CT-based metabolic imaging, for example, has been demonstrated to accurately define patient prognosis and chances of benefit from preoperative treatment (4-6). However, this tool remains largely neglected by most research teams, probably reflecting the perceived difficulty of setting up a multicenter trial focused on metabolic imaging.

On the basic science level, The Esophageal Adenocarcinoma Genetics Consortium and The Wellcome Trust Centre for Human Genetics, Oxford, UK, have demonstrated the susceptibility to Barrett's esophagus for common variants at the Major Histocompatibility Complex locus and at chromosome 16q24.1, which represent a first step towards the decryption of the Barret-ADC sequence.

Gastric Cancer : Unlike breast cancer, HER2 positivity has no prognostic value in gastric cancer, despite being present in about 20% of this tumor type (8). This finding offers very little rationale to explain the observed benefit in one study favoring the use of anti-HER2 antibodies in advanced gastric cancer (9). Based on these results, numerous pharmaceutical-industry sponsored trials have nonetheless been initiated to assess newer HER2-blocking or dual HER1&2-inhibiting agents.

Concern has been increasing about these new trials, following the presentation of the results of two randomized trials exploring the addition of anti-HER1 antibodies to standard chemotherapy in advanced gastric cancer. The REAL 3 trial, presented at the 2012 ASCO meeting, seemingly showed worse, even if not statistically significant, Progression-Free Survival (PFS) and OS for patients treated in the chemotherapy plus panitumumab arm than for those in the chemotherapy alone arm (10). Similarly, the EXPAND trial, presented during the 2012 ESMO meeting (11), failed to show any advantage of cetuximab when added to a cape-cisplatin combination in first-line metastatic gastric cancer.

In fact, few biological agents have become stars on the gastric cancer podium during the last few years. Bevacizumab failed to improve patient outcome when added to a first-line cisplatin-capecitabin combination, as shown in the AVAGAST trial (12), and everolimus monotherapy (GRANITE trial) was not superior to placebo in advanced chemorefractory gastric cancer (13).

By contrast, the benefit of chemotherapeutic agents to treat gastric cancer in the adjuvant setting has been confirmed, with significant improvement (HR 0.56, 95%CI 0.44-0.72, $p < 0.0001$) in 3 year disease-free survival determined for postoperative capecitabine plus oxaliplatin over surgery alone (14).

Conclusions : In 2012, the use of preoperative radiochemotherapy with a carboplatin-taxol regimen was confirmed as a promising approach to treat locally advanced esophageal cancer. The adjuvant capecitabine and oxaliplatin regimen also gained ground for treating locally advanced GI cancer. However, no significant breakthrough was achieved in either metastatic esophageal or in metastatic gastric cancer during this time period, despite progress made in the genomic characterization of the Barett-ADC transition.

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- O13 -

CLINICAL PATTERNS AND PANITUMUMAB IN WILD-TYPE KRAS METASTATIC CRC FAILING CHEMOTHERAPY. M. De Man (1), A. Bols (2), J.L. Canon (3), T. Delaunoit (4), M. Polus (5). (1) OLV Hospital, Aalst, Belgium ; (2) AZ Sint-Jan Brugge-Oostende, Brugge, Belgium ; (3) Notre Dame, Charleroi, Belgium ; (4) Centre Hospitalier de Jolimont-Lobbes., La Louvière, Belgium ; (5) Centre Hospitalier Universitaire de Liège, Liège, Belgium.

Introduction : Panitumumab has been found to improve PFS with manageable toxicity in patients with KRAS wild-type metastatic colorectal cancer who have progressed after standard therapy. Little is known about characteristics of chemotherapy refractory patients and response and safety of treatment with panitumumab in routine clinical practice.
Aim : The aim of the study was to characterise the population of KRAS wild type metastatic colorectal cancer patients failing chemotherapy, to analyse factors that influence decision on treatment choice in this situation and evaluate effectiveness and safety of those patients treated with panitumumab in daily practice in Belgium.
Methods : In this multi-centre observational trial patients with wild-type KRAS metastatic colorectal cancer progressing after 5FU-, oxaliplatin-, and irinotecan-based chemotherapy were included. Patient and tumour characteristics and further treatment decision were recorded. Patient treated with panitumumab monotherapy were prospectively followed for effectiveness and safety.
Results : 144 patients were included in the registry part of the study. Median age was 69 year (42-88). Majority of patients (87%) had a good performance status ECOG < 2, and 73.6% had liver metastasis with a median of two metastatic sites involved. 60.4% had metastatic disease at the time of diagnosis of the primary cancer and 80% of patients had resection of the primary tumour. Most patients (97%) were treated with anti-EGFR targeted antibodies. The main reason (75%) for choosing panitumumab was the assumption that single agent targeted treatment had the best risk/benefit ratio. When combination with chemotherapy was preferred cetuximab irinotecan was prescribed (25%).
101 patients were treated with panitumumab and included in the prospective part of the study : characteristics of this patient group were comparable with those of the registry group. Best response evaluated by the investigator was 2 CR (2%), 24 PR (24%), 31 SD (31%), 32 PD (32%) and 12 unavailable (12%). The median PFS was 14 weeks and in the anti-EGFR naive group 15 weeks. Grade 3 toxicities occurred in 13% of patients.
Conclusion :
(1) Patients with wild-type KRAS metastatatic colorectal cancer progressive after second line chemotherapy are often elderly patients with good performance status and with more than one metastatic site.
(2) Most patients are treated with anti-EGFR antibodies in further line therapy : the choice of panitumumab mainly depends on preference of monotherapy.
(3) Treatment with panitumumab monotherapy after failure of previous lines including oxaliplatin an irinotecan is in real life practice effective and safe with disease control rate and PFS similar to those observed in the phase 3 clinical trial (Amado et al : JCO 2008, 26, 1626-1634) and manageable toxicity.

- O14 -

SEMIMECHANICAL ANASTOMOSIS VERSUS HANDSEWN ANASTOMOSIS AFTER ESOPHAGECTOMY WITH GASTRIC TUBULISATION. P. Nafteux, T. Lerut, J. Moons, W. Coosemans, H. Decaluwe, G. Decker, D. Van Raemdonck, H. Van Veer, P. De Leyn. University Hospitals Leuven, Leuven, Belgium.

Introduction : Semimechanical side-to-side stapled anastomosis is thought to reduce frequency of leaks and strictures when using whole stomach. Scarce data are available when using gastric tubulisation.
Methods : From a prospective database two matched groups, operated between 2005 and 2008, were retrieved of cancer patients receiving a cervical esophagogastrostomy on a gastric tubulisation : 92 semimechanical anastomosis (SMA), 41 handsewn anastomosis (HSA). EORTC QLC-30 and OES-18 questionnaires were used to score anastomosis related symptoms. A difference of 10 points or 10% was, as described in literature, considered clinically significant.
Results : Overall incidence of anastomotic leaks was 4.5%. There was no fistula related mortality. Leakage rate in SMA was 2.17% versus 4.88% in HSA ($p = 0.587$). All fistula were treated conservatively. Dilatation occurred in 30% of SMA and 61% of HSA ($p < 0.001$), 15% and 49% respectively needing = 3 dilatations ($p < 0.001$). Both groups demonstrate an initial increase of dysphagia score until 3 months postoperatively. The initial increase is steeper for patients with HSA (mean score 31 versus 26).
Also the dysphagia subscales revealed at 3 months higher mean scores for solids (HSA 38 and SMA 31) than for semi-solids (HSA30 and SMA 20) and for liquids (HSA 25 and SMA 26). When dichotomizing these results in symptomatic/asymptomatic, at 3 months a significant higher percentage of HSA patients (33%) reported being symptomatic for difficulties swallowing solids compared to SMA patients (22%).
Patients with HSA also had a significant higher score for swallowing saliva (30 versus 20). No significant differences in mean scores were seen in choking nor reflux symptoms. After 3 months postoperative no more significant differences

were seen between both groups except for reflux at 1 year being 27% in HSA versus 16% for SMA. Patients in both groups gave a similar global HRQL score at all timepoints.

Conclusion : Semimechanical anastomosis results in a better dysphagia score for both solids and semisolids and reduces significantly the need for dilatations, in particular repeat dilatations. The negative effect of dysphagia in the HSA group fade out over time, probably due to the treatment, i.c. the dilatations.

Semimechanical anastomosis can be safely used after gastric tubulisation allowing thus resection of the lesser curvature, an important oncologic principle for distal half tumours.

RADIOLOGY, PATHOLOGY AND NUCLEAR MEDICINE

Invited Lecture

- P01 -

TUMOR BUDDING IN COLORECTAL CANCER : HISTOLOGY AND IMPACT ON STAGING. G. Puppa. Geneva, Switzerland.

- P02-

IMPAIRED ANTITUMOR IMMUNITY IN BARRETT'S ESOPHAGUS CARCINOGENESIS. J. Somja (1), S. Demoulin (2), P. Roncarati (2), E. Dortu (2), N. Blétard (2), P. Delvenne (1), P. Hubert (2). (1) Centre Hospitalier Universitaire de Liège, Liège, Belgium ; (2) University of Liege, Liège, Belgium.

Introduction : Barret's esophagus (BE) corresponds to the replacement of the normal esophageal squamous epithelium by a metaplastic columnar epithelium through a process called intestinal metaplasia. This epithelial tissue remodeling is associated with a chronic gastroesophageal reflux and constitutes a premalignant lesion leading to a 30-60 fold increase in the risk to develop an esophageal adenocarcinoma (EAC). Dendritic cells (DCs) represent major players in the induction of an antitumor immune response. However, tumors are capable of disabling DCs in order to avoid immune surveillance. We hypothesized that the progression of cancer in BE is associated with modifications of the density and functions of immunocompetent DCs due to a change of the local microenvironment within the metaplastic epithelium.
Aim : To investigate a possible immune evasion favoring EAC development and progression by analysing the distribution and function of myeloid (mDCs) and plasmacytoid (pDCs) dendritic cells in the metaplasia-dysplasia-cancer (MDC) sequence. A special attention will be paid to the interactions between DCs and regulatory T cells (Treg cells) because of their importance in the regulation of immune reactions.
Methods : Specimens were obtained from patients with BE, low grade BE (LGB), high grade BE (HGB) and EAC. Immunohistochemical stainings using CD1a (dendritic cells), FoxP3 (Tregulatory cells), BDCA2 (plasmacytoid dendritic cells),MIP3a and chemerin antibodies were performed. mDCs and pDCs were co-cultivated with several tumor cell lines including BE, HGB and EAC cell lines using 0.4 mm pore size inserts. After incubation with LPS, DC were analysed for maturation (CD80, CD83, CD86, HLA-DR, HLA-ABC) and migratory (CCR7) markers expression. Supernatants collected from coculture experiments using mDCs were assessed for IL-10, IL-12p70 concentrations.
Results : mDCs and pDCs density, examined by immunohistochemistry, was increased in vivo during the MDC sequence. Evolution of HGB into EAC was correlated with a higher amount of CD1a+ mDCs. The expression of chemokines such as MIP3 alpha and chemerin which control the migration of respectively immature mDCs and pDCs, was also shown to be increased during the MDC sequence suggesting that the distribution of mDCs and pDCs is directed by local epithelial chemokine expression. We next demonstrated that mDCs cultivated in the presence of BE and EAC cell lines display a tolerogenic phenotype characterized by a low expression of CD80, CD83, CD86, HLA-DR and HLA-ABC and an IL10High and IL-12 low cytokine secretion profile compared to control.
Conclusion : In conclusion, soluble factors secreted by epithelial cells into the esophageal microenvironment may influence DCs distribution and modify their functionality in order to render them tolerogenic and to promote tumor progression. In agreement with those results, we observed that both metaplastic areas and (pre)malignant lesions of the esophagus are infiltrated by Treg cells and a colocalization was observed between mDCs and Treg cells during esophageal carcinogenesis.

- P03 -

EPITHELIAL EXPRESSION OF FHL2 IS ASSOCIATED WITH METASTASIS-FREE SURVIVAL IN COLORECTAL CANCER. L. Verset (1), J. Tommelein (2), X. Moles Lopez (1), C. Decaestecker (1), M. Mareel (2), M. Bracke (2), I. Salmon (1), O. De Wever (2), P. Demetter (1). (1) Erasme Hospital, Brussels, Belgium ; (2) Ghent University Hospital, Gent, Belgium.

Introduction : Four and a half LIM domains protein 2 (FHL2) is a component of the focal adhesion structures and has been suggested to play a role in cancer progression. It has been shown to be overexpressed in colorectal cancer (CRC).
Aim : To examine a possible prognostic value of FHL2 in CRC.
Methods : Immunohistochemistry for FHL2 was performed on 296 CRCs without distant metastases at time of surgery. Staining in the epithelial compartment was quantitatively evaluated using image analysis, and results were related to clinical variables. Antibody specificity was tested using siRNA transfection in hTERT-immortalised myofibroblasts.

Results : Varying degrees of cytoplasmic FHL2 expression by neoplastic epithelial cells were detectable in all cases. Higher FHL2 expression in the epithelial compartment was an independent adverse prognostic factor. Expression in the tumour invasion front ($P < 0.001$) as well as in the centre of the tumour ($P < 0.001$) was associated with metachronous metastases. No relation between FHL2 expression and microsatellite instability could be demonstrated.
Conclusion : Higher FHL2 expression is involved in CRC progression and correlates with the development of metachronous metastases, suggesting that FHL2 is an independent adverse prognostic indicator for CRC.

- P04 -

KRAS MUTATION REFLECTS THE DIFFERENT CHOLANGIOCYTES PHENO- AND GENOTYPE IN CHOLANGIOCARCINOMA. M. Komuta (1), C. Verslype (1), S. Vander Borght (2), O. Govaere (1), V. Vandecaveye (1), W. Laleman (2), W. Van Steenbergen (1), R. Aerts (1), J. Pirenne (1), B. Topal (1), F. Nevens (1), T. Roskams (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) UZ Leuven, Leuven, Belgium.

Introduction : Intrahepatic cholangiocarcinoma (ICC) originates from cholangiocytes that show topographical heterogeneity within the different levels of the biliary tree. Recently, we reported a new classification of ICCs based on their possible cell of origin (ref) ; Pure mucin-producing ICC (muc-ICC) similar to mucin-producing cholangiocytes lining large bile ducts, and ICC with histological diversity (mixed-ICC) presumably originating from the smallest bile ducts containing hepatic progenitor cells (HPCs), which can differentiate into either hepatocytes or cholangiocytes. Large bile ducts and the pancreas have a common embryologic origin and with regards to the later malignancy is often associated with KRAS mutation.
Aim : Therefore we investigated KRAS and BRAF mutation status in 63 resected ICCs (29 muc-ICCs, 34 mixed-ICCs) and compared their mutation status with respect to the different phenotypes of ICCs. Histological diagnosis and tumor classification were made according to the WHO classification and our latest publication (ref).
Methods : After DNA extraction from paraffin embedded and formalin-fixed tissues, KRAS mutations were determined by mutation specific PCR (TheraScreen KRAS Mutation Kit), according to the manufacturer's instruction. BRAF mutations (codon 600) were determined by a mutation-enriched PCR on a light cycler 480II instrument followed by sequencing. Microsatellite instability (MSI) was immunohistochemically assessed by using MSI markers (MSH2, MSH6, MLH1, and PMS2).
Results : Amongst the 63 ICCs, KRAS mutations were found in 12 (19%) ICCs [p.G12D (n = 7), p.G12V (n = 4), p.G12R (n = 1)]. In contrast, BRAF mutation was not detected in any ICCs. Importantly, KRAS mutations were only observed in the muc-ICCs, representing 41.4% of that group. No immunohistochemical difference of MSI markers was seen between KRAS mutated muc-ICCs and wild-type muc-ICCs. Clinicopathologically, within this group the 12 KRAS mutated muc-ICCs showed a predominantly perihilar location, smaller tumor size, better tumor differentiation, without underling liver disease compared with 17 wild-type muc-ICCs. KRAS mutated ICCs tended to show a better overall survival and recurrence free survival than wild-type ICCs.
Conclusion : KRAS mutations were only observed in muc-ICCs that have a similar pheno/genotype as mucin-producing cholangiocytes, but not in mixed-ICCs that have a HPC-pheno/genetype. This confirms the validity of the classification and gives new therapeutic possibilities in ICCs.
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- P05 -

ANAL SMEARS AND THE DETECTION OF INTRAEPITHELIAL NEOPLASIA. A. Camboni, D. Hoton, Y. Guiot, A. Jouret-Mourin, M.A. Denis, C. Galant. Université Catholique de Louvain, Brussels, Belgium.

Introduction : Anal smears are increasingly being used as a screening test for anal intraepithelial lesions (AINs). The disease is increasing in both incidence and prevalence, especially among patients with the following risk factors : homosexual men, acquired or iatrogenic immunosuppression, and presence of other HPV-related diseases. However, standardized guidelines have not been yet established in order to manage these lesions.
Aim : This prospective study was undertaken to assess the usefulness and limitations of anal smears in detection of AIN in combination with human papillomavirus testing.
Methods : The cytological features of 48 anal smears collected in a liquid medium in the last twelve months from 44 patients were analyzed and the characterization of human papillomavirus (HPV) types of high oncogenic potential was performed. Cases showing a cytological diagnosis of atypical squamous cells which cannot rule out a high grade

lesion (ASC-H) or low-grade squamous intraepithelial lesions (L-SIL) were all investigated by biopsy except for two cases.
Results : One specimen was unsatisfactory for diagnosis. For the remaining 47 cases, the diagnosis was negative in 22 patients, atypical squamous cells of undetermined significance (ASC-US) in 13 patients, ASC-H in 1 and L-SIL in 11 patients. Parakeratosis without atypia was referred as negative whereas atypical parakeratosis diagnosed as ASC-US. L-SIL cases did not present typical koilocytosis, but squamous cells showing enlarged and hyperchromatic nuclei (AIN 1). High oncogenic HPV typing was positive in the case of ASC-H (1/1), in all cases of L-SIL (9/9), in 72% of ASC-US (8/11) and also in 40% of negative smears (9/22). All the biopsies performed (1 ASC-H and 8 L-SIL) showed an anal intraepithelial neoplasm (AIN 1 and AIN2).
Conclusion : For detection of anal dysplasia of any grade, anal smears in combination with HPV typing present a good sensitivity, but a low specificity. The low specificity can partially be explained by slightly differences in cytological criteria with vaginal cytology and mostly the difficulty to distinguish atypical and normal parakaratosis. Patients with any cytological abnormality but with an HPV positive testing require further follow-up and investigations.

- P06 -

NEOPLASTIC THROMBOSIS OF THE INFERIOR MESENTERIC VEIN : A ANATOMO-RADIOLOGICAL CONFRONTATION. I. Ferreira, C.A. Dragean, C. Remue, A. Kartheuser, E. Danse, A. Jouret-Mourin. Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : The venous thromboembolic disease is a common complication of cancer. It is estimated that this risk is between 15 and 25%. However, tumoral thrombosis of the inferior mesenteric vein in the context of neoplasia has been rarely described.
The pathophysiologic mechanism is well represented by Virchow's triad which includes venous stasis, hypercoagulability and endothelial injury.
Results : A 70 year old woman comes to the consultation for bloody stools. Colonoscopy revealed the presence of a stenosis of the sigmoid in the shape of an apple core.
The investigation included a CT abdominal scan. During this test, the radiologist discovered a colon tumor infiltrating the left adnexal parameter accompanied by a tubular elongated structure of 4.5 cm long. This image was highly suggestive of a neoplastic thrombosis of the inferior mesenteric vein.
The patient underwent surgery. The lesion was characterized as a mucoid adenocarcinoma of high grade (TNM 5th edition : pT4N2 [14/29] M1).
The elongated structure was clearly identified macroscopically and microscopically as a neoplastic thrombosis, confirming the radiological image.
Conclusion : This case illustrates an uncommon form of mesenteric vein thrombosis caused by tumoral tissue.

- P07 -

PERCUTANEOUS CHOLECYSTOSTOMY : SINGLE CENTRE EXPERIENCE IN 111 PATIENTS WITH AN ACUTE CHOLECYSTITIS. R. Peters, M. Simoens, S. Braak, S. Rakic. Zgt Almelo, Almelo, Netherlands.

Introduction : Percutaneous cholecystostomy is an alternative nonsurgical therapeutic option for critically ill patients with acute cholecystitis.
Aim : To evaluate the safety and long-term outcome of percutaneous cholecystostomy (PC) under radiologic guidance for acute calculeous (ACC) and acalculeous cholecystitis (AAC) in all patients undergoing that procedure at our institution.
Methods : We performed a retrospective analysis of 111 patients who underwent PC from 2005 to 2011. Patients were divided into two groups : AAC and ACC. For all patients, comorbidity and American Society of Anesthesiologists (ASA) classification were determined. The indications, outcomes of the procedure (PC) complications, recurrence rate, and long-term outcome for both groups were analysed. The mean follow-up was 55 months.
Results : Twenty-four patients with AAC and 87 patients with ACC underwent PC. The most common sonographic appearance for acute calculeous and acalculeous cholecystitis was gallbladder wall thickening (90,9%) and hydrops (72,9%). Twelve of 24 patients with AAC (50%) were hospitalized at the Intensive Care. The procedure failed in 2 (1,8%) patients. There were 4 (3,6%) abscesses and 2 (1,8%) fistulas post PC. Drain dislodgment was found without sequelae in 8 (7,2%) patients.
Elective cholecystectomy was performed in 35/111 (31,5%). Fifty-one of 87 (58,6%) patients with gallstones underwent cholecystectomy ; 36/87 (41,3%) did not undergo surgery either due to a too short follow-up or they died of non-biliary

disease. In the AAC group, there was no recurrent cholecystitis in 17/24 (70,8%) patients ; 3/24 (12,5%) needed surgery later on and 4/24 (16,6%) patients died at the Intensive Care.
Conclusion : PC is a minimally-invasive treatment with a low complication rate for patients with acute cholecystits who are considered to be at a high-risk for cholecystectomy. In patients with ACC, PC seems to be a valuable bridge to surgery, which will be required in the majority of these patients later on. In most of AAC-patients no further treatment seems to be needed.

- P08 -

PREEMPTIVE TIPSS FOR PORTAL HYPERTENSION FROM CYSTIC FIBROSIS LIVER DISEASE : 5 YEARS EXPERIENCE. E. Delanghe, L. Defreyne, E. Robberecht. Ghent University Hospital, Gent, Belgium.

Introduction : Liver disease is the second cause of death in cystic fibrosis (CF). Liver insufficiency mostly occurs late and is preceded by liver fibrosis (LF). Liver fibrosis causes portal hypertension (PH) with splenomegaly and complications such as ascites and variceal haemorrhage.
Aim : To prevent the complications of portal hypertension, a TIPSS procedure (transjugular intrahepatic portosystemic shunt) was performed.
Materials and methods : Between 2007 and 2012 five pediatric patients with CF-related liver disease underwent TIPSS placement and were followed for a median of 50 months. Patients had a yearly control in the cystic fibrosis centre, where blood analysis, abdominal ultrasound, fibroscan, lung test and nutrition analysis were performed. Splenomegaly was evaluated with palpation of the abdomen, measurements on ultrasound and evaluation of labs (blood platelets and blood cells).
This study was approved by the Ethics Committee of the Ghent University Hospital.

Results :

Patients	1	2**	3	4	5
Age detection LF (y)	4	4.9	6.5	4.5	7.5
Age TIPSS (y)	8.5	9	9	6.4	8.8
Spleen size*	3+/2+	3+/3+**	0/+	0/0	0/+
Platelets* (10E3/μL)	55/72	62/50**	264/177	242/157	186/137
WBC* (10E3/μL)	2.7/3.6	3.7/4.19**	8.5/6.2	9.6/7.7	7.7/7.9
RBC* (10E6/μL)	4.3/4.0	4.0/4.2**	4.7/4.9	4.6/5.4	4.6/4.3

* : Before placement TIPSS/ at present.
** : Patient 2 underwent liver transplantation (LTX). The results were collected until LTX.

The table summarizes the evolution in spleen size and function before TIPSS placement and at present. One patient underwent liver transplantation 2 years after TIPSS placement for unrelated reasons. After TIPSS placement 6 revisions were performed and revealed 1 early occlusion, 1 late occlusion and 4 late stenoses, which were resolved by dilatation and/or stenting. During observation time, all patients were in good condition and had no gastrointestinal complaints or variceal haemorrhage. No encephalopathy occurred.
Conclusions : Preemptive TIPSS seems to postpone development or to slow down progression of PH, but cannot reverse symptoms of PH in CF-related liver disease.

- P09 -

HEPATIC EPITHELIOID HEMANGIOENDOTHELIOMA : REVIEW OF 10 CASES WITH RADIOLOGIC-PATHOLOGIC CORRELATION. K. Boeren (1), D. Strybol (2), M. Lefere (1), D. Bielen (1), W. Van Steenbergen (3), T. Roskams (2), G. De Hertogh (2), D. Vanbeckevoort (1). (1) Radiology, UZ Leuven, Leuven, Belgium ; (2) Pathology, UZ Leuven, Leuven, Belgium ; (3) Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

Introduction : Hepatic epithelioid hemangioendothelioma (HEHE) of the liver is a rare vascular tumor, arising from vascular elements of mesenchymal tissue with intermediate malignant potential. On imaging studies, the lesions have a solid and frequently a multifocal appearance that may mimic metastatic disease or intrahepatic cholangiocarcinoma with satellite lesions. We attempt to give a brief overview of some typical imaging characteristics, in correlation with their histological presentation.
Aim : Retrospective analysis of the imaging characteristics in 10 patients (7 women and 3 men) with pathologically confirmed HEHE.
Methods : CT and/or MRI images of 10 patients (presenting between 2006 and 2012) with pathologically confirmed HEHE were retrospectively reviewed. Description of typical and atypical imaging characteristics, correlated with the histology, with emphasis on the differential diagnosis.
Results : The clinical manifestations are often nonspecific and variable. Presenting signs and symptoms were right hypochondrial pain, epigastric discomfort, hepatomegaly and elevated liver function tests. In 9 patients, various imaging modalities, particularly CT and/or MRI showed multiple liver lesions, mostly in the periphery and extended to the liver margin. Retraction of the liver capsule overlying tumor nodules was detected in 8 patients. Confluence of tumor nodules was seen in 7 patients. In at least half of the patients a target-like appearance of tumor nodules was observed with intravenous contrast, resembling the histological pattern of a fibrous hypocellular central stroma with hypercellular margins composed of dentritic and epithelioid cells. Only 1 patient presented with an atypical large right hepatic mass with foci of intraluminal bleeding. Calcifications were rarely seen, only in 2 patients.
Conclusion : Definitive diagnosis of HEHE requires histopathological confirmation, but radiologists should be aware of the imaging findings associated with these tumors in order to facilitate an accurate diagnostic work-up. Our findings support earlier reports that the most typical imaging features of HEHE are multifocal lesions, peripheral localization, capsular retraction and the target sign.

- P10 -

THE CT IMAGING FEATURES OF PURE AND SEGMENTAL GROOVE PANCREATITIS. S. Traen, S. Dekeyzer, P. Smeets. Ghent University Hospital, Gent, Belgium.

Groove pancreatitis is a rare form of chronic pancreatitis affecting the pancreaticoduodenal groove, a potential space bordered by the pancreatic head, duodenum and common bile duct. Two forms of groove pancreatitis have been desribed : a segmental form, which involves the groove and the pancreatic head ; and a pure form, which affects the groove only. Differentiation between groove pancreatitis and pancreatic head carcinoma can be difficult, both clinically and radiologically. The aim of this poster is to illustrate the CT imaging features of pure and segmental groove pancreatitis.

- P11 -

UNUSUAL HEPATOCELLULAR TUMOUR PRESENTATION AT IMAGING. L. Annet, M. Mertens, C. Dragean, P. Trefois, E. Danse, O. Ciccarelli, C. Sempoux. Université Catholique De Louvain, Brussels, Belgium.

Introduction : A 21-year-old woman with abdominal pain underwent magnetic resonance imaging (MRI).
Aim : It showed a large hypervascular tumour involving the entire right liver lobe with a radiological central scar, slightly hypointense on T1 before injection and on 3 minutes after injection, but isointense on T2, on diffusion and on the late phase after gadobenate dimeglumine injection. These features suggested the diagnosis of an atypical FNH or a well-differentiated liver carcinoma. As biopsy remained doubtful for liver carcinoma, this patient underwent a liver transplantation.
Methods : On the explanted liver, a large tumour characterised by small well-differentiated liver cells with B-catenine activation and diffuse glutamine synthase staining was observed. No vascular involvement and no cellular atypia were found.
Results : The pathologist concluded to a borderline lesion : atypical hepatocellular adenoma-like neoplasm / very-well differentiated hepatocellular carcinoma.

Conclusion : MRI has made important progress in characterising liver tumours using liver specific contrast agent, diffusion and apparent diffusion coefficient. Classification of some liver adenomas as steatosic or inflammatory is possible. However, some tumours remain difficult to differentiate.
Atypical hepatocellular adenoma-like neoplasm represents a challenging sub-classification of liver adenoma with morphologic features close to the liver carcinoma. These entities are currently under investigation.

- P12 -

COMPUTED TOMOGRAPHY DERIVED LIVER VOLUME : A PROGNOSTIC FACTOR IN DECOMPENSATED ALCOHOLIC CIRRHOSIS ? N. Lanthier (1), S. Terraz (2), R. Breguet (2), Y. Chalandon (3), L. Rubbia-Brandt (4), L. Spahr (1). (1) Gastroenterology And Hepatology, University Hospitals Of Geneva, Geneva, Switzerland ; (2) Radiology, University Hospitals Of Geneva, Geneva, Switzerland ; (3) Hematology, University Hospitals of Geneva, Geneva, Switzerland ; (4) Pathology, University Hospitals of Geneva, Geneva, Switzerland.

Introduction : Decompensated alcoholic liver disease (ALD) causes high morbidity and mortality. Factors associated with a poor clinical evolution, as well as the impact of liver atrophy linked with cirrhosis are poorly determined. The goal of this study is to explore the link between the liver volumetry calculated by computed tomography (CT) and the evolution of liver insufficiency in patients admitted for decompensated ALD.
Materials and methods : Non-abstinent patients with acute decompensation of ALD (increase in serum bilirubin and deterioration of liver function) from a prospective studywere included. This trial did not evidence any benefice of cellular therapy during the 3 months of follow up (Spahr L *et al*. *Autologous bone marrow stem cell transplantation versus standard of care in patients with decompensated alcoholic liver disease : interim analysis of a RCT*. Hepatology, 2011, 54 : A62). Liver volumes evaluated by CT performed rapidly after patient admission were compared to further evolution of hepatic insufficiency, irrespective of treatment allocation. We defined patients with a ≥ 3 points decrease in MELD compared to baseline value as “improvers”, and those with less than 3 points decrease in MELD as “non-improvers”.
Results : Fifty-eight patients were included. At admission, mean MELD score was 20.6 points. All patients had cirrhosis. Biopsy proven alcoholic steatohepatitis was diagnosed in 81% of cases. Patients with severe steatohepatitis (Maddrey score ≥32) were treated by steroids (66%). 34 patients (59%) with a good evolution at 3 months (decrease in MELD score of 3 points or more) were classified as improvers meanwhile 24 patients (41%) were classified as non-improvers. Patients’characteristics at admission depending on the 3 month evolution are reported in this table.
Conclusion : This study highlights the link between the severity of hepatic atrophy associated with cirrhosis and the prognosis in case of liver decompensation. Indeed, in patients with decompensated ALD, a low liver volume evaluated by CT is associated with a bad evolution of hepatic function evaluated by MELD score.

- P13 -

HETEROTOPIC OSSIFICATION OF A RECTAL TUMOR : RADIOLOGICAL AND PATHOLOGIC CORRELATION. S. Smasjda (1), E. Danse (2), L. Annet (1), M. Mertens (1), A. Kartheuser (3), C. Remue (4), D. Leonard (4), P. Scalliet (4), A. Jouret-Mourin (5). (1) Cliniques Universitaires St-Luc, City Of Brussels, Belgium ; (2) Université Catholique De Louvain, Brussels, Belgium ; (3) Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (4) Cliniques Universitaires St Luc, City Of Brussels, Belgium ; (5) Ucl Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : We report a case of a 29 year old woman who presented with a colorectal cancer detected by colonoscopy and staged with the classical guidelines (endoscopic US, biopsy, CT and MRI). The staging was T4N+M0. The carcino-embryonic antigen (CEA) was 7,8ng/ml (normal <) and CA19-9 935 U/ml (normal < 37).
The patient rapidly developped an occlusion and a derivation-surgery was performed. Neoadjuvant chemotherapy and radiotherapy was administrated to the patient. Three cures of FOLFOX were started associated with radiotherapy (45 gray) and chemotherapy (Xeloda). Resection surgery of the rectal tumor was then planned as the patient was in a good general condition (OMS O 52 kg for 164 cm). A CT and MRI were performed before the surgery.

These demonstrated the global stability of the mass but important increase in its mineralised component. Peritoneal nodules were also noted. A complete resection of the tumor was carried out accompanied by intra-peritoneal hyperthermy chemotherapy (CHIP). The histology of the surgical resection confirmed a muco d adenocarcinoma with ossified matrix. Osteocytes were identified in ossified rows. The tumor was classified as ypT3N2M1 according to the TNM classification (5th edition). Immuno-histochemistry of the tumor cells revealed a mutation of the codon 12 of the K-ras gene and no sign of micro-satellite instability.

Conclusion : This case illustrates heterotopic ossification of a rectal tumor which is quite uncommon. The mechanism of heterotopic bone formation within colorectal adenocarcinoma is not yet totally understood and is therefore to be debated. A potential action of radiotherapy on metaplasia should be considered.

Invited Lecture

- P14 -

THE EFFECT OF CIGARETTE SMOKE ON THE SMALL INTESTINE. Stephanie Verschuere. Pathology department, Ghent University Hospital.

Smoking is a worldwide epidemic, causing considerable morbidity and mortality. Next to the airways, it affects many organs and functions including systemic and mucosal immunity. Also inflammatory bowel disease (IBD) is affected by smoking. Epidemiological evidence shows that cigarette smoke has a dual effect on IBD, worsening the outcome of Crohn's disease (CD) but positively influencing the course of ulcerative colitis (UC). However, the underlying molecular and cellular mechanisms through which smoking interferes with IBD pathogenesis are still unidentified. Moreover, little is known about the interaction of cigarette smoke with the healthy gut and the intestinal mucosal immune system, the GALT (gut-associated lymphoid tissue).
GALT consists of several organs, of which Peyer's patches (PPs) are the best studied. PPs are located in the small intestine, and are concentrated in the terminal ileum in humans. Lymphoid aggregations in the terminal ileum, including PPs, are the onset site of ileal CD. Smoking is known to primarily affect ileal CD, rather than Crohn's colitis. However, the interaction of cigarette smoke with the terminal ileum or PPs is poorly understood up till now. The aim of this presentation is to discuss current knowledge on the effect of cigarette smoke on the small intestine.
Firstly, the published experience from animal smoke models on the interaction smoking – gut is summarized. Several methods for simulation of human smoking are discussed. Furthermore, the divergent effects of cigarette smoke on the small intestine and colon are described. Smoking is shown to negatively affect the small intestine and enteritis, but has an ambiguous effect on the colon with both immunosuppressive and immunogenic effects.
Secondly, our findings on the effect of chronic cigarette smoke exposure on the GALT in a murine smoke model are discussed. A first finding is that smoking increases apoptosis in the follicle-associated epithelium of PPs. Furthermore, the immune cell composition of PPs demonstrated shifts in immune cell populations. Especially dendritic cells and several T-cell subsets accumulate in PPs following smoke exposure. This is accompanied by a higher expression of chemokines in PPs. We hypothesize that smoking first causes alterations in the epithelial barrier, which results in increased antigen entry in PPs and subsequent immune activation with recruitment of various immune cell populations.
A last part decribes the effect of chronic cigarette smoke exposure on autophagy in the follicle-associated epithelium and PPs, again in a murine smoke model. A significant increase in the number of autophagic vesicles per epithelial cell is observed. Also M-cells are involved in smoke-induced autophagy. In the underlying PP increased mRNA levels of several autophagy-related genes are observed, suggesting that also PP immune cells undergo increased autophagy following smoke exposure. Smoke-induced autophagy might represent a defence mechanism to repair smoke-related cellular damage.
In conclusion, chronic cigarette smoke causes considerable alterations in ileal mucosal immunology. The follicle-associated epithelium experiences smoke-induced apoptosis and autophagy. As these mechanisms are both involved in the pathogenesis of CD, this can implicate a first mechanism through which smoking exerts its detrimental effect on CD. Furthermore, the immune cell alteration in PPs may point to a second mechanism by which smoking predisposes the ileum towards inflammation and CD.

- P15 -

COLLAGENOUS GASTRITIS IN PEDIATRIC PATIENTS : REPORT OF ONE CASE. A. Camboni (1), S. Fonseca (1), C. Sempoux (1), L. Collin (2), H. Piessevaux (1), A. Jouret-Mourin (1). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) CH Wapi Notre Dame, Tournai, Belgium.

Collagenous gastritis is a rare entity with less than 60 cases reported so far since the first description in 1989 by Coletti and Trainer. Half of reported cases concerned children. It is characterized by the deposition of a thickened subepithelial collagen band in the gastric mucosa associated with an inflammatory infiltrate. Two distinctive clinicopathological subtypes of collagenous gastritis have been described : 1) a paediatric form presenting with severe anemia, a nodular pattern on endoscopy, and a disease limited to gastric mucosa without evidence of colonic involvement, and 2) an adult form associated with collagenous colitis presenting with chronic diarrhea. Specific therapy has not been established and the disease can relapse or persist for years.

We described a case of a paediatric collagenous gastritis in a 15-year-old-boy presenting with iron-deficiency anemia. The patient was no affected by auto-immune diseases. The gastric endoscopy revealed an hyperplasic and erythematic mucosa with a diffuse nodular appearance. Pathologic examination of the gastric biopsies from antrum, body and fundus showed a subepithelial collagen deposition associate with a moderate infiltrate of lymphoplasma cells, neutrophils and eosinophils. No intraepithelial lymphocytes were observed. The collagen deposition appeared as a discontinuous thick bands confirmed by Masson's trichrome stain. A focal atrophy was described in biopsies from fundus. Helicobacter pylori infection was not detected by immunohistochemistry. No significant subepithelial collagen deposition was identified in duodenal biopsies.
Although some cases of collagenous gastritis are associated with other well characterised diseases, such as microscopic colitis (collagenous or lymphocytic), celiac sprue and collagenous sprue, the etiology, the pathogenesis and the prognosis of collagenous gastritis remain unknown.
This disorder, although rare, should be considered in children and young patients with anemia and or epigatric pain. The identification and the follow-up of cases may help to better elucidate this rare and enigmatic disease.

- P16 -

EFFECT OF H PYLORI ON GASTRIC MUCOSAL CHANGES AMONGST PATIENTS ON LONG TERM ACID SUPPRESSANTS. A. Krishnan, J. Venkataraman. Stanley Medical College, Chennai, India.

Introduction : Long term acid suppressants are known to have adverse effects. s. Gastric acid secretion influences the density of H.pylori colonisation, its distribution within the stomach and the severity of the mucosal inflammatory response to the infection. Its effect on gastric mucosa is still under debate.
Aim : To study the effect of H pylori on gastric mucosa amongst patients on long term acid suppressants
Methods : 126 patients with symptoms of ulcer type dyspepsia and reflux type dyspepsia for more than a year and on acid suppressants for at least a year were included in the study. Biopsy was obtained from the stomach for demonstration of H pylori and the histological changes. The duration of treatment and presence of H pylori was correlated with the histological changes.
Results : 66 patients were on omeprazole at a dose of 20 mg a day (Gp I) and the rest were on ranitidine 150 mg twice a day (Gp II). Demography and the duration of treatment was comparable in both groups. Gastric mucosa was normal in 18 (27.3%) and 30 (60%) patients in Gp I and Gp II respectively, which was statistically significant ($p < 0.01$). Intestinal metaplasia was significantly more common amongst those on PPI ($p < 0.05$). None had dysplasia or carcinoma. The colonization of H pylori correlated with the duration of therapy in each of the two groups but was not statistically significant ($p > 0.05$).
Conclusion : Long term acid suppressants were associated with *H. pylori* related gastric mucosal changes mainly in fundus and body. Histological worsening correlated with increasing duration of PPI when compared to H2RA. This had provided a new insight towards the management of *H. pylori* in such cases. i.e, Antral predominant gastritis would benefit from PPI containing anti H pylori regimen, which may be harmful in corpus predominant gastritis requiring H2RA containing regimen as an alternative.

- P17 -

STRESS RESPONSE OF DUODENO-PANCREATIC CELLS IN RATS WITH DYSBIOSIS AND NORMOBIOSIS UPON HYPOACIDITY. S. Vakal, U. Savko, K. Dvorshchenko, T. Borodina, L. Ostapchenko. Taras Shevchenko National University Of Kyiv, Kiev, Ukraine.

Introduction : Long-term suppression of gastric acid secretion (GAS) is associated with a range of negative consequences for the gastrointestinal tract (GIT). One of them is dysbiosis development that leads to colonization of GIT by opportunistic microbiota, thus favoring inflammatory processes both in GIT and associated organs, such as pancreas. Oxidative stress (OS) is a terminal non-specific stage of duodeno-pancreatic cells damage in these conditions. Cure of dysbiosis with probiotics can give us valuable information about role of dysbiotic pathway in OS development in duodeno-pancreatic cells upon long-term inhibition of GAS.
Aim : To establish the role of dysbiotic pathway in development of OS in pancreatic and duodenal cells of rats upon long-term suppression of GAS.
Methods : Experiments were performed on white non-strain male rats. Animals were divided into 4 groups. First group (control) obtained water (0,2 ml abdominally and 0,5 ml *per os*), second - omeprazole (abdominally 14 mg/kg) during 28 days, third group along with omeprazole obtained multiprobiotic "Symbiter" *per os* (0,14 ml/kg), and fourth group obtained only "Symbiter" at the same dose.

Duodenal cells were isolated by low-temperature method with following separation of crypt and villus cells. Pancreatic homogenates were prepared with potassium buffered saline by standard assay. The level of hydrogen peroxide (H2O2), TBA-reactive substances (TBA-RS), reduced glutathione (GSH) and total glutathione peroxidase (GPx) activity were determined spectrophotometrically. Cu,Zn-superoxide dismutase (SOD) and Mn-SOD activities, as well as content of metallothioneins (MT) were measured with standard spectrophotometric assays in appropriate cellular fractions. Oxidative stress indices (OSI) were calculated from the ratio between above mentioned pro- and antioxidant factors.
Results : It was observed that content of TBA-RS significantly increased, while GSH level significantly reduced in all investigated tissues of animals of the second group in comparison with control. The concentration of MT, as well as Cu,Zn-SOD and GPx activity were diminished in pancreatic and duodenal crypt cells at these conditions. At the same time, the H2O2 level was essentially higher in these cells. The increased activity of Mn-SOD in pancreatic and villus cells of animals treated with omeprazole was established, while in crypt cells its activity was lower than control values. Calculated OSI were dramatically changed in animals of the second group, thus suggesting OS development. All above mentioned indices was closer to control values in animals with normobiosis and hypoacidity (third group) in comparison with animals of the second group. There were no statistically significant differences between parameters of the fourth group and control ones.
Conclusion : Dysbiotic pathway plays a significant role in the development of OS in pancreatic and duodenal epithelial cells, since its treatment with multiprobiotic is associated with amelioration of OSI. At the same time, other pathways are also important because indices were recovered to control values only partially upon dysbiosis cure.

- P18 -

A CASE SERIES OF SUSPECTED AUTO-IMMUNE PANCREATITIS : HISTOLOGY AND IMMUNOHISTOCHEMISTRY FOR IGG4. I. Renckens (1), S. Verbeek (1), T. Roskams (1), W. Van Steenbergen (2), G. De Hertogh (1). (1) Pathology, UZ Leuven, Leuven, Belgium ; (2) Hepatology, UZLeuven, Leuven, Belgium.

Introduction : Auto-immune pancreatitis (AIP) may present as 2 entities. The lymfoplasmacytic sclerosing form (LPSP) is supposedly a T-helper2 mediated disease, is positive for IgG4 and may have multiple other organ manifestations such as ulcerative colitis (UC). The idiopathic duct-centric type (IDCP) is negative for IgG4 in serum and is only associated with UC. We selected a series of suspected AIP cases from a single academic center for histological and immunohistochemical review.
Aim : 1) to determine the proportions of LPSP and IDCP ; 2) to count IgG4 positive plasma cells and eosinophils in pancreatic and other inflamed tissues and to evaluate their usefulness in the distinction between LPSP and IDCP.
Methods : We selected 31 suspected AIP patients (21 M, mean age 47) for whom tissue samples (pancreas 18, colon 11, other 11) were available. All biopsies were reviewed and tissues were stained for IgG4. Eosinophils and IgG4 positive plasma cells were counted in 5 HPFs and mean and peak values were determined. We also investigated biopsies from 2 control groups : 38 UC cases and 31 colon carcinomas.
Results : Eight pancreas cases were excluded because the diagnosis of AIP was not withheld, or for lack of tissue for further staining. Of the remaining 10 cases, 6 were categorized as LPSP (4 M, mean 68 yrs) and 4 as IDCP (3 M, mean 29 yrs). Both the eosinophil and IgG4 counts were higher in LPSP (mean IgG4 count : 61 vs 32, $P = 0.09$). For the non-pancreatic tissues, meaningful results were obtained only in the colon biopsies. Suspected AIP cases with UC segregated in 2 groups, with either 10 or more IgG4 positive cells / HPF (3/4 M, possible LPSP, mean 44 yrs) or less than 10 (1/7 M, possible and in 1 case biopsy-proven IDCP, mean 27 yrs). Eosinophil counts were higher in the first group. All AIP-associated UC cases had higher eosinophil counts than the colon carcinoma controls, but only the possible LPSP group had significantly elevated peak IgG4 counts (55 vs 3 ; $P = 0.004$). Eosinophil counts were similarly elevated in AIP-associated LPSP and control UC cases, but IgG4 counts were at least twice higher in the AIP patients.
Conclusion : AIP can best be diagnosed on surgical specimens. In our group, LPSP was more frequent than IDCP, with LPSP patients being older at diagnosis. IgG4 and eosinophil counts in the pancreas are higher in LPSP. When a patient with suspected AIP has UC, a high IgG4 count in the colon biopsies suggests LPSP while a low eosinophil number is probably indicative for IDCP.

- P19 -

CHRONIC CIGARETTE SMOKE EXPOSURE ALTERS THE MURINE GUT MICROBIOME. L. Allais, F.M. Kerckhof, S. Verschuere, K. Bracke, R. De Smet, D. Laukens, M. Devos, N. Boon, G. Brusselle, T. Van De Wiele, C. Cuvelier. Ghent University, Ghent, Belgium.

Introduction : The microbiome plays a crucial role in maintaining intestinal homeostasis. Disruption of this homeostatic environment leads to destabilisation of the gut immune system and aberrant immune responses against harmless microbiota, which may be involved in the development of Crohn's disease (CD). The most prominent environmental risk factor for CD is smoking.
Aim : The present study aims to investigate the influence of cigarette smoke on the microbiome, in particular the mucosa-adherent microbiota, and how this is linked to changes in mucin production.
Methods : C57BL/6 mice were exposed to cigarette smoke (n = 6) or air (n = 6) according to a well-established protocol. After 24 weeks, the animals were sacrificed and multiple parts of the gut (ileum, proximal and distal colon) were collected. The microbial composition was analysed using denaturing gradient gel electrophoresis (DGGE) and 454 pyrosequencing. Furthermore, the expression of mucins was determined at both the mRNA level (real-time PCR) and the protein level (Alcian Blue (AB)/Periodic Acid Schiff (PAS), High Iron Diamine (HID)/AB staining).
Results : Analysis of the microbiome in smoke- and air-exposed mice revealed that microbial community structures changed significantly after smoke exposure in all parts of the gut. In addition, the abundance of specific species, in particular Bifidobacterium sp. and Clostridium sp., tended to decrease in response to cigarette smoke in both colonic and ileal samples. A general analysis of mucin expression, using AB/PAS and HID/AB, could not demonstrate an altered expression of acidic and neutral mucins, nor changes in sulphated and sialylated mucins after cigarette smoke exposure. However, in the ileum, mRNA expression of MUC2 and MUC3 significantly increased after cigarette smoke exposure ($p = 0,04$ and $p = 0,03$ respectively). In contrast, colonic expression of MUC2 and MUC3 was unaltered, but an increased expression of MUC4 was observed ($p = 0,04$).
Conclusion : Comparative microbial analysis in mice showed a general shift in the mucosa-adherent bacterial population, as well as specific changes in *Bifidobacterium sp*. and *Clostridium sp*., in response to cigarette smoke. In addition, smoking alters intestinal mucins, which play an important role in the gut barrier, but also in the colonization efficiency of specific gut microbiota. These findings may point to a role for altered interactions between the microbiome and intestinal mucosa contributing to the effect of smoking on intestinal homeostasis.

- P20 -

HOLOTRANSCOBALAMIN VERSUS TOTAL VIT B12 AS INDICATORS OF VIT B12 DEFICIENCY IN THYROGASTRIC SYNDROME. L. Vranken, E. Cavalier, H. Valdes-Socin. Centre Hospitalier Universitaire de Liège, Liège, Belgium.

Introduction : The autoimmune association of Hashimoto thyroiditis and atrophic body gastritis (ABG) is known as thyrogastric syndrome which occurs in at least 14% of autoimmune thyroid disease (ATD). ABG is characterized by the loss of HCl and intrinsic factor (IF) production and is often undiagnosed. It is important to make an early diagnosis because patients with ABG have greater risk for developing gastric cancer and is responsible for anemia. Holotranscobalamin (HoloTC) is the biologically active form of vitamin B12 (cobalamin) and represents only around 20% of total vitamin B12. HoloTC is believed to be an earlier detection marker then total vitamin B12 to diagnose cobalamin deficiency which is especially frequent when gastritis manifestations are present.
Aim : In this study, we want to compare two markers to determine the vitamin B12 status of our population : total vitamin B12 (Roche) and HoloTC (Abbott).
Methods : A total of 118 patients have been separated in 3 groups : "controls" (n = 35), "autoimmune thyroiditis" (n = 60) and "thyrogastric syndrome" (n = 23). Major criteria for thyrogastric syndrome were evidence of ATD as well as cobalamin deficiency and/or ABG in gastroscopy/biopsy and/or hypergastrinemia and/or parietal cell autoantibodies (PCA)/intrinsic factor antibodies (IFA) (neither taking gastric acid antisecretory drugs nor Metformin). We excluded patients with *H.Pylori* infection as well as patients with estimated glomerular filtration rate (eGFR) calculated using MDRD equation < 30ml/min/1.73m2 because HoloTC concentrations may be affected by renal function. Most patients underwent HoloTC, total vitamin B12, TSH, FT4, FT3, ATPO, ATG, TBII, PCA, IFA and gastrin determinations to determine their auto-immune status. All patients supplemented with vitamin B12 (per os or IM) were excluded of the study.
Results : We determined that the correlation between HoloTC and total vitamin B12, HoloTC was significantly but not strongly associated with total vitamin B12 ($r = 0,5414$; $p < 0,0001$). The vitamin B12 concentration (both total and HoloTC) was significantly lower in "thyrogastric syndrome" group ($p < 0,0001$) that confirms there is vitamin B12 malabsorption during gastritis. However, more patients were deficient using HoloTC (73,9%) than with total vitamin B12

(47,8%) in the “thyrogastric syndrome” group. A total of 6 patients (26%) affected by thyrogastric syndrome were < 40 years old.

Conclusion : HoloTC is as suitable as total vitamin B12 for diagnosis of cobalamin deficiency and tends to be lower than total vitamin B12 in thyrogastric patient. So, these patients would be more quickly supplemented if their vitamin B12 status is determined by HoloTC. Using holotranscobalamin as a diagnostic marker would allow early therapy before neurological irreversible damage occurs. The initial symptoms of cobalamin deficiency are insidious and could easily be overlooked, especially as the serum concentration of total vitamin B12 can lie within the reference range. Thyrogastric syndrome can be present even in young patients (< 40 years).

SEVEN SOCIETIES POSTGRADUATE COURSE

- S01 -

THE MANAGEMENT OF PRECANCEROUS CONDITIONS AND LESIONS OF THE STOMACH. M. Dinis Ribeiro. Porto, Portugal.

Invited lecture

- S02 -

THE FUTURE DEVELOPMENTS REGARDING DETECTION AND RESECTION OF COLONIC POLYPS. J. East. John Radcliffe Hospital, Oxford, U.K.

The current model of colonoscopy is not likely to be sustainable in the long term as case volumes increase and resources are squeezed. Detection will need to be optimised to deliver more cancer prevention value per examination, and therapy for comprehensive resection of advanced lesions and early cancers must become less technically demanding. It is likely that the role of medically trained endoscopists will move away from routine diagnostics to high value activities such as advanced imaging for difficult condition e.g. colitis or Lynch syndrome, and advanced therapeutic procedures. Routine diagnostics and population screening are likely to be delivered by nurse endoscopists and radiological colonic imaging (CT and MR colonography).
For detection changes in optics including super-wide fields of view and super high definition with image enhancement will become standard, not necessarily mounted on standard “push” colonoscopes. Coupled with this computer aided detection will support endoscopists with concurrent reading for both detection and characterisation. Molecular techniques with small molecules and antibodies may enhance high value surveillance or ensure comprehensive lesion resection.
In therapy the need for comprehensive resection will become more important with routine use of en bloc techniques for larger lesions, supported by new devices and instruments. Early cancers found in screening programmes will be resected with minimally invasive techniques including flexible staplers and laparo-endoscopic hybrid procedures combined with chemotherapy “lite” to minimise local lymph node risk in an aging and co-morbid population.
Critically to deliver practice change in colonoscopy in the next 20 years high quality collaborative international studies will be needed to deliver a truly robust evidence base if we are to move forwards as our oncology and cardiology colleagues have done in previous decades.

- S03 -

PANCREATIC IMAGING : PAST, PRESENT AND FUTURE. M. Bali. ULB Erasme.

Invited lecture

- S04 -

CHOLINERGIC MODULATION OF INTESTINAL INFLAMMATION. G. Boeckxstaens. University Hospitals Leuven, Leuven, Belgium.

It is well accepted that the immune system has a major impact on neural function, contributing to increased pain perception and motility abnormalities in a variety of functional and inflammatory disorders. The communication between the immune system and the gut innervation is however bidirectional, i.e. also the autonomic nervous system has a major impact on the immune system. In addition to its contribution in stress-induced immune activation/dysregulation in functional bowel disorders, the autonomic innervation of the gut is increasingly recognized as an important player in immune homeostasis dampening both the mucosal and muscular immune system. Through the vagus nerve, the brain is informed on gut inflammation and subsequently modulates the immune response in order to prevent excessive inflammation and subsequent tissue damage. Mainly through the enteric nervous system, the vagus nerve interacts with the immune system not only dampening ongoing inflammation, but is also contributing to maintenance of oral tolerance, a key mechanism of immune homeostasis in the gut. The potential mechanisms and receptors involved will be discussed and the evidence indicating the therapeutic effect of vagus nerve stimulation will be summarized. Finally, the current data supporting its relevance to human disease and its therapeutic potential will be discussed.

Insight in the mechanisms underlying these crucial properties may indeed lead to better understanding of immune-mediated diseases of the gut and to improved anti-inflammatory therapies

- S05 -

SECOND GENERATION OF BIOLOGICS IN IBD : INHIBITING MONONUCLEAR CELLS HOMING IN THE INTESTINE. E . Louis. ULG Liege.

Invited lecture

- S06-

INTERFERON FREE TREATMENT OF HEPATITIS. Graham R. Foster – Professor of Hepatology. Queen Marys University of London.

For the last 20 years interferon has been the backbone of therapy for patients with chronic hepatitis C infection. The development of new direct acting antiviral agents leads us to hope that this difficult to use drug may soon be replaced by all oral combination regimes. To-date a large number of different antiviral agents are in clinical trials and combinations that are effective are beginning to be developed. It is now clear that some combinations are brittle and have high failure rates (except in well-defined patient cohorts) whereas other combinations are more robust and patients are likely respond without the development of drug resistance. The optimal, most cost effective regime remains to be determined but drugs with a high barrier to resistance, high potency and user friendly regimes will be of increasing importance.

- S07 -

GUT MICROBIOTA : PERSPECTIVES IN DIAGNOSTICS AND THERAPEUTICS. N. Delzenne. UCL St-Luc.

Invited Lecture

- S08 -

INFLUENCE OF EARLY NUTRITION ON LATER HEALTH - STATE OF THE ART OF GROWTH CHARTS. L. Weaver, School of Medicine, Glasgow, Great Britain (UK).

Growth charts have become widely used, if not universal, tools for the assessment of the growth and health of children. In 2006, the WHO published a set of charts designed to represent standards to which all the world's children should aspire. They were produced in response to the apparent variability in the patterns of child growth documented world-wide, with the aim of creating a prescriptive standard based on best feeding advice. Our modern understanding and use of growth references arose out of the application of technology, mathematics and charting to the biology of growth in the 19th century. As means of summarizing normal development, modern growth standards have replaced Renaissance conceptions of human form based on idealized proportions in harmony with the cosmos, and the simple reference to key developmental milestones first noted by the ancients. The WHO growth standards are the culmination of a search for a human ideal based on 20th century biology. However, while they may be the 'best' standards based on contemporary feeding advice, they are 'provisional' because all developmental processes in biology, including body growth, are plastic and permit a flexibility of life course trajectories in response to epigenetic, nutritional and other environmental conditions.

- S09 -

HOW WILL WE BE ABLE TO AFFORD ALL THE EXPENSIVE TREATMENTS ? L. Annemans. UGhent & VUB.

- S10 -

CONVERGENCE BETWEEN GI -SURGEONS AND ENDOSCOPISTS : A CHALLENGE TO FACE. M. Morino. Torino, Italy.

BELGIAN PANCREATIC CLUB (BPC)

Invited Lecture

- T01 -

OPTIMAL DIAGNOSIS AND STAGING OF FOCAL PANCREATIC MASS. J.L. Van Laethem, ULB Erasme.

- T02 -

A SUSPICIOUS PANCREATIC MASS IS NOT ALWAYS PANCREATIC CANCER, EVEN WITH VERY HIGH LEVELS OF CA 19-9. E. Cesmeli (1), F. Hublé (2), A. De Backer (2), W. Pauwels (2), C. Vandenbroecke (2), M. Kint (2). (1) Ghent University Hospital, Gent, Belgium ; (2) AZ St Lucas, Ghent, Belgium.

Introduction : Serum CA 19-9 is the most extensively used biomarker for pancreatic cancer, but slight elevations are not uncommon in a broad range of benign and malignant conditions. A higher cut-off level (CA 19-9 > 100 U/mL) in absence of biliary obstruction seems to have a high sensitivity and specificity for pancreatic cancer.
Aim : We discuss three cases of pancreatic masses with high serum levels of CA 19-9(> 100 IU/mL) without the diagnosis of pancreatic cancer.
Results : The first patient was a 64 year old woman with type 2 diabetes who was admitted to the hospital with anorexia,weight loss and upper abdominal complaints.The bilirubin level was normal. Lipase was elevated : 1074 U/L, but with normal CRP. CA 19-9 was very high : 596 U/mL. CT-scan showed a cystic mass of 4 cm at the uncinate process of pancreas without signs of pancreatitis. Abdominal MRI and EUS-FNA couldn't differentiate between a pseudocyst and a cystic malignant mass.The patient was sent to surgery and a Whipple procedure was performed. Histological examination showed chronic fibrosing pancreatitis with a retention cyst.
The second patient was a 59 year old woman hospitalized with deep vein thrombosis and pulmonary embolism but without abdominal complaints. Liver tests and lipase were normal. There was a very high CA 19-9 level : 1930 IU/mL and slightly elevated CEA : 9.5 mg/L. CT abdomen and MRI showed a cystic mass of 5 cm in the corpus of the pancreas. Multiple small nodular pancreatic lesions could also been seen. EUS-FNA with cyst fluid analysis was performed and we found a high CEA in cyst fluid : 4344 ng/mL suggesting a non-benign lesion. The patient underwent a corpo-caudal pancreatectomy. The final diagnosis was a mucinous cystadenoma besides multiple well differentiated neuroendocrine tumors of the pancreas. The third patient was a 76 year old woman presenting with acute abdominal pain, elevated lipase and slightly elevated liver tests. Bilirubin was normal. CA 19-9 level was high : 1100 U/mL. CT abdomen showed a large and suspicious mass in the head of the pancreas. The patient wasn't sent to surgery after a decline of CA 19-9 level to 77 U/mL and a regression of the pancreatic mass on MRI. EUS-FNA confirmed the diagnosis of an inflammatory mass.
Conclusion : These cases demonstrate that even a high cut-off level of CA 19-9 cannot always discriminate between pancreatic neoplasia and non-malignant lesions. CA 19-9 levels have to be used with caution in the management of patients with (cystic) pancreatic masses.

- T03 -

ETIOLOGY OF CHRONIC PANCREATITIS : TWO-YR SINGLE-CENTRE EXPERIENCE IN A TERTIARY HOSPITAL IN BELGIUM. W. Van Steenbergen (1), C. Verslype (1), W. Laleman (1), S. Vandermerwe (1), D. Cassiman (1), D. Vanbeckevoort (2), V. Vandecaveye (2), G. De Hertogh (3), B. Topal (4), R. Aerts (4), A. Corveleyn (5), F. Nevens (1). (1) Hepatology, Uzleuven, Leuven, Belgium ; (2) Radiology, UZ Leuven, Leuven, Belgium ; (3) Pathology, UZ Leuven, Leuven, Belgium ; (4) UZ Leuven, Leuven, Belgium ; (5) University Hospital Leuven, Center For Human Genetics, Leuven, Belgium.

Introduction : Chronic pancreatitis (CP) may result from environmental, genetic, immunologic, metabolic, and morphologic risk factors. Population studies related to the frequency of these factors are rare.
Aim : Our aim was to study factors leading to CP in a Belgian population.
Methods : 314 consecutive pts seen in a tertiary referral center between January 2010 and December 2011 were studied. Data were gathered on alcohol and smoking behavior, gene mutations, autoimmunity, and morphologic anomalies such as pancreas divisum and groove pancreatitis.
Results : Patients comprised 91 women and 223 (71.1%) men. Mean age at evaluation was 53.4 yrs with ages between 10 and 87. Alcohol was considered the major risk factor in 190 pts (60,5%) ; in this group women comprised 16.3% and

men 83.7% of pts, and 95.8% were smokers. Nineteen of these 190 pts had Groove pancreatitis and all of them were males with a history of alcohol abuse and smoking.
Genetic analysis for CFTR and SPINK1 mutations was performed in 70 out of the 190 pts with alcohol-induced CP, and in 14 of them (20%) a mutation was found with a SPINK1 mutation in 8, a T5 CFTR allele in 3 and a heterozygous CFTR mutation in another 3 cases.
A diagnosis of idiopathic CP was made in 44 (14.0%) ; 24 (54.5%) were women and 18 (40.9%) were smokers. Genetic analysis was performed in 32/44 pts and in 8 of them (25%) a mutation was found with a heterozygous SPINK1 mutation in 4. A diagnosis of apparent autoimmune pancreatitis (AIP) was made in 35 pts (11.1%). Seventeen, with mean age 58 yrs, with 14 (82.4%) men, and with a 53% smoking behavior were classified into the type 1 or LPSP form although 6 of them had IgG4 < 2.8 g/l. Sixteen, with mean age 32 yrs, with 10 (62.5%) men, and with an 12.5% smoking behavior were classified into the type 2 or IDCP form.
Familial pancreatitis was diagnosed in 6 pts. Three of them were sisters who developed CP at an early age, and the pancreatitis was related to the combination of SPINK1 mutation and the T5 CFTR allele. Pancreas divisum (PD) was found in 33 (10.5%) ; in 19 alcohol and smoking was considered the primary etiology ; in 13 (4%) PD was the only etiologic factor, whereas in one pt PD was combined with SPINK1 mutation.
Conclusion :
1. Alcohol/smoking was considered the main mechanism in 60% ; in this group all pts with groove pancreatitis were found.
2. In alcoholic and in idiopathic CP, CFTR and SPINK1 mutations may play a role in 20-25%.
3. Familial CP may also be related to mutations in CFTR/SPINK1.
4. AIP is found in 11% with an even distribution between the IgG4 positive and negative group.

- T04 -

EUS GUIDED NECROSECTOMY TEMPORARY CYSTOGASTROSTOMY WITH COVERED STENT FOR PANCREATIC NECROSIS.A. Krishnan, R. Ramakrishnan. Apollo 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 : we report our experience using pseudocyst drainage with cystogastrostomy and endoscopic necrosectomy for infected pancreatic necrosis with fully covered self-expanding metallic stents (CSEMS).
Methods : 12 patients underwent endoultrsound guided endoscopic necrosectomy and temporary cystogastrostomy for infected pancreatic necrosis by using CSEMSs. Patient details, disease severity scores, scores for severity assessed at CT, treatment procedures, length of hospital stay, and outcome for patients undergoing endoscopic therapy were recorded. Patients proceed to intervention if infection is strongly suspected on clinical and radiological grounds or is confirmed bacteriologically.
After the necrosis cavity had been accessed, with the assistance of endoscopic ultrasound, a large orifice was created and necrotic debris was removed using special short fully covered 15 mm diameter SEMS with large flares was deployed across the tract under radiological control. Completeness of the necrosectomy procedure was ascertained by visualization of a clear pseudocyst cavity on endoscopy.
Results : A total of 12 patients (10 men, 2 women ; median age 39, range 19-76) who were treated successfully. Median APACHE 2 score on presentation was 11 (range 3-18). Two patients presented with organ failure and needed intensive care.
Necrosis was successfully treated endoscopically in all patients, requiring a median of 2 endoscopic interventions (range 1-4). The tissue samples obtained at the first necrosectomy confirmed infection in 12 patients. Complication included superinfection in patient 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 (2-11) 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 endoscopic approach using fully covered self-expandable metallic stent in terms of less morbidity is conceivable and our report demonstrates that such an approach is feasible.

- T05 -

DOES EUS-FNA OF PANCREATIC CYSTS PROVIDES ADEQUATE MATERIAL FOR CYTOLOGY AND LABORATORY ANALYSIS ? G. Mavrogenis (1), B. Weynand (2), A. Sibille (1), G. Longheval (1), P. Warzee (1). (1) Notre Dame, Charleroi, Belgium ; (2) UCL, Mont-Godinne, Belgium.

Introduction : Recent data suggest that endoscopic ultrasound fine needle aspiration (EUS-FNA) is of limited value in the diagnostic approach of pancreatic cysts due to a low rate of definite cytological diagnosis and a failure to obtain sufficient material for biochemical analysis.
Aim : The aim of the study was to address the question whether EUS-FNA aspiration of pancreatic cysts provides sufficient material for cytology and laboratory analysis.
Methods : Retrospective analysis of all EUS-FNA guided examinations for pancreatic cysts performed in our center between the period 2000-2011.Collected samples were processed as liquid based cytological preparations (ThinPrep method).
Results : A total of 68 patients were included (43 females, 25 males) with a median age of 63 years (range : 33-86). In total, 70 cysts were punctured. Eight were located at the uncinated process (11.4%), 30 at the head (42.9%), 24 at the corpus (34.3%) and 8 at the tail of the pancreas (11.4%). The median size of the cysts was 22.2 mm (range 4-68). In 10 out of 70 cases the cytological examination was not conclusive while in 60/70 (85.7%) of cases a cytological diagnosis was obtained. Moreover, in 43/70 (61.4%) the pathologist could identify specific cytological criteria that further enhanced his diagnostic accuracy. Predictive factor of a conclusive cytological examination was a bigger cyst size (23.75 mm (range 4-68) versus 13.35 mm (range 8-31), $p = 0.023$, Mann-Whitney test, SPSS 17). Fluid biochemical analysis succeeded in 42/70 cases (60%).
Conclusion : Our data, are in accordance with a previous study published by Frossard *et al*. who reported a classifying diagnosis in 98 out of 127 cases (77.1%) of pancreatic cysts by cytology, and suggest that the characterization of pancreatic cysts by EUS FNA is possible in the majority of patients. In addition, our data show that EUS FNA may contribute to the detection of a significant number of malignant cysts, since in 11.4% of our cases the diagnosis of adenocarcinoma or neuroendocrine tumor was established by cytological analysis.
The higher rate of sufficient material for cytological analysis found in our study may be attributed to several reasons :
a) Collected samples were processed as liquid based cytological preparations (ThinPrep method)
b) Aspirated fluid was preferentially sent for cytological instead of laboratory analysis
c) Cytological analysis was performed by a cytopathologist with a high volume experience in pancreatic fluid analysis.

- T06 -

EUS FINE NEEDLE ASPIRATION AND BIOPSY SAMPLE QUALITY ANALYSIS FOR HENT1 IMMUNO-HISTOCHEMICAL STAINING. F. Puleo (1), R. Marechal (1), P. Demetter (1), L. Verset (1), P. Eisendrath (1), A. Calomme (1), E. Toussaint (2), J.B. Bachet (3), M. Arvanitakis (1), J. Deviere (1), J.L. Van Laethem (1). (1) Erasme Hospital, Brussels, Belgium ; (2) Institut Jules Bordet, Brussels, Belgium ; (3) Hôpital Pitié-Salpêtrière, Paris, France.

Introduction : In the new era of biomarkers studies and biomarker-driven protocols, tissue acquisition is the limiting factor. In pancreatic cancer (PAC) in particular, this represents a challenge. Data from several retrospective studies suggest human Equilibrative Nucleotide Transporter-1 (hENT1) as a biomarker to select gemcitabine responders in the adjuvant setting. No studies have been published on the impact of hENT1 status in neoadjuvant, locally advanced and metastatic settings, the limiting factors being PAC tissue availability in patients who will not undergo surgery. Endoscopic ultrasound (EUS) fine needle aspiration (FNA) and biopsy (FNB) are the gold standards for tissue acquisition in PAC. We aimed to evaluate the feasibility of immunohistochemical (lHC) staining for hENT1 on EUS FNA/FNB and provide information on predictive factors of samples quality.
Aim : We aimed to evaluate the feasibility of immunohistochemical (lHC) staining for hENT1 on EUS FNA/FNB and provide information on predictive factors of samples quality.
Methods : In our institutional database of PAC patients we identified 192 patients who underwent EUS-FNA (1/2006-5/2011) (Cohort 1). We prospectively collected tissues from 38 patients who underwent EUS FNA or FNB for pancreatic masses with a pathologically confirmed diagnosis of PAC from 6/2011 to 11/2012 (Cohort 2). We compared the two cohorts in terms of type of needle, cellblock preparation, presence of neoplastic cells in cellblock and specifically, yield to perform IHC staining for hENT1. We then evaluated factors correlated to the success or failure to perform IHC. hENT1 staining was evaluated semi-quantitatively by two pathologists.
Results : The two cohorts were comparable in terms of age, sex, localisation of the lesion, presence of associated-chronic pancreatitis (CP), rapid on-site cytopathological examination (ROSE), tumor stage and number of passes.

For 53 patients in cohort 1 and 4 patients in cohort 2, no cellblocks were available (p = 0.017). No neoplastic cells were found in 71 and 10 cellblocks in cohort 1 and 2 respectively. hENT1 IHC was possible in 68 of 192 EUS-FNA in cohort 1 and 24 of 34 EUS-FNA/FNB in cohort 2 (36,98% vs 63,16%, p = 0.015).
Factors associated with the possibility to perform IHC on cellblocks were : prospective collection of sample (p = 0.015), absence of CP (p = 0.024), > = 3 passes or more (p = 0.0001). Size, type of needle, localisation, ROSE and tumor stage was not predictive of the ability to perform IHC. Overall, IHC staining for hENT1 was negative, positive and inconclusive in 71.4%, 18,2% and 10,4% of patients.
Conclusion : PAC tissue availability still remains a challenge, especially for inoperable and locally advanced disease. In the prospective arm of this study, we obtained enough tissue to perform hENT1 IHC staining in 63% of patients. Predictive factors include a higher number of passes and the absence of CP. We should take this into account in future tissue biomarker-driven protocols and efforts should be focused on increasing the yield of tissue availability, not only for diagnostic but also for research and therapy-guided purpose.

Invited Lecture
- T07 -

MECHANISMS OF CELL DIFFERENTIATION IN PANCREATIC EXOCRINE METAPLASIA. F. Lemaigre. Liver and Pancreas Development Unit, de Duve Institute, Université Catholique de Louvain, City of Brussels, Belgium.

Pancreatic exocrine metaplasia is a histological lesion in which pancreatic acini are progressively replaced by duct-like structures. The observation that metaplastic cells co-express acinar and ductal proteins, and the recent use of mouse models allowing to trace the fate of cells, demonstrate that acinar cells are converted to –and not replaced by- duct-like cells, and subsequently evolve to pancreatic intraepithelial neoplasia (PanIN). Therefore, exocrine metaplasia results from transdifferentiation of acinar cells, and is considered a premalignant lesion of pancreatic ductal adenocarcinoma.
Identification of tumor-initiating events is essential for developing early diagnosis methods, a critical issue for pancreatic ductal adenocarcinoma, whose high mortality rate results in part from late detection of the disease. Our laboratory has expertise in the study of pancreas development and has identified critical regulators of pancreatic duct development in the embryo. We reasoned that during metaplasia, adult acinar cells might activate the embryonic gene expression program promoting duct differentiation. Therefore, we looked for expression of duct-promoting genes in metaplasia and found that transcription factors known to be required for duct development in the embryo are ectopically activated in human and mouse acinar cells and contribute to generate metaplastic lesions. Such ectopic activation of duct-promoting genes depends in part on deregulated expression of microRNAs.
We conclude that transcription factors and microRNAs form a regulatory network involved in exocrine metaplasia, and that members of the network constitute potential biomarkers for premalignant lesions

Invited Lecture
- T11 -

LESIONS OF THE PANCREATIC TAIL : A DIFFERENTIAL DIAGNOSIS. J. Decock, D. Vanbeckevoort, D. Bielen. UZ Leuven.

We present a challenging differential diagnosis of lesions in het pancreatic tail, with the emphasis on auto-immune pancreatitis. The imaging characteristics of auto-immune pancreatitis on MRI are compared with those of adenocarcinoma, acute pancreatitis and chronic pancreatitis. The focus will be the differences distinguishing the pathologies mentioned above, leading to a correct diagnosis of a lesion in the pancreatic tail. All pathology is extensively illustrated with beautiful MR-imaging.

Reviewer index to volume 75 (2012)

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