

ABSTRACTS

XXIIth Belgian Week of Gastroenterology 2011

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- A01 — A30 Belgian Association for the Study of the Liver (BASL)
- B01 — B22 Research Group “Gastrointestinal Regulatory Mechanisms (OG-FWO)”
- D01 — D64 Joint Meeting of Gastroenterology
- E01 — E14 Belgian Group of Pediatric Gastroenterology, Hepatology and Nutrition
- I01 — I13 IBD Research Group
- N01 — N11 Research Group of Clinical Nutrition and Metabolism (SBNC and VVKVM)
- P01 — P35 Gastro-intestinal Pathology Club & the Belgian Group for Digestive Oncology and Research Group
“Digestive and Abdominal Imaging”
- S01 — S11 Symposium of the six societies
- T01 — T10 Research Group “Belgian Pancreatic Club”
- V01 — V11 Belgian Videocapsule Group

BASL

- A01 -

HSC-SPECIFIC DELIVERY OF DOXORUBICIN : A POSSIBLE ANTI-FIBROTIC THERAPY ? L. Peeters, A. El Taghdouini, L. Van Grunsven. UZ Brussel Vrije Universiteit, Brussels, Belgium.

Introduction : Doxorubicin (Adriamycin) is a commonly used chemotherapy agent that is very effective in both Hodgkin and Non-Hodgkin lymphomas. Recently it has been reported that application of Doxorubicin can decrease liver fibrosis in the BDL rat model. Serious adverse effects, *i.e.* cardiotoxicity, associated with the use of this cytostatic drug precludes its clinical use for the treatment of liver fibrosis. However, specific targeting of Doxorubicin to HSCs by using vitamin A coupled liposomes containing Doxorubicin may reduce the toxicity in non-target tissues.

Aim : In the present study, we further evaluated the anti-fibrotic effect of Doxorubicin and tested the possibility of HSC specific Doxorubicin delivery by the incorporation of the compound in vitamin A coupled liposomes (VA-lipDox).

Methods : BALB/c mice were injected twice a week with CCl₄ during 4 weeks. During the last week we also injected the mice 3 times with vitamin A coupled Doxorubicin liposomes. 24 hrs after the CCl₄ injection, the mice were euthanized. Tissue samples of liver, hart and kidney were taken and analyzed for matrix deposition and cell toxicity. The collagen positive area was quantified after Picrosirius staining using NIH ImageJ software. Total RNA from tissue was extracted using Trizol, reversed-transcribed and subjected to quantitative PCR (qPCR).

Results : The animals co-treated with VA-lipDox showed less fibrosis evidenced by a reduced collagen stained area in the co-treated animals. Moreover, qPCR results showed a decreased expression of \pm -smooth muscle actin.

Conclusion : These results show that the Doxorubicin containing vitamin A-coupled liposomes are able to reduce fibrosis in the CCl₄ liver injury model.

- A02 -

DIFFERENTIAL EFFECTS OF FATTY ACIDS OR TUNICAMYCIN-INDUCED ENDOPLASMIC RETICULUM STRESS ON INFLAMMATION AND INSULIN RESISTANCE IN HEPG2 CELLS. V. Legry, O. Molendi-Coste, I. Leclercq. Université Catholique de Louvain, Brussels, Belgium.

Introduction : Recent findings suggest that endoplasmic reticulum (ER) stress is a key link between fat overload, inflammation and insulin resistance.

Aim : Our aim was to investigate the effects of various fatty acids and a classical ER stress-inducer on inflammation and insulin resistance in cultured hepatocytes.

Methods : HepG₂ cells were exposed to oleic acid (1 mM), palmitic acid (0.5 mM), a mixture of oleic :palmitic acids (ratio 1:5 ; 1 mM) or tunicamycin (2 μ g/ml) during 8 hours. We evaluated lipid droplets accumulation by oil red O staining. The splicing of XBP1 and the expression of ER stress markers, including GRP78, ATF4, GADD34 and CHOP, and of inflammatory markers, such as TNF and IL-6, were investigated by RT-PCR. NF κ B, JNK activation and insulin signalling were monitored by western blot.

Results : As expected, we observed lipid droplets accumulation in HepG₂ cells treated with oleic and palmitic acids but not tunicamycin. Treatment by tunicamycin induced ER stress as evidenced by increased expression of GRP78, ATF4, GADD34, CHOP and splicing of XBP1. While it induced TNF expression, it failed to activate JNK and NF κ B inflammatory pathways or to interfere with insulin signalling.

Exposure to palmitic acid, alone or together with oleic acid, induced ER stress, with higher expression of GRP78, GADD34, CHOP and the splicing of XBP1, and increased the expression of TNF and IL-6. Palmitic acid also induced the phosphorylation of JNK and NF κ B p65 and decreased dramatically AKT phosphorylation. By contrast, oleic acid, while inducing the expression of TNF and IL-6, did not induce ER stress, pro-inflammatory pathways or alter insulin signalling in HepG₂.

Conclusion : Our present results show that exposure to palmitic acid induces ER stress, activation of pro-inflammatory pathways and insulin resistance. However, ER stress does not appear as the causative link. More in-depth analysis of specific pathways in ER stress and further studies using ER stress inhibitors will help in resolving this issue.

LIVER MESENCHYMAL CELLS CONTRIBUTE TO IMPROVEMENT OF HUMAN CRYOPRESERVED HEPATOCYTES SURVIVAL AND FUNCTION. A.C. Dusabineza (1), D. Ngoc Khuu (1), L. Dolle (2), J. Abarca-Quinones (1), E. Sokal (1), L. van Grunsven (2), M. Najimi (1), I. Leclercq (1). (1) UCL Saint-Luc, Brussels, Belgium ; (2) UZ Brussel Vrije Universiteit, Brussels, Belgium.

Introduction : Clinical development of hepatocyte transplantation, an alternative to orthotopic liver transplantation for inborn errors of metabolism, is hampered by the low quality of cryo-preserved hepatocytes and early graft loss.

Aim : We analyze whether interactions with a mesenchymal environment will improve stability and function of cryo-hepatocytes.

Methods : *In vitro*, human cryo-hepatocytes were cultured either alone or with hepatic stellate cells (human primary, pHSC or a human HSC-derived cell line, LX2) directly or in a transwell manner. Hepatocyte attachment was indirectly evaluated by the number of floating cells and hepatocyte function by urea concentration in the medium, 24 h and 8 days post-plating, respectively.

Hepatocytes alone or with pHSC or LX2 (ratio 40 to 2) were transplanted into immunodeficient (SCID) mice and livers examined 4 weeks later. We used immunohistochemistry and morphometry to assess hepatocyte engraftment [anti-human albumin (hAlb), OTC (hOTC)], proliferation (hKi67) and integration into host liver parenchyma (CD10/hAlb). Anti- α SMA and Sirius red staining were used to track transplanted HSC and extracellular matrix (ECM) deposition.

Results : Better attachment (3.5 times less floating cells) and function (2 to 4-fold increase of urea production) were reached by cryo-hepatocytes on collagen matrix co-cultivated with either LX2 or pHSC in contact or transwell mode. *In vivo*, hAlb+ cells were scattered in the lobules of mice transplanted with cryo-hepatocytes only. When co-transplanted with pHSC or LX2, hAlb+ hepatocytes were respectively 2.6 or 4.6 times more numerous and formed clusters. Engrafted hepatocytes co-expressed hAlb and hOTC, and membranar CD10 (a canalicular marker) in a polarized fashion. They formed hybrid canaliculi with adjacent endogenous mouse hepatocytes showing their full integration into liver parenchyma. No hKi67+/hAlb+ cells were found. Collagen fibres and α SMA+ cells were similarly restricted to vessels in CTL and transplanted livers.

Conclusion : We demonstrate that *in vitro* survival and function of cryo-hepatocytes are improved by interactions with HSC, through ECM and paracrin factors they provide. *In vivo*, the increased number of hepatocytes incorporated in the hepatic cords together with the absence of fibrosis and lack of proliferation of transplanted hepatocytes, suggest that HSC have contributed to improved homing/engraftment of hepatocytes. This will be confirmed in further experiments.

MITOCHONDRIAL UNCOUPLING INHIBITS ACTIVATION OF HEPATIC STELLATE CELLS. E. Guimarães, L.A. van Grunsven. Free University (VUB), Brussels, Belgium.

Introduction : Mitochondrial dysfunction has been linked to the development of non-alcoholic fat liver disease (NAFLD) and progression to liver fibrosis. Although there is increasing evidence for a mitochondrial role in liver disease, little is known about its role during hepatic stellate cell (HSC) activation.

Aim : We investigated the role of mitochondrial activity during HSC activation through treatment with two mitochondrial uncouplers.

Methods : Cultured primary mouse HSCs were treated with the chemical uncouplers Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and Valinomycin. ATP levels were measured by luciferase assays. Production of reactive oxygen species (ROS) was measured using the fluorescent probe DCFH-DA. Activation of HSCs was evaluated by gene expression of activation markers using RT-qPCR, proliferation by measurement of DNA synthesis and protein expression of alpha-smooth muscle actin (α -SMA) by immunocytochemistry. Response to the pro-fibrogenic cytokine TGF β 2 was evaluated by gene expression of the inhibitory SMADs by RT-qPCR.

Results : Uncouplers treatment mildly decreased (20%) the levels of ATP. ROS levels were also affected, decreasing to 50% after 30 minutes treatment with FCCP. Uncouplers treatment increased the expression of several mitochondrial genes e.g., Tfam and COXIV up to 2-fold. Together, these data suggest a mild mitochondrial uncoupling. Treatment of cultured primary mouse HSCs with FCCP and Valinomycin for seven days resulted in morphological features of quiescent HSCs, such as accumulation of lipid droplets, while control groups showed the typical activated myofibroblastic phenotype. The gene expression of several pro-fibrogenic markers was reduced in treated cells compared to non-treated HSCs (e.g., 5-fold decreased expression of PDGF receptor-2 and α -SMA). HSC proliferation and α -SMA protein expression were also reduced in mildly-uncoupled HSCs. The treatment with chemical uncouplers abrogated the induction of pro-fibrogenic genes and SMADs 6 and 7 by TGF β 2, an important cytokine in the development of liver fibrosis.

Conclusion : We showed in this study that mild mitochondrial uncoupling can inhibit culture-induced HSC activation and the response to pro-fibrogenic cytokines like TGF β 2. These results indicate a role for mitochondrial activity during HSC activation and the progression of liver fibrosis. Further experiments will address the effects of mitochondrial uncoupling on the inhibition of *in vivo* models of liver fibrosis.

FARNESYLTHIOSALICYLIC ACID INDUCES APOPTOSIS AND SENSITIZES HEPATOCARCINOMA CELL LINES TO DOXORUBICIN AND TRAIL-INDUCED CELL DEATH. N. Charette (1), C. De Saeger (2), Y. Horsmans (1), I. Leclercq (1), P. Starkel (1). (1) UCL Saint-Luc, Brussels, Belgium ; (2) Université Catholique de Louvain, Brussels, Belgium.

Introduction : Ras activation is a frequent event in hepatocarcinoma (HCC) and is thought to contribute to the resistance of HCC cells to apoptosis. Farnesylthiosalicylic acid (FTS) inhibits ras by dislodging its activated form from its membrane docking sites.

Aim : The aim of this study was to evaluate whether FTS could induce apoptosis in HCC cell lines (HepG2, Huh7, and Hep3B), and whether it could sensitize those cells to doxorubicin and/or TRAIL.

Methods : Cell viability was assessed by a WST-1 assay in cells treated with DMSO or FTS, alone or in combination with various concentrations of doxorubicin or TRAIL, for 1 or 2 days. Apoptosis induction was evaluated by a caspase 3/7 activity assay after 24 hours of treatment with DMSO or FTS. Expression of mediators and inhibitors of apoptosis was determined by immunoblotting (p53, cytochrome c, Mcl-1, Bcl-XL) or quantitative PCR (DR4, DR5, survivin, cFLIP, TNF α , Fas).

Results : FTS reduced cell viability in all tested cell lines, and induced caspase 3 activation in HepG2 and Hep3B cells. This was related to a global pro-apoptotic balance with death receptor upregulation, cytochrome c release, and a reduced mRNA expression of the apoptosis inhibitors cFLIP and survivin. Furthermore, Mcl-1 expression was slightly down-regulated in Huh7 and Hep3B cells, while Fas was upregulated in HepG2 cells and a strong induction of TNF α was observed in Hep3B cells. These observations were independent of p53, since both HepG2 (p53 wild-type) and Hep3B (p53-null) had a similar behavior upon FTS treatment. Finally, FTS enhanced the reduction in cell viability induced by both doxorubicin and TRAIL treatment.

Conclusion : FTS induces apoptosis and globally shifts the balance between pro- and anti-apoptotic factors towards cell death in a p53-independent way in human HCC cell lines. The induction of this pro-apoptotic phenotype leads to sensitization of those cells to doxorubicin and TRAIL-induced cell death.

A MURINE AND HUMAN STUDY DEMONSTRATING THE ROLE OF PLGF IN HEPATOCELLULAR CARCINOMA. F. Heindryckx, S. Coulon, L. Libbrecht, C. Van Steenkiste, A. Geerts, I. Colle, H. Van Vlierberghe. UZ, Gent, Belgium.

Introduction : Inhibition of angiogenesis is currently hot topic in the search for an effective treatment for hepatocellular carcinoma (HCC). At the moment, the focus is mainly on the vascular endothelial growth factor (VEGF), one of the key mediators in physiologic and pathologic angiogenesis. However, the placental growth factor (PIGF) is a VEGF homologue only involved in pathologic angiogenesis. Its inhibition could be used as a valuable therapeutic approach, since it would not affect healthy organs and could result in less negative side-effects compared to VEGF-inhibitors. To see whether PLGF plays a role in the development of HCC, we measured PLGF-serum levels in HCC patients, cirrhotic patients and healthy control patients. To assess if PIGF inhibition could influence HCC-progression, PIGF knock out mice (PLGF $^{-/-}$) were treated with diethylnitrosamine (DEN) and compared to wild type (WT) mice at several time points.

Aim : To demonstrate the role of PLGF in HCC and to assess if this molecule could serve as a potential therapeutic target.

Methods : Serum was collected from HCC-patients (n = 15), cirrhotic patients (n = 17) and healthy patients (n = 10) and analysed using the R&D PIGF ELISA kit. HCC-patients and cirrhotic patients were matched according to gender, etiology and age. 5-week-old male mice (PIGF $^{-/-}$ and WT) received intraperitoneal injections once a week with DEN (35 mg/kg bodyweight), which gives rise to HCC after 25W. Mice were sacrificed after 20W, 25W and 30W of DEN administration. Tumour burden was quantified on haematoxylin-eosin stained slides and immunohistochemical stainings were performed to assess macrophage recruitment (F4/80), vascularisation (endoglin), hepatic stellate cell (HSC) activation (aSMA) and hypoxia (HIF1a).

Results : Serum levels of PIGF were significantly increased in patients with cirrhosis (P < 0,05) and HCC (P < 0,01) compared to healthy patients. In addition, HCC-patients had higher serum levels of PIGF compared to cirrhotic patients without HCC (P < 0,05). PIGF inhibition significantly improved survival in mice with HCC (p < 0,05) and PIGF $^{-/-}$ had a significant lower tumour burden than WT at 25W (P < 0,001) and 30W (P < 0,05). PLGF $^{-/-}$ tumours were less vascularised (P < 0,05) and had a more uniform blood vessel distribution (P < 0,001). This uniform vessel distribution and the smaller tumours, resulted in less tumour hypoxia (P < 0,05) compared to WT tumours. Furthermore, tumour environment was characterised by less activated HSC (P < 0,05) and less macrophages (P < 0,05).

Conclusion : The expression levels of PIGF in patients confirm the importance of PIGF in the human hepatocarcinogenesis. Furthermore, inhibition of PIGF in a knock out model has a positive effect on survival in mice with HCC, it decreases tumour burden, restrains angiogenesis and reduces hepatic inflammation. The decreased tumour hypoxia suggests that there is less activation of HIF-mediated pro-metastatic pathways, possibly resulting in a less aggressive tumour phenotype. Furthermore, inhibition of PLGF does not affect healthy tissue and could therefore serve as a promising therapeutic target in HCC patients.

- A07 -

LONG-TERM SORAFENIB EXPOSURE IN HEPATOCELLULAR CANCER CELL LINES : RESISTANCE, RISK OF REBOUND GROWTH AND EPITHELIAL TO MESENCHYMAL TRANSITION. H. Van Malenstein (1), C. Verslype (1), P. Windmolders (1), L. Libbrecht (2), F. Nevens (1), J. Van Pelt (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) UZ, Gent, Belgium.

Introduction : Patients with advanced hepatocellular carcinoma (HCC) are eligible for treatment with the tyrosine kinase inhibitor sorafenib. Sorafenib leads to a survival benefit, but treatment is hampered by two phenomena ; many patients have important side-effects and eventually all patients show progression.

Aim : We aimed to determine the effects of long-term exposure to sorafenib and its withdrawal.

Methods : We developed liver cancer cell lines (HepG2 and WRL-68) that are resistant to sorafenib. We studied the effect of sorafenib withdrawal on proliferation and metabolism with XTT- and BrdU-assay. And we examined the morphological changes with immunocytochemistry, gene expression changes with RT-PCR and the invasive potential with matrigel invasion chambers. Furthermore, we tested the effect on proliferation and invasion of mTOR- and PI3K inhibition in the HepG2 resistant cell line.

Results : HepG2 cells (6 μ M and 8 μ M) and WRL-68 cells (6 μ M) became resistant to sorafenib. Resistance was confirmed with a shift of the IC50 from 5.5 μ M to 16 μ M. Furthermore, the resistant cell lines showed ongoing phosphorylation of ERK during sorafenib exposure. After withdrawal of sorafenib, these resistant cells show increased proliferation and metabolic activity. The HepG2 resistant cell lines have undergone an epithelial to mesenchymal transition (EMT) with loss of E-cadherin and high expression of vimentin, both on RNA- and protein level. The cells that displayed EMT became spindle shaped and were highly invasive. The resistant HepG2 cell line showed cross resistance for the mTOR inhibitor everolimus, but not for the PI3K-inhibitor LY294002. LY294002 also inhibited invasive potential at high dose.

Conclusion : Long-term treatment with sorafenib can lead to the development of resistant cells with an aggressive phenotype, because the cells undergo EMT. Furthermore, we demonstrated that abrogation of treatment leads to rebound growth, suggesting the importance of aggressive management of side-effects. In addition, the resistant cell lines can be used to identify new therapies, for example PI3K-inhibitors, after progression with sorafenib. To understand the mechanism of resistance and find new therapeutic targets, we are currently performing microarray on the resistant cells.

COMMON POLYMORPHISM IN THE PNPLA3/ADIPONUTRIN GENE CONFERS HIGHER RISK OF CIRRHOSIS AND LIVER DAMAGE IN ALCOHOLIC LIVER DISEASE. E. Trépo, T. Gustot, D. Degré, A. Lemmers, L. Verset, P. Demetter, R. Ouziel, E. Quertinmont, V. Vercruyse, L. Amininejad, O. Le Moine, J. Devière, D. Franchimont, C. Moreno. ULB Erasme, Brussels, Belgium.

Introduction : A recent genome-wide association study identified genetic polymorphism rs738409 C > G in the PNPLA3/adiponutrin gene associated with liver steatosis. This variant has also been linked to increased risk of alcoholic liver disease (ALD) and cirrhosis in Mestizo Mexicans with excessive alcohol intake.

Aim : Our aim was to study the influence of this polymorphism on European Caucasian patients with histologically suggestive ALD.

Methods : Three-hundred-twenty-eight healthy controls and 330 ALD patients, among whom 265 had cirrhosis, were genotyped for the rs738409 C > G polymorphism. We studied the impact of rs738409 C > G on clinical and biological parameters, together with histological staging of steatosis and fibrosis. PNPLA3 messenger RNA (mRNA) levels were measured by quantitative real-time PCR according to the patient's phenotype.

Results : The mutant G allele was significantly more frequent in ALD patients than in controls (odds ratio [OR] = 1.54, 95% confidence interval [CI] = 1.12-2.11 p = 0.008) and was significantly associated with steatosis (p = 0.048), fibrosis (p = 0.001) and greater risk of cirrhosis (p = 0.001). In multivariate analysis, rs738409 C > G remained the only independent factor associated with risk of cirrhosis (OR = 2.40 ; 95% CI = 1.08-5.35 ; p = 0.03). Furthermore, the PNPLA3 mRNA liver expression level was significantly lower in patients with more advanced fibrosis (p = 0.041) and negatively correlated with the hepatic venous pressure gradient (p = 0.005).

Conclusion : In European Caucasians, the rs738409 C > G PNPLA3 variant is associated with increased risk of ALD, liver damage and cirrhosis. Further prospective studies are required to confirm these results and to evaluate the potential of PNPLA3 as both a predictor and a therapeutic target in ALD.

INSULIN RESISTANCE CONFERS A HIGHER RISK OF PORTAL HYPERTENSION, CIRRHOSIS AND EARLY MORTALITY TO ALCOHOLIC LIVER DISEASE PATIENTS. E. Trépo (1), D. Degré (1), A. Lemmers (1), T. Gustot (1), A. Gerkens (1), S. Evrard (1), R. Ouziel (1), P. Deltenre (2), M. Adler (1), J. Devière (1), C. Moreno (1). (1) ULB Erasme, Brussels, Belgium ; (2) Hôpital de Jolimont, Haine-Saint-Paul, Belgium.

Introduction : Insulin resistance (IR) plays an important role in various chronic liver diseases. In alcoholic liver disease (ALD), BMI and blood glucose have been identified as risk factors for fibrosis. However, the impact of IR in ALD is currently unknown.

Aim : The aim of our study was to evaluate the relationship between IR and histological damage, portal hypertension and severity of liver disease in ALD.

Methods : From April 2009 to September 2010, 103 consecutive patients with excessive alcohol intake (> 40 g/day) undergoing transjugular liver biopsy for suspected liver disease were included in the study. IR was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR). Patients with a history of diabetes or other cause of liver disease were excluded.

Results : 103 patients (64% males, mean age 53.45 ± 9.79 years, mean BMI 25.41 ± 5.38 kg/m², 69% with cirrhosis, 12% died within 6 months 40% had histological alcoholic hepatitis [AH], median MELD score 12.09 [7.99-20.18], median hepatic venous pressure gradient [HPVG] 13 [6-18] mmHg , median HOMA-IR 2.76 [1.33-5.29]) were included with a median follow-up of 3 months. HOMA-IR showed a significant correlation with HVP (r = 0.29 p = 0.004), BMI (r = 0.37, p < 0.001), INR (r = 0.20, p = 0.049) and platelets (r = -0.43, p < 0.001). Conversely, HOMA-IR was not correlated with steatosis, the presence and severity of AH or with the MELD score. A ROC curve analysis (AUC = 0.67, p = 0.005) showed that the optimal HOMA-IR cut-off to identify patients at risk of cirrhosis was 2.69 (sensitivity = 0.60, specificity = 0.69, positive and negative predictive value were 0.81 and 0.44 respectively). After adjustment for age, gender, BMI and presence of AH, HOMA-IR above 2.69 remained an independent predictor of cirrhosis (OR = 3.43, 95% CI = 1.97-10.96, p = 0.038). Moreover, this threshold was associated to a higher risk of 6 months' mortality (p = 0.043).

Conclusion : In ALD, IR is correlated with the severity of portal hypertension. Furthermore, IR predicts 6 months' mortality and is independently associated to cirrhosis. IR may represent both a new predictor of severity and a new therapeutic target in ALD.

DONATION AFTER CARDIAC DEATH INCREASES THE NUMBER OF LIVER GRAFTS FOR LIVER TRANSPLANTATION. H. Le Dinh, M. Hans, J. Monnard, M.H. Delbouille, A. Deroover, J. Delwaide, A. Lamproye, S. Lauwick, P. Honoré, O. Detry. CHU de Liège, Liège, Belgium.

Introduction : Liver transplantation from donors after cardiac death (DCD) has been shown to increase the rate of primary graft dysfunction and ischemic cholangiopathy due to the unavoidable warm ischemia time prior to organ procurement and preservation. We evaluated the results of DCD liver transplants in a Belgian liver transplantation group.

Aim : Medical records of a consecutive series 41 DCD liver recipients from 2003 to 2010 were retrospectively reviewed with regards to patient and graft survivals and biliary complications. Mean follow-up was 24.4 months (range 1-90 months).

Methods : Mean donor age was 57.8 ± 14.7 years (mean \pm SD) with 48.8% of donors at the age of 60 or over. Donor causes of death were anoxia (43.9%), stroke (39%) and head trauma (14.6%). Mean time of treatment withdrawal to aortic cold perfusion was 20.1 ± 7.0 min (range : 10-39), mean cold ischemia time was 266.6 ± 90.7 min (range : 105-576) and mean suture time was 39.9 ± 7.6 min (range : 25-57). Liver grafts were transplanted locally in most cases (92.7%). HTK was the most used perfusion solution (82.9%).

Results : Mean recipient age was 55.5 ± 11.5 years (range : 29-73). Hepatocellular carcinoma (HCC) was the most frequent indication for DCD liver transplant (51.2%). Mean MELD score was 15.8 ± 6.7 (range : 6-40). Postoperatively, there was no primary nonfunction. Mean peak serum aspartate aminotransaminase level was 2,538.8 UI/mL, mean peak serum bilirubin was 54.6 mg/L. Six patients (14.6%) developed biliary complications and underwent endoscopic or surgery management. No patient developed symptomatic intra-hepatic bile duct strictures or needed a second transplantation. Patient and graft survival was 95% at 1 year. Nine liver grafts were lost during follow-up due to recipient deaths which included 3 multiple organ failures due to sepsis, 5 tumoral diseases and 1 death in the context of Alzheimer disease.

Conclusion : This experience confirms that controlled DCD donors may be a valuable source of transplantable liver grafts if short warm ischemia at procurement, minimal cold ischemia time and appropriate recipient selection are insured.

99MTC-MEBROFENIN CLEARANCE OF THE FUTURE REMNANT LIVER IS AN ACCURATE TOOL TO PREDICT LIVER FAILURE AFTER HEPATECTOMY. T. Chapelle, G. Roeyen, K. De Greef, N. Boeykens, B. Op De beeck, I. Hyughe, T. Lambrechts, A. Verbruggen, D. Ysebaert. UZ Antwerpen, Edegem, Belgium.

Introduction : Correct assessment of the remnant liver function is still difficult and acute liver failure after major hepatectomy is a serious complication in these patients. It is therefore important to develop accurate diagnostic tools that can predict the risk of liver resection-related morbidity.

Aim : The purpose of this study was to evaluate preoperative hepatobiliary scintigraphy of the future remnant liver in patients undergoing liver resection.

Methods : Postoperative morbidity was evaluated using the "Clavien Complication Score". Liver failure was defined as PT < 50 ; bilirubin > 50 μ mol/l, ammonium > 50 μ mol/l and therapy-resistant ascites, from day 5 until 3 months after hepatectomy. Preoperative hepatobiliary scintigraphy was performed by using 99m Tc-mebrofenin. Uptake in the liver is expressed as %/min/BSA.

Preoperative MRI volumetry was used to measure the volume of the total liver (TLV), the tumor volume and the future remnant liver volume (FRLV) and expressed in ml. Receiver-operating characteristic analysis was performed to assess cutoff values for risk assessment of morbidity and liver failure.

Results : Between October 2008 and August 2010, 61 patients were included in the study. Liver failure occurred in 8 patients. The volume of the future remnant was not significantly associated with liver failure and severe complications. FRLV below 40% had a low positive predictive value of 25% and a negative predictive value of 94% to predict liver failure and a low positive predictive value of 16% to predict serious liver-related complications. In contrast, patients with liver failure had significant lower 99m Tc-mebrofenin clearance than patients without liver failure (2.39 ± 0.78 vs 4.45 ± 1.63 respectively, $p < 0.001$). Fifteen patients had severe complications. Patients with severe complications had significant lower 99m Tc-mebrofenin clearance than patients without severe complications (2.67 ± 0.90 vs 4.67 ± 1.65 respectively ; $p < 0.01$). ROC curve analysis showed that a clearance below 2.1%/min/BSA of FRLV had a positive predictive value of 72% and a negative predictive value of 95% for the development of liver failure.

Conclusion : Hence, preoperative measurement of 99m Tc-mebrofenin uptake in the future remnant liver on hepatobiliary scintigraphy proved more valuable than measurement of the volume of the future remnant on MRI in assessing the risk of liver failure and liver related severe complications after partial liver resection. Therefore, preoperative measurement of 99m Tc-mebrofenin uptake in the future remnant liver on hepatobiliary scintigraphy is a valuable tool to predict liver-resection related morbidity.

DIAGNOSIS OF HEPATIC NODULES < 20MM IN CHRONIC LIVER DISEASE. HISTOLOGIC VALIDATION OF CT AND MRI CRITERIA. T. Serste (1), V. Barrau (2), V. Ozenne (2), M.P. Vuilermé (2), O. Farges (2), D.C. Valla (2), V. Paradis (2), V. Vilgrain (2), F. Degos (2). (1) ULB Saint-Pierre, Brussels, Belgium ; (2) Hôpital Beaujon, Clichy, France.

Introduction : The diagnosis of nodules of 20 mm or smaller detected during US surveillance in patients with chronic liver disease remains difficult and histology is still recommended. However, this procedure may be risky or inconclusive. The accuracy of non invasive radiological examinations therefore needs updated evaluation.

Methods : 50 consecutive patients in whom US detected a small nodule (mean diameter 17 ± 3 mm) with advanced liver disease (Metavir F3 or F4), without prior Hepatocellular Carcinoma (HCC) underwent 3D-CT, MRI and liver biopsy of the nodule, all performed within 1 month.

Results : **Results** : sex ratio : M/F 82/18%, mean age 63.5 ± 11.6 years, HBV 37%, HCV 39%, alcohol 16%, Mean alpha foetoprotein : 34.3 ± 80.4 ng/ml. HCC was demonstrated on biopsy in 34 patients (fine needle aspiration in 2 patients). The biopsy demonstrated a regenerative macronodules in 7 cases, dysplasia in 4 cases and a cholangiocarcinoma in 1 case. In 4 patients, biopsy of the nodule found cirrhosis. The 4 patients with dysplasia at initial biopsy were carefully followed up (2-8 months) : a second biopsy showed HCC in 3 patients, and one patient was transplanted while HCC was demonstrated on the explant. On CT, the nodules were hypervascular in 33 cases (= suspicious diagnosis) ; among them 28 showed wash-out during portal or late phase (= conclusive diagnosis). On MRI, the nodules were hypervascular in 38 cases (suspicious) ; among them 32 showed wash-out during portal or late phase (conclusive). Thirty-six patients had a wash-out at CT and/or at MRI (= at least one Typical vascular pattern). Twenty-three patients had coincidental typical vascular pattern on both CT and MRI (= AASLD 2005 Criteria). In 20/34 patients (59%) a wash-out was detected on coincidental CT and MRI while in 14/34, it was detected in only one procedure. The presence of a wash-out on at least one of the two imaging techniques had a 92% positive predictive value for HCC diagnosis and reached a sensibility of 100% and a specificity of 87%.

Conclusion : In patients with chronic liver disease, the diagnostic performance of dynamic radiological procedures in small nodules US detected, is excellent. The presence of a wash out on at least one imaging techniques is highly evocative of HCC.

OUTCOME OF PATIENTS WITH HEPATOCELLULAR CARCINOMA LISTED FOR LIVER TRANSPLANTATION BEFORE AND AFTER THE MELD-BASED ALLOCATION SYSTEM WITHIN EUROTRANSPLANT. A BELGIAN MULTICENTRE RETROSPECTIVE STUDY. B. Vos (1), S. Rogge (2), F. Nevens (3), J. Pirenne (3), J. Lerut (4), O. Ciccarelli (4), H. Van Vlierberghe (2), R. Troisi (2), C. Moreno (1), V. Lucidi (1), V. Donckier (1), J. Delwaide (5), O. Detry (5), P. Michielsen (6), T. Chapelle (6), M. Adler (1). (1) ULB Erasme, Brussels, Belgium ; (2) Ghent University, Ghent, Belgium ; (3) University of Leuven, Leuven, Belgium ; (4) Université Catholique de Louvain, Brussels, Belgium ; (5) CHU Liège, Liège, Belgium ; (6) UZ, Antwerpen, Belgium.

Introduction : Since 16th December 2006, Eurotransplant (ET) implemented the MELD system for allocation of liver grafts, hepatocellular carcinoma (HCC) within the Milan criteria (MC) receiving 22 MELD points. This has modified the priorities for liver allocation in Belgium.

Aim : The aim of our study was to analyse the effects of this new rule on the outcome of patients listed for liver transplantation (LT) for HCC in Belgium.

Methods : We compared, on an intention-to-treat (ITT) basis, 226 patients listed for HCC as first diagnosis in the pre-MELD era (October 1999 to October 2004) with 191 patients with the same indications in the post-MELD era (16th December 2006 to June 2009).

Results : The 2 groups were identical for age, gender, median MELD score (9 vs. 10) and median alpha-foetoprotein level at listing but in the post-MELD era, median Child-Pugh score was significantly lower : 7 vs. 6, $p = 0.001$, as well as median tumor nodal number : 2 vs. 1, $p = 0.003$. Treatment before listing was similar between both groups : 54% vs. 61%, $p = 0.16$. Delisting rates were similar for the two eras (12%) whereas death while waiting decreased : 10% vs. 3%, $p < 0.001$ and transplantation rate increased : 140 (62%) vs. 163 (85%), $p < 0.001$. Median waiting time until LT was shorter in post-MELD era : 4 vs. 3 months, $p = 0.001$. At transplantation, patients within MC were more numerous in post-MELD era on the explant : 66 (47%) vs. 134 (82%), $p < 0.001$. After transplantation, HCC recurrence at 2 years was similar in both groups : 17 (12%) vs. 21 (13%), $p = 0.236$ and, the one year ITT mortality, was significantly lower in post-MELD era : 114/226 (50%) vs. 56/191 (29%), $p < 0.001$. Multivariate analysis post-LT on the 417 patients disclosed that a tumor above MC on the explant was the best predictor factor of post-LT mortality (RR 1.9, CI : 1.1-3.6, $p = 0.035$) whereas the best predictor factor of post LT HCC recurrence was vascular involvement on the explant (RR 3.2, CI : 1.7-7.6, $p = 0.04$).

Conclusion : The implementation of MELD for liver allocation by ET has decreased the delay for LT as well as the one-year ITT mortality and increased the LT rate for patients listed for HCC in Belgium.

ALTERED EXPRESSION OF GLUCOSE AND LIPID REGULATORY GENES IN LIVER TISSUE IN RELATION TO THE SEVERITY OF NON-ALCOHOLIC FATTY LIVER DISEASE IN A LARGE PATIENT COHORT. S. Francque (1), A. Verrijken (1), J. Prawitt (2), S. Caron (2), I. Mertens (1), E. Van Marck (1), G. Hubens (1), P. Pelckmans (1), P. Michielsen (1), B. Staels (2), L. Van Gaal (1). (1) Antwerp University Hospital, Antwerp, Belgium ; (2) Institut Pasteur, Lille, France.

Introduction : Genes involved in glucose and lipid metabolism, inflammation and apoptosis have been implicated in the pathogenesis of NASH based on animal model experience. Data on gene expression in liver tissue of NASH patients are, however, very limited or non-existing.

Aim : To study the expression of genes related to glucose and lipid metabolism, inflammation and apoptosis in liver tissue of a large cohort of obese patients with prospective assessment of 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 quantification of mRNA levels by real time RT-PCR after total RNA extraction from liver homogenates and reverse transcription into cDNA. Liver histology was scored using the NASH Clinical Research Network Scoring System.

Results : 138 patients were prospectively included with a mean age of 44.6 ± 12.5 y (range 17-69) and a mean BMI of 39.1 ± 7.0 kg/m² (range 28-39) ; 60% fulfilled the criteria of the metabolic syndrome (IDF criteria). IL-6 and TNF α (inflammation) and Fatty Acid Synthase gene expression did not correlate with NASH features. PPAR α correlated with all features of NASH (NASH activity score : $R = 0.284$, $p = 0.001$, fibrosis grade : $R = 0.198$, $p = 0.023$). PPAR γ correlated negatively with steatosis degree ($R = -0.349$, $p = 0.008$), PEPCK with inflammation ($R = 0.234$, $p = 0.007$). PGC1 and ACC correlated with fibrosis ($R = -0.241$, $p = 0.006$ and $R = 0.382$, $p = 0.006$ respectively). After correction for BMI, only PEPCK ($R = 0.321$, $p = 0.019$ with steatosis ; $R = 0.423$, $p = 0.002$ with inflammation ; $R = 0.372$, $p = 0.006$ with NASH activity score) and PPAR γ ($R = -0.317$, $p = 0.021$ with steatosis, $R = -0.280$, $p = 0.043$ with ballooning, $R = -0.336$, $p = 0.014$) correlated with NASH features.

Conclusion : Liver tissue expression of glucose homeostatis genes (PEPCK and PPAR γ) are altered in human NASH and correlate with its severity, regardless of BMI. These findings points toward a primary role for NASH in the pathophysiology of glucose homeostasis disturbances. They also substantiate the rationale for glitazone treatment in NASH.

HIGHLY EXPRESSED CCL-2 IN SEVERE FORM OF ALCOHOLIC HEPATITIS : A POTENTIAL ENHANCER OF THE IL-17- NEUTROPHIL LIVER RECRUITMENT PATHWAY. D. Degré, A. Lemmers, T. Gustot, E. Trépo, R. Ouziel, P. Demetter, L. Verset, E. Quertinmont, V. Vercruysse, O. Le Moine, J. Devière, C. Moreno. ULB Erasme, Brussels, Belgium.

Introduction : Immune dysregulations, including cytokines and chemokines secretions, occurs in alcoholic liver disease (ALD). Serum levels and liver expression of CCL2 are increased in patients with alcoholic hepatitis (AH) but the role of this chemokine in the pathogenesis of ALD is nevertheless unknown. Our group has recently shown that liver of patients with ALD showed IL-17⁺ cells particularly in patients with AH and as Th-17 cells express CCR2, we hypothesized that CCL2 might play a role in the severity of ALD by recruiting Th-17 cells known to attract neutrophils through IL-8 action.

Aim : Our aim was to study the correlation between CCL2 plasma and liver expression and disease severity and liver inflammatory infiltrates. We also studied the association between -2518 CCL2 polymorphism associated with higher CCL2 production and severity of ALD.

Methods : We studied 123 plasma samples and 74 biopsy specimens of ALD patients undergoing a liver biopsy for diagnosis of alcoholic liver disease. CCL2 plasma levels were assayed by enzyme-linked immunosorbent assay and liver expression of chemokines was measured by qRT-PCR. Inflammatory infiltrates were assessed by immunohistochemistry. -2518 CCL2 rs1024611 A > G genotyping was performed in 235 patients with biopsy proven ALD and 224 controls with TaqMan assay.

Results : CCL2 plasma levels were increased in patients with ALD compared with healthy subjects ($p = 0.003$). Among ALD patients, those with AH had significantly higher plasma levels ($p < 0.001$) and hepatic expression ($p < 0.005$) of CCL2 than patients without AH. Plasma levels and hepatic expression of CCL2 were correlated with different parameters of disease severity including hepatic venous pressure gradient, MELD score and modified Discriminant Function. Liver CCL2 mRNA levels were correlated to neutrophilic infiltrates ($r = 0.411$ $p < 0.005$) but neither to T lymphocytes nor to mononuclear cells infiltrates. In the liver, we observed that CCL2 expression was correlated to IL-17⁺ cells infiltrates ($r = 0.339$ $p = 0.013$) and IL-8 expression ($r = 0.834$ $p < 0.001$). Moreover, CCL20, another Th-17 recruiting chemokine, expression was increased in the liver of the patients with AH. Finally, we showed that there were more -2518

CCL2 G allele carriers, corresponding to higher *CCL2* secretion, in the group of severe alcoholic hepatitis compared to other ALD patients ($p = 0.037$).

Conclusion : *CCL2* is increased in ALD particularly in severe forms and our results suggest a potential role of this chemokine in neutrophils recruitment through IL-17 pathway. *CCL2* and its receptor *CCR2* may thus become in the future an interesting therapeutic target in patients with ALD.

- A16 -

MIR141 AND MIR200C REGULATE PROGENITOR CELL FEATURES IN HEPATOCELLULAR CARCINOMAS. O. Govaere (1), J. Berkers (1), L. Van Kempen (2), K. Van Den eynde (1), B. Spee (1), R. Aerts (1), B. Topal (1), F. Nevens (1), E. Lerut (1), J. Van Den oord (1), T. Roskams (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) Jewish General Hospital, Montreal, Canada.

Introduction : The heterogeneous nature of hepatocellular carcinomas (HCCs) is reflected by a variable clinical outcome. Several HCC subtypes have been described based on the expression of progenitor cell markers. Keratin (K) 19 positivity in HCC has been correlated with a higher recurrence and shorter overall survival. Nevertheless the underlying mechanisms that regulate progenitor cell features in HCCs still remain unclear. In this study we want to unravel those mechanisms on an epigenetic level through miRNA profiling.

Methods : Genome wide RT² miRNA PCR Arrays were performed on frozen K19 positive and negative HCC liver biopsies ($n = 5$ per group), diagnosed between 1992 and 2008. Synthetic miRNAs were transfected ($n = 3$) into PLC/PRF/5 cell line for transient overexpression and knockdown. The biological effect was objectivised by means of qPCR and correlated with the mRNA expression of human HCC samples ($n = 14$). Localization of miRNAs and target proteins was obtained by means of in situ hybridization and immunohistochemistry on FFPE samples ($n = 20$).

Results : MicroRNA profiling of K19 positive HCCs, as compared to K19 negative HCCs, reveals a signature involving metastasis pathways and cholangiocyte/hepatoblastoma characteristics. In addition there is a strong decline in the expression of mir-122, which is known to be enriched in healthy liver tissue. Mir-141, mir-200c and mir-429 are strongly up-regulated in K19 positive HCCs, whereas mir-885-5p was significantly reduced. Overexpression of mir-141 as well as mir-200c in a PLC/PRF/5 cell line induced the expression of several known progenitor cell markers (such as *KRT19*, *KRT7*, *EPCAM*) and reduced typical hepatocytic markers (such as *ALB*, *HNF4A*). Knockdown of mir-885-5p only induced progenitor markers. In situ hybridization on human FFPE samples revealed that mir-141 and 200c are located in cholangiocytes and hepatic progenitor cells, which supports the idea of progenitor-derived origin of K19 positive HCCs.

Conclusion : The microRNA profile of K19 positive HCCs poses new insights into the pathogenesis of this aggressive subtype of HCCs. Several microRNAs regulate the progenitor features in HCCs and are also found in the non-neoplastic progenitor cells, indicating that the same mechanisms are active in human progenitor cells and K19 positive HCCs.

THE BASL-BLIC SPRING MEETING LECTURE : ETHICS IN CLINICAL RESEARCH. Vittorio Bertele (Milan, Italy).

Posters

- A18 -

NONCIRRHOTIC PRESINUSOIDAL PORTAL HYPERTENSION IS COMMON IN CYSTIC FIBROSIS-ASSOCIATED LIVER DISEASE. P. Witters (1), L. Libbrecht (2), T. Roskams (1), K. De Boeck (1), L. Dupont (1), M. Proesmans (1), F. Vermeulen (1), B. Strandvik (3), A. Lindblad (3), X. Stéphenne (4), E. Sokal (4), S. Gosseye (4), S. Heye (1), G. Maleux (1), R. Aerts (1), D. Monbaliu (1), J. Pirenne (1), I. Hoffman (1), F. Nevens (1), D. Cassiman (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) UZ, Gent, Belgium ; (3) Sahlgrenska Hospital, Göteborg, Sweden ; (4) Université Catholique de Louvain, Brussels, Belgium.

Introduction : Liver disease is the second cause of mortality in cystic fibrosis (CF). The current hypothesis gives a central role to bile inspissations and hence obstructed bile flow, but is unlikely to be the sole explanation.

Aim : The aim of this study was to further elucidate the clinicopathological features of CF liver disease.

Methods : 39 liver specimens (30 needle biopsies, 9 explants), of 32 patients were analyzed and correlated with clinical characteristics. Fibrosis, biliary and vascular changes were evaluated according to prespecified criteria.

Results : Cirrhosis was present in 9% (or 3/32 patients, only present in 3/9 explants). Classical biliary changes were present in 53% (17/32). The most striking histological abnormalities were portal vascular changes in 72% (23/32) and paraportal shunt vessels in 69% (22/32). The absence of cirrhosis in 5 patients with classical portal hypertension fits with noncirrhotic portal hypertension which was confirmed by hepatic venous pressure gradient measurements in 2 patients.

Conclusion : Our results suggest that CF related liver disease predominantly presents as noncirrhotic, presinusoidal portal hypertension with portal venopathy. Shunting procedures to alleviate this portal hypertension should probably be considered before liver transplantation.

HCV BURDEN IN EUROPE : IMPACT OF NATIONAL TREATMENT PRACTICES ON FUTURE HCV-RELATED MORBIDITY AND MORTALITY THROUGH A MODELING APPROACH. P. Deltenre (1), C. Moreno (2), M. Buti (3), T. Stroffolini (4), J. Parkes (5), N. Mühlberger (6), U. Siebert (6), A. Hatzakis (7), W. Rosenberg (8), S. Zeuzem (9), P. Mathurin (10), S. Deuffic-Burban (11). (1) Hôpital de Jolimont, Haine-Saint-Paul, Belgium ; (2) ULB Erasme, Brussels, Belgium ; (3) Hospital General Universitario Valle Hebrón and Ciber-ehd del Instituto Carlos III, Barcelona, Spain ; (4) Policlinico Umberto 1, Roma, Italy ; (5) School of Medicine, University of Southampton, Southampton, United Kingdom ; (6) University for Health Sciences, Medical Informatics and Technology, Innsbruck, Austria ; (7) Athens University Medical School, Athens, Greece ; (8) University College London, London, United Kingdom ; (9) Goethe University, Frankfurt, Germany ; (10) Centre Hospitalier Régional Universitaire, Lille, France ; (11) INSERM U995, Lille, France.

Introduction : Screening and accessibility to anti-viral therapy for HCV patients differ among European countries. It is unknown if such differences affect HCV-related mortality rates.

Aim : To compare the impact of treatment practices on HCV burden in Europe using a modeling approach.

Methods : A country-specific Markov model of HCV progression was based on published epidemiological data and reports of competitive and hepatocellular carcinoma mortality for France, Belgium, Germany, Italy, Spain and UK. It predicted HCV-related morbidity and mortality until 2025 in each country considering HCV prevalence, HCV screening, genotype, alcohol consumption, treatment practice and therapeutic progresses.

Results : In 2010, 33% of the patients were HCV RNA negative (ranging from 31% in Italy to 42% in France), of whom 20% after a successful treatment. 49% were aware of their infection. Cirrhosis occurred in 15% (in 31% decompensated). 5% had HCC. Compared to a scenario without treatment, current treatment practice will reduce HCV mortality by 13% until 2025 (65,600 deaths, 95% CI : 60,800-71,200). This impact will be highest in France (24% ; 15,500 deaths, 95% CI : 15,000-15,900), lowest in Italy (9% ; 20,400 deaths, 95% CI : 20,200-23,700) and intermediate in other countries (12% in Belgium, 1,600 deaths, 95% CI : 500-2600, 20% in Germany, 14,900 deaths, 95% CI : 13,100-15,000, 11% in UK, 2,000 deaths, 95% CI : 1,100-2,900 ; 12% in Spain, 11,200 deaths, 95% CI : 10,800-11,600). Cirrhosis incidence will also be reduced by 21% until 2025 (130,500 cirrhosis, 95% CI : 120,200-136,100) with similar differences between countries. If, in 2012, all naïve patients and 70% of non responders were treated and if a protease inhibitor (increasing SVR rates to 75 and 51% in naïve and non-responders HCV-1 patients) became available, HCV mortality would be reduced by 15 additional % (76,000 deaths, 95% CI : 71,600-76,200). This impact would be highest in Spain and UK (19%), lowest in Italy (13%), and intermediate in France (14%), Belgium (14%), and Germany (16%).

Conclusion : Antiviral treatment will reduce HCV-related mortality by 13% in Europe in 2025, with marked heterogeneities between countries due to differences in treatment access. Ambitious strategies and the availability of a protease inhibitor may have a major impact on HCV-related mortality.

PURIFICATION OF LIVER PROGENITOR CELLS BY USING ALDEHYDE DEHYDROGENASE ACTIVITY. L. Dollé (1), J. Best (1), J. Mei (1), C. Empsen (1), E. Van Rossen (1), M. Najimi (2), S. Snykers (1), K. Streetz (3), E. Sokal (2), V. Rogiers (1), I. Leclercq (2), L. Van Grunsven (1). (1) Free University (VUB), Brussels, Belgium ; (2) Université Catholique de Louvain, Brussels, Belgium ; (3) University Hospital Aachen, Aachen, Germany.

Introduction : In vivo, an oval cell response or liver progenitor cell (LPC) activation can be observed in situations where hepatocytes are incapable of restoring liver function by proliferation. One of the promises of these LPCs is linked to their potential to provide a renewable source of hepatocytes.

Aim : We have optimized a functional assay based on ALDH activity to enrich LPCs from normal mouse and human livers.

Methods : Mouse livers were perfused and LPCs were isolated from the non-parenchymal fraction by means of the ALDEFLUOR substrate using Flow Cytometry-Activated Cell Sorting. Isolated cells were characterized by Immunohistochemistry and qPCR. The hepatocyte differentiation capacity was tested in vitro using a time-dependent exposure to insulin, HGF, OSM and dexamethasone. For human material, the non-parenchymal fraction was sorted for high ALDH activity and analyzed similarly. Immunohistochemistry for ALDH1A1 was performed on paraffin-embedded mouse and human liver tissue.

Results : Hepatic progenitors were isolated from Balb/C mice based on high ALDH activity and represent a small population (2-2.5%) with a high percentage of LPC markers like CK19 (35%), EpCAM (40%) and ABCG2 (30%). *In vitro*, the ALDH^{HIGH} cells are able to give rise to hepatocyte-like cells as demonstrated by glycogen storage, lipid metabolism and albumin secretion. In human liver samples, we can identify a similar ALDH^{HIGH} population expressing human stem cell markers. ALDH1A1 expression can be detected in CK19 positive cells in bile ducts and the “Canal of Hering” in mouse. Liver injury due to different hepatic insults leads to a rapid increase of ALDH1A1 expression in CK19 positive cells.

Conclusion : ALDH^{HIGH} cells are able to differentiate into hepatocytes, found in Canal of Hering and CK19+ cells when there is LPC expansion, thus ALDH^{HIGH} fraction might contain liver progenitors. The functional LPC isolation procedure based on ALDH activity is highly reproducible, applicable to human material and does not involve antibody recognition nor the use of DNA intercalating dyes. Furthermore, it will allow the isolation of human LPCs and the study of their activation in vitro. Comparison with other LPC isolation techniques and the potential of the ALDH^{HIGH} population to rescue a damage liver will be addressed.

ARE PATIENTS WITH HBV AND HCV INFECTION DIFFERENT ? COMPARISON BETWEEN 2 COHORTS OF NEWLY DIAGNOSED CASES INCLUDED IN PROSPECTIVE REGISTRIES OF THE BELGIAN ASSOCIATION FOR THE STUDY OF THE LIVER (BASL). B. De Vroey (1), C. Moreno (2), W. Laleman (3), M. Van Gossum (4), I. Colle (5), C. De Galocsy (6), P. Langlet (7), G. Robaey (8), H. Orlent (9), P. Michiels (10), J. Delwaide (11), H. Reynaert (12), M. Adler (2), J. Henrion (1), P. Deltenre (1). (1) Hôpital de Jolimont, Haine-Saint-Paul, Belgium ; (2) ULB Erasme, Brussels, Belgium ; (3) KUL, Leuven, Belgium ; (4) CHU Saint-Pierre, Brussels, Belgium ; (5) UZ, Gent, Belgium ; (6) Hôpital Iris Sud Bracops, Brussels, Belgium ; (7) CHU Brugmann, Brussels, Belgium ; (8) Ziekenhuis Oost-Limburg, Genk, Belgium ; (9) AZ Sint-Jan, Brugge, Belgium ; (10) UZ Antwerpen, Edegem, Belgium ; (11) CHU Liège, Liège, Belgium ; (12) UZ, Brussels, Belgium.

Introduction : Hepatitis B (HBV) and C (HCV) infections share many epidemiological and clinical similarities but exhibit also important differences. Moreover, their epidemiological characteristics are evolving in western countries. Nationwide studies comparing representative samples of patients newly diagnosed with HBV or HCV infections have not been reported.

Aim : To compare the main epidemiological, biological and histological characteristics of patients with newly diagnosed HBV or HCV infection in Belgium, and to compare their management.

Methods : Data of patients with newly diagnosed HBV or HVC infection were extracted from two Belgian registries (HBsAg carriers registry, 2008-2009 and observational survey of hepatitis C, 2003-2004).

Results : 705 patients (387 with HBV and 318 with HCV) were included. Compared to HCV patients, HBV patients were younger (36 vs. 44 years, $p < 0.0001$), more frequently male (69 vs. 56%, $p < 0.0003$), less frequently of Caucasian origin (43 vs. 86%, $p < 0.0001$), more frequently black Africans (32 vs. 9%, $p < 0.0001$), less frequently contaminated by transfusion or IV drug use (9 and 6% vs. 33 and 43%, respectively, $p < 0.0001$), more frequently contaminated by sexual or familial transmission (40 and 30% vs. 1 and 1% respectively, $p < 0.0001$). HBV patients had higher rates of normal ALT (65 vs. 36%, $p < 0.0001$), lower rates of ALT > 2ULN (15 vs. 38%, $p < 0.0001$), and lower rates of detectable viral nucleic acid by PCR (70 vs. 84%, $p < 0.0001$) than HCV patients. A liver biopsy was performed in 303 patients (in 29% of HBV patients and in 61% of HCV patients, $p < 0.0001$). Twenty-five percents of the patients had extensive fibrosis or cirrhosis (F3/4) (32% of HBV patients, 21% of HCV patients, $p = 0.04$). In multivariate

analysis, significant predictors of F3/4 were : older age ($p = 0.003$), male sex ($p = 0.02$), HBV infection ($p = 0.03$), ALT $> 2ULN$ ($p = 0.01$) and activity score ≥ 2 ($p = 0.004$). HBV patients were less frequently considered for treatment (25 vs. 47%, $p < 0.0001$) than HCV patients.

Conclusion : Newly diagnosed HBV and HCV patients disclosed different epidemiological characteristics that should be taken into account for screening. Management of HBV and HCV patients differed, HBV patients undergoing less frequently a liver biopsy and being less frequently considered for treatment.

- A22 -

POST TRANSPLANT IMMUNISATION FOR HEPATITIS B VIRUS. RATHER IM THAN IV. X. Verhelst, I. Colle, A. Geerts, R. Troisi, X. Rogiers, H. Van Vlierberghe. UZ, Gent, Belgium.

Introduction : Post transplantation immunisation with Hepatitis B immunoglobulin (HBIg) is indispensable after liver transplantation for Hepatitis B virus (HBV) infection. Intravenous (IV) administration once monthly used to be the agent of choice. However there is growing evidence that the intramuscular (IM) route is equivalent in preventing relapse and is more convenient and less expensive.

Aim : We wanted to evaluate the efficacy of IM HBIg in the prevention of relapse of hepatitis B virus infection after liver transplantation and identify characteristics of the relapsers.

Methods : Between 1999 and 2009 13 patients underwent liver transplantation related to a hepatitis B virus infection. During follow up 11 of them were switched from IV (Hepacaf® 5000 IU) to IM (HepBquin® 500 IU) administration in the second half of 2009. The number of relapsers and their characteristics were evaluated.

Results : Two out of eleven patients (18,18%) developed a relapse after switch from IV to IM administration, respectively after 4 and 7 months. The first one was a 55 year old male transplanted for a HBV related hepatocellular carcinoma (Child A5, Meld 8, HBs and HBe Ag positive, viral load 2983 IU/ml). Lamivudin was initiated on the waiting list. He relapsed 7 months after switch to the IM route, HBsAb titer was 19 and viral load was 90 IU/ml. He was treated with IV HBIg and tenofovir but viral load remains 73 IU/ml. The second patient, a 60 year old male, was transplanted because of a decompensated liver cirrhosis (Child B9, Meld 12, precore mutant, viral load 9020×10^5 IU/ml). Lamivudin was initiated before transplantation with adefovir add-on therapy after transplantation. He relapsed 4 months after switch to the IM route. HBsAb was 44 and HBV DNA $4,44 \times 10^7$ IU/ml. He was successfully treated with switch to the IV route. We could not identify specific characteristic of the relapsers, although the first patient was the one with the lowest HBsAb titer of the cohort.

Conclusion : IM administration of HBIg after transplantation is an efficient, cost-effective and convenient alternative for the IV route. In this small cohort two patients out of 11 patients did relapse without affecting mortality or morbidity. We could not identify prognostic markers for relapse.

A PROSPECTIVE STUDY COMPARING THE DIFFERENT INDIRECT METHODS TO MEASURE PORTAL PRESSURE. G. Maleux (1), E. Willems (1), S. Heye (1), W. Laleman (2), D. Cassiman (2), C. Verslype (2), F. Nevens (2). (1) UZ Leuven Departement of Radiology, Leuven, Belgium ; (2) UZ Leuven Departement of Hepatology, Leuven, Belgium.

Introduction : Wedged hepatic pressure (WHVP) measurement is a reliable technique to estimate portal pressure in patients with cirrhosis. WHVP can be registered by occluding the hepatic vein either by an end-hole catheter (WHVP-cath) or by balloon catheter (WHVP-balloon).

Aim : The aim of the present study was to compare the different methods of portal pressure measurement and to investigate the factors which affects the discrepancies.

Methods : This is a prospective trial from 2007-2010 which compare the values obtained by WHVP-cath and WHVP-balloon with direct portal pressure measurement at the moment of TIPS placement in patients with cirrhosis. The latter was confirmed by transjugular biopsy which was performed at the same moment.

Results : The study population consists of 181 patients : mean age $56,5 \pm 12,7$, male/females : 119/62, Child-Pugh class (A/B/C) : 29/81/71 and mean MELD 14 ± 7 . Indications for TIPS were refractory ascites (n = 98), variceal bleeding (n = 78) or a combination (n = 5). The etiology of cirrhosis was : alcohol n = 135, NASH n = 14, viral hepatitis = 10, others = 14 and PCB n = 8. These last patients were analyzed separately because a pre-sinusoidal component of portal hypertension was expected. The results are summarized in the table

| | |
|------------------|-------------------------------------|
| FHVP (mmHg) | 10.3 ± 5.5 (Q1 : 7 - Q3 : 14) |
| WHVP | |
| • Cath (mmHg) | 31.3 ± 14.3 (Q1 : 22 - Q3 : 37) |
| • Balloon (mmHg) | 25.8 ± 7.9 (Q1 : 21 - Q3 : 30) |
| PP-direct (mmHg) | 24.5 ± 7.6 (Q1 : 21 - Q3 : 30) |

Q1 = percentile 25, Q3 = percentile 75

In patients with cirrhosis due to PBC the hemodynamic data were : FHVP (mmHg) : 11.5 ± 7.0 , WHVP-cath (mmHg) : 23.0 ± 8.1 , WHVP-balloon (mmHg) : 24.3 ± 9.9 and PP-direct (mmHg) : 28.6 ± 10.1 .

The median absolute differences in comparison with the direct PP measurement was 6.0 with the end-hole catheter and 2.0 with the occlusion balloon ($P < 0.0001$ signed rank test). With the balloon catheter an overestimation was seen in case of higher MELD and higher Child Pugh score ($P = 0.0005$) and an underestimation in case of PBC.

Conclusion : The best method to estimate portal pressure is with the balloon-catheter. However, with this technique in case of patients with advanced liver disease there is an overestimation of the portal pressure and in case of PBC there is an underestimation.

LIVER PROGENITOR CELL EXPANSION IN THE DISEASED LIVER : DO ASSOCIATED MYOFIBROBLASTS DERIVE FROM PROGENITOR CELLS ? R. Español Suárez, N. Van Hul, J. Abarca-Quiñones, I. Leclercq. UCL Saint-Luc, Brussels, Belgium.

Introduction : In diseased liver, expansion of the liver progenitor cell (LPC) compartment is associated with and appears to be dependent on deposition of extracellular matrix and myofibroblasts (MF) activation.

Aim : Our aim was to clarify whether those MF may derive from phenotypic conversion of LPCs through epithelial mesenchymal transition (EMT) mechanism.

Methods : We evaluated the dynamics of expression and topographical localization of EMT markers (fibroblast-specific protein 1 FSP-1, Snail, nuclear β -catenin...) in the murine CDE (Choline-Deficient, Ethionine-supplemented diet) model of LPC expansion by immunohistochemistry, immunofluorescence and RT-PCR analyses.

Results : In control mice, FSP1 is expressed in MF of blood vessels. After 5 days of CDE diet when LPCs start to expand, we observed numerous small FSP-1+ cells in parenchymal zone 2 forming a ring at a distance from the portal tract. Those cells had two distinct phenotypes : some were round and intensely stained while other were elongated and located in front of CK19+ LPCs. At 14 days of CDE diet, numerous FSP1-rounded shape cells were observed, but the elongated (peri-)sinusoidal cells were no longer present. At all times, FSP-1+ cells were found outside the area of LPCs proliferation. We were unable to find CK19/FSP1 double positive cells whether in controls, at an early or more advanced stage of LPCs expansion. Also, along the timeline, no nuclear β -catenin accumulation was seen nor did we observe a significant change in expression of EMT transcription factor Snail by RT-PCR compared to controls.

Conclusion : Our data do not support that, in this CDE model, MF around LPCs derive from a conversion of LPCs by EMT but rather from expansion of the existing portal MF and stellate cells.

UTILITY OF GAS CHROMATOGRAPHY/MASS SPECTROMETRY TO DETECT BREATH BIOMARKERS IN PATIENTS WITH LIVER IMPAIRMENT : PRELIMINARY DATA. J. Dadamio (1), W. Laleman (2), S. Van Den velde (1), D. Cassiman (2), P. Van Hee (3), M. Coucke (4), M. Quirynen (1), F. Nevens (2). (1) UZ Leuven Department of Periodontology, Leuven, Belgium ; (2) UZ Leuven Department of Hepatology, Leuven, Belgium ; (3) ZNA Laboratory for Biochemistry and Toxicology, Antwerpen, Belgium ; (4) Department of Clinical Biology - Scientific Institute of Public Health, Brussels, Belgium.

Introduction : Adequately assessing liver function remains a difficult clinical issue.

Aim : This study aimed to determine whether a specific pattern of biomarkers can be found in breath samples of liver patients using gas chromatography – mass spectrometry (GC/MS).

Methods : Alveolar air samples were collected from 3 independent groups : 49 healthy volunteers (29 ♀ , 8 smokers), 35 out patients established liver cirrhosis (14 ♀ , 9 smokers, alcoholic n = 26, mean Child quantitative 8.8 ± 2.1 , mean MELD 16.6 ± 7.8) and 12 patients with indication of transjugular intrahepatic portosystemic stent shunt (TIPSS) (4 ♀ , 5 smokers, mean MELD 14.3 ± 4.8 , mean Child 7.6 ± 1.2 , etiology post-alcoholic n = 11). The breath samples were analyzed by means of thermal desorption coupled with GC/MS.

Results : From the 881 compounds detected at least once, 67 were presents in at least half of the patients and 28 of them showed a significant difference between healthy volunteers and liver patients (Mann Whitney U-test with Bonferroni correction). A linear discriminant analysis model was built to discriminate between healthy volunteers and patients with established liver cirrhosis by means of the detected compounds. Volunteers and patients were randomly assigned to either a training set in order to create a model, or to a prediction set to validate the model. The set of patients with TIPSS indication was considered as an independent group and used to test the model(s). A total of 5959 models of 8 independent compounds were identified that discriminate well between the groups. When the models were applied to the independent data set, 24 models showed a specificity of 100% and a sensitivity of 83.3%. Considering the initial data set sensitivity and specificity of the models vary between 82% and 88%, and 96% and 100%, respectively. The compounds that appear on these models include : ketones (acetone and 2-butanone), sulphur compounds (dimethyl sulphide), alkanes (octane, nonane, and branched chain alkanes), alkenes (2-methyl-1propene), terpenes and terpenoides (isoprene

and pinenes), aromatics (styrene and indole), alcohols (phenol) and dimethylselenide. All compounds with exception of indole, phenol and dimethyl selenide were significantly higher in breath of liver patients than in the healthy population. **Conclusion** : Breath-analysis by means of GC-MS represent a potential new non-invasive tool to evaluate liver disease/insufficiency by means of a currently identified panel of highly sensitive and liver-specific signature of bio-markers. Studies to assess the real utility of these markers for diagnosis and follow-up of liver pathologies are in progress.

- A26 -

HEPATITIS B REACTIVATION FOLLOWING KIDNEY TRANSPLANTATION : INCIDENCE AND RISK FACTORS. Z. Hassoun, B. Kabamba, C. Maréchal, E. Goffin, N. Kanaan. Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Introduction : Limited data is available on the risk of hepatitis B virus (HBV) reactivation in patients with resolved HBV infection undergoing renal transplantation.

Aim : The aim of this study was to evaluate the incidence of HBV reactivation in such patients, and to identify potential risk factors for reactivation.

Methods : We retrospectively reviewed the charts of all adult patients who underwent kidney transplantation between January 1995 and December 2007. Diagnostic criteria for resolved HBV infection were : HbsAg negative, anti-HBc Ab positive, anti-HBs Ab positive or negative, and normal liver enzymes. HBsAg reversion was defined as the appearance of HbsAg during follow-up. HBV reactivation was defined as HbsAg reversion with serum HBV DNA > 2,000 IU/mL.

Results : Ninety three patients with resolved hepatitis B at time of transplantation were included. Mean age was 53 (\pm 12) years, and 61% were male. Mean duration of follow-up was 6.5 (\pm 3.5) years. Four patients experienced HBsAg reversion followed by HBV reactivation. HBsAg reversion occurred within the first year following transplantation in 3 patients. Graft survival was not affected by reactivation. Immunosuppression therapy was similar in patients with and without reactivation. The incidence of acute rejection was significantly higher in patients with HBV reactivation ($p = 0.005$). Two patients with HBV reactivation (50%) were born in North Africa and 2 were Caucasian, compared to 72% Caucasians and 10% North Africans in the group without reactivation ($p < 0.05$). HBV genotype D was identified in two patients with reactivation and genotype A in one. Among the 4 patients with HBV reactivation, only one (25%) was anti-HBs Ab positive before transplantation, compared to 82% of patients without reactivation ($p = 0.03$). In this patient, anti-HBs Ab titre decreased progressively and HBsAg reversion occurred when anti-HBs Ab disappeared.

Conclusion : HBsAg reversion occurs after renal transplantation with an incidence of 0.7 per 100 patient-year and is followed by reactivation. This intervenes mainly in the first post transplantation year. Ethnic background, acute rejection episodes, and absence of anti-HBs Ab before transplantation appear as risk factors for reactivation. Patients with resolved hepatitis B but without protective anti-HBs Ab should receive vaccination.

DIAGNOSTIC PERFORMANCE OF TRANSIENT ELASTOGRAPHY (FIBROSCAN) TO IDENTIFY PATIENTS ELIGIBLE FOR ANTIVIRAL TREATMENT IN CHRONIC HEPATITIS B. V. Leroy (1), M. Adler (2), P. Marcellin (3), N. Sturm (1), C. Moreno (2), P. Bedossa (3), A. Cheveau (1), J.P. Zarski (1), T. Asselah (3). (1) CHU, Grenoble, France ; (2) ULB Erasme, Brussels, Belgium ; (3) INSERM Bichat-Beaujon, Paris, France.

Introduction : According to EASL guidelines, antiviral treatment is recommended when histological lesions are greater than A1 or F1. Non-invasive methods to identify such patients were not evaluated.

Aim : To evaluate the diagnostic performance of TE to identify patients eligible for antiviral treatment.

Methods : 418 CHB patients were recruited from 3 centres (Grenoble, Beaujon and Brussel). Criteria for inclusion were : ALT > N or HBV DNA > 2,000 IU/ml, available liver biopsy, and valid TE. Exclusion criteria were antiviral treatment, HDV, HIV and HCV co-infections, and other liver diseases.

Results : Characteristics of patients were : age 42 years, men 72%, HBeAg(+) 28%, ALT 92 IU/ml (> N :78%), HBV DNA 6.4 log IU/ml, > F1 51%, F4 11%. Eligibility to treatment was met in 62% of patients. Diagnostic performance of TE (AUROC) was 0.78 (CI95% 0.75-0.81). Using a low cut-off of 5 kPa, sensitivity was 83% and NPV was 63% to rule out indication of treatment. Using a high cut-off of 8 kPa, specificity was 91% and PPV was 92%. In order to optimize the diagnostic performance, we stratified patients according to HBV DNA and ALT levels. For HBV DNA < 4 log IU/ml, 5-6 log and > 6 log the rates of treatment eligibility were 53%, 66% and 63% respectively (NS). By contrast, ALT levels were associated to histological lesions with rates of treatment eligibility of 38% (ALT < N), 62% (N < ALT < 2N) and 74% (ALT > 2N), $p < 0.001$. For patients with ALT < N, sensitivity (82%) and NPV (59%) did not improve using the lower cut-off, while specificity (82%) and PPV (81%) worsen. By contrast, in patients with ALT > 2N, higher cut-off (8 kPa) gave a 100% specificity and a 100% PPV.

Conclusion : Sixty percent of patients who meet the EASL criteria for liver biopsy have significant histological lesions (> A1 and/or F1). The sensitivity of TE is not sufficient to rule out such lesions even in patients with ALT < N and/or HBV DNA < 4 log, and a liver biopsy remains mandatory in this situation. By contrast, specificity of TE (> 8 kPa) to diagnose significant lesions is excellent especially in patients with ALT > 2N.

RISK ASSESSMENT OF POSTHEPATECTOMY LIVER FAILURE USING PREOPERATIVE SERUM CHOLINESTERASE. T. Chapelle, G. Roeyen, K. De Greef, B. Op De beeck, I. Hyughe, A. Verbruggen, T. Lambrechts, N. Boeykens, D. Ysebaert. UZ Antwerpen, Edegem, Belgium.

Introduction : Postoperative acute liver failure is a life-threatening complication inpatients, undergoing major hepatectomy. Not the future remnant liver volume as such but the function of the remnant liver is important in the prevention of complications after hepatectomy. However, accurate diagnostic tools that can predict the risk of liver resection-related morbidity are still lacking.

Aim : The purpose of this study was to evaluate preoperative serum cholinesterase of the future remnant liver in patients undergoing liver resection. Postoperative morbidity and liver failure were evaluated in these patients.

Methods : Between October 2008 and August 2010, 61 patients were included. Liver failure was defined as PT < 50 ; bilirubin > 50 μ mol/l, ammonium > 50 μ mol/l and therapy-resistant ascites, from day 5 until 3 months after hepatectomy. Preoperative MRI volumetry was used to measure the volume of the total liver (TLV), the tumor volume and the future remnant liver volume (FRLV) and expressed in ml. Preoperative serum cholinesterase recalculated to the remnant liver volume (FRL-cholinesterase). Receiver-operating characteristic analysis was performed to assess cutoff values for risk assessment of morbidity and liver failure.

Results : Liver failure occurred in 8 patients. The volume of the future remnant was not significantly associated with liver failure and severe complications. FRLV below 40% had a low positive predictive value of 25% and a negative predictive value of 94% to predict liver failure and a low positive predictive value of 16 % to predict serious liver-related complications. In contrast, patients with liver failure had significant lower FRL-cholinesterase levels than patients without liver failure (2532 ± 1277 vs 5635 ± 2917 respectively, $p < 0.001$). Fifteen patients had severe complications. Patients with severe complications had significant lower FRL-cholinesterase levels than patients without severe complications (3894 ± 2286 vs 5635 ± 2917 respectively ; $p < 0.05$). ROC curve analysis showed that FRL-cholinesterase below 2100 had a positive predictive value of 80% and a negative predictive value of 93% for the development of liver failure. Likelihood ratio for a positive test result was 25.

Conclusion : Hence, preoperative measurement of serum cholinesterase recalculated for the future remnant liver proved more valuable than measurement of the volume of the future remnant on MRI in assessing the risk of liver failure and liver related severe complications after partial liver resection. Therefore, preoperative measurement of serum cholinesterase recalculated for the future remnant liver is a valuable tool to predict liver-resection related morbidity.

INTEGRATED ANALYSIS OF MIRNA AND MRNA EXPRESSION DURING IN VITRO MOUSE HEPATIC STELLATE CELL ACTIVATION. B. Schroyen (1), I. Mannaerts (1), A. Noetel (2), M. Odenthal (2), L.A. van Grunsven (1). (1) UZ Brussel Vrije Universiteit, Brussels, Belgium ; (2) University Hospital of Cologne, Cologne, Germany.

Introduction : Hepatic stellate cell (HSC) activation is a transdifferentiation from a quiescent to a myofibroblastic phenotype as a consequence of chronic liver injury. The main phenotypical changes characterizing the HSC activation are loss of vitamin A containing lipid droplets and extensive production of extracellular matrix. The clarification of this process is important for the development of effective therapies for fibrosis. A large set of genes are differentially expressed when quiescent HSCs are compared with activated myofibroblasts. In an earlier study, we have shown that inhibition of class I HDACs by valproic acid (VPA) inhibits HSC activation in vitro and in vivo (Mannaerts I., *et al.* (2010) *Hepatology* 51, 603-614).

Aim : Here, we evaluated the gene expression and miRNA profile of control and VPA-treated cells, using respectively the mouse GeneChip Mouse Gene 1.0 ST Array (Affimetrix) and the TaqMan Rodent MicroRNA Array v2.0 (Applied Biosystems) in order to further identify genes that play a role during HSC activation.

Results : In order to identify direct and/or indirect VPA target genes in HSCs, we compared the gene expression profile of freshly isolated mouse HSCs cultured in control conditions with the expression profile of cells cultured in the presence of VPA for 4 and 64 hours. Gene expression patterns of the different samples were generated using the (GeneChip Mouse Gene 1.0 ST Array (Affimetrix)). Analysis of these patterns was performed using the Agilent GeneSpring GX v11 software in combination with Microsoft Excel 2007. Direct targets are genes not differentially expressed during HSC activation and up-regulated by VPA or genes down-regulated during HSC activation and not down-regulated after VPA treatment. Indirect targets were defined as genes that are up-regulated/not changed during activation and down-regulated by VPA. GeneSpring GX was used to define groups of genes that were unchanged or differentially expressed during HSC activation or by VPA. Basic functions of Excel combined with virtual basic scripting were used to filter out potential target genes. For miRNA profiling, the TaqMan Rodent MicroRNA Array v2.0 was used on control- and VPA-treated primary hepatic stellate cells cultured for 4 h, 1 day and 5 days. Analysis was performed with the Real Time Statminer program. Potential targets of differentially expressed microRNAs were identified using Targetscan and DIANA - mirPath. The expression pattern of these genes was verified in our microarray data and eventually confirmed by RTQ-PCR on in vitro and in vivo activated HSCs.

Conclusion : The functional relationship of the miRNAs with their target genes will be further investigated by influencing endogenous miRNA levels using either miRNA mimics or antagomirs or regulate the target genes by siRNA mediated knock-down in order to evaluate involvement during HSC transdifferentiation.

LAPAROSCOPIC LIVER RESECTION : MONOCENTRIC UNIVERSITY EXPERIENCE. D. Szeceł, M. Arenas, A. Deroover, J. Delwaide, M. Polus, A. Kaba, P. Honoré, O. Detry. CHU de Liège, Liège, Belgium.

Introduction : Laparoscopic liver resection (LLR) is becoming more frequent for benign and malignant liver tumors. Indications and outcomes still need to be evaluated.

Aim : The aim of this study was to review the results in LLR of a Belgian university hospital.

Methods : We performed a retrospective analysis of a continuous series of LLR performed between 2003 and July 2010. This series did not include any liver biopsies of radiofrequency ablation (RFA) without associated hepatic resection.

Results : There were 42 LLR in 41 patients, including 25 (59,5%) benign lesions (14 HNF, 4 hydatid cysts, 3 polyadenomatosis, 1 angioma, 1 adenoma, 1 inflammatory pseudo-tumor and 1 other) and 17 malignant lesions (40,5%) (10 metastases and 7 hepatocarcinoma). The mean age was 50.2 years (26-80). LLR constituted in a minor resection in 35 patients (83,3%) (14 tumorectomies or wedge resections, 10 segmentectomies, 2 bisegmentectomies except II-III, 9 bisegmentectomies II-III) and a major resection, including 5 right hepatectomies, in 7 patients. In 18 patients (42,9%), LLR was associated with an other surgery (cholecystectomy, adrenalectomy, appendicectomy, ileostomy reintegration, incisionnal hernia, ovarian kystectomy, right annexectomy, rectosigmoïdectomy, sigmoïdectomy, and tubal ligation) and in 2 patients with radiofrequency. There were 3 conversions and 1 per-operative and 13 post-operative complications (31%) (1 bile leak). None needed intensive care. Median OR time was 163 minutes. Transfusion was required in 3 patients (7,1%). Median hospital length of stay was 6 days (3 – 36). Negative margins were achieved in 100%. Overall survival at 1 and 3-years was 100% in benign lesions and respectively 91,7 % and 73,3% in malignant lesions.

Conclusion : LLR is safe and effective for benign lesions as well as malignant ones, even when associated with other surgeries, when performed by experimented surgeons. The interest of LLR has to be further studied, especially for right hepatectomies.

“GASTROINTESTINAL REGULATORY MECHANISMS”

Invited lecture

- B01 -

PHYSIO(PATHO)LOGY OF THE GASTROINTESTINAL MUSCULATURE, A DEVELOPMENTAL PERSPECTIVE. P. De Santa Barbara. Arnaud de Villeneuve, Montpellier, France.

Introduction : The motility of the digestive tract is ensured by the correct coordination of the autonomous enteric nervous system (ENS), the interstitial cells of Cajal (ICC) and the visceral smooth muscle cells (SMC). During the development, these different cell types develop in coordination in order to create a functional bowel. The maldevelopment of at least one cell type will result in gastrointestinal neuromuscular pathologies (Knowles *et al.*, 2010).

Aim : At early developmental stage, the visceral SMCs originate first from the splanchnopleural mesoderm that forms the primitive visceral mesenchyme via Hedgehog/Bone Morphogenetic Protein (BMP) signaling pathway. Under specific signals, the visceral precursor mesenchymal cells start to differentiate and the earliest morphological signs of visceral SMC differentiation are their elongation and clustering organization. Their subsequent differentiation is characterized by the expression of SMC-specific lineage markers such as Smooth Muscle α -Actin (α SMA), SM22, calponin, smoothelin and smooth muscle myosin heavy chain (SM-MHC), which precedes contractile function.

Methods : These last years, the molecular mechanisms that control the differentiation of visceral mesenchyme into SMCs have been investigated using different animal models.

Results : Endodermal Hedgehog signaling activation promotes through receptor Patched and BMP4 expression the mesenchymal growth of visceral mesenchyme that conducts to their SMC differentiation (de Santa Barbara *et al.*, 2005). FGF signaling pathway is activated in the visceral mesenchymal cells and specifically down-regulated during the differentiation of the visceral SMCs. Sustained activation of the FGF pathway *in vivo* blocks the visceral SMC differentiation (Le Guen *et al.*, 2009). These data highlight that different signaling pathways tightly regulate visceral SMC differentiation process.

Conclusions : These studies have shown that the molecular mechanisms that control the visceral SMC differentiation are conserved in vertebrates. Alterations in these molecular controls are associated with perturbations of the visceral smooth muscle integrity and could be responsible for human gastrointestinal dysmotility.

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EXPRESSION AND DISTRIBUTION OF MAS-RELATED GENE RECEPTORS IN THE NON-INFLAMED AND INFLAMED MURINE ILEUM. L. Avula (1), L. Van Nassauw (2), E. Stuyven (3), R. Buckinx (1), K. Alpaerts (1), D. Adriaensen (1), H. Favoreel (3), E. Cox (3), J.P. Timmermans (1). (1) Laboratory of Cell Biology and Histology, Antwerp, Belgium ; (2) Laboratory of Cell Biology and Histology/Laboratory of Human Anatomy and Embryology, Antwerp, Belgium ; (3) Laboratory of Immunology, Ghent, Belgium.

Introduction : Mas-related gene receptors (Mrgs) constitute a complex family of orphan G protein-coupled receptors, some of which are predominantly expressed in spinal sensory neurons. Some Mrgs have also been suggested to participate in the IgE-independent activation of mast cells and mast cell-sensory nerve communication. There is a lack of data concerning the expression and function of Mrgs in the gastrointestinal tract during physiological and pathophysiological conditions.

Aim : To unravel the expression, distribution and possible role of Mrgs in the healthy non-inflamed and *Schistosoma mansoni*-infected murine ileum.

Methods : Immunohistochemical analyses were performed on cryosections and whole-mounts with custom-made polyclonal antisera directed against MrgA4, MrgB8 and MrgB10 as well as a commercially available antiserum directed against MrgD. The relative expression levels of MrgA4, MrgB8, MrgB10 and MrgD were quantitatively analysed using real-time PCR.

Results : Immunohistochemistry revealed no MrgB10 and MrgD expression in the non-inflamed ileum, whereas a moderate MrgA4 and MrgB8 immunoreactivity was detected in a few neuronal cell bodies and nerve fibres in the enteric plexuses. In the inflamed ileum, MrgA4, MrgB8, MrgB10 and MrgD were clearly observed on an increased no. of neuronal somata and nerve fibres in both enteric plexuses ($P < 0.05$), and also on nerve fibres in the tunica muscularis and the lamina propria. Colocalisation studies using antibodies directed against several neurochemical markers, demonstrated that MrgB4-, MrgB8-, MrgB10 and MrgD-immunoreactive neurons were predominantly intrinsic primary afferent neurons. In the inflamed ileum, MrgB10 and MrgD were also detected on mucosal mast cells (MMC). The immunohistochemical results were corroborated by real-time PCR, which demonstrated the upregulation of MrgA4, MrgB8, MrgB10 and MrgD mRNAs in the inflamed ileum. MrgB10 and MrgD mRNAs were exclusively detected in the inflamed ileum.

Conclusion : The increased expression of MrgA4, MrgB8, MrgB10 and MrgD in neurons and nerve fibres, as well as the presence of MrgB10 and MrgD on MMCs during inflammation support the hypothesis that Mrgs are involved in neuronal and mast-cell responses during intestinal inflammation.

IMMUNOHISTOCHEMICAL AND FUNCTIONAL EVIDENCE OF VAGAL IMMUNOMODULATION IN A MOUSE MODEL OF POSTOPERATIVE ILEUS. P.J. Gomez-Pinilla (1), M. Di Giovangiulio (1), G. Matteoli (1), C. Van Heijningen (1), C. Cailotto (1), J. Van Der vliet (2), G.E. Boeckxstaens (1). (1) Department of Gastroenterology University hospital Leuven KUL, Leuven, Belgium ; (2) Academic Medical Center, Amsterdam, Netherlands.

Introduction : Postoperative ileus (POI) is defined as the transient inhibition of co-ordinated bowel motility after abdominal surgery. The cholinergic anti-inflammatory pathway through vagus nerve exerts beneficial effects in a septic model and also in peritoneal macrophages after intestinal manipulation but the impact of the vagus nerve stimulation on gastrointestinal tract is less known.

Aim : To evaluate the effects of vagus nerve on POI. In addition, the distribution of vagus nerve terminals within the small intestine wall was investigated.

Methods : Vagus nerve was electrically stimulated (square pulse of 1ms duration at 5 Volt and 5 Hz) for 5 minutes before intestinal manipulation of the small intestine. Three hours after IM, the small intestine was harvested and the upregulation of pro-inflammatory genes (Tnf- α and Il 6) was evaluated using qPCR. Twenty-four hours after IM, gastrointestinal transit was determined using a 70KD fluorescent dextran and the number of mieloperoxidase (MPO) positive cells in the muscularis was quantified. Anterograde labeling of motor neurons in the dorsal motor nucleus of the vagus (DMV) was achieved by stereotactic injection of biotin-conjugated dextran amine (5%) in the DMV. Vagus nerve fibers were visualized using fluorescence-conjugated streptavidin alone or in combination with neuronal and/or macrophages markers (PGP, Chat, nNOS, F4/80) in full thickness small intestine sections or in whole mount devoid of mucosa.

Results : In our mouse model of POI, stimulation of the vagus nerve ameliorated surgery-induced inflammation detected as a reduction in Tnf- α and Il 6 genes expression (Tnf- α ; 3.90 vs 1.90, Il 6 ; 10 vs 5.4 ; IM vs VNS) and reduction of MPO positive cells in the muscularis after VNS (67.50 vs 17.33 ; IM vs VNS). Reduced inflammation was accompanied by a normalization of gastrointestinal transit measured as an increase in geometrical center (GC) (5.87 vs 8.67 ; IM vs VNS). Biotin-labeled vagal nerve fibers were identified in the myenteric plexus, but not the submucosal plexus. The vagus nerve was found within myenteric ganglia and vagus terminals innervate cholinergic neuronal bodies. A

dense network of F4/80-positive resident macrophages was identified surrounding myenteric ganglia but not in close association with vagus.

Conclusion : We confirm that the vagal anti-inflammatory pathway exerts beneficial effects reducing inflammation (*Tnf- α* and *Il 6*) and the number of MPO positive cells and normalize gastrointestinal transit. Also we present morphological evidences that vagus nerve synapses with cholinergic myenteric neurons suggesting an amplification of the vagal signal through the enteric neuronal network without direct contact between vagus and resident macrophages.

- B04 -

INFLAMMATORY NITRERGIC DYSFUNCTION EVOKES INTESTINAL DYSMOTILITY. T. Masaoka, T. Vanuytsel, S. Salim Rasoel, S. Kindt, W. Boesmans, G. De Hertogh, P. Vanden Berghe, J. Tack. KULeuven, Leuven, Belgium.

Introduction : The Biobreeding (BB) rat consists of a diabetes-resistant (BBDR) and a diabetes-prone (BBDP) strain, which is characterized by spontaneous development of diabetes in about half of the population. Loss of intestinal nitrergic motor control has been reported in BBDP (Zandecki 2008). We previously demonstrated that loss of jejunal neuronal nitric oxide synthase (nNOS) mRNA expression occurs in the BBDP, which is related to transient intestinal inflammation (myeloperoxidase : MPO activity) and inducible nitric oxide synthase (iNOS) expression, and which is independent from the development of hyperglycemia (Kindt DDW 2009). Studies in brain and enteric neuron cultures suggest that iNOS overexpression downregulates nNOS expression through oxidative stress (DeAlba 1999, Zandecki 2006).

Aim : As aminoguanidine (AG) is known to inhibit iNOS, investigated the effect of adding 5 g/l AG in the drinking water (20 weeks) in Normoglycemic BBDP (BBDP-N) rats and BBDR rats.

Methods : Jejunal inflammation, spontaneous motility both in the longitudinal and circular axis, and the number of nitrergic neuron were analyzed respectively by MPO measurements, organ bath (37°C) video analysis, NADPH-diaphorase staining and immunohistochemistry, respectively in control and AG treated groups.

Results : Compared to untreated BBDR rats (n = 7), untreated BBDP-N rats (n = 9) had higher MPO levels (1.1 ± 0.4 vs. 5.7 ± 1.4 U/mg, $p < 0.05$) and, fewer nitrergic neurons as identified by nNOS immunoreactivity (25.1 ± 5.4 vs. 6.3 ± 3.1 /10 ganglia, $p < 0.05$) and confirmed by NADPH-diaphorase staining (27.0 ± 1.4 vs. 5.9 ± 1.6 neurons /10 ganglia, $p < 0.01$). A 3 cm H₂O pressure stimulus caused elongation (8.2 ± 1.8 vs. 1.4 ± 0.4 mm, $p < 0.001$) and dilatation (5.7 ± 0.2 vs. 3.8 ± 0.3 mm, $p < 0.001$) of a 5-cm jejunum segment *in vitro* in BBDP-N but not BBDR rats. AG treatment in BBDP-N (n = 7) decreased MPO activity (5.7 ± 1.4 vs. 2.2 ± 0.3 U/mg, $p = 0.16$) and partially restored the number of nNOS immunoreactive neurons (6.3 ± 3.1 to 21.0 ± 7.4 /10ganglia), which did not differ significantly from untreated BBDP-N, ($p = 0.32$). AG treatment in BBDP-N also prevented the elongation (2.0 ± 0.4 vs. 8.2 ± 1.8 mm, $p < 0.05$) and limited the dilatation (5.0 ± 0.1 vs. 5.7 ± 0.2 mm, $p < 0.05$) observed in response to a pressure stimulus.

Conclusion : AG treatment counteracts the inflammation-induced nitrergic dysfunction and prevents intestinal dysmotility in BBDP-N.

Invited lecture

- B05 -

CAJAL BEYOND THE GUT : INTERSTITIAL CELLS IN THE URINARY SYSTEM. TOWARDS GENERAL REGULATORY MECHANISMS OF SMOOTH MUSCLE CONTRACTILITY ? K. Thornbury, G. Sergeant, M. Hollywood, N. Mchale. Dundalk Institute of Technology, Dundalk, Ireland.

Introduction : It is now widely held that the peristaltic movements of the gastrointestinal tract are initiated and coordinated by specialized pacemaker cells called interstitial cells of Cajal (ICC). ICC have also been identified throughout the urinary system, including the renal pelvis/proximal ureter, bladder and urethra. Although each part of the system serves a different function, ranging from peristalsis of the ureters, storage of urine by the bladder, and a sphincteric action by the urethra, they share a common mechanism in being able to generate phasic myogenic contractions. Even the urethra, often considered to be a 'tonic' smooth muscle, achieves an apparently sustained contraction by averaging numerous small asynchronous 'phasic' contractions in a manner that has been compared to the asynchronous recruitment of motor units in skeletal muscle. Unlike skeletal muscle, this activity can occur in the absence of any neural input, therefore implying the presence of an intrinsic pacemaker. That this pacemaker seems to have an electrical component is suggested by intracellular microelectrode recordings from urethral smooth muscle, which reveal the presence of electrical slow waves similar to those of the gastrointestinal tract (Hashitani *et al.*, 1996, Br. J. Pharmacol. 118 : 1627-32). To study the pacemaker mechanism further, we isolated single cells from the smooth muscle layer of the rabbit urethra and were surprised to find not only smooth muscle cells (SMC), but a second cell type comprising ~10% of the total (Sergeant *et al.*, 2000, J. Physiol. 526 : 359-66). The latter cells were branched and non-contractile and closely resembled published pictures of intestinal ICC (Langton *et al.*, 1989, PNAS 86 : 7280-84). Electrophysiological studies revealed that, while the isolated smooth muscle cells were electrically quiescent, the 'ICC' fired electrical slow waves that were strikingly similar to those observed in the whole tissue. The basis of this difference was the presence of a large pacemaker current involving the activation of calcium-activated Cl⁻ channels by spontaneous intracellular Ca²⁺ waves. These, in turn, have been shown to be modulated by neurotransmitters such as nitric oxide, noradrenaline and ATP, thus providing a possible mechanism whereby neural regulation of the urethra, as well as spontaneous tone, may be mediated via ICC.

- B06 -

THE FACILITATING EFFECT OF PRUCALOPRIDE ON CHOLINERGIC NEUROTRANSMISSION IN PIG GASTRIC CIRCULAR MUSCLE IS CONTROLLED BY PHOSPHODIESTERASES. E. Priem. Heymans Institute of Pharmacology, Gent, Belgium.

Introduction : The inotropic response to 5-HT₄ receptor stimulation with prucalopride in pig left atrium is very transient due to cAMP metabolism by phosphodiesterases (PDEs). The facilitating effect of prucalopride on cholinergic neurotransmission in pig gastric longitudinal muscle is sustained. Still, PDEs have been shown to be present in enteric neurons.

Aim : To investigate whether PDEs are controlling the signal transduction pathway of the facilitating 5-HT₄ receptors on cholinergic neurons in pig gastric circular muscle.

Methods : After removal of the mucosa, circular smooth muscle strips were prepared from the proximal stomach of male pigs. Submaximal cholinergic contractions or tritium outflow after incubation with [³H]-choline as a measure for acetylcholine release was induced by electrical field stimulation (EFS). For contractility : 10 s trains at 0.5 ms and 4 Hz ; for acetylcholine release : 2 min trains at 1 ms and 4 Hz.

Results : Prucalopride increased the amplitude of submaximal cholinergic contractions as well as acetylcholine release induced by EFS in a concentration-dependent way. The effect of prucalopride on cholinergic contractions was antagonized by the selective 5-HT₄ receptor antagonist GR113808, but not by the 5-HT₃ receptor antagonist granisetron or the 5-HT₁, 5-HT₂, 5-HT₅ and 5-HT₇ receptor antagonist methysergide ; the antagonism of prucalopride by GR113808 was confirmed in the release assay. The phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX) reduced the amplitude of the cholinergic contractions in a concentration-dependent way ; IBMX (3 μM) reduced the cholinergic contractions by 16% but it enhanced the facilitating effect of prucalopride from 51 to 83%. IBMX (10 μM) had no influence per se on the electrically induced acetylcholine release but it induced and enhanced the facilitating effect of prucalopride.

Conclusion : Prucalopride enhances acetylcholine release in the porcine gastric circular muscle via 5-HT₄ receptors ; this facilitating effect of prucalopride is enhanced by IBMX, indicating that the porcine gastric 5-HT₄ receptors are also under regulatory control of PDEs.

RELAXANT EFFECT OF BAY 41-2272 AND BAY 58-2667 IN THE GASTRIC FUNDUS OF 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 Molecular Biomedical Research, VIB, Zwijnaarde, Belgium.

Introduction : In vascular tissue, the relaxant effect of the NO-independent but heme-dependent sGC stimulator BAY 41-2272 is reduced in mice with oxidized sGC, while that of the NO- and heme-independent sGC activator BAY 58-2667 is increased. Whether this also applies in the gastrointestinal tract has not been investigated. In apo-sGC mice, his at position 105 of sGC β 1 is mutated to phe ; both sGC isoforms (α 1 β 1 and α 2 β 1) are heme-deficient and can no longer be activated by NO ; this can be considered as a model for oxidized sGC.

Aim : To investigate the relaxant effect of BAY 41-2272 and BAY 58-2667 in the gastric fundus of wild-type (WT) and apo-sGC mice.

Methods : Homozygous apo-sGC mice and wild type (WT) controls were derived from a heterozygous breeding (mixed 129/SvJ-C57BL/6J, 11-15 weeks). Circular smooth muscle strips of the fundus were mounted in organ baths with aerated Krebs solution (NANC conditions) and incubated with the sGC inhibitor 1H[1,2,4,]oxadiazolo [4,3-a]quinoxalin-1-one (ODQ, 10 mM) or its solvent for 30 min. Strips were then pre-contracted with PGF_{2a} (3×10^{-7} M) and the relaxant effect of cumulatively administered BAY 41-2272 (10^{-8} M to 10^{-5} M) and BAY 58-2667 (10^{-9} M to 10^{-7} M) was examined.

Results : BAY 41-2272 (from 10^{-6} M on) and BAY 58-2667 (from 10^{-9} M on) induced a sustained relaxing response, consisting of a decrease in tone. The relaxing effect of BAY 41-2272 was decreased in fundus strips of apo-sGC mice. In WT fundus strips, ODQ reduced the relaxant effect of BAY 41-2272 ; in fundus strips of apo-sGC mice, ODQ had no influence on BAY 41-2272. The relaxant effect of BAY 58-2667 was more pronounced in fundus strips of apo-sGC mice. ODQ increased the relaxant effect of BAY 58-2667 in WT fundus strips, but it had no influence on the relaxant effect of BAY 58-2667 in fundus strips of apo-sGC mice.

Conclusion : At the level of the gastric fundus, BAY 58-2667 is more efficient when sGC is in the heme-free condition.

DEOXYCHOLIC ACID IMPAIRS ESOPHAGEAL MUCOSA INTEGRITY VIA APOPTOSIS INDUCTION. N. Pardon (1), M. Vicario (2), M. Vieth (3), H. Vanheel (1), T. Vanuytsel (1), K. Blondeau (1), D. Sifrim (4), M. Jimenez (5), J. Tack (1), R. Farré (1). (1) Translational Research Center for Gastrointestinal Disorders KU Leuven, Leuven, Belgium ; (2) Hospital General Universitario Valle Hebrón and Ciber-ehd del Instituto Carlos III, Barcelona, Spain ; (3) Bayreuth Hospital, Bayreuth, Germany ; (4) Barts and the London School of Medicine and Dentistry, London, United Kingdom ; (5) Universitat Autònoma de Barcelona Department of Physiology, Barcelona, Spain.

Introduction : The esophageal squamous epithelium consists of the basal or proliferative layer, the intermediate or prickly layer and the superficial layer. Non-erosive reflux disease (NERD) is the most common phenotype of gastro-esophageal reflux disease characterized by the presence of dilated intercellular spaces (DIS) mainly in basal cell layer that may lead to heartburn perception. Up to 50% of NERD patients fail to respond to acid suppressive therapy indicating that other gastric components may be responsible for symptom generation. In *in vitro* studies, deoxycholic acid (DCA) 2 mM at pH 5.0 disrupts esophageal mucosal integrity and provokes DIS (Farré 2008). Bile acids can also induce apoptosis in esophageal epithelial cell lines in a weakly acidic environment. However, the effect of bile acids *in vivo* has not been addressed.

Aim : The aim of the present study is to assess the effect of DCA on mucosal integrity and apoptosis induction in an *in vivo* esophageal perfusion model in rabbits.

Methods : New Zealand rabbits were anesthetized and esophageal perfusion was performed for 30 min with saline solutions at pH 7.2, pH 1.0, pH 5.0 and pH 5.0 + DCA 200 μ M and 500 μ M. Thereafter, rabbits were sacrificed and transepithelial mucosa resistance (TER) was determined in Ussing chambers. Finally, the mucosa was assessed by light and electron microscopy to determine diameter of intercellular spaces. Apoptosis was evaluated by TUNEL staining.

Results : After esophageal perfusion with control solution (pH 7.2) the TER was $2106 \pm 91 \Omega/\text{cm}^2$. Acidic solution (pH 1.0) decreased TER to $563 \pm 129 \Omega/\text{cm}^2$ ($p < 0.0001$, $N = 7$). Similar changes were provoked after perfusion with the solution at pH 5.0 + DCA 500 μ M ($367 \pm 45 \Omega/\text{cm}^2$, $p < 0.0001$, $N = 9$). Significant changes were also induced by the lowest concentration of DCA 200 μ M ($1412 \pm 114 \Omega/\text{cm}^2$, $p < 0.0001$, $N = 8$). None of the tissues showed erosions under light microscopy. Nevertheless, solutions at pH 1.0 provoked DIS in the basal and in the prickly cell layers, intercellular spaces widths at the basal layer were $0.01 \pm 0.002 \mu\text{m}$ after perfusion with pH 7.2 and were $0.28 \pm 0.06 \mu\text{m}$

($p < 0.05$, $N = 3$) after pH 1.0. In contrast, the solution at pH 5.0 + DCA 500 μM only provoked DIS in the basal cell layer ($0.15 \pm 0.02 \mu\text{m}$, $p < 0.05$, $N = 3$). In the prickle cell layer, ultrastructural abnormalities consistent with apoptosis (margination and condensation of chromatin, intact cell membrane and empty cytoplasm), which was confirmed with the TUNEL staining, were induced by DCA, but not by acidic solution.

Conclusion : Acid and deoxycholic acid in weakly acidic conditions impair mucosal integrity via a different underlying mechanism. The unconjugated deoxycholic acid in weakly acidic conditions disrupts esophageal mucosa integrity *in vivo* via induction of apoptosis, while acid induces DIS without apoptosis. The presence of apoptosis in the esophageal mucosa of NERD patients needs to be evaluated.

- B09 -

MEASURING NERVE ACTIVITY IN ROUTINE HUMAN INTESTINAL BIOPSIES. C. Cirillo, J. Tack, P. Vanden Berghe. Department of gastroenterology Catholic University Leuven, Leuven, Belgium.

Introduction : Morphological and functional abnormalities of the human enteric nervous system (ENS) have been described both in general neurological disorders and in gastrointestinal (GI) diseases. In particular, changes affecting neurons of the submucosal plexus (SMP) have been demonstrated in several inflammatory and motility disorders of the upper GI tract.

Aim : Therefore, we aimed to set up a new method to measure neuronal activity in human duodenal biopsies taken during routine endoscopy.

Methods : Biopsies were taken from macroscopically normal duodenal mucosa in 23 subjects (13 females, mean age 47 ± 21 ; 10 males, mean age 51 ± 21) during routine endoscopy of the upper GI tract using standard forceps with needle. To verify the presence of ganglia and assess the number of neurons present in these biopsies, the preparations were processed for immunocytochemistry, after removal of the mucosa. Neurons were identified by the expression of typical neuronal markers (neurofilament 200 (NF 200), Hu C/D). To confirm that we were able to collect viable neurons we designed a specialized chamber and measured changes in intracellular Ca^{2+} concentration with Fluo-4. To identify the neurons we used a brief depolarizing stimulus (high K^+ , 75 mM ; 5 s) that triggered the opening of voltage operated Ca^{2+} channels.

Results : The average size of duodenal biopsies was of about $6 \pm 1 \text{ mm}^2$ (63 biopsies, 23 subjects). The SMP architecture, as revealed by NF-200 immunostaining, was characterized by the presence of both isolated neurons and ganglia interconnected by typical fiber bundles. The ganglionic density was 2.4 ± 0.69 per mm^2 and each ganglion contained on average 3 ± 1 Hu C/D positive neurons (25 biopsies, 15 subjects). Using Ca^{2+} imaging, we were able to record transient ($[\text{Ca}^{2+}]_i$) changes in the neurons upon depolarization with high K^+ solution, proving their neuronal identity and viability. The transient changes had the typical fast linear upstroke and a multi-exponential decay, with a maximal amplitude of 1.08 ± 0.01 ($n = 13$ neurons from 5 ganglia, 5 biopsies, 2 subjects). Some of these neurons also displayed spontaneous activity before the stimulus was given, indicating that they were still receiving input from other neurons in the SMP network.

Conclusion : We developed a suitable method to measure nerve activity in the ENS using human routine duodenal biopsies. This new approach has an important potential to assess, in a relatively easy way, ENS alterations in patients with GI or more general neurological disorders.

Invited lecture

- B10 -

ENTERIC SMOOTH MUSCLE IN HEALTH AND DIGESTIVE MOTILITY DISORDERS. C. Knowles. Barts and the London School of Medicine and Dentistry, London, United Kingdom.

Introduction : Smooth muscle is the final downstream effector of gastrointestinal motility. Dysfunction of smooth muscle is implicated in several gastrointestinal neuromuscular diseases (GINMD) of children and adults but is still relatively neglected in comparison to the role of nerve injury.

Aim : This presentation will briefly address the normal structure of the muscularis propria (excluding discussion of developmental biology) and then overview histopathological phenotypes present in GINMD, in particular those included within the recent London Classification. Technical aspects in relation to diagnostic credibility will be critically reviewed for each. The talk will highlight recent developments in the field and areas for future research.

- B11 -

ROLE OF THE ENERGY SENSOR AMP-ACTIVATED PROTEIN KINASE (AMPK) AND ITS DOWNSTREAM EFFECTOR UNCOUPLING PROTEIN 2 (UCP2) IN THE OREXIGENIC EFFECT OF ENDOGENOUS GHRELIN. P.J. Verhulst, S. Janssen, J. Tack, I. Depoortere. Department of Gastroenterology University hospital Leuven KUL, Leuven, Belgium.

Introduction : Ghrelin released from the stomach, stimulates food intake through stimulation of neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons in the hypothalamus. Several studies proposed a pivotal role for the energy sensor AMPK and UCP2 in ghrelin's effects on NPY/AgRP expression and food intake stimulation, although most of these studies focused on the effects of exogenous ghrelin.

Aim : To investigate whether a rise in endogenous ghrelin levels is able to influence NPY/AgRP expression via activation of AMPK activity and UCP2 expression.

Methods : Endogenous ghrelin levels were increased in wildtype (GHS-R^{+/+}) and ghrelin receptor knockout (GHS-R^{-/-}) mice by fasting (24 h) or by induction of streptozotocin (STZ)-diabetes (15 days). Plasma octanoylated ghrelin levels were determined by radio-immunoassay. The mRNA expression of AgRP, NPY and UCP2 in the hypothalamus was measured by real-time PCR. Hypothalamic AMPK activity in tissue lysates was measured with an immunoprecipitation kinase assay.

Results : Octanoylated ghrelin levels peaked (4.5-fold, $P < 0.01$) after 24 h of fasting and declined thereafter due to a decrease (1.7-fold, $P < 0.01$) in the mRNA expression of ghrelin-O-acyl transferase (GOAT). GHS-R^{+/+} mice showed a significant increase in AgRP mRNA (from 0.33 ± 0.03 to 1.03 ± 0.09 ; $P < 0.001$) and NPY mRNA (from 0.54 ± 0.08 to 0.88 ± 0.05 ; $P < 0.01$) expression after 24 h-fasting. In GHS-R^{-/-} mice no significant increase in AgRP and NPY mRNA expression was observed. Fasting did not affect AMPK activity in both genotypes but increased UCP2 mRNA expression (GHS-R^{+/+} : from 0.35 ± 0.05 to 0.50 ± 0.04 ; $P < 0.05$ and GHS-R^{-/-} : from 0.41 ± 0.04 to 0.55 ± 0.08 ; $P = 0.058$). The hyperghrelinemia associated with the induction of STZ-diabetes was accompanied by a significant increase in the expression of NPY and AgRP in GHS-R^{+/+} mice compared to non-diabetic controls (AgRP : from 0.22 ± 0.12 to 2.74 ± 0.45 ; $P < 0.001$ and NPY : from 0.73 ± 0.06 to 2.23 ± 0.64 ; $P < 0.05$) but not in GHS-R^{-/-} mice. AMPK activity in the hypothalamus of GHS-R^{+/+} mice after induction of diabetes (20.2 ± 1.6 pmol/min/mg) was decreased ($P < 0.05$) compared to non-diabetic littermates (16.3 ± 1.3 pmol/min/mg) and there was no genotypic difference. Similarly, UCP2

mRNA levels were decreased after the induction of STZ-diabetes compared to control mice in both genotypes (GHS-R^{+/+} : from 0.68 ± 0.02 to 0.26 ± 0.03 ; $P < 0.001$ and GHS-R^{-/-} : from 0.61 ± 0.05 to 0.28 ± 0.05 ; $P < 0.01$).

Conclusion : Increasing endogenous ghrelin levels by fasting and by induction of diabetes stimulates the expression of AgRP and NPY in the hypothalamus via interaction with the GHS-R. The change in AMPK activity and its downstream effector, UCP2, accompanying these changes in endogenous ghrelin levels occur independently from the GHS-R suggesting that AMPK and UCP2 do not play a major role in the orexigenic effect of endogenous ghrelin.

- B12 -

THE EFFECT OF PANCREATIC POLYPEPTIDE ON GASTRIC ACCOMMODATION IN RATS. S. Verschueren, C. Van Heijningen, J. Tack, P. Janssen. Department of Gastroenterology University hospital Leuven KUL, Leuven, Belgium.

Introduction : Pancreatic polypeptide (PP) is an anorexigenic hormone released from pancreatic F-cells upon food intake. It is unclear whether PP influences gastric motor function during food intake.

Aim : We set out to determine the effect of PP on gastric accommodation in conscious Wistar HAN rats by measuring intragastric pressure (IGP) during intragastric nutrient infusion.

Methods : After an overnight fast, a chronically implanted gastric fistula was connected to a custom-made nutrient drink infusion system and a water-perfused catheter to measure IGP (n = 23). IGP was measured before and during the infusion of a nutrient drink (Nutridrink® ; 0.5 ml min^{-1} ; $150 \text{ kcal } 100 \text{ ml}^{-1}$). Rats were treated with PP ($0, 33$ and $100 \text{ pmol kg}^{-1} \text{ min}^{-1}$) from 15 minutes before the nutrient drink infusion started until the end of the experiment in combination with the nitric oxide synthase inhibitor L-NG^G-arginine methyl ester (L-NAME ; $180 \text{ mg kg}^{-1} \text{ h}^{-1}$), the muscarinic antagonist atropine ($3 \text{ mg kg}^{-1} \text{ h}^{-1}$) or vehicle. Furthermore, the effect of PP was tested after subdiaphragmatic vagotomy of the stomach. Data were represented as mean \pm S.E.M. and compared using ANOVA and Tukey post test ($p < 0.05$ was considered significant).

Results : During nutrient infusion IGP initially increased to reach an inflection point, after which the IGP stabilized despite further test-meal infusion. The maximal pressure increase during control experiments was $5.09 \pm 0.38 \text{ mmHg}$. PP had no effect on baseline pressure, but during nutrient drink infusion IGP was significantly increased in the high-dose group compared to vehicle ($p < 0.01$). Both after L-NAME and atropine treatment IGP was significantly increased during nutrient drink infusion as compared to vehicle treatment ($p < 0.005$ and < 0.01 respectively). In the presence of atropine, the effect of PP persisted ($p < 0.05$), while it was abolished in the presence of L-NAME. In vagotomized rats the effect of PP was still present compared to vehicle, although this was only significant during the first ten minutes of nutrient infusion ($p < 0.05$).

Conclusion : PP enhanced the IGP increase during nutrient drink infusion. The effect of PP persists after vagotomy and atropine treatment while it is suppressed in the presence of L-NAME, indicating that PP inhibits gastric accommodation through inhibition of nitric oxide release.

BCTC, A TRPV1 ANTAGONIST, DECREASES COLORECTAL DISTENSION-EVOKED VISCEROMOTOR RESPONSES IN CONSCIOUS TNBS COLITIS RATS. W. Vermeulen (1), J.G. De Man (1), H.U. De Schepper (2), T.G. Moreels (2), P.A. Pelckmans (2), B.Y. De Winter (1). (1) University of Antwerp, Antwerp, Belgium ; (2) Antwerp University Hospital, Antwerp, Belgium.

Introduction : The transient receptor potential of the vanilloid type 1 (TRPV1) receptor is a polymodal nociceptor involved in visceral inflammatory pain, predominantly expressed on primary afferent neurons and cross-sensitized by many inflammatory mediators.

Aim : The aim of the current study was to investigate whether blocking TRPV1-mediated afferent sensitization alters visceromotor responses (VMR) to graded distension of the colon in unanesthetized rats with TNBS colitis.

Methods : Female Wistar rats were intrarectally instilled with TNBS and colitis was monitored with a baby upper gastrointestinal endoscope at day 3 after TNBS instillation. A barostat was used to induce graded colorectal distension (CRD, 10-20-30-40-60 mmHg, 20 s stimulus, 4 min interval). The VMR to CRD was studied in conscious rats at day 0 (baseline response) and during acute colitis at day 3, before and after administration of the selective TRPV1 antagonist N-(4-Tertiarybutylphenyl)-4(3-cholorpyridin-2-yl)-tetrahydro-pyrazine1(2H)-carboxamide (BCTC 5, 10 mg.kg-1 i.p., 1 h) or its vehicle (25% hydroxypropyl- β -cyclodextrin). Quantitative RT-PCR was used to assess TRPV1 mRNA expression in dorsal root ganglia (DRG) at the lumbosacral level (L6-S1) in TNBS colitis rats.

Results : Colonoscopy showed signs of severe mucosal inflammation in the distal colon 3 days after induction of TNBS colitis. CRD-evoked visceromotor reflexes were enhanced during acute colitis (day 3) compared with baseline responses measured at day 0. Intraperitoneal injection of the TRPV1 antagonist BCTC (5 mg.kg-1) significantly decreased the enhanced VMR to colorectal distension at day 3 in rats with colitis (repeated measurements two-way ANOVA, $P < 0.05$). A higher dose of BCTC (10 mg.kg-1) did not further decrease the VMR response. However, treatment with vehicle did not influence the VMR. Quantitative RT-PCR demonstrated that TNBS colitis induced a significant upregulation of TRPV1 mRNA in the pelvic nerve DRG L6-S1 (unpaired Student's t-test, $P < 0.05$).

Conclusion : The selective TRPV1 antagonist BCTC significantly decreased the magnitude of enhanced CRD-evoked VMR in unanesthetized rats with TNBS colitis, suggesting modulation of TRPV1 receptors during inflammation. These results support a role for TRPV1 antagonist therapy for the treatment of inflammatory diseases characterized by visceral hyperalgesia.

VALIDATION OF A POST-INFECTIOUS IBS MOUSE MODEL USING CAMPYLOBACTER JEJUNI. M.M. Wouters (1), N. Kotman (1), C. Van Heijningen (1), K. Lambaerts (1), G.E. Boeckxstaens (2). (1) KULeuven, Leuven, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Post-infectious irritable bowel syndrome (PI-IBS) occurs in 10 to 30% of patients who suffered from bacterial gastroenteritis. Insight in the underlying pathophysiological mechanisms of PI-IBS is however mainly based on studies using rodents infected with parasites or helminths. These infections trigger a different immune response compared to bacterial infections potentially leading to different pathophysiological mechanisms.

Aim : The aim of the study is to develop a PI-IBS mouse model using *C. jejuni* to unravel the mechanisms underlying PI-IBS.

Methods : Six weeks old balb/c were orally gavaged with 5×10^{10} CFU *Campylobacter jejuni*. To study the inflammatory response, mice were killed at PI day 3 and 10. Quantitative PCR for inflammatory markers was performed on total RNA (RNeasy ; Qiagen, reverse transcriptase using QuantaScript ; Quanta) from jejunum, colon and mesenteric lymph nodes (MLN). To evaluate visceral pain, electrodes (Nichrome 80, Pelican Wire) were implanted in the abdominal muscularis and EMG was recorded. Colorectal distention (CRD) was performed by inflating a Fogarty probe 4.0 (0.02-0.1 ml) at 3 weeks post infection. EMG spikes were analyzed with Spike2. Statistical analysis was performed using a paired Student's t-test.

Results : *C. jejuni* was cultured from stool samples up to 8 days post infection. At PI day 3, inflammatory markers (IL4 (fold change 3.01 ± 0.66 ; $p < 0.02$), IFN γ (4.66 ± 0.64 ; $p < 0.02$), TNF α (4.35 ± 0.87 ; $p < 0.02$), MCP1 (3.78 ± 0.91 ; $p < 0.03$), IL1b (3.18 ± 0.66 ; $p < 0.03$), IL10 (3.14 ± 0.61 ; $p < 0.01$) and IL6 (3.48 ± 0.75 ; $p < 0.02$)) were increased in the jejunum of infected mice compared to controls. TGF β , IL9 and IL17a and IL17f were unaffected. At PI day 10, all inflammatory markers in jejunum and MLN returned to normal. CRD in control balb/c resulted in a volume dependent increase in visceromotor response (0.02 ml 1.79 ± 1.78 ; 0.04 ml 2.25 ± 0.29 ; 0.06 ml 4.3 ± 0.93 ; 0.08 ml 4.21 ± 0.57 ; 0.1 ml 2.81 ± 0.53 spikes per second). After infection, the pain response to CRD was increased (0.02 ml 0.4 ± 0.36 ; 0.04 ml 4.31 ± 1.30 ; 0.06 ml 12.95 ± 1.72 ; 0.08 ml 29.53 ± 5.94 ; 0.1 ml 38.45 ± 3.91 spikes per second) compared to control animals. Seven out of 10 PI mice showed an increased pain response (threshold for hypersensitivity : 5 spikes/second at 0.1 ml) to colorectal distention.

Conclusion : *C. jejuni* induced a weak and transient infection in balb/c mice resulting in post infectious visceral hypersensitivity in 7 out of 10 animals at 3 weeks PI. These results demonstrate that *C. jejuni* infection in balb/c mice may be a useful model to unravel the mechanisms underlying bacterial PI-IBS.

- B15 -

A PILOT STUDY ON DUODENAL MUCOSAL INTEGRITY IN FUNCTIONAL DYSPEPSIA. H. Vanheel (1), T. Vanuytsel (1), L. Holvoet (1), N. Pardon (1), M. Vicario (2), S. Kindt (1), J. Söderholm (3), J. Tack (1), R. Farré (1). (1) Department of Gastroenterology University hospital Leuven KUL, Leuven, Belgium ; (2) Hospital General Universitario Valle Hebrón and Ciber-ehd del Instituto Carlos III, Barcelona, Spain ; (3) Department of Surgery University Hospital, Linköping, Sweden.

Introduction : Functional dyspepsia (FD) is an extremely common disorder of gastrointestinal function characterized by chronic upper abdominal symptoms. Despite intensive research, the pathogenesis remains obscure. However, it has been proposed that low grade mucosal inflammation in the duodenum may contribute to symptom generation, especially in presumed post-infectious FD (Kindt 2009). A key role underlying this low grade inflammation is increasingly being attributed to enhanced mucosal permeability.

Aim : The aim of this study was to evaluate the possible impairment of duodenal mucosal integrity in FD patients.

Methods : Duodenal biopsies were obtained from FD patients fulfilling the Rome III criteria and from age- and gender-matched healthy volunteers. *In vitro* transepithelial resistance (TER) was measured in four duodenal biopsies per subject during 120 minutes with the Ussing chamber technique. At the same time, paracellular permeability was assessed using fluorescein isothiocyanate dextran (MW 4000Da, FITC-D4). Time curves were compared using two-way repeated measures ANOVA followed by a Bonferroni test. Results are shown as mean \pm SEM.

Results : 6 presumed post-infectious FD patients (2 men, mean age 29 ± 5 years, weight loss 7.7 ± 1.3 kg) and 4 healthy volunteers (1 man, mean age 27 ± 5 years) were included. Ussing chamber results show that the TER values of FD patients are significantly lower than those of healthy controls (ANOVA $p = 0.03$). FD patients have a significantly decreased TER at 0 (21.6 ± 1.5 &/cm² vs. 28.3 ± 1.8 &/cm², $p < 0.05$), 30 (18.5 ± 1.3 &/cm² vs. 24.8 ± 2.3 &/cm², $p < 0.05$) and 60 minutes (16.8 ± 1.0 &/cm² vs. 22.4 ± 2.3 &/cm², $p < 0.05$) compared to healthy controls. The paracellular permeability in FD patients is significantly higher than in healthy volunteers (ANOVA $p = 0.02$). FD patients have a significantly increased FITC-D4 passage at 90 (51.4 ± 3.6 pmol vs. 31.6 ± 6 pmol, $p < 0.01$) and 120 min (82.7 ± 6.7 pmol vs. 51.2 ± 8.1 pmol, $p < 0.001$) compared to healthy controls. A significant correlation is present between TER values and FITC-D4 passage at 60 ($R = -0.473$; $p = 0.003$), 90 ($R = -0.564$; $p = 0.0003$), and 120 min ($R = -0.600$; $p < 0.0001$) indicating the reliability of the measurements.

Conclusion : These preliminary results show that an impaired duodenal integrity can be present in presumed post-infectious FD.

A DISTINCT MECHANISM FOR REFLUX IN PATIENTS WITH CYSTIC FIBROSIS : A STUDY USING HIGH RESOLUTION MANOMETRY-IMPEDANCE. A. Pauwels (1), K. Blondeau (1), V. Mertens (1), R. Farre (1), L.J. Dupont (2), D. Sifrim (3). (1) UZ Leuven Department of Gastroenterology, Leuven, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) Barts and the London School of Medicine and Dentistry, London, United Kingdom.

Introduction : Up to 80% of patients with cystic fibrosis (CF) have increased gastro-esophageal reflux (GER). Recent impedance-pH monitoring studies showed that acid reflux is predominant in these patients. It has been suggested that increased reflux in CF is due to low basal lower esophageal sphincter (LES) pressure and a high number of transient lower esophageal sphincter relaxations (TLESRs).

Aim : The aim of our study was to reassess mechanisms of reflux and gastro-esophageal pressure gradients in adult CF patients using state of the art upper-GI physiology techniques : high resolution manometry-impedance (HRM-imp).

Methods : We studied 10 CF patients [age 29 (19-58), 5m/5f] and 11 age-matched healthy volunteers [age 27 (20-36), 4m/7f]. HRM-imp was performed in a semi-recumbent position for 30 minutes during fasting and 2 hours after a standard meal (1000 kcal). We measured total reflux and proximal extent of reflux with impedance ; basal LES pressure, TLESRs and gastro-esophageal pressure gradients with HRM.

Results : Compared to controls, CF patients had significantly higher number of total reflux episodes [19 (13-26) vs. 7 (4-17), $p = 0.03$] ; most increased reflux was liquid or mixed [16 (7-24) vs. 3 (1-7), $p = 0.007$] and CF patients had more often reflux episodes with a high proximal extent [6 (3-9) vs. 0 (0-1), $p = 0.003$]. The postprandial LES basal pressure was lower in CF patients [postprandial hour 1 10 (7-12) vs. 16 (9-32), $p = 0.03$] and TLESR was the main mechanism for reflux both in CF and controls. The number of TLESRs was similar in CF patients and controls [21 (13-21) vs. 16 (12-26), $p = \text{NS}$]. However, reflux during TLESRs was more common [75 (60-86) vs. 24 (6-50) %, $p = 0.003$] in CF patients compared to the healthy volunteers. The gastro-esophageal pressure gradient during TLESRs was significantly higher in CF [2.3 (1.8-7.4) vs. 0 (-2-2.5) mm Hg, $p = 0.01$] than in controls. Such difference was due to lower esophageal pressure in CF patients [-1.5 (-4.3-1.1) vs. 5 (-1-6) mm Hg, $p = 0.02$].

Conclusion : CF patients have increased GER with high proximal extent. The increased reflux is due to higher proportion of TLESRs associated with reflux. Unlike non-CF GERD patients (with increased intra-abdominal pressure), reflux during TLESRs in CF is probably due to an increased gastro-esophageal pressure gradient mainly generated by the reduction in esophageal pressure.

ADDED VALUE OF COMBINED HIGH RESOLUTION MANOMETRY-IMPEDANCE RECORDINGS IN PATIENTS WITH POSTPRANDIAL BELCHING OR REGURGITATION. V. Boecxstaens (1), N. Rommel (1), K. Blondeau (1), J. Tack (2). (1) KULeuven, Leuven, Belgium ; (2) UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Stationary manometry has been applied in the diagnostic work-up of patients with postprandial belching or regurgitation, but adding impedance monitoring (MII) increases the diagnostic accuracy (Rommel *et al.*, 2010). Combined high resolution manometry (HRM) and MII is now available and allows a superiorly detailed characterization of esophageal flow and pressure events.

Aim : The aim of our study was to compare the diagnostic yield of HRM-MII to standard manometry, HRM and MII in patients with postprandial regurgitation or belching.

Methods : 12 women [age 31 (16-89)], with postprandial belching/regurgitation underwent stationary combined HRM-MII. After 30 minutes of baseline recordings, patients received a 1000kcal solid meal. Recordings were continued 1 h postrandially. Combined HRM-MII, standard manometry, HRM, and MII tracings were evaluated by separate experienced investigators. Individual flow events were identified and categorized as : 1) reflux, 2) rumination, 3) supragastric belching, 4) aerophagia or 5) gastric belching. A final diagnosis for each patient was provided by each investigator. HRM-MII recordings were used as the gold standard and compared to HRM, MII and standard manometry alone.

Results : Combined HRM-MII identified 322 flow events of which 26 (8.1%) were correctly identified using standard manometry, 150 (46.5%) using HRM and 212 (65.6%) using MII alone (Table 1). Using combined HRM-MII, 6 patients were diagnosed with supragastric belching, 3 with rumination and 3 with GERD. Traditional manometry and HRM showed 50% discordance in final diagnosis, whereas MII showed 25% discordance.

| HRM-MII | | Standard Manometry | | HRM | | MII | |
|-----------------------|--------|--------------------|-------|---------|-------|---------|-------|
| Type | Number | correct | wrong | correct | wrong | correct | wrong |
| Aerophagia | 11 | 1 | 10 | 0 | 11 | 5 | 6 |
| Belching | 6 | 4 | 2 | 0 | 6 | 0 | 6 |
| Reflux | 24 | 0 | 24 | 5 | 19 | 13 | 11 |
| Rumination | 68 | 17 | 51 | 35 | 33 | 0 | 68 |
| Supragastric belching | 213 | 4 | 209 | 110 | 103 | 194 | 19 |

Conclusion : Combined HRM-MII is superior to traditional manometry, HRM or MII alone in distinguishing between rumination, supragastric belching or GERD in patients with symptoms of postprandial belching/regurgitation. The distinction is relevant as each of these entities require specific therapeutic approaches. Table 1.

- B18 -

DETERMINANTS OF SYMPTOM PERCEPTION DURING IMPEDANCE-PH MONITORING : DO PSYCHOSOCIAL FACTORS AND ‘SOMATIZATION’ PLAY A ROLE ? C. Caenepeel (1), L. Van Oudenhove (1), V. Boecxstaens (1), S. Depeyter (2), L. Holvoet (2), G. Boeckxstaens (2), J. Tack (2), K. Blondeau (1). (1) KULeuven, Leuven, Belgium ; (2) UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Symptom association analysis during 24hour reflux monitoring is an important diagnostic tool in GERD patients which requires accurate symptom registration. Psychosocial factors and ‘somatization’ play an important role in functional GI disorders, but their contribution to GERD symptom burden is understudied

Aim : We aimed to study the association between symptom events, reflux parameters, psychosocial factors and ‘somatization’ in patients with GERD.

Methods : Patients with symptoms suggestive of GERD (‘on’ or ‘off’ PPI), undergoing 24hrs impedance-pH (MII-pH) recordings were prospectively recruited for the study. Patients were instructed to carefully register reflux symptoms (typical and atypical) using an event marker. On the day of the MII-pH monitoring subjects completed a series of questionnaires assessing psychosocial factors. Analysis : MII-pH recordings were manually analysed according to previously published guidelines. Depression and ‘somatization’ were measured using two modules of the Patient Health Questionnaire (PHQ-9 and PHQ-15, respectively). Different forms of anxiety were measured using validated self-report questionnaires, including trait anxiety, post-traumatic stress (PTS), social anxiety and gastrointestinal-specific anxiety (GSA). Finally, pain coping mechanisms, pain catastrophizing and sexual and physical abuse history were assessed. Correlation analysis and multiple linear regression were used to study the association of these patient characteristics with the number of GERD symptoms registered using the event marker.

Results : 118 patients participated in this ongoing study (mean age 48.5 ± 14.7 , 63 women, 54 ‘on’ PPI). Each patient recorded 4(1-15) GERD symptoms during the study. There was a significant correlation between the number of GERD symptoms recorded and the proximal extent of reflux ($r = 0.40$, $p < 0.0001$) but not with the total number of reflux events ($r = 0.18$, $p = 0.09$). Correlations are shown in *Table 1*. Several psychosocial factors and ‘somatization’ correlated significantly with the number of GERD symptoms registered. In multiple linear regression, the proximal extent of reflux ($B = 0.33$, $p = 0.002$) and pain coping ($B = 0.23$, $p = 0.03$) were significantly associated with number of GERD symptoms registered, whereas ‘somatization’ ($B = 0.17$, $p = 0.12$), physical abuse history ($r^2 = 0.68 \pm 0.36$) and trait

anxiety ($B = 0.17$, $p = 0.10$) were not independently associated (model $R^2 = 0.31$, $p < 0.0001$). The association between symptoms and psychosocial factors was only found for typical GERD symptoms.

Table 1

| | Proximally extending reflux | 'somatization' | GSA | depression | trait anxiety | social anxiety | pain coping | pain catastrophizing | physical abuse history |
|------------------|-----------------------------|----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| # symptom events | $r = 0.40$ $p < .0001$ | $r = 0.37$ $p = 0.0004$ | $r = 0.13$ $p = 0.23$ | $r = 0.24$ $p = 0.03$ | $r = 0.21$ $p = 0.05$ | $r = 0.18$ $p = 0.12$ | $r = 0.18$ $p = 0.10$ | $r = 0.10$ $p = 0.36$ | $t = 2.62$ $p = 0.01$ |

Conclusion : Beside proximally extending reflux events, psychosocial factors play an important role in the occurrence of GERD symptoms during 24 hr MII-pH monitoring. Further studies will need to confirm their impact on reflux-symptom association.

EFFECT OF PEPPERMINT OIL ON INTRAGASTRIC PRESSURE DURING FASTING AND INTRAGASTRIC INFUSION OF A LIQUID MEAL IN HEALTH. A. Papathanasopoulos, A. Rotondo, R. Vos, P. Janssen, E. Scarpellini, P. Vanden Berghe, W. Boesmans, J. Tack. UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Animal studies have shown that menthol reduces intestinal segmentation/motility, an effect that is probably mediated by transient receptor potential (TRP) channels. Peppermint oil (PO), with menthol as a major constituent, is widely used as a spasmolytic agent in irritable bowel syndrome. Recent data have implicated tone of the proximal stomach, through its effects on intragastric pressure (IGP), in the modulation of satiation during meal ingestion.

Aim : We set out to investigate the effect of single-dose PO on IGP and satiation in health.

Methods : In ten fasted healthy volunteers (HVs ; mean age \pm SEM : 32.5 ± 3.2 , BMI : 23.1 ± 0.8 , 4 female) a manometry and an infusion catheter were positioned in the proximal stomach. Baseline intragastric pressure was determined over 20 min before administering an enteric-release PO capsule (182 mg) or placebo, in a randomized controlled crossover design. Sixty min post-medication a nutrient drink was intragastrically infused at 60 ml min^{-1} . HVs scored satiation on a 6-grade Likert scale every min until maximum, when the infusion was stopped. IGP was presented as a change from baseline (mean \pm SEM), and the areas under the IGP and satiation curves (AUC) were compared with paired t-test.

Results : IGP AUC decreased significantly from baseline 40 to 60 min after PO administration, as compared to placebo (-1.9 ± 1.5 vs. $2.0 \pm 0.6 \text{ mmHg min}^{-1}$, $p = 0.02$, see Figure). In contrast, IGP AUC did not differ between PO and placebo during the 20-min period post-medication ($p = 0.14$) and 20 to 40 min post-medication ($p = 0.10$). During intragastric infusion, the maximum infused volume was lower (only significant at the 10% level) after PO as compared to placebo (647.5 ± 52.1 vs. $735.3 \pm 29.2 \text{ mL}$, $p = 0.06$). Accordingly, satiation scores 6 to 8 minutes after the start of the infusion were significantly higher after PO vs. placebo (3.3 ± 0.3 vs. 2.4 ± 0.3 units min, $p = 0.01$). No significant differences occurred between PO and placebo in infusion-induced relaxation (2.7 ± 1.3 vs. $4.2 \pm 0.7 \text{ mmHg}$, $p = 0.23$), time-to-nadir-pressure (4.1 ± 0.7 vs. $4.5 \pm 0.7 \text{ min}$, $p = 0.69$), and IGP recovery slope (0.36 ± 0.10 vs. $0.27 \pm 0.06 \text{ mmHg min}^{-1}$, $p = 0.41$).

Conclusion : A single dose of PO reduces fasting IGP in health and enhances satiation during nutrient drink infusion with negligible effects on IGP profiles. Further studies will need to elucidate whether these effects involve members of the TRP channel family in the upper g.i. tract.

Posters

- B20 -

EFFECT OF THE GLP-1 ANALOGUE LIRAGLUTIDE ON INTRAGASTRIC PRESSURE AND SATIATION DURING NUTRIENT DRINK INFUSION IN HEALTHY VOLUNTEERS. A. Rotondo, A. Papathanasopoulos, V. Rita, P. Janssen, J. Tack. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : The gastric relaxation upon food intake, called gastric accommodation (GA), is involved in food intake. Liraglutide is a glucagon-like peptide-1 (GLP-1) analog that has a spectrum and magnitude of action different than GLP-1.

Aim : We set out to investigate whether liraglutide, just as GLP-1, can affect the relation between GA and satiation by measuring satiation and IGP during intragastric nutrient drink infusion.

Methods : 12 healthy volunteers (HV's) have participated. Each subject was tested 12-20 h after subcutaneous injection with placebo, 0.6 or 1.2 mg liraglutide in a randomized blinded fashion. An infusion catheter and a manometry probe were positioned with the tip 5-10 cm in the proximal stomach. After a 15 minutes stabilization period intragastric infusion of a nutrient drink (Nutridrink 1.5 kcal/ml) started (speed of 60 mL/min). During the nutrient drink infusion the HV's scored their satiation every minute on a graded scale (0-5) until maximum, when the infusion was stopped. Maximal IGP decrease and area under the IGP curve during nutrient drink infusion (corrected for the length of the experiment) were compared using ANOVA ($p < 0.05$ was considered significant). Data is presented as mean \pm SEM.

Results : Placebo and 0.6 mg liraglutide were well-tolerated while 1.2 mg liraglutide induced nausea. Liraglutide dose-dependently increased satiation. After placebo, 0.6 and 1.2 mg liraglutide the maximum volume HV's drank was 1060 ± 136 , 945 ± 143 and 880 ± 126 ml, respectively ($p < 0.05$). In all groups IGP decrease initially during nutrient drink infusion and gradually increase thereafter. During nutrient drink infusion the maximum pressure decrease was -3.9 ± 0.5 , -5.0 ± 0.8 and -2.5 ± 0.6 mmHg and the area under the curve was -2.5 ± 0.3 , -3.3 ± 0.6 and -1.1 ± 0.5 mmHg (for placebo, 0.6 and 1.2 mg liraglutide respectively ; $p < 0.05$). In all groups IGP decreased initially during nutrient drink infusion and gradually increased thereafter.

Conclusion : Liraglutide increased satiation in a dose-dependent manner. 0.6 mg liraglutide enhanced GA during nutrient drink infusion, while 1.2 mg liraglutide seemed to decrease GA. The latter unexpected result might be explained by the side-effects observed using this relative high dose.

- B21 -

THE POSSIBLE ROLE OF FETAL ESOPHAGUS GRAFT IN ESOPHAGEAL CIRCULAR DEFECT REPAIR. S. Vandaele (1), P. Delrée (2), C. Deprez (1), S. Najar (1), V. Coulic (3). (1) CHU Brugmann, Brussels, Belgium ; (2) Institut de Pathologie et de Génétique, Gosselies, Belgium ; (3) CHU Brugmann ULB Dpt Experimental Medicine, Brussels, Belgium.

Introduction : Previous experiments have shown that fetal esophagus grafted at the neck site gives growth to cysts, the wall of which present all the features of the adult organ. But even when the grafts tightly surround the scaffold used for keeping the esophagus lumen open, the cysts have their mucosa inside and the adventitial layer turned to the prosthesis.

Aim : So the aims of the study were 1. to test the possibility of using a grown fetal esophageal graft together with a resorbable chitosan scaffold to bridge circular defects of the esophagus ; 2. to evaluate the role of the fetal esophagus graft in the repair process.

Methods : 20 Fischer and Wistar rats mainly males, body weight /BW/300 \pm 50 g, were used (Bioethic agreement accorded). A 2-stage operation was performed : first – implantation of fetal esophagus (age 14-19 days of development) at the neck site under the salivary gland, second – when cyst was formed (ultrasonic control), creation of a circular defect 5-8 mm long in the cervical esophagus of the graft recipient, bridging of this defect by a chitosan tube and suturing a well irrigated cyst flap with the recipient esophagus. As control circular esophageal defects were bridged by chitosan tube alone. After the operation BW was regularly measured, histological study of the operated esophagus was performed (necropsy or biopsy material).

Results : After avoiding the per and early postoperative complications (sudden death, leakage, bezoar, malnutrition) the highest survival rates were obtained using combined prosthesis (up to 2 months and more against 12-17 days in control group). Histology has shown a satisfactory repair of the wounded esophagus one month after the operation, though the morphology of the repaired segment was still different from the normal one (many not yet fully differentiated cells, difficulty to visualize the typical layers of the wall structure).

Conclusion : It is supposed that the grown fetal esophageal flap enhances the reconstruction of esophageal continuity thanks to the presence of young highly proliferating cells in it as well as the stimulation of regenerative properties of the operated esophagus. Questions remain < about the connection of intramural nervous systems of the graft and the recipient esophagus, their capacity to regulate the food transit and may be to prevent stenosis in late delays, the inhibition of the regeneration process when the mucosal reconstitution is achieved.

INFLUENCE OF NALOXONE AND METHYLNALTREXONE ON INTERDIGESTIVE GASTROINTESTINAL MOTILITY AND HUNGER SCORES IN MAN. E. Scarpellini, R. Vos, E. Deloouse, A. Papathanasopoulos, A. Rotondo, I. Depoortere, J. Tack. Department of Gastroenterology University hospital Leuven KUL, Leuven, Belgium.

Introduction : We recently reported that hunger ratings in the fasting state in man were closely correlated with interdigestive motor activity, in particular that hunger peaks coincided with gastric phase 3 (GF3) of the migrating motor complex (MMC) (Scarpellini *et al.*, DDW 2009). Recent evidence from animal studies suggests that endogenous opioids are also involved in nutrient acquisition and energy regulation. It is still unclear whether the peripheral opioid receptors alone are involved in gastric motility regulation and hunger ratings in man.

Aim : To investigate the influence of methylnaltrexone (MNTX) and naloxone (NA), peripherally selective and non-selective $\frac{1}{4}$ -opioid receptor antagonists, respectively, on the interdigestive motor activity of the proximal gastrointestinal tract and hunger scores in man.

Methods : Eleven fasted healthy subjects (2 males ; 29.5 ± 3.5 years) underwent four antroduodenojejunal manometry studies at least one week apart in a randomized single-blinded order : twenty minutes after a full MMC cycle intravenous or subcutaneous infusion/injection of saline or intravenous infusion of NA or subcutaneous injection MNTX were performed. Phases of the MMC were visually identified. Computer-aided baseline reconstruction was used to quantify phasic contractions as a motility index (MI), reflecting the area between signal and baseline normalized over time. Hunger scores (on 100 mm visual analogue scales (VAS expressed as AUC)) were measured throughout the study.

Results : A total of 25 F3s before and 16 F3s after drug administration were registered. Compared to pre-treatment, GF3s were significantly suppressed by MNTX (6/12 vs. 0/10, $p = 0.0152$) but not by NA (6/13 vs. 0/6, $p = 0.1093$) ; all post-treatment F3s lacked a gastric component. Administration of MNTX was followed by SBF3 in all subjects after 80 ± 11 mins, while NA induced SBF3 only in 6 subjects, after 83 ± 14 mins ($p < 0.05$). After NA, a drop in SB and antral MI occurred compared to pre-treatment (3.43 ± 0.37 vs 2.96 ± 0.64 and 1.96 ± 1.20 vs 1.06 ± 0.47 , both $p = 0.0001$), and this was prevented by MNTX (3.26 ± 1.03 vs 3.05 ± 0.62 and 1.72 ± 1.61 vs 1.58 ± 0.91 , both $p = \text{NS}$). The hunger scores 20 min before and after the F3s were significantly higher after MNTX administration compared to the spontaneous F3 (before F3 147 ± 18.3 vs 81.6 ± 23.9 , $p = 0.02$; after F3 156.4 ± 14.9 vs 122 ± 18.7 , $p = 0.05$). With NA differences in hunger scores before and after F3 did not reach statistical significance (respectively 148.4 ± 15.9 vs 111.8 ± 21.9 , $p = 0.07$ and 118.2 ± 27.3 vs 144.9 ± 13.1 , $p = 0.47$).

Conclusion : Peripheral $\frac{1}{4}$ -opioid receptors are involved in the control of GF3. Compared to non-selective inhibition, inhibition of peripheral $\frac{1}{4}$ -opioid receptors stimulates interdigestive phasic contractions and hunger ratings in man.

JOINT MEETING

- D01 -

PRACTICE - AN OBSERVATIONAL TRIAL ASSESSING THE EFFICACY OF PEGINTRON® AND REBETOL® ACCORDING TO LIVER FIBROSIS IN NAIVE GENOTYPE 1/4/5/6 CHRONIC HEPATITIS C PATIENTS. S. Bourgeois (1), J. Delwaide (2), P. Deltenre (3), J. Henrion (3), M. Adler (4), P. Langlet (5), J. Mulkay (6), F. Nevens (7), C. Brixko (8), N. Botembe (9), I. Colle (10), H. Reynaert (11), G. Robaey (12), P. Golstein (4), C. Moreno (4). (1) ZNA Stuivenberg, Antwerpen, Belgium ; (2) CHU Sart Tilman, Liège, Belgium ; (3) Hôpital de Jolimont, Haine-Saint-Paul, Belgium ; (4) ULB Erasme, Brussels, Belgium ; (5) CHU Brugmann, Brussels, Belgium ; (6) CHU Saint-Pierre, Brussels, Belgium ; (7) University Hospital Gasthuisberg, Leuven, Belgium ; (8) CHC de la Citadelle, Liège, Belgium ; (9) Centre Hospitalier des Ardennes, Libramont, Belgium ; (10) UZ, Gent, Belgium ; (11) UZ Brussel, Brussels, Belgium ; (12) Ziekenhuis Oost-Limburg, Genk, Belgium.

Introduction : The guidelines for CHC therapy did not recommend treating patients with low fibrosis progression rate. However, this rate is unlinear and difficult to predict with the available tools. Delaying time to treatment or considering it only for METAVIR score above stage F2 may reduce the chance of virological cure.

Aim : PRACTICE study, a multicenter phase IV Belgian trial, had as primary objective evaluating the fibrosis impact on the virological response in naïve CHC genotype 1,4,5,6 patients treated with pegylated interferon alpha-2b and ribavirin in clinical practice.

Methods : Patients were treated with pegylated interferon alpha-2b 1.5 mcg/kg and with ribavirin according to body weight (< 65 kg : 800 mg, 65-85 kg : 1000 mg, > 85 kg : 1200 mg) between January 2005 and November 2009. Virological responses were assessed at week 12, 24, 48 and 24 weeks following therapy (sustained virological response, SVR). Fibrosis stage was assessed by a liver biopsy (Metavir score) in all patients.

Results : Five hundred and five patients were included (60% male, median age 46 years, median weight 75 kg, median BMI : 25,3 kg/m²), 75% of the patients were infected with genotype 1 (n = 376) and 62 % (n = 315) had a METAVIR score e F2 (11% F0, 27% F1, 36% F2, 17% F3, 9% F4). High (e 400.000 IU/mL) baseline viral load was present in 71% (n = 361). Ninety four patients (17%) discontinued treatment before month 3 because of lost to follow-up 34, patient preference 19, investigator decision 7, disease complications 3 and side effects 31 patients. SVR in the ITT analysis was 35%. Of the F0F1 patients 43% achieved SVR, significantly more than in the advanced fibrosis group with 30% SVR (p = 0.0045). The Relapse Rate in F0F1 patients was 19% compared with 33% in e F2 patients. The SVR predictors in the univariate analysis were : age (p = 0.008), EVR (p < 0, 0001), low fibrosis level F0F1 (p = 0.0045) and low baseline viral load < 400.000IU/mL (p = 0.0258) and in the stepwise multivariate analysis EVR (p < 0.0001) and Metavir score (p = 0.0049).

Conclusion : Higher SVR rates were reached in patients with low hepatic fibrosis, confirming that treatment in CHC patients is more successful at an earlier fibrosis progression stage.

BETA-BLOCKERS CAUSE PARACENTESIS-INDUCED CIRCULATORY DYSFUNCTION IN PATIENTS WITH CIRRHOSIS AND REFRACTORY ASCITES. T. Serste (1), C. Francoz (2), F. Durand (2), P.E. Rautou (2), C. Mélot (3), D.C. Valla (2), R. Moreau (2), D. Lebrech (2). (1) ULB Saint-Pierre, Brussels, Belgium ; (2) Hôpital Beaujon, Clichy, France ; (3) ULB Erasme, Brussels, Belgium

Background : Beta-blockers may play a role in the development of paracentesis-induced circulatory dysfunction (PICD) and have a negative impact on survival in patients with cirrhosis and refractory ascites.

Aim : The aim of this study was to investigate the incidence of PICD before and after discontinuation of beta-blockers in patients with cirrhosis and refractory ascites.

Methods : Patients with cirrhosis and refractory ascites treated with beta-blockers were selected. Heart rate, arterial pressure and plasma renin concentrations (PRC) were collected before, immediately after and 1 week after large-volume paracentesis associated with intravenous albumin administration. Beta-blocker therapy was progressively discontinued after complete endoscopic eradication of varices. The clinical and biological evaluation was then repeated. The presence of PICD was defined as an increase in PRC of at least 50% above baseline one week after paracentesis.

Results : Ten patients were included. Nine men, mean age 59.1 ± 10.7 years old. The MELD score was 17.7 ± 4.4 and 8 patients were Child-Pugh C. When patients were given beta-blockers, the heart rate did not change immediately after paracentesis while mean arterial pressure significantly decreased ; PICD developed in 8 patients. After beta-blockers were discontinued, the heart rate significantly increased immediately after paracentesis and mean arterial pressure significantly decreased ; PICD only developed in 1 patient ; the difference in the incidence of PICD was significant when these same patients were treated with beta-blockers.

Conclusion : Use of beta-blockers is associated with a high risk of PICD in patients with cirrhosis and refractory ascites.

MAGNETIC RESONANCE IMAGING FINDINGS CORRELATES WITH PATHOLOGICAL SUBTYPE CLASSIFICATION OF HEPATOCELLULAR ADENOMAS. S. Van Aalten, M. Thomeer, T. Terkivatan, R. Dwarkasing, J. Verheij, R. De Man, J. Ijzermans. Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : Recently a molecular and pathological classification system for hepatocellular adenomas (HCA) was introduced and four major subgroups were identified.

Aim : We studied the correlation between magnetic resonance (MR) imaging findings and pathological subtype classification of HCA in a large single centre study in the Netherlands and proposed guidelines for follow-up and management.

Methods : Seventy-one tumours previously diagnosed as HCA were classified on the basis of pathological findings and immunohistochemical analysis : liver-fatty acid binding protein (L-FABP) negative HCA, inflammatory HCA, β -catenin positive HCA and unclassified HCA. MR images were independently reviewed by two abdominal radiologists, thereafter consensus was obtained.

Results : We included MR images of 61 lesions (48 patients) resected from 2000 to 2010. Forty-eight lesions were characterized as HCA : 9 L-FABP-negative HCA, 30 inflammatory HCA, 4 β -catenin positive HCA, and 5 unclassified HCA. Focal positivity for β -catenin with concomitant homogeneous staining of GS was seen in 3 inflammatory HCA. Thirteen lesions were characterized as focal nodular hyperplasia (FNH) and presented a characteristic “map-like” pattern of glutamine synthetase (GS). MR imaging signs of diffuse intratumoural fat deposition was present in 78% of L-FABP-negative HCA compared to 17% of inflammatory HCA ($P = 0.001$). Steatosis within the non-tumoural liver was present in 17% of inflammatory HCA compared to none of the L-FABP-negative HCA ($P = 0.001$). A characteristic “atoll” sign was only seen in the inflammatory group ($P = 0.027$). Presence of a typical vaguely defined type of scar was seen in 5 out of 7 β -catenin positive HCA ($P = 0.003$). GS positivity in HCA and FNH lesions showed presence of a scar in 14 out of 18 GS positive lesions ($P = < 0.001$). No specific MR imaging features were identified for the unclassified group of HCA

Conclusion : L-FABP-negative HCA, inflammatory HCA and β -catenin positive HCA were associated with specific MR patterns. Intratumoural fat deposition and presence of an “atoll” sign and/or a typical vaguely defines scar can help to distinguish between these subtypes. Since β -catenin positive HCA are considered premalignant, resection or closer follow-up on MR imaging may be preferred in these cases.

EMBOLIZATION OF SPONTANEOUS PORTOSYSTEMIC SHUNTS IN CIRRHOTIC PATIENTS WITH REFRACTORY HEPATIC ENCEPHALOPATHY : RESULTS OF A SINGLE CENTER PROSPECTIVE EXPLORATORY STUDY. K. Ameloot (1), W. Laleman (1), S. Heye (2), J. Vaninbrouckx (2), C. Verslype (1), D. Cassiman (1), J. Fevery (1), G. Maleux (2), F. Nevens (1). (1) UZ Leuven Department of Hepatology, Leuven, Belgium ; (2) UZ Leuven Department of Interventional Radiology, Leuven, Belgium.

Introduction : Therapy-refractory hepatic encephalopathy remains a major cause of morbidity in cirrhotic patients. A frequently encountered phenomenon in these patients, when actively screened for, is the observation of natural large spontaneous portosystemic shunts (SPSSs).

Aim : We hypothesized that embolization of these SPSSs in cirrhotic patients might lead to a reduction in HE and subsequent improvement in quality of life and health-economic burden.

Methods : Between June 2008 and June 2010, 10 patients (7 women, mean age 63 years, predominantly post-alcoholic cirrhosis, mean Child B8, mean MELD 14) with refractory hepatic encephalopathy were found to have SPSSs on angio-MRI of the abdomen, eligible for embolization. Refractory HE was defined as recurrent episodes of HE (\geq grade 2 according to New Haven-classification) with at least 2 hospital admissions after start of standard therapy (n = 8, 80%), which consisted of daily lactulose \pm selective intestinal decontamination, or as persisting HE 30 days after start of standard therapy during the first hospital admission (n = 2, 20%) (maximal grade of HE \geq III in 90%). Considered culprit SPSSs were recanalized paraumbilical veins (n = 6), splenorenal shunts (n = 3) and a shunt between the superior mesenteric vein and the right ovarian vein (n = 1). Both efficacy (assessed by grade of HE and number & duration of hospitalisation within 100 days pre- and post-treatment) and complications (procedural and portal hypertensive rebound-related (de novo/aggravated ascites, increase in grade or bleeding of gastro-oesophageal varices)) were analyzed.

Results : All procedures were technically successful and without immediate complications. All patients are still alive and none transplanted (if eligible). Since embolization, both the number of hospitalizations (2 episodes per 100 days pre-embolisation vs. 0.15 episodes per 100 days post-embolization, P = 0.01) and days-spent-in-hospital because of HE (47 days pre vs. 10 days per 100 days post-embolization, P = 0.04) were significantly reduced. Sub-analysis of all patients with episodes of grade III-IV HE before embolization showed that 75% of them did not experience any episode of HE after embolization. The grade of ascites (assessed at 1 and 3 months) and of gastro-oesophageal varices (assessed endoscopically after 3 months post-SPSS-embolization) was comparable to before radiological intervention. No episodes of variceal hemorrhage or renal function deterioration was observed.

Conclusion : Selective embolization of SPSSs in a group of cirrhotic patients with low MELD (and therefore low priority for transplantation) with invalidating therapy-refractory recurrent or persistent HE leads to a maintained and improved quality of life and health-economic balance, assessed by a significant reduction in degree of HE and reduced number and days of hospitalisations for this reason. Moreover, this procedure is without a potentially expected aggravation of the portal hypertensive syndrome (with a minimum follow-up for now of 6 months). Long-term effects are being evaluated.

INTRA-ARTERIAL TREATMENT WITH YTTRIUM-90 MICROSPHERES FOR HEPATOCELLULAR CARCINOMA : 4 YEARS EXPERIENCE IN UZ GENT. B. Lambert, E. Sturm, H. Van Vlierberghe, J. Mertens, P. Smeets, R. Troisi, L. Defreyne. UZ, Gent, Belgium.

Introduction : At the UZ Gent we treat patients suffering hepatocellular carcinoma (HCC), who are not eligible for surgery or radiofrequent ablation, by means of intra-arterial Yttrium-90 microspheres. We present our 4 years experience using TheraSphere (Nordion).

Methods : 44 HCC patients were scheduled for a preparatory liver angiography and intra-arterial Tc99MAA-scintigraphy. Of these patients, 14 patients were excluded of Y90 radioembolization, mainly because of extrahepatic captation and unfavourable deposition of ^{99m}Tc-MAA in the liver, suggesting inadequate targeting of tumours. 30 patients received Y90-radioembolization (mean activity 2,13 GBq). 90% of them suffered from cirrhosis (Child A in 25 patients, Child B7 in 2 patients). We assessed the tolerance of Y90 radioembolization by means of the CTCAE v3.0. Tumour response on imaging was assessed using the modified RECIST criteria. Survival was estimated by Kaplan-Meier (SPSSv15.0)

Results : A median survival of 368 dd, (CI 227-459) was estimated. On imaging resp. 17%, 26% and 30% of 23 treatments showed complete response, partial response or stable disease. Disease progression was present in 26% and in half the cases this was limited to the untreated liver lobe. All six patients with elevated alfa fetoprotein (> 400 ng/ml) before treatment showed a significant disease afterwards. Adverse events typically presented as transient fatigue and minor abdominal discomfort and biochemically as a rise in bilirubin : at week 2 this was the case in 53% of treatment sessions and at week 6-12 in 38% of treatments. 1 out of 2 patients with a steep rise was offered transplantation since he showed tumour response on imaging. 2 patients presented with a subacute GI bleeding, the relation to the treatment remains unclear. One patient died 58 days after treatment due to spontaneous bacterial peritonitis and subsequent liver failure.

Conclusion : Yttrium-90 radioembolization for HCC is effective and well tolerated. By optimizing patient selection criteria and angiographic techniques, more patients might be eligible for treatment

IN VITRO HEPATOGENIC DIFFERENTIATION INCREASES ADULT HUMAN LIVER PROGENITOR CELL SUSCEPTIBILITY TO HEPATITIS B VIRUS INFECTION. M. Paganelli, D.N. Khuu, N. Jazouli, F. André, B. Kabamba, P. Goubau, M. Najimi, E.M. Sokal. UCL Saint-Luc, Brussels, Belgium.

Introduction : The response of liver stem and progenitor cells to hepatitis B virus (HBV) infection is unknown. Understanding such a response is important for the comprehension of hepatitis B pathogenesis.

Aim : To assess whether mesenchymal progenitor cells derived from adult human livers are infectable by HBV in vitro and to understand the role of differentiation status upon their susceptibility towards infection.

Methods : Adult human liver progenitor cells (AHLPCs) were cultured and differentiated to hepatocyte-like cells in vitro as previously described (Cell Transplant. 2007 ; 16 : 717-28). Quality of differentiation was assessed analysing morphology change, expression of mature hepatocytes markers (RT-PCR), glycogen production (PAS staining) and CYP3A4 activity. A hepatitis B virus (HBV)-infected human hepatoma cell line (HepAD38) was used for the production of infective virions. Undifferentiated AHLPCs (UD-AHLPCs) and differentiated AHLPCs (D-AHLPCs) were incubated for 72 h with HBV (100 gen.equiv/cell), then washed and cultured in normal conditions. Total viral DNA, HBsAg and HBeAg were dosed in supernatant using qPCR and ELISA. The presence of intracellular cccDNA was evaluated by real time PCR after digestion by Plasmid-Safe DNase. Intracellular viral mRNA was dosed by real time RT-PCR. The study was supported by the Fonds National de la Recherche Scientifique Belge.

Results : At the end of the differentiation process, AHLPCs showed a polygonal shape with granular cytoplasm. Expression of mature hepatocyte-specific marker genes (TDO, TAT, CYP3A4, CYP2B6), as well as glycogen production and CYP3A4 activity, increased significantly after differentiation as compared to UD-AHLPCs. After incubation with HBV and subsequent extensive washing, fluorescence immunostaining for both HBsAg and HBeAg showed an uptake of viral particles by the cells. Total HBV DNA was detected in culture supernatant from day 3 up to day 35 post-infection (p-i). cccDNA was detected inside the cells from day 4 up to day 28 p-i ($3.6 \pm 0.6\%$ of cccDNA found in HepAD38s). Significantly higher levels of cccDNA were present in D-AHLPCs as compared to UD-AHLPCs ($12 \pm 2.5\%$ higher, $p < .05$). An extremely small amount of viral mRNA was found in UD-AHLPCs 4-14 days p-i (0.01% of mRNA found in HepAD38s). D-AHLPCs showed significantly higher levels of both total and precore mRNA as

compared to UD-AHLPCs (4.5 ± 1.1 and 2.5 ± 0.8 times higher respectively, $p < .05$). HBsAg in supernatant became detectable from day 3 to day 10 p-i in both UD-AHLPCs and D-AHLPCs, while HBeAg was always negative.

Conclusion : AHLPCs are capable of HBV uptake, as shown by the presence of viral proteins and cccDNA inside the cells after incubation with the virus. Nevertheless, the absence of HBeAg in culture supernatant and the extremely low levels of viral mRNA suggest that UD-AHLPC are not permissive for HBV replication. After in vitro differentiation to hepatocyte-like cells, cccDNA amplification and viral genes expression significantly increased, confirming that the stage of differentiation plays a central role in cell susceptibility to HBV infection.

INVITED LECTURE

- D07 -

Marc Hautekeete Lecture : "Hepatitis E"
N. KAMAR (Toulouse, France)

INCREASING THE TOLERANCE FOR WARM ISCHEMIA OF LIVERS IN TRANSPLANTATION BY THE LEUVEN DRUG PROTOCOL. K. Vekemans (1), D. Monbaliu (2), E. Balligand (1), V. Heedfeld (1), J. Pirenne (2), J.F. Van Pelt (2). (1) Liver Research Facility, Leuven, Belgium; (2) University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Liver Transplantation (LTx) from non-heart-beating donors (NHBD) is associated with an increased risk of primary nonfunction (PNF). In porcine NHBD-LTx, our previous reported multifactorial biological modulation approach (Leuven Drug Protocol) can eliminate PNF, improve liver function and increases survival (D. Monbaliu *et al.* Ann. Surg., 2009).

Aim : Defining the molecular basis (genes, pathways) of this multifactorial drug protocol that gives protection of porcine liver grafts from NHBD against warm ischemia (WI) - reperfusion injury and the rational identification of additional drug targets based on detailed microarray analysis.

Methods : Porcine livers exposed to 45' WI were cold stored, transplanted and either modulated (n = 3) or not (controls, n = 4). In the modulation group, donor livers were treated with 7 drugs (streptokinase and epoprostenol prior to cold storage. In recipients, glycine, alpha1-acid-glycoprotein, MAPKinase-inhibitor FR167653, alpha-tocopherol, glutathione, and apotransferrin were administrated intravenously and PNF and serum parameters were monitored. Liver biopsies were taken at baseline and 1 hour after reperfusion. Gene expression was determined on Affymetrix GeneChip Porcine Genome MicroArray. Using bioinformatic software, molecular pathways and key regulatory genes were identified.

Results : Functionally, modulation protected the livers from PNF. Differential gene expression (2log fold change > +/-1) between baseline and 1 hr reperfusion : modulation 386 genes and control 244 genes. The extra genes in the modulation group belonged predominantly to molecular pathways related to cytokine activity, apoptosis, stress and cell proliferation. We identified 7 genes (IL6, IL8, JUN, MMP1, PTGS2/COX2, SERPINE1 and STAT3) that were induced in the control and repressed by the drugs. These genes could be linked to the 7 drugs applied. New potential drug targets were further identified based on induction in the control, unaffected by the drugs in the modulation group and predicted interactions according data mining.

Conclusion : Biomodulation in LTx using the Leuven Drug Protocol is protective against PNF. Treatment primarily results in the induction of a group of genes and the suppression of inflammation regulating genes. These findings will help us to make the multifactorial Drug Protocol more robust and applicable in the clinic.

HEPATOCYTE TRANSPLANTATION TRANSFORMS SEVERE PHENYLKETONURIA TO MILD HYPER-PHENYLALANINEMIA. X. Stephenne (1), F. Smets (1), G. Debray (2), R. Menten (1), R. Reding (1), M. Najimi (3), E.M. Sokal (1). (1) Cliniques Universitaires Saint-Luc, Brussels, Belgium; (2) University of Liège, Liège, Belgium; (3) Université Catholique de Louvain, Brussels, Belgium.

Introduction : In phenylketonuria (PKU) patients, the intellectual quotient outcome is directly related to the levels of phenylalanine during infancy. Best prognosis is associated with maintenance of average phenylalanine levels below 400 $\mu\text{mol/L}$ (6.6 mg/dl) in children less than 10 years of age. There is currently no specific treatment beside protein restriction and phenylalanine free products. The metabolism of phenylalanine is exclusively liver based.

Aim : Hence, liver cell transplantation is an attractive and logical option to help controlling phenylalanine levels in the most severely affected patients. Best results of liver cell transplantation are achieved with good quality cells, freshly isolated, from young donors and short ischemic time. This is made extremely infrequent due to lack of organs offered for liver cell transplantation.

Methods : The candidate patient was a 6 years old male with severe PKU. He was poorly equilibrated despite a close medical, dietary and psychosocial follow up. Genotype P281L / IVS10-11G > A confirmed the diagnosis of severe PKU with no residual activity of PAH, and no improvement was obtained under tetrahydrobiopterin treatment. The child had frequent elevated levels of phenylalanine above the safe limit of 400 $\mu\text{mol/litre}$ for the last three years. Tolerance to Phenylalanine was very low, and normal levels could only be reached when the child was hospitalized. In this context, we performed liver cell transplantation, using 1.7 billion fresh cells from a 14 months old girl with type 1b glycogen storage. The child received a second infusion of 0.8 billion fresh cells seven and a half months later from a healthy donor.

Results : Following this, mean phenylalanine level 3 months before transplantation, 11.1 ± 3.8 mg/dl (n = 11), decreased up to 3.5 ± 1.8 mg/dl (n = 11) after the 2 cell infusions, returning within normal limits. In parallel, the half life of phenylalanine, as evaluated by a loading test, decreased from 41.6 hours before cell therapy, to 19.1 hours after second cell transplantation.

Conclusion : This is the first demonstration in man that liver cell therapy can significantly improve phenylketonuria in severe patients.

CHOLESTASIS-ASSOCIATED PRURITUS TREATED WITH UVB PHOTOTHERAPY : REPORT OF 13 CASES. S. Decock, P. Roelandt, W. Van Steenberghe, J. Fevery, J. Van Pelt, W. Laleman, D. Cassiman, C. Verslype, F. Nevens. UZ Leuven Department of Hepatology, Leuven, Belgium.

Introduction : Intractable pruritus is a frequent and disabling complication of cholestasis. The management of cholestasis-associated pruritus remains challenging. Especially the pruritus related to ischemic strictures after liver transplantation is difficult to treat and can be one of the reasons for retransplantation. AASLD guidelines recommend first line therapy of cholestasis-associated pruritus with cholestyramin, second line therapy with rifampicin and third line therapy with opiate antagonists. Ultraviolet B (UVB) phototherapy is successfully used to treat pruritus in patients with atopic dermatitis, aquagenic pruritus, systemic mastocytosis, chronic renal failure,

Aim : We report our experience on treatment of cholestasis-associated pruritus with UVB phototherapy.

Methods : Thirteen patients (3 men, 10 women, mean age 52 years, range 28-73 years) with pruritus from cholestatic liver disease were treated with UVB phototherapy. Six patients had a chronic cholestatic liver disease (4 PBC, 2 PSC), four patients had cholestatic liver disease post liver transplantation (3 ischemic bile duct strictures and 1 vanishing bile duct syndrome due to ductopenic rejection), three patients had a drug-induced cholestatic hepatitis. In all patients conventional medical treatment had failed to control symptoms of pruritus. All patients were treated with UVB phototherapy. The perception of pruritus was recorded with the aid of a visual analogue scale (VAS) where 0 represented no pruritus and 10 the worst imaginable pruritus.

Results : Ten patients (77%) had a more than 60% reduction in perceived pruritus. Four patients (30%) had a more than 80% reduction in perceived pruritus. Mean VAS score before and at the end of treatment decreased from 8.5 to 2.3. The number of phototherapy sessions required varied between 12 to 60 with a mean of 26. Four patients started a second phototherapy session because of recurrence of pruritus. In all of them again a marked improvement of pruritus was observed. Side effects seen with UVB phototherapy were erythema and paraesthesias. Treatment had to be interrupted in two patients for that reason.

Conclusion : UVB phototherapy appears to be a promising and well tolerated treatment in the therapeutic armamentarium for cholestasis-associated pruritus. The treatment is also successful in patients with intractable pruritus after liver transplantation.

QUANTITATIVE HBV-DNA AND AST ARE STRONG PREDICTORS FOR SURVIVAL AFTER DETECTION OF HEPATOCELLULAR CARCINOMA. C. Witjes, J. Ijzermans, A. Van Der eijk, B. Hansen, C. Verhoef, R. De Man. Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : Hepatitis B virus infection (HBV) is an important co-factor in the development of hepatocellular carcinoma (HCC).

Aim : We studied whether quantitative HBV DNA at time of HCC detection influences survival of HCC patients.

Methods : All diagnosed HCC cases between 2000-2008 at our University based reference center were analysed to determine the influence of hepatitis B viral load on overall survival. Clinical and virological findings were evaluated in univariate and multivariate analyses, survival rates were assessed for HCC patients with high viral load (HBV DNA $\geq 10^5$ copies/mL) and low viral load (HBV DNA $< 10^5$ copies/mL).

Results : HCC was diagnosed in 597 patients, including 98 patients with HBV infection. The group of 37 patients (38%) who had high viral load contained more HBeAg-positive patients, had lower serum albumin level and higher serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level. The 1- and 5-year survival rates of HCC patients with high viral load were 58% and 11% and for HCC patients with low viral load 70% and 35%, respectively. A higher AST level and higher viral load were in multivariate analysis significantly associated with shorter overall survival (HR = 2.30 ; p = 0,018, HR = 1.22 ; p = 0,015, respectively).

Conclusion : HBeAg positivity, low albumin level or a high AST or ALT level in HCC patients is associated with higher mortality. HBV DNA level at detection is associated with overall survival of HCC patients. These findings support the concept that after HCC detection adequate suppression of HBV DNA by nucleoside analogue therapy may improve survival.

PREDICTIVENESS OF WASH-OUT TIME INTENSITY CURVE ON HEPATOCELLULAR CARCINOMA RECURRENCE. C. Witjes, F. Willemsen, J. Verheij, S. Van Der veer, B. Hansen, C. Verhoef, R. De Man, J. IJzermans. Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : The differentiation grade and presence of microvascular invasion predicts long-term survival after surgical treatment of a hepatocellular carcinoma (HCC).

Aim : We examined whether pre-operative MRI characteristics can predict differentiation grade and presence of microvascular invasion in HCC before hepatic resection.

Methods : Date of 597 patients with HCC were analysed, all slides of the resected specimen and the pre-operative MRI of all HCC patients treated with curative intent in a single reference center between 2000-2008 were prospectively analysed. Clinical, pathological and imaging findings were evaluated in uni- and multivariate analyses, a wash-out time intensity curve (TIC) was assessed.

Results : 87 patients with 104 nodules had at least one pre-operative MRI before surgical treatment with curative intent. According to the Lauwers classification ; 15 nodules (14%) were differentiated as good, 50 nodules (48%) as moderate and 34 nodules (32%) as poor. 55 nodules (53%) showed microvascular invasion. 28 patients with recurrence of HCC, had a significant higher alpha-fetoprotein (AFP), a larger tumour size, more often microvascular invasion and more often a moderate or poor differentiated tumour. In 85 nodules (88%) there was wash-out of contrast. HCCs well differentiated showed significantly less wash-out compared to HCCs moderate or poor differentiated ($p < 0.001$). HCCs without microvascular invasion also showed significant less wash-out ($p = 0.032$). The shape of the TIC of patients with and without recurrence did not differ significantly. There was no significant difference in TIC shape between patients with a good, moderate and poor differentiated HCC. The TIC of patients with and without microvascular invasion did not differ significantly.

Conclusion : Our data confirm that differentiation grade of HCC and presence of microvascular invasion predicts long-term survival. Microvascular invasion and a moderate or poor differentiation are associated with the presence of wash-out on MRI, which can be visualized with a TIC. The TIC shape can not differentiate between HCC with and without microvascular invasion. Neither can differentiated between a HCC differentiated as good, moderate or poor, neither can predict recurrence of HCC.

LONG-TERM OUTCOME OF PATIENTS WITH POLYCYSTIC LIVER DISEASE TREATED WITH LANREOTIDE. F. Nevens (1), M. Chrispijn (2), R. Vanslebrouck (3), M.H. Van Oijen (2), W. Coudyzer (3), A. Hoffmann (2), H. Dekker (2), R.A. De Man (2), L. Van Keimpema (2), J.H. Drenth (2). (1) UZ Leuven Departement of Hepatology, Leuven, Belgium ; (2) UMC St. Radboud, Nijmegen, Netherlands ; (3) UZ Leuven Departement of Radiology, Leuven, Belgium.

Introduction : Polycystic livers are phenotypical expression of autosomal dominant polycystic kidney disease (ADPKD) and isolated polycystic liver disease (PCLD). Symptoms result from a mass effect of the liver. In a randomized clinical trial we recently demonstrated that 6 months lanreotide 120 mg, effectively reduces polycystic liver volume.

Aim : The purpose of this 1-year study was establish the long-term efficacy and effect of discontinuation of lanreotide in the treatment of PLD.

Methods : This study was an open, observational, extension study of a 6-month, randomized, placebo-controlled trial, that studied the effect of lanreotide 120 mg, administered every 28 days in patients with a polycystic liver. Patients who initially had placebo received a total of 12 months of lanreotide (plac/lan/lan), and those who received lanreotide in the initial trial were treated with another 6 months of lanreotide (lan/lan). All patients were invited for a post-treatment follow-up after 6 months. Primary endpoint was change in total liver volume, as determined by CT-volumetry, after 6 and 12 months of treatment, compared to baseline.

Results : The 19 patients from the plac/lan/lan and the 22 from the lan/lan group had similar results after 12 months of treatment with lanreotide ($5.3\% \pm 7.0\%$ vs. $2.6\% \pm 6.7\%$, NS). For the collective 41 patients there was a decrease of mean liver volume $3.9\% \pm 6.9\%$ ($p < 0.01$) after 12 months. After 6 months of lanreotide, mean liver volume in this group decreased by $4.1\% \pm 5.6\%$ ($p < 0.01$), and remained unchanged during the following 6 months. Overall 24/41 (59%) were considered as responders : they had a reduction of TLV. Some 22/41 patients accepted a 6 month post-treatment CT, and there was an increase of liver volume of $2.9\% \pm 5.4\%$ ($p < 0.01$).

Conclusion : Lanreotide reduces liver volume after 6 months of treatment, and the beneficial effect is maintained in the following 6 months. Discontinuation results in recurrence. The majority of the responders can be identified at 6 months. These observations support the long-term use of lanreotide for the treatment of PLD.

DREAM 2020 : DEVELOPMENT OF LAPAROSCOPIC SURGERY AND ENDOSCOPY IN THE UNIVERSITY HOSPITAL OF KINSHASA, DRC. B. Nsadi (1), D. Veyi (1), J. Kazadi (1), D. Sanduku (1), P. Mukaba (1), Y. Kosi (1), P. Mukuna (1), A. Kaba (2), O. Detry (2). (1) Cliniques Universitaires de Kinshasa (CUK), Kinshasa, Belgium ; (2) CHU de Liège, Liège, Belgium.

Introduction : Nowadays, laparoscopic surgery is widely disseminated in developed countries, even in small primary hospitals. The spreading of laparoscopy in developing countries has not been as successful. The technological nature of laparoscopy, and the required specific laparoscopic tools and medical skills, may render the initiation of this approach difficult for the developing countries.

Aim : We hypothesized that laparoscopy may be developed in the Cliniques Universitaires de Kinshasa (CUK), University of Kinshasa (UNIKIN) and may be cost-effective for the population, considering the reduction of the cost of the associated medications and of the length of hospital stay. The final aim of this program is to bring the benefits of laparoscopy to the population of DRC, by allowance of adequate training of the UNIKIN anaesthetists and surgical trainees, who in the future might have the opportunity to apply their knowledge in their own professional practice. We hypothesized also that the availability of adequate and modern equipment may reduce the incentive for the brain drain and may help to keep some highly trained doctors within DRC.

Methods : In partnership with the university of Liège and the financial support of Wallonia region, a complete CUK team, including a surgeon (2 years training in Belgium), an anaesthetist and nurses, was trained in Belgium and then after in DRC. The laparoscopic equipment, adapted to the African conditions, was sent to Kinshasa, and three theoretical and practical missions of a Belgian team were organised over one year.

Results : The CUK team performed 57 surgical laparoscopic procedures in the first 12 months, including 18 appendectomies, 13 cholecystectomies, 8 hernia repairs, 5 laparoscopy explorations for peritoneal carcinoma assessment and biopsy, 3 procedures for catheter of peritoneal dialysis, 2 minors gynecologic procedures, 2 management of generalized peritonitis, 2 adhesiolysis, 2 abscess drainages, 1 rectal prolapse, and 1 cystocoele. No mortality and no postoperative infection were observed. After 12 months of local use, all the medical material was in perfect condition. In addition to surgery, this program allowed the improvement of the quality of anaesthesia for non laparoscopic procedure.

Conclusion : This program demonstrated the development, accessibility and durability of such new approaches in developing countries. The interest for laparoscopy was demonstrated in several hospitals of Kinshasa. The next step will include the development of GI endoscopy and abdominal imaging. The needs of the DRC population are profound. The DRC medical schools, isolated from the developed countries for more than 30 years, are in great need for support. All the University and non-University GI teams or individuals willing to join such a project, are welcome.

THE EFFECT OF SHORT-TERM PREOPERATIVE WEIGHT LOSS USING VERY LOW ENERGY DIET ON OPERATIVE OUTCOME OF LAPAROSCOPIC GASTRIC BYPASS SURGERY. A MULTICENTER PROSPECTIVE RANDOMISED STUDY (SHOPWEL). Y. Van Nieuwenhove, E. Shopwel-study Group, european obesity academy, UZ, Gent, Belgium.

Introduction : Obesity with its associated disorders is an increasing health problem in the western society. Due to disappointing results after conservative treatment modalities, surgical therapy for morbid obesity (bariatric surgery), with its well documented long-lasting effects, has become the therapy of choice for an increasing number of patients with extreme overweight. Although morbidity and mortality rates associated with bariatric surgery are low, the need for measures to reduce perioperative complication rates is high. Excess body fat and, due to hepatosteatosis, enlarged liver complicate the technical aspects of the surgical procedure. It is known that, in response to a 2-weeks period of energy restriction, liver volume is decreased. Therefore, in many centres recommendation of a short period of preoperative very low energy diet (VLED) has become routine, since this procedure has been hypothesised to facilitate the surgical procedure and thus, resulting in less operative time and complications. However, this has never been evaluated in a controlled study.

Aim : To compare the results of a 2 week preoperative VLED on operative time and perioperative complication rates in laparoscopic Roux-en-Y gastric bypass (LRYGB).

Methods : International, prospective, randomised multicentre clinical trial. Patients scheduled for LRYGB were randomly assigned for a two weeks period of preoperative VLED or no dietary restriction. Primary endpoint was operative time ; the secondary endpoints are estimated perioperative blood loss, number of liver lacerations, complexity of the procedure, perioperative events/complications and the length of stay. Data were analyzed on an intention to treat basis.

Results : A total of 294 patients were randomized to either the control (n = 145) or diet group (n = 149). Nine patients in the control 12 patients in the study group dropped out during the study. Sixteen patients (10.7%) of the study group did not finish the two week diet because of intolerance. While the mean (s.d.) weight the day before surgery was not different in the groups, the mean weight loss during the weeks prior to surgery was 0.40 (3.24) kg in the control group and 4.97 (3.60) kg in subjects who followed the VLCD regimen (p < 0.0001). Similarly, BMI on the day before surgery

was not different, but the drop in BMI was significantly higher in the diet ($1.69 \pm 1.25 \text{ kg/m}^2$) than in the control group ($0.11 \pm 1.06 \text{ kg/m}^2$; $p < 0.0001$).

The mean (s.d.) operating time was 81.18 (21.24) minutes in the control patients, which was not significantly different from the 80.20 (23.23) minutes of the study group. The mean VAS of difficulty was significantly higher in the control group ($38.17 \pm 35.95 \text{ mm}$) than compared to the group who had the diet (30.91 ± 20.94 ; $p = 0.04$). However, no differences could be observed in mean blood loss, and number or degree of liver lacerations. The effect of a higher BMI did not seem to influence the outcome. Both patients who had a BMI less than 48 kg/m^2 or above 48 kg/m^2 , there was no statistically significant difference in operating time, VAS of difficulty, blood loss or number and score of liver lacerations. 18 (13.2%) complications were observed in the control group, and 8 (5.8%) in the diet group ($p = 0.037$).

Conclusion : A VLCD prior to gastric bypass surgery for morbid obesity induced a significant weight loss after 2 weeks. This weight loss did not result in a reduced operating time but did decrease perceived difficulty and morbidity. These results support the routine use of this diet 2 weeks prior to gastric bypass surgery.

-D16-

TREATMENT OF GASTRIC LEAKS WITH SELF EXPANDING FULLY COVERED STENTS AFTER BARIATRIC SURGERY. G. Hubens, T. Moreels, E. Macken, M. Ruppert, W. Vaneerdeweg. Antwerp University, Antwerp, Belgium.

Introduction : Anastomotic or gastric leakage after bariatric surgery occurs in 1-6%. Redosurgery is associated with a substantial morbidity and even mortality. Classic conservative treatment at the best condemns patients to a very long postoperative hospitalisation period and often fails (especially after sleeve gastrectomies) due to the anatomical construction of the bariatric procedure.

Aim : To study whether the use of self-expanding fully covered stents is beneficial in the treatment of these difficult postoperative complications.

Methods : Ten morbid obese patients with persisting leaks after a bariatric procedure were treated with a self expanding fully covered stent. Five patients had a sleeve gastrectomy, two patients a primary Roux en Y gastric bypass procedure (RYGBP) and three patients had a redo procedure (lap band to RYGBP, Mason gastroplasty to RYGBP, Scopinaro to RYGBP). Four patients had at least one surgical attempt at fistula closure before endoscopic treatment. The leaks were situated at the angle of Hiss (all after primary sleeve procedures), the gastroenterostomy ($n = 3$) or the pouch stapler-line ($n = 2$).

Results : Successful closure of the fistula was obtained in 70% of the cases after a mean time of 9 weeks. All these patients were able to be sent home a few days after placement of the stent. Two patients had persistent fistulisation after removal of the stent and were further treated conservatively with closure of the fistula. In one patient a reintervention (total gastrectomy) was necessary to obtain healing but placement of the stent permitted local healing allowing us to operate in more favourable circumstances. Early dislocation (after a few days) occurred in 40% of the cases. All these dislocations were treated with reposition of the stent with a 50% success rate in fistula closure. Late dislocation was seen in 20%. One of these patients had a successful introduction of a new stent while the other patient underwent redosurgery.

Conclusion : Treatment of leakage after bariatric procedures with the use of self-expanding fully covered stents was successful in 70% of the cases avoiding difficult reinterventions or long postoperative hospitalisation periods. Dislocation of the stents occurs frequently (60%) due to the anatomical situation after surgery but in 50% of these cases reposition of the stent or placement of a new stent resulted in closure of the leak.

LYMPH NODE RATIO AS PROGNOSTIC FACTOR IN COLORECTAL CARCINOMA : AN ITALIAN STUDY.
S. Rossi Del monte, A. Scarinci, G. Marino, M. Ferri, M. La Torre, V. Ziparo. Sapienza University, Rome, Italy.

Introduction : The most important prognostic factor in colorectal cancer is the involvement of the lymph nodes. It has been demonstrated that a high numbers of examined lymph nodes leads to a better prognosis even in patients without lymph node metastases, and current guidelines recommend the excision a minimum of 12 lymph nodes to adequately assess the tumor stage.

Aim : The aim of this study was to determine the prognostic value of lymph node ratio (LNR) that is the quotient between the number of positive lymph nodes and the total number of lymph nodes examined.

Methods : A total of 822 cases were retrospectively reviewed from the database of three different Centers. Six-hundred-twentyfour patients underwent radical colorectal surgery for colorectal carcinoma at the Surgery Department, Sant' Andrea Hospital, University of Rome La Sapienza in the period 2000-2008 and 198 at the Department of Surgical Oncology, IRCSS, Rionero in Vulture in the period 2005-2008. Patients were divided into five groups : LNR-0 for patients with no lymph nodes involved ; LNR-1, LNR-2, LNR-3 and LNR-4 respectively for patients with LNR between 0,01 and 0,17, between 0,18 and 0,41, between 0,42 and 0,69, and greater than 0,69. The 5-years survival was determined using the Kaplan-Meier method and the log-rank test was used to compare survival curves. A Cox regression model was used for multivariate analyses.

Results : Multivariate analysis showed that stage, N status, M status, vascular invasion and LNR were all independent prognostic factors. The 5-year overall survival and disease-free survival were respectively 90% and 82% for LNR-0 patients, 72% and 69% for LNR-1 patients, 48% and 40% for LNR-2 patients, 36% and 28% for LNR-3 patients and 20% and 15% for LNR-4 patients ($p < 0,001$). a subgroup analysis of patients with less than 12 lymph nodes examined (159 cases), showed that both N stage and LNR were significant indicators of prognosis ($p = NS$).

Conclusion : LNR is a strong and independent prognostic factor in colorectal carcinoma and may optimise patient stratification for prognosis. A minimum of 12 lymph nodes should be harvested and examined in order to assess correctly the lymph node status and the LNR. LNR should be routinely reported and included in the current staging systems.

PREOPERATIVE BEVACIZUMAB LOWERS TUMOR INTERSTITIAL FLUID PRESSURE IN A RODENT MODEL OF INTRAPERITONEAL CHEMOPERFUSION FOR COLORECTAL PERITONEAL METASTASIS.
J. Verhulst, N. Van Damme, W. Ceelen. UZ, Gent, Belgium.

Introduction : Intraperitoneal chemoperfusion is increasingly used in patients with peritoneal surface malignancy. Adequate penetration of the instilled agent(s) into tumor tissue is a prerequisite for antitumor efficacy. It is known that drug delivery to solid tumors is impeded by the pathologically elevated interstitial fluid pressure (IFP) associated with neoplastic tissue.

Aim : We aimed to verify whether vascular normalization by administration of bevacizumab would result in a decreased IFP in isolated peritoneal metastasis from colorectal cancer treated with intraperitoneal chemoperfusion (IPC).

Methods : HT29 colorectal tumors were grown in athymic rats and isolated 5-7 mm diameter fragments transplanted in the peritoneal cavity of acceptor rats. Two weeks after tumor implantation, animals underwent IPC with oxaliplatin (460 mg/m²) during 60 minutes. Tumor IFP was measured continuously using a fiberoptic probe based on the Fabry Perot interferometer (Samba Preclin®, Samba Sensors). Animals were treated with saline, bevacizumab (5 mg/kg) once one week before IPC, or bevacuzimab daily during 7 days before IPC. Each treatment was followed by IPC at 37°C or 42°C, totaling six groups (N = 6 per group).

Results : The IFP at the start of IPC was significantly lower in all bevacizumab treated groups compared to the control group. At 37°C, the IFP was 22.8 ± 19 mmHg, -10.5 ± 20 mmHg, and -2.4 ± 3.3 mmHg in the control, bevacizumab 1×, and bevacizumab 7× groups respectively ($P < 0.001$, Kruskal Wallis test). A similarly significantly decreased IFP was observed in bevacizumab treated animals who underwent hyperthermic IPC. Interestingly, IFP decreased progressively during the chemoperfusion in the control group at 37°C (22.8 versus 5.7 mmHg, $P = 0.046$, Mann Whitney U test) but remained stable in the other treatment groups.

Conclusion : Single or repeated administration of bevacizumab results in a significantly lower IFP in colorectal peritoneal metastases. This may result in enhanced cytotoxic drug delivery during IPC and translate in improved antitumor efficacy.

ESOPHAGEAL CANCER SURGERY IN PATIENTS OLDER THAN 75 : LONG TERM RESULTS. C. Honoré, A. De Roover, A. Al-Azzeh, N. Gilson, P. Honoré, M. Meurisse. University of Liège, Liège, Belgium.

Aim : The purpose of this study was to evaluate short and long term results after esophageal resection for cancer in patients older than 75 which are often denied surgery because of a suspected increased morbidity and a lesser life expectancy.

Methods : We retrospectively analyzed the prospective electronic database of esophageal cancer surgically treated in our department between January 2003 and December 2009 to identify patients older than 75. The preoperative (denutrition, ASA score, WHO general status, Charlson Comorbidity Index, histological subtype, neoadjuvant treatment), operative and postoperative characteristics (morbidity, mortality, disease free and overall survival) were analyzed.

Results : Among the 137 patient surgically treated during that period, 23 patients were older than 75. The preoperative characteristics' analysis showed 39% of severe/moderate denutrition, a mean ASA score of 2 (ASA1 : 4%, ASA2 : 87%, ASA3 : 9%), a mean WHO general status of 1 (WHO 0 : 65%, WHO 1 : 13%, WHO 2 : 22%), a mean Charlson Comorbidity Index of 3 (Range : 2-5). 4% of patients received a preoperative chemoradiotherapy, 26% received a preoperative chemotherapy. The histological subtype was adenocarcinoma in 100%. The surgical techniques were a "Lewis-Santý" procedure in 43%, a trans-hiatal resection in 22%, a "Sweet" procedure in 13%, a stripping in 13% and a "McKeown" procedure in 9%. The in-hospital postoperative mortality was 13%. The in-hospital postoperative morbidity (Dindo-Clavien Grade > 2, deceased patients included) was 26%. In univariate analysis, no statistically significant risk factor of morbidity was found. A Charlson Comorbidity Index > 2 was, in univariate analysis, associated to postoperative death (p = 0.0362). The mean hospital stay was 22 + 12 days. The median survival was 24.2 months. The 5-year overall survival was 39% and the 5-year disease free survival was 26%. 57% of long-term deaths were not cancer related.

Conclusion : Esophageal surgery performed in selected patients older than 75 has an acceptable morbidity and mortality. Anyway, when a severe complication occurs, it leads to death in half of the cases, mostly in patients with associated comorbidities. The long term survival after surgery is comparable to a younger population. This study confirmed our attitude of not considering age as a contra-indication for esophageal surgery but rather considering general status, self-reliance and associated comorbidities for the patients' selection.

COMBINATION OF GEMCITABINE AND CETUXIMAB IN PATIENTS WITH ADVANCED CHOLANGIO-CARCINOMA : A PHASE II STUDY. A. Ceratti (1), I. Borbath (1), C. Verslype (2), T. Delaunoy (3), M. Van Den Eynde (1), S. Laurent (4), M. Peeters (4), A. Hendlisz (5), A. Deleporte (5), P. Vergauwe (6), B. Delhougne (7), M. Polus (8), E. Van Cutsem (2), J.L. Van Laethem (9). (1) Cliniques Universitaires Saint-Luc, Brussels, Belgium ; (2) University of Leuven, Leuven, Belgium ; (3) Hôpital de Jolimont, Haine-Saint-Paul, Belgium ; (4) UZ, Gent, Belgium ; (5) Institut Jules Bordet, Brussels, Belgium ; (6) AZ Groeninge, Kortrijk, Belgium ; (7) CHC de la Citadelle, Liège, Belgium ; (8) CHU Liège, Liège, Belgium ; (9) ULB Erasme, Brussels, Belgium.

Introduction : Cholangiocarcinomas (CCK) are uncommon tumors with an increasing incidence and a poor prognosis. Epidermal growth factor receptor (EGFR) expression and activation in CCK have been demonstrated.

Aim : The aim of this trial was to assess the efficacy of combining cetuximab (Ctx) and gemcitabine (Gem) in front line treatment of advanced unresectable CCK. The primary endpoint was to determine the progression-free survival (PFS) rate at 6 months with this regimen. Secondary endpoints were to assess response rate, safety profile and overall survival (OS).

Methods : We conducted a multicenter phase II trial combining Ctx, an anti-EGFR chimerized IgG1 monoclonal antibody, to Gem. Patients with either locally advanced (LA) or metastatic (M) measurable CCK (excluding gallbladder cancer) were included ; no prior systemic therapy was allowed. Ctx was administered at the initial dose of 400 mg/m² and further injections at 250 mg/m² every 7 days, and Gem was administered at 1000 mg/m² on day 1, 8 and 15 every 4 weeks. The primary endpoint was the 6 month-PFS rate. A Simon 2-stage design was used. We hypothesized that the combination therapy would improve 6 month-PFS rate from 20% to 40% . We therefore needed 3 patients with PFS > / = 6 months from the first 13 to further include a total of 43 patients.

Results : A total of 44 patients with advanced CCK (41% LA/59%M) was enrolled from September 2008 to January 2010. The median age was 61.5 years (range : 40 to 86 years) and baseline ECOG performance status was 0 for 68% and 1 for 32% of the patients. Forty-three percent of the patients had prior surgery. Forty-six percent of the patients were free from progression at 6 months. Median PFS was 5.8 months (95% CI, 4.4-7.4 months) and median OS was 11.6 months (95% CI, 8.7-14.6 months). Nine patients (20.9%) had partial response with a median duration of 5 months (range, 2-10 months). Disease control rate (PR + SD > 8 weeks) was 81.4%. The most common grades 3/4 related-

toxicities were haematological abnormalities (47.7%), skin rash (13.6%) and fatigue (11.3%). Due to toxicity, 6 patients discontinued study treatment ; 14 patients had a Gem dose reduction and 3 patients had a Ctx dose reduction. Among the nine responders, 8 experienced a skin rash of at least grade 2, suggesting a relationship between skin toxicity and efficacy.

Conclusion : Our study met its endpoint, i.e. a PFS rate of 46% at 6 months, suggesting that Gem-Ctx combination had promising activity with a manageable toxicity profile. Adding Ctx to the new standard of care Gem-cisplatin deserves further investigations in CCK.

- D21 -

SHORT TERM SIDE EFFECTS AFTER RADIOFREQUENCY ABLATION. ARE WE READY TO ABLATE NON-DYSPLASTIC BARRETT ? Y.N. Choi, H. Willekens, G. Coremans, S. Depeyper, R. Bisschops. UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Radiofrequency ablation (RFA) is an effective treatment for Barrett's associated dysplasia and eradication of intestinal metaplasia. Due to its efficacy and good safety profile it has been suggested that RFA of non-dysplastic Barrett's might be cost-effective. Although major complications are well studied and limited, little is known on RFA induced symptoms.

Aim : We aimed to assess the short-term side effects after circumferential (HALO 360) or focal (HALO 90) RFA.

Methods : RFA was performed to obtain total eradication of intestinal metaplasia in patients with low grade of high grade dysplasia, after endoscopic resection of any visible lesion. Post RFA, patients who were on pantoprazole 40 mg bid and an evening dose of ranitidine 300 mg, were asked to fill out a 30-day diary in which they had to score for the following 8 symptoms on a 7 point Likert scale (0 = no symptoms to 7 = unbearable, rendering normal daily activity impossible) : retrosternal pain and burning, dysphagia, epigastric pain, decreased appetite, sour throat, nausea and vomiting. A score above or equal to 3 was defined as symptoms that cannot be ignored and were regarded as significant side-effect. Mean scores were calculated for symptomatic patients as well as the time until the mean score dropped below 1.

Results : Data for 13 Halo 360 and 22 Halo 90 procedures were available. Retrosternal pain, retrosternal burning, dysphagia and decreased appetite were the most common symptoms after RFA occurring in 70-92% of the patients. These symptoms did not significantly differ between the Halo 360 and 90 group. Retrosternal pain was a highly prevalent and the most severe symptom with a maximum mean score of 4 and 3.76 and lasting 14 and 13 days in the HALO 360 and HALO 90 group respectively. Dysphagia was the most prevalent symptom but was rather limited in severity. It was present in 92% and 91% of cases but only significant in 23% and 27% of Halo 360 and Halo 90 cases respectively. Decreased appetite occurred in 69% and 63% of HALO 360 and HALO 90 patients respectively, but was only severe in 15% and 14%. Nausea and vomiting was not so prevalent (18-38%) and was only significant in 8-15% of patients. Fever occurred in 31% and 19% after Halo 360 and Halo 90 respectively. No cases of melena were reported. After 16 days the mean score for all symptoms dropped below 1.

Conclusion : The majority of patients report short term side effects after RFA. In more than 25% of patients these symptoms are considered significant to severe and last for about two weeks. These findings are important when consenting patients for RFA and should be taken into account when considering RFA for non-dysplastic Barrett.

LONG-TERM OUTCOME OF ENDOSCOPIC STENTING FOR OBSTRUCTIVE COLORECTAL CANCER AS BRIDGE-TO-SURGERY. J. Vandervoort, P. Van Der spek, M. De Man, K. Hendrickx, Y. Van Molhem, L. Lepoutre. OLV Ziekenhuis, Aalst, Belgium.

Introduction : Stenting in the treatment of obstructive colorectal cancer (OCRC) as a bridge-to-surgery (BTS) remains controversial because no data are available on long-term survival in these patients with possible curable disease.

Aim : We report our results and long-term outcome with stenting in OCRC as BTS and its implications on further surgical therapy.

Methods : From 269 stenting procedures in colonic obstruction entered in our database since 1998, we identified 84 (31%) procedures in BTS setting. All were included for short-term analysis. Nineteen patients were excluded from long-term analysis because of benign disease, incomplete tumorstaging or follow-up. Accurate pTNM-classification and long-term follow-up was available for 65 patients. The need for chemotherapy was decided at multidisciplinary meetings. Surgical resection was performed according to the surgeons preference.

Results : Mean age was 69yrs. (range 40-85), 43 males and 22 females. Technical success rate was 83/84 (98%) and clinical success 81/84 (96%). Stent migration occurred in 7 patients (8%) and perforation in 3 (3,5%). One critically ill patient at the time of the procedure died within 24 h (1,2%). Mean time to surgery was 17 days (range 1-119). Laparoscopic resection was performed in 54 (83%), open surgery in 9 (14%) and stoma in 2 (3%). Post-operative complications were hyperglycaemic coma (1 pt), urinary tract infection (pt), post-operative bleeding (2 pt), ischemic colon (1 pt), pancreatitis (1 pt), catheter sepsis (1 pt) and anastomotic leakage (1 pt).

Table 1 : Longterm survival for different TNM-classifications :

| | <i>T2N0</i> | <i>T2N1</i> | <i>T3N0</i> | <i>T4N0</i> | <i>T3N1</i> | <i>T3N2</i> | <i>T4N1</i> | <i>T4N2</i> | <i>TxNxM1</i> |
|---------------|---------------|---------------|----------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|
| | <i>1 pt.</i> | <i>1 pt.</i> | <i>19 pt.</i> | <i>5 pt.</i> | <i>6 pt.</i> | <i>8 pt.</i> | <i>9 pt.</i> | <i>2 pt.</i> | <i>14 pt.</i> |
| 1 yr survival | 1/1 100% | 1/1 100% | 18/19 95% | 5/5 100% | 6/6 100% | 8/8 100% | 9/9 100% | 2/2 100% | 12/14 85% |
| 3 yr survival | n.a. | n.a. | 16/18 88% | 2/3 66% | 5/5 100% | 2/4 50% | 6/7 85% | 0/2 0% | 4/12 33% |
| 5 yr survival | n.a. (76%) | n.a. (71%) | 8/10 80% (67%) | 1/2 50% (55%) | 2/3 66% (55%) | 1/3 33% (38%) | 1/4 25% (39%) | 0/2 0% (21%) | 2/9 22% n.a. |

n.a. = not available

(x%) = observed 5-yr survival Surveillance Epidemiology and End Results (SEER) program of the National Cancer Institute.

Conclusion : This single center experience reports on the longest current available length of follow-up in a large cohort of patients treated with a stent as BTS for OCRC. There is a high success rate with low morbidity and mortality. Surgical technique used to remove the tumor is not influenced by the presence of a stent. Survival rates compare favourable with data from the SEER program of the National Cancer Institute. This suggests no negative impact of BTS stenting on prognosis. Data collection will continue to update these results in the future.

LOW MOLECULAR WEIGHT PROTEIN PLASMA SIGNATURE FOR DISCRIMINATION BETWEEN COLORECTAL CARCINOMA, ADENOMA AND HEALTHY CONTROLS SUBJECTS. M.A. Meuwis (1), P. Leclercq (2), M. Fillet (3), R. Marée (4), M. Polus (2), D. Van Daele (2), M. Malaise (5), E. Louis (2), M.P. Merville (3). (1) GIGA Proteomic Platform, ULg, Liège, Belgium ; (2) Gastroenterology, hepatology and digestive oncology, GIGA R, CHU Liège., Liège, Belgium ; (3) Medical chemistry, GIGA-R, ULg, Liège, Belgium ; (4) GIGA Bioinformatic platform, Liège, Belgium ; (5) Rheumatology, GIGA R, CHU Liège, Liège, Belgium.

Introduction : Colorectal carcinoma (CRC) early stage and adenoma (A), discriminations from healthy controls (HC) is still not possible based on a single biomarker. Hence we investigated the possibility to discriminate these 3 groups on the basis of their plasma signature obtained by Surface Enhanced Laser Desorption Ionization-Time of Flight-Mass Spectrometer (SELDI-TOF-MS) and multivariate analysis.

Aim : The selection of potential biomarker discriminating CRC, A and HC.

Methods : We collected 50 patients' plasma (17 CRC, 16 A and 17 HC) and further equalized each sample protein content individually with the proteominer® kit (BioRad). The pretreated fractions were loaded in triplicate on SELDI chips according to a standardized protocol. Every patient's low molecular weight protein signatures were obtained on a SELDI-TOF-Mass spectrometer (BioRad) and treated using univariate and multivariate statistical analysis.

Results : Univariate analysis allowed selection of 48 proteins or peptides peaks for which distributions were significantly different between diseases groups (even after P-values Benjamini-Hochberg correction). By multivariate analysis, we obtained a model (Extra Tree) able to discriminate CRC from A with 93.8% sensitivity and specificity and 91% accuracy. Adenoma could be discriminated from HC with 81.3% sensitivity, 70.6% specificity and 75.8% accuracy using a boosting method. When discriminating CRC from HC, a boosting model reached 70.6% sensitivity, 82.4% specificity and 76.5% accuracy. Some potential biomarkers identification is currently under investigation.

Conclusion : Based on plasma proteomic signatures, we generated models able to discriminate CRC, from adenoma and from HC. Although these models need confirmation by other proteomic and non proteomic approaches on another cohort of patients, the selected biomarkers might represent new candidates for CRC early diagnosis.

EARLY GENE EXPRESSION CHANGES INDUCED BY OXALIPLATIN PREDICTING A RESPONSE TO CHEMOTHERAPY. IN VIVO STUDY ON A TISSULAR MODEL OF HUMAN COLORECTAL CANCER. C. Honoré, D. Goéré, D. Allard, V. Boige, V. Lazar, D. Elias. Institut Gustave Roussy, Villejuif, France.

Aim : The purpose of this study was to search on a tissular model of human colorectal cancer for early gene expression changes induced by oxaliplatin predicting a response to chemotherapy.

Methods : A tumoral tissular model was created from operative specimen of colorectal cancer, grafted in the flank of «swiss nude» mice. Chemotherapy response was experimentally determined for each model and 10 models were selected, 5 responder and 5 resistant. Among each model, 1 group of mice was not treated, being taken as a control and 5 groups were treated and euthanatized at 6, 12, 24, 48 and 72 h. The graft was surgically removed, RNA were extracted and hybridized on pangenomic microarray, each treated time with the control from the same model, to identify the gene expression changes induced by oxaliplatin. Correlating these results with the experimentally known chemotherapy response, discriminating gene expression changes predicting a response were isolated. Another analysis focused on gene expression changes trough time to identify the most accurate moment to search for these changes.

Results : Thirteen models were made (6 responder and 7 resistant) in 57 + 18 days. The analysis of the 10 selected models identified 39 genes whose expression was modified after giving oxaliplatin. The analysis correlating these changes to the experimental chemotherapy response of the model identified 15 discriminating genes : TTN, MYL2, TNNT3, MYOZ1, MMP14, HLA-DRB1, PLEKHA2, BRD3, SARDH, MPHOSPH8, ENST00000368917, FOXR1, AK021866, SPATA13, FAM123A. Concerning the time analysis, genes expressions were recorded after 6 h but the global level of expression remained plain until 72 h and no peak was identified.

Conclusion : We identified an induced signature of 15 genes predicting the response to oxaliplatin on a tissular model of colorectal cancer. The next step is to validate this signature on a larger cohort of model before starting a clinical application.

SYSTEMATIC SECOND-LOOK SURGERY PLUS HIPEC IN ASYMPTOMATIC PATIENTS AT HIGH RISK OF PERITONEAL RECURRENCE OF COLORECTAL CANCER. C. Honoré, D. Goéré, F. Dumont, C. Dromain, V. Boige, D. Malka, P. Burtin, M. Ducreux, D. Elias. Institut Gustave Roussy, Villejuif, France.

Aim : The purpose of this study was to analyse the impact of a systematic second-look surgery plus hyperthermic intraperitoneal chemotherapy (HIPEC) performed one year after resection of the primary tumor in asymptomatic patients at high risk of developing a peritoneal carcinomatosis

Methods : From 1999 to 2009, 41 patients with a colorectal cancer at high risk of peritoneal recurrence, without any clinical, radiological or biological signs of recurrence underwent a second-look surgery one year after resection of the primary tumor and 6 months after the end of the adjuvant chemotherapy. The selection's criteria of high risk patients were : a minimal synchronous macroscopic PC or ovarian metastases resected during the primary tumor surgery or a perforated primary tumor.

Results : PC was found and treated with complete surgery plus HIPEC in 23 patients (56%). The other 18 patients underwent complete abdominal exploration plus systematic HIPEC. Median follow-up was 30 [9-109] months. One patient died postoperatively (2%). A severe complication (grade > 2) occurred in 4 patients (9.7%). The 5-year overall survival rate was 90% and the 5-year disease-free survival rate was 44%. Peritoneal recurrences occurred in 7 patients (17%), 6 of whom had macroscopic PC discovered during the second-look (26%), and one patient had no macroscopic PC (6%). In the univariate analysis, the presence of PC at second-look surgery was a significant risk factor for peritoneal recurrence (p = 0.006).

Conclusion : In high risk patients, the systematic second-look surgery plus HIPEC strategy diagnosed more than 50% of asymptomatic peritoneal recurrence and achieved a 90% 5-year overall survival rate. This original approach will, to be validated, be compared in a randomized trial to a standard follow-up, the actual reference of care.

CROHN'S DISEASE : INFLIXIMAB TROUGH LEVELS AND CRP DURING INFLIXIMAB-IMMUNOMODULATOR COMBINATION TREATMENT ARE ASSOCIATED WITH CLINICAL OUTCOME AFTER IMMUNOMODULATOR WITHDRAWAL. P. Bossuyt (1), D. Drobne (2), C. Breynaert (2), N. Vande Casteele (2), G. Compennolle (2), M. Juergens (2), V. Ballet (2), I. Cleynen (2), W. Wollants (2), P. Rutgeerts (2), S. Vermeire (2), A. Gils (2), G. Van Assche (2). (1) Imelda GI Clinical Research Centre, Bonheiden, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Combining immunomodulators (IMM) with scheduled maintenance infliximab (IFX) has superior efficacy in IMM naive patients out to 1 year, but withdrawal of IMM later on is an issue of debate.

Aim : To study the influence of IMM withdrawal on IFX trough levels (IFX TLs) and to identify predictors of disease flare and loss of response to IFX after withdrawal of IMM.

Methods : 223 patients on IFX maintenance therapy for Crohn's disease were studied. 158 patients were co-treated with IMM. In 117 IMM were discontinued when durable clinical remission was achieved after > 6 months of co-treatment. With an in-house developed ELISA we measured a total of 1.055 IFX TLs at different time points. Antibodies against IFX (ATIs) were measured in samples with undetectable IFX TLs. Clinical response and CRP were assessed before every IFX infusion. Potential independent predictors of disease flares and loss of response were subjected to univariate analysis and significant variables were integrated into Cox proportional-hazards regression model using a forward selection.

Results : During the follow-up 49% of the whole cohort needed IFX dose optimisation and 26% stopped IFX – of these 15% stopped within 1 year. 20% of patients had already been dose optimized before IMM was withdrawn. After withdrawal of IMM 31/117 (26%) patients had a disease flare necessitating IFX dose optimisation and 14/117 (12%) patients lost response to IFX during the median follow-up of 29 months. Overall, IFX TLs remained stable after withdrawal. However, combination treatment strategy resulted in higher IFX TLs (3.4 µg/ml) as compared to IFX monotherapy (2.5 µg/ml) (p < 0,001). Two independent predictors for complete loss of response to IFX after withdrawal of IMM were identified : undetectable IFX TLs at the time of IMM withdrawal (hazard ratio = 6.2, p = 0.027) and CRP > 5 mg/l at the time of IMM withdrawal (hazard ratio = 7.6, p = 0.010). Undetectable IFX TLs at withdrawal (but not CRP) were also predictive for disease flare without the need to stop IFX (hazard ratio = 8.6, p = 0.003). In contrast, the duration of

IFX-IMM co-treatment and IFX dose optimisation before stop of IMM were not associated with an increased risk for disease flare and loss of response after withdrawal of IMM. IFX TLs at disease flare were lower in patients who later lost response to IFX compared to patients who did not ($0.38 \mu\text{g/ml}$ vs. $4.8 \mu\text{g/ml}$, $p = 0.013$). ATIs were found in 87% of samples with undetectable IFX TLs.

Conclusion : Undetectable IFX TLs and $\text{CRP} > 5 \text{ mg/l}$ during IFX-IMM combination therapy are associated with an increased loss of response to IFX after IMM withdrawal and should be implemented in future treatment algorithms.

- D27 -

OBESITY-RELATED INCREASE IN SERUM PLASMINOGEN ACTIVATOR INHIBITOR-1 IS INDEPENDENTLY CORRELATED TO THE PRESENCE AND SEVERITY OF NON-ALCOHOLIC STEATOHEPATITIS. S. Francque, A. Verrijken, I. Mertens, E. Van Marck, G. Hubens, P. Pelckmans, L. Van Gaal, P. Michielsen. Antwerp University Hospital, Antwerp, Belgium.

Introduction : Obesity is associated with an increased risk of thromboembolic complications. Recently increased levels of plasminogen activator inhibitor-1 (PAI-1) have been associated with visceral adiposity and inflammation. The role of NAFLD/NASH in the increased risk for thromboembolism is unknown.

Aim : To study the role of NASH in obesity-related changes in hemostatic and fibrinolytic factors.

Methods : Patients presenting to the obesity clinic underwent a metabolic and liver assessment. If NAFLD/NASH was suspected, a liver biopsy was proposed. Liver biopsy was scored using the NASH Clinical Research Network System. Hemostatic and fibrinolytic factors were determined.

Results : 198 patients were prospectively included. Mean age was $45 \pm 13 \text{ y}$; 66% were female, mean BMI was $38.3 \pm 6.4 \text{ kg/m}^2$. PAI-1 is significantly associated with BMI ($R = 0.281$, $p < 0.001$), visceral adipose tissue (VAT) ($R = 0.227$, $p < 0.001$) and HOMA-IR ($R = 0.425$, $p < 0.001$) confirming previous data. Also other factors (e.g. Von Willebrand factor, protein S) correlated with BMI, VAT and/or HOMA-IR. Most of these coagulation factors, however, did not correlate with NASH features. PAI-1 levels, by contrast, were significantly different between patients with NASH compared to patients without NASH (3.27 ± 2.29 vs. $2.32 \pm 2.68 \text{ ng/mL}$, $p = 0.003$). PAI-1 correlated with steatosis ($R = 0.327$, $p < 0.001$) and NASH activity score ($R = 0.284$, $p < 0.001$) and to a lesser extent also to fibrosis score ($R = 0.193$, $p < 0.001$). After correction for BMI, VAT and HOMA-IR, PAI-1 remained significantly correlated to the degree of steatosis ($R = 0.209$, $p < 0.001$) and to the NASH activity score ($R = 0.188$, $p < 0.001$), but not to fibrosis.

Conclusion : NASH is independently associated with an increase in PAI-1, suggesting that NASH contributes to an increased thromboembolic risk independently of obesity.

BEST RESPONSE DISTRIBUTION OF 12-WEEK TREATMENT WITH PRUCALOPRIDE (RESOLOR) IN PATIENTS WITH CHRONIC CONSTIPATION : COMBINED RESULTS OF THREE RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE III TRIALS. L. Vandeplassche. Movetis, Turnhout, Belgium.

Introduction : Treatment of severe chronic constipation (CC) is suboptimal. Prucalopride (PRU) is a selective high affinity 5-HT₄ receptor agonist, first representative of new chemical class (dihydro-benzofurancarboxamide compounds), developed for CC treatment. This study evaluates the combined efficacy results of PRU in 3 identical pivotal, randomised, placebo (PLA)-controlled trials.

Aim : The objective of each trial was to compare the efficacy and safety of a 12-week once daily treatment of 2 mg or 4 mg PRU with PLA in CC.

Methods : All 3 trials, 2 in US and one in Europe, Canada, Australia and South Africa, were of identical design with 3 parallel treatment groups : PLA, PRU 2 mg and PRU 4 mg. A 12-week treatment phase followed a 2-week run-in. For research purposes a most stringent primary endpoint was selected : the % of patients with an average of e 3 spontaneous complete bowel movements (SCBM) per week over a 12-week treatment period (i.e. normalisation of bowel movements). Clinical benefit could also be present in patients who did not meet this criterion but satisfied other efficacy endpoints. Patients' best response was derived from (in order of importance) : an average increase of e 1 SCBM/week, an improvement of e 1 point on a satisfaction scale (validated PAC-QOL 5-point subscale), an increase of e 1 SBM or an increase of e 1 BM.

Results : A total of 1924 ITT patients were included ; 89% female, average age 47 years, average duration of constipation 20 years. During the 2-week run-in 57% of the patients had no SCBM. In each individual trial the results for the primary and secondary endpoints were significantly better for both PRU groups compared to PLA.

| | Placebo (N = 645) | | | PRU 2 mg (N = 640) | | | PRU 4 mg (N = 639) | | |
|----------------------|-------------------|------|-------|--------------------|------|-------|--------------------|------|-------|
| | n | % | Cum % | n | % | Cum % | n | % | Cum % |
| e3 SCBM/Week | 73 | 11.3 | 11.3 | 151 | 23.6 | 23.6 | 158 | 24.7 | 24.7 |
| Increase avg SCBM e1 | 82 | 12.7 | 24.0 | 115 | 18.0 | 41.6 | 124 | 19.4 | 44.1 |
| Impr e1 Satisfaction | 54 | 8.4 | 32.4 | 92 | 14.4 | 55.9 | 92 | 14.4 | 58.5 |
| Increase avg SBM e1 | 106 | 16.4 | 48.8 | 109 | 17.0 | 73.0 | 103 | 16.1 | 74.6 |
| Increase avg BM e1 | 64 | 9.9 | 58.8 | 31 | 4.8 | 77.8 | 35 | 5.5 | 80.1 |

Thus, in addition to the response on the primary endpoint almost 75% of patients had clinical benefit from treatment with PRU with at least an increase of e 1 SBM.

Conclusion : In addition to primary response rates of about 24% with both 2 and 4 mg PRU, another 50% of patients had clinical benefit of at least an increase of e 1 SBM per week.

LABORATORY MELD-BASED ALLOCATION OF LIVER GRAFTS : IS THE "SICKEST FIRST" PRINCIPLE JUSTIFIED ? B. Van Geluwe, I. Jochmans, N. Meurisse, B. Desschans, K. Vromman, W. Van Steenberghe, D. Cassiman, C. Verslype, P. Ferdinande, G. Meyfroidt, D. Mesotten, R. Aerts, W. Laleman, D. Monbaliu, F. Nevens, J. Pirenne. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : The gap between the supply and the demand of liver grafts, and the increased waiting list mortality has led many countries to prioritize Liver Transplant (LTx) candidates according to their Model for End-stage Liver Disease (MELD) score (creatinine, INR, bilirubine). This 'Sickest first' allocation is seriously brought into question because LTx in (very) high MELD patients may cause unacceptable mortality, particularly early post-LTx, resulting in the *futile* use of scarce liver grafts and resources.

Aim : We sought to determine the impact of MELD on the short-term patient survival and hospital stay post-LTx.

Methods : Data of all patients transplanted between 01/2006 & 09/2010 were analyzed. LTx for acute liver failure, multiorgan, and re-Tx were excluded. Lab MELD was calculated immediately pre-LTx. Patients were categorized according to the ET-MELD classes (6-19 ; 20-24 ; 25-29 ; 30-34 ; > 35) and 3 & 12 mth survival and length of ICU/hospital stay were analyzed.

Results : During the study period, 221 isolated first LTx for non acute liver disease were performed. Mean Lab MELD was :16.5 ± 8.8. Mean age was :59.1 ± 13.8 yo. Indications were : ethyl (41%), viral (17%), cholestatic (12%), metabolic (11%), other (36%). HCC was present in 36%.

| <i>RESULTS</i> | <i>3 months Patient Survival</i> | <i>12 months Patient Survival</i> | <i>ICU stay (days)</i> | <i>Hospital stay (days)</i> |
|---------------------|----------------------------------|-----------------------------------|------------------------|-----------------------------|
| MELD 6-19 (n = 155) | 96% | 94.6% | 3 (1-57) | 18 (5-134) |
| MELD 20-24 (n = 26) | 88.1% | 79.7% | 9.5 (1-94) | 30.5 (13-109) |
| MELD 25-29 (n = 20) | 90% | 65% | 8.5 (1-54) | 33 (3-240) |
| MELD 30-34 (n = 12) | 100% | 77.8% | 7 (2-39) | 43 (14-72) |
| MELD > 35 (n = 8) | 87.5% | 87.5% | 8 (4-39) | 33 (21-167) |

MELD had no effect on 3 mth but on 12 mth survival (Cox proportional hazards model p :.03). Above MELD 19, survival did not differ among various MELD classes (Log rank/Bonferroni correction). ICU/hospital stay was prolonged by a factor 2-to-3 in > 19 MELD patients (Kruskal-Wallis p : .00).

Conclusion : MELD has no impact on early post-LTx mortality, but it does on 1 yr survival. Higher MELD implies higher resource utilization. Nevertheless, MELD-based allocation seems justified considering the favourable results reached even in (very) high (and probablyselected) MELD recipients and their dismal prognosis without LTx. Long-term data are warranted.

ANTI-TNF INDUCED SKIN MANIFESTATIONS IN IBD PATIENTS : CHARACTERIZATION AND SEARCH FOR PREDISPOSING FACTORS. I. Cleynen (1), W. Van Moerkercke (1), N. Vande Casteele (2), V. Ballet (1), M. Juergens (1), C. Breynaert (1), D. Drobne (1), P. Vandecandelaere (1), A. Gils (2), K. Van Steen (3), M. Noman (1), G. Van Assche (1), P. Rutgeerts (1), S. Segaert (1), S. Vermeire (1). (1) University Hospitals Leuven, Leuven, Belgium ; (2) Lab of Pharmaceutical Biology, Leuven, Belgium ; (3) Montefiore Institute, Liège, Belgium.

Introduction : Despite the efficacy of antitumor necrosis factor (anti-TNF) alpha therapy in the treatment of immune-mediated inflammatory diseases as inflammatory bowel disease (IBD) and psoriasis, paradoxical inflammation of the skin has been reported as side-effect during therapy.

Aim : We aimed to investigate in a consecutive cohort of IBD patients treated with anti-TNF : the cumulative incidence and type of skin lesions, the outcome, and possible risk factors.

Methods : All 922 consecutive IBD patients treated with infliximab that reported skin manifestations were referred to one dermatologist. The predominant skin lesion was classified in one of six categories : (1) xerosis cutis, (2) eczema, (3) psoriasis, (4) psoriasiform eczema, (5) palmoplantar pustulosis, and (6) other. Over time, 33% were switched to another TNF antagonist (adalimumab, ADA and/or certolizumab, CZP) for loss of response and/or intolerance. Studied risk factors included : development of anti-nuclear antibodies (ANA), relationship with infliximab serum levels, and genetic risk factors of IBD and psoriasis/atopic eczema. For the latter, 56 single nucleotide polymorphisms (SNPs) were genotyped in *IL23R*, *IL12B*, *NOD2*, *TNFAIP3*, *FLG*, *IL12RB2*, HLA, *C8ORF13*, *15q21*, *4q27*, *COG6*, and *LCE3B-LCE3C*.

Results : Of 922 patients, 206 (22.3%) developed skin lesions related to anti-TNF therapy. 22 patients (11%) were classified as xerosis cutis, 46 (23%) as eczema, 10 (5%) as psoriasis, 62 (30%) as psoriasiform eczema, 9 (4%) as palmoplantar pustulosis, and 54 (27%) as other. 74.3% of patients developed skin lesions under infliximab after a median time of 1.9 years, 23.8% of patients only after switch to ADA or CZP. The vast majority of patients responded well to topical and/or systemic treatment, with only 12 patients (6.3%) that needed to stop anti TNF because of the skin lesion. The most typically affected locations were the face (40.3%), scalp (28.2%) and pubic region (12.2%). The percentage of patients developing skin lesions was the same in male and female patients (20.1% and 24.1% respectively, $p = 0.15$). Induction of ANAs (ANA-titer $\geq 1/80$) was associated with a 1.3 fold increased risk for skin related adverse events (54% versus 43% ($p = 0.03$)). 25/145 (17.2%) of patients developing a skin lesion had undetectable infliximab serum levels, compared to 64/243 (26.3%) of patients not developing skin lesions at the moment of analysis ($p = 0.04$). Logistic regression showed that the risk to develop a skin lesion increased with increasing number of risk alleles for SNPs in *IL23R*, *IL12B* and *COG6* ($p < 0.0001$, OR = 1.35 [1.16-1.59]).

Conclusion : Anti-TNF induced cutaneous side-effects occur in as many as 22% of patients treated with anti-TNF and are a class-effect, but rarely necessitate interrupting therapy. The most frequently observed type of skin lesion is psoriasiform eczema. Auto-immune phenomena, high infliximab serum level and cumulative dose, and genetic make-up at loci common to, and specific for IBD and psoriasis/atopic eczema are risk factors for the development of these skin lesions in IBD patients.

RISK FACTORS FOR ANTIMICROBIAL RESISTANCE OF *HELICOBACTER PYLORI* STRAINS IN BELGIUM : EVOLUTION DURING THE LAST 20 YEARS. V.Y. Miendje Deyi (1), P. Bontems (2), J. Vanderpas (3), E. De Koster (1), R. Ntounda (4), M. Scaillon (2), C. Van Den borre (1), S. Cadranel (2), A. Burette (5). (1) CHU Brugmann, Brussels, Belgium ; (2) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (3) Scientific Institute of Public Health, Brussels, Belgium ; (4) CHU Saint-Pierre, Brussels, Belgium ; (5) CHIREC – Basilique, Brussels, Belgium.

Introduction : Antimicrobial resistance is a major factor jeopardizing *Helicobacter pylori* eradication therapy that needs epidemiologic surveillance.

Aim : We analysed the rate of *H. pylori* resistance to currently used drugs and identified risk factors associated with resistance.

Methods : Gastric biopsies were collected between January 1990 and December 2009 from several digestive diseases centres in the region of Brussels. We routinely performed antimicrobial susceptibility test using disk diffusion for clarithromycin (CLA), metronidazole (MET), ciprofloxacin (CIP), amoxicillin (AMO) and tetracycline (TET). Both Univariate and Multivariate analysis were performed : antibiotic susceptibility was introduced as dependent variable ; covariates were gender, age groups, time periods, previous eradication therapy and ethnic background of the patients. Finally resistance rates were compared to antimicrobials use in Belgium.

Results : Over the 2 decades, 10825 isolates were collected but antimicrobial susceptibility testing failed in 155 cases. Among the 10670 evaluable results, 9430 strains were isolated from patients who were not previously treated for *H. pylori* (1527 from children and 7903 from adults), 1371 strains were isolated from patients who were unsuccessfully treated for *H. pylori* infection (162 from children and 1209 from adults). For 24 strains we had no information concerning previous eradication attempt. *H. pylori* strains were susceptible to all tested antimicrobials in 62.1% and 23.3%, resistant to one drug in 31.6% and 57.5% and to multiple drugs in 4.7% and 18.5% of cases, before and after treatment respectively ($p < 0.05$). No resistance was observed for AMO and TET-resistance was very rare (2/10670). Primary MET resistance (METr) remains stable over the year with significant lower rates among children isolates. CIP-resistance (CIPr) remained rare in children, while it increased significantly over the years in adults. We recently observed a significant decrease in primary CLA-resistance (CLAr) : 9.9% in 2009 compared to a peak of 19.2% in 2003 that correlates with lesser macrolides use. For both CLA, MET and CIP : female gender was a significant independent risk factor of resistance ; patients aged from 40 to 64 years exhibited higher resistance rates ; resistance rates increased significantly with the number of previous eradication attempts and we observed significant increase of resistance rates over the years for patients which were previously treated. A significant difference in CLAr was found between North European patients and Middle East group. Higher level of METr and CIPr were observed in sub-Saharan African patients.

Conclusion : Female gender, age groups, ethnical background, previous unsuccessful eradication attempt and time periods are independent risk factors of resistance to CLA, MET and CIP. This study highlights the need to update local epidemiological data of *H. pylori* antimicrobials resistance to optimize efficacy of eradication strategies. Originally, after an alarming period up to 2003, we found a significant decrease in primary CLAr in *H. pylori* strains that seems correlated with the decrease of macrolides use.

PROGNOSTIC AND PREDICTIVE VALUE OF CXCR4, SMAD4 AND S100A2 PROTEIN EXPRESSION IN RESECTED PANCREATIC ADENOCARCINOMA. R. Maréchal (1), J.B. Bachet (2), P. Demetter (1), F. Bonnetain (3), A. Couvelard (4), M. Svrcek (5), P. Hammel (4), C. Louvet (5), T. André (2), J. Closset (1), I. Salmon (1), J.F. Emile (6), J.L. Van Laethem (1). (1) ULB Erasme, Brussels, Belgium ; (2) Hôpital Pitié-Salpêtrière, Paris, France ; (3) Centre Georges François Leclerc Unité de Biostatistique et d'épidémiologie, Dijon, France ; (4) Hôpital Beaujon, Paris, France ; (5) Hôpital Saint Antoine, Paris, France ; (6) Hôpital Ambroise Paré, Paris, France.

Introduction : Prognosis of pancreatic cancer (PC) needs to be refined before offering specific treatment strategies. CXCR4 is a chemoreceptor implicated in the proliferation, migration, angiogenesis and homing metastasis of pancreatic cancer cells. Recently, smad4, TGF β R2 and S100A2 have been proposed as predictor for overall survival (OS) in resected PC patients (pts).

Aim : We sought to confirm the value of CXCR4 as predictor for survival and to evaluate whether those 4 biomarkers impact on adjuvant chemotherapy benefit.

Methods : Resected PC pts from 5 centres have been included in this retrospective study. Expression of CXCR4, Smad4, S100A2 and TGF β R2 were assessed by IHC using the TMA technology. Protein expression was semi-quantitatively assessed applying scoring systems reported in previous studies. Pts were classified as followed : CXCR4 low/high, smad4 positive/negative, S100A2 low/high, TGF β R2 low/high. Clinical information and OS were evaluated with respect to the expression of CXCR4, TGF β R2, smad4 and S100A2.

Results : 471 tissue specimens were examined (surgery alone : n = 142, adjuvant gemcitabine-based therapy : n = 259, radiochemotherapy (5FU) : n = 49, other : n = 21). Median follow-up duration was 21.1 months (range :1.1-143.7 months) and median patient age was 63 years (range 34-87). In univariate analysis, CXCR4 expression is a negative prognostic factor for OS (CXCR4^{high} median OS = 21.9 months (95%CI :17.3-26.4), CXCR4^{low} : median OS = 35.0 months (95%CI :30.3-39.7) ; p < 0.0001). In a multivariate model adjusted for centre, R0/R1, tumor differentiation, maximal tumor size, T stage, N0/N+, adjuvant CT and smad4 expression ; CXCR4 was an independent factor associated with distant metastases occurrence and OS (Hazard ratio (HR) = 1.84 (95% CI : 1.39-2.34), p < 0.001). In this model, R1 resection (p = 0.002), undifferentiated tumor (p = 0.02), N+ status (p = 0.002), no adjuvant CT (p = 0.001) were additional independent variables affecting OS. Pts with smad4 negative (n = 287 ; HR :1.51 (95%CI :1.12-2.05), p = 0.007) and S100A2 high (n = 186 ; HR :1.99 (95% CI :1.35-2.93), p < 0.001) tumors showed improved OS after adjuvant gemcitabine CT. These results were maintained in multivariate model.

Conclusion : We confirmed that CXCR4 is a strong prognostic factor for distant relapse and OS in resected PC. Expression of S100A2 and smad4 may predict benefit of adjuvant chemotherapy.

A PROSPECTIVE RANDOMIZED CONTROLLED MULTICENTER STUDY OF EXTRACORPOREAL LIVER SUPPORT BY FRACTIONATED PLASMA SEPARATION AND ADSORPTION (PROMETHEUS®) IN PATIENTS WITH ACUTE - ON - CHRONIC LIVER FAILURE (HELIOS STUDY). H. Van Vlierberghe (1), K. Rifai (2), A. Kribben (3), G. Gerken (3), S. Haag (3), S. Herget-Rosenthal (3), U. Treichel (3), C. Betz (4), C. Sarrazin (4), E. Hoste (1), A. Escorsell (5), P. Gines (5), C. Hafer (2), M. Schuchmann (6), P.R. Galle (6), M. Bernardi (7), P. Caraceni (7). (1) UZ, Gent, Belgium ; (2) Medizinische Hochschule Hannover, Hannover, Germany ; (3) University Duisburg-Essen, Duisburg-Essen, Germany ; (4) Goethe University, Frankfurt, Germany ; (5) Hospital Clinic, Barcelona, Spain ; (6) University of Mainz, Mainz, Germany ; (7) University, Bologna, Italy.

Introduction : Prometheus® is an extracorporeal liver support system for patients with liver failure allowing removal of protein bound and water soluble toxins by Fractionated Plasma Separation and Adsorption (FPSA). This is the first large prospective randomized controlled trial on the survival of patients with acute-on-chronic liver failure (AOCLF) under FPSA therapy.

Methods : 145 patients with AOCLF recruited in 10 centers from 7 European countries were randomized either to standard medical therapy (SMT ; n = 68) or to SMT+FPSA therapy (n = 77). FPSA therapy was intended for 8-11 treatments (minimum duration 4 hours each) during the first 3 weeks of the study. Primary endpoints were survival at days 28 and 90 irrespective of liver transplantation.

Results : Chronic liver disease was due to alcohol abuse in 56% of patients and in 20% of patients due to viral hepatitis. 63% of patients were male, mean age was 51 \pm 10 years. Average MELD score was 27 \pm 10 and Child Pugh Score (CPS) 12 \pm 1. The two study arms did not differ in age, sex, etiology of chronic liver disease, MELD score, CPS and SMT. All 145 patients were included in the intention-to-treat analysis. Survival on day 28 was 66 vs. 63% (p = 0.70 ; SMT+FPSA vs. SMT) and on day 90 47 vs. 38% (p = 0.35), respectively. The difference in the overall survival did not reach statistical significance (p = 0.38, log rank test). During the trial, 28 patients underwent liver transplantation : 15 patients in SMT + FPSA (19.5%) and 13 in SMT (19.1%). In a predefined subgroup analysis, a significant survival benefit in the range of 30% was observed under FPSA therapy in patients with a MELD score > 30 (p = 0.02) or in those with hepatorenal syndrome type I (p = 0.04).

Conclusion : Extracorporeal liver support therapy by FPSA (Prometheus®) was not associated with an improved overall survival in patients with AOCLF compared to standard medical therapy alone. However, a survival advantage was observed in patients with severe AOCLF and MELD score > 30 or hepatorenal syndrome type I.

77% PATIENT SURVIVAL AFTER INTESTINAL TRANSPLANTATION : A 10 YEAR EXPERIENCE. J. Pirenne, R. Aerts, D. Monbaliu, F. Nevens, M. Hiele, G. Van Assche, K. Geboes, N. Ectors, P. Ferdinande, L. Desmet, D. Vlasselaers, I. Hoffman, R. Lombaerts. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Obstacles to Intestinal Transplantation (ITx) are risks associated to i) complex surgery in sick patients ; ii) rejection ; iii) immunosuppression (IS) ; iiiii) postTx infection/malignancy. For these reasons, ITx is only performed in patients with life-threatening complications from Parenteral Nutrition (TPN) and whose natural prognosis is dismal.

Aim : We review our 10 yrs ITx experience.

Methods : ITx was done in 9 patients (8 deceased & 1 living donor) for the following indications : liver failure in 4 who received liver/bowel ; electrolyte dysbalance-catheter shortage/sepsis in 3 who received isolated ITx ; combined bowel/renal failure in 1 who received kidney/bowel ; splanchnic thrombosis in 1 who received Multivisceral Tx (MVTx). Liver/bowel was procured/transplanted *en bloc* with pancreas. Isolated bowel was procured with superior mesenteric artery/vein and implanted on aorta/vena cava. Distal ileostomies were constructed. Intensive bacterial, fungal viral prophylaxis was administered. Graft monitoring was via transstomal biopsies. To promote engraftment and reduce IS, a tolerogenic protocol {Donor-Specific Blood Transfusion ; use of low-dose Tacrolimus/steroids & anti-inflammatory maneuvers (*Jl 2005, Tx 2005, Tx 2009*)} was used in 7/8 deceased ITx.

Results : *Case 1.* 56 yo fem ; bowel infarct/TPN liver failure received liver/bowel. PostTx morbidity : pancreatitis & abdominal abscesses. She is well 10 yrs postTx. *Case 2.* 57 yo fem ; bowel infarct/TPN liver failure received liver/bowel. PostTx morbidity : intestinal obstruction. She is well 9 yrs postTx. *Case 3.* 25 yo fem with pseudoobstruction. She is 5 yrs postTx doing well but had recurrent episodes of subobstruction. *Case 4.* 2 yo boy with volvulus/liver failure received liver/bowel. PostTx morbidity : wound defect, bacterial, fungal sepsis, pericarditis. He is 5 yrs postTx doing well. *Case 5.* 30 yo fem with volvulus/renal failure received combined bowel/kidney. After psychological problems, she is well 4 yrs postTx. *Case 6.* 33 yo fem ; bowel infarct received bowel. Succumbed to aspergillus at 4 mth. *Case 7.* 7 yo girl ; cirrhosis/neonatal volvulus received liver/bowel. PostTx morbidity : portal hypertension, native & Tx colitis. She is well 3 yrs postTx. *Case 8.* 42 yo male ; splanchnic thrombosis received MVTx & succumbed to aspergillus at 6 mth. *Case 9.* 27 yo female with Churg-Strauss syndrome received an ileal segment from mother. Transplantectomy for rejection was done at 8 mths postTx. Overall patient survival is 77% follow-up 2 to 10 yrs. Under the tolerogenic protocol, complete freedom from rejection was achieved the first 3 mths postTx in all deceased ITx.

Conclusion : ITx has evolved and provides now an excellent chance of survival and an excellent quality of life in patients suffering from life-threatening complications of short bowel syndrome.

EFFICACY AND SAFETY OF RETREATMENT WITH INFLIXIMAB AFTER WITHDRAWAL IN PATIENTS IN LONG-TERM REMISSION UNDER COMBINED THERAPY WITH IMMUNOSUPPRESSANT. A SUBANALYSIS OF THE STORI COHORT STUDY. E. Louis (1), G. Vernier-Massouille (2), J.C. Grimaud (3), Y. Bouhnik (4), D. Laharie (5), J.L. Dupas (6), H. Pillant (4), L. Picon (7), M. Veyrac (8), M. Flamant (9), G. Savoye (10), R. Jian (4), M. De Vos (11), G. Paintaud (12), E. Piver (13), J.Y. Mary (4), J.F. Colombel (2), M. Lemann (4). (1) CHU Liège, Liège, Belgium ; (2) GETAID, Lille, France ; (3) GETAID, Marseille, France ; (4) GETAID, Paris, France ; (5) GETAID, Bordeaux, France ; (6) GETAID, Amien, France ; (7) GETAID, Tours, France ; (8) GETAID, Montpellier, France ; (9) GETAID, Nantes, France ; (10) GETAID, Rouen, France ; (11) University Hospital, Ghent, Belgium ; (12) Université François Rabelais, Tours, France ; (13) Centre hospitalier Universitaire, Tours, France.

Introduction : The GETAID-STORI cohort study has recently shown a relapse rate around 50% over 30 months in 115 patients with Crohn's disease who stopped infliximab while being in long-term remission under combined therapy with immunosuppressant.

Aim : A secondary aim of the trial was to assess efficacy and safety of infliximab retreatment in patients who relapsed after IFX cessation.

Methods : Patients who relapsed were retreated using infliximab scheduled treatment (5 mg/Kg every 6 to 12 weeks). Response to retreatment and remission were prospectively assessed 4 weeks after first retreatment infusion and before third retreatment infusion representing the last visit of the protocol. Safety was investigated up to third retreatment infusion. Infliximab trough levels were measured just before first and third retreatment infusions. At the end of the trial, a retrospective questionnaire on clinical and treatment status of the retreated patients was filled in by investigators.

Results : Among 57 patients in relapse, 52 were retreated with infliximab after a median drug holiday of 6.6 months. At week 4, 40 patients were adequately assessed : 39 were in clinical response and 37 in remission. Just before third retreatment infusion (80-160 days), 43 patients were adequately assessed : 42 were in clinical response and 38 in remission. Median (IQR) trough levels was 3.7 mg/L (1.5-6.3) (n = 29) just before third retreatment. Median variation (IQR) as compared to trough levels before treatment cessation was 0.1 mg/L (-0.55-2.1) (n = 23). No acute or delayed infusion reaction occurred. Over long term retrospective follow-up, after a median follow-up time from first retreatment of

24 months, only 12/52 patients had stopped infliximab due to loss of response (n = 6), intolerance (n = 1), pregnancy (2), patient decision (3). The proportion (+/-SE) of loss of response after retreatment was 12+/-5 % at 2 years.

Conclusion : Infliximab retreatment appeared safe and effective after a drug holiday in patients previously in long-term remission under combined treatment with immunosuppressant.

- D36 -

A DOUBLE-BLIND PLACEBO-CONTROLLED STUDY EVALUATING PROBIOTICAL® IN THE TREATMENT OF ACUTE GASTROENTERITIS IN CHILDREN. Y. Vandenplas. UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

Introduction : There is general consensus that all (combination of) probiotic strains need clinical studies in order to acknowledge claims.

Aim : We aimed to test the efficacy of one probiotic product in the treatment of acute gastroenteritis in children.

Methods : A double-blind, placebo-controlled prospective (DBPCP) trial was performed in ambulatory primary health care in Belgium in 111 healthy, except for the episode of acute gastroenteritis, children (3 months-15 years) comparing placebo to Probiotal® (Streptococcus thermophilus, Lactobacillus (L.) rhamnosus, L. acidophilus, Bifidobacterium (B.) lactis, B. infantis, fructooligosaccharides). In order to be eligible for inclusion, patients needed to have > 4 stools/day since > 1 day (and < 5 days) with a Bristol score > 6 ((semi-)watery stools). The primary endpoint was defined as the time needed for the first formed soft stool (Bristol score < 4) ; secondary endpoints were stool consistency, tolerance and safety.

Results : The number of patients/physician recruited varied between 3 and 10. At admission, all patients were estimated to be dehydrated between 3 and 5 %, except 7 patients that were 10% dehydrated. ORS (Soparyx®) was given at libitum, exclusive during 4-6 hours and whenever there was a watery stool. Other medication (mainly antipyretics and domperidone) were administered in 49/111 (44%) children. One patient of the placebo group was hospitalized. Although the number of stools per day did not differ between both groups (secondary outcome), significantly more patients had formed stools in the treatment than in the placebo group on day 2 and 3. Placebo and product were well tolerated and no side-effects were reported. Physicians reported to be satisfied with the results, equally distributed over both groups.

| | Probiotical® | Placebo | P |
|---|--------------|------------|-------|
| Number | 57 | 54 | NS |
| Age (year) (mean ± 1 SD) | 4.7 ± 4.2 | 4.9 ± 4.2 | NS |
| Weight (kg) (mean ± 1 SD) | 19.7 ± 14.3 | 17.8 ± 8.2 | NS |
| Duration diarrhea before inclusion (median (range)) | 1 (0-7) | 1 (0-6) | NS |
| Bristol score ≥ 4 | | | |
| Day 1 | 56/57 | 54/54 | NS |
| Day 2 | 45/57 | 52/52 | 0.005 |
| Day 3 | 27/51 | 41/53 | 0.009 |
| Day 4 | 11/36 | 18/38 | NS |
| Stool frequency | | | |
| Day 1 (median (range)) | 7 (3-7) | 7 (6-7) | NS |
| Day 2 | 5 (3-7) | 6 (3-7) | NS |
| Day 3 | 5 (3-6) | 5 (2-7) | NS |
| Day 4 | 4 (3-5) | 4 (3-6) | NS |
| Other medication | 23 (40%) | 26 (48%) | NS |

Conclusion : According to this double-blind placebo-controlled prospective trial performed in primary health care in children with acute gastroenteritis, the symbiotic Probiotical® normalizes stool consistency 48 hours sooner than placebo.

- D37 -

ADALIMUMAB RESPONSE DOSE ESCALATION AND DOSE DE-ESCALATION SUCCESS RATE IN A LARGE NATIONAL COHORT OF CROHNS PATIENTS. F. Baert (1), E. Glorieus (2), C. Reenaers (3), G. D Haens (4), H. Peeters (5), D. Franchimont (6), O. Dewit (7), P. Caenepeel (8), E. Louis (9), G. Van Assche (10). (1) Heilig Hartziekenhuis, Roeselare, Belgium ; (2) UZ, Gent, Belgium ; (3) CHU Sart Tilman, Liège, Belgium ; (4) Imelda GI Clinical Research Centre, Bonheiden, Belgium ; (5) University Hospital, Ghent, Belgium ; (6) ULB Erasme, Brussels, Belgium ; (7) UCL Saint-Luc, Brussels, Belgium ; (8) Ziekenhuis Oost-Limburg, Genk, Belgium ; (9) CHU Liège, Liège, Belgium ; (10) UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Adalimumab is efficacious in inducing and maintaining remission in Crohn's disease (CD). Nevertheless, loss of response with need for dose escalation occurs in 30-40% of pts after one year. The long term need for dose escalation is unknown moreover attempts for dose de-escalation have not been studied.

Aim : We assessed the need and outcome of dose escalation and de-escalation in a large cohort of adalimumab treated Crohn's patients (pts).

Methods : This observational retrospective cohort study included all consecutive pts treated with open label adalimumab for active CD from the participating BIRD centers. A detailed chart review was performed to look for demographics, disease characteristics, smoking, CRP, prior and concomitant medication at the different time points as possible factors predicting outcome.

Results : A total of 720 consecutive pts (61% F, median age 36 yrs (13 to 91) from 26 centers (9 academic centers)) were included in this study. The median time of follow-up was 18 months (range 3 to 73 months). 603 (84%) pts had a primary response. The response was 95% (191/201) in anti TNF naïve pts versus 83% (329/395) in pts with prior anti TNF treatment. ($p < 0.001$). 206/603 (34%) patients needed adalimumab dose escalation to a weekly injection of 40 mg SC to maintain clinical response after a median of 7 months (0-55 months). Predictors for need to dose escalation were perianal disease ($p = 0.009$) and prior anti TNF use. Dose escalation reinduced clinical response for at least 6 months in 138/206 (67%) pts. Predictors for successful dose escalation in univariate analysis were prior Crohn resection (OR 2.123 95%CI (1.090-4.134) $p = 0.025$) and no concomitant medication at dose escalation (OR 4.47 (0.237-0.846) $p = 0.013$).

Dose de-escalation to 40 mg e.o.w. again was attempted in 72/138 (52%) patients after a median of 3 months and was successful (i.e. no loss of response again for a period of at least 6 months) in 62% (45/72). The only predictor for unsuccessful dose de-escalation was abnormal CRP at time of dose escalation (OR 1.909 (1.431-2.547) $p = 0.001$). Overall after a median follow-up of 18m adalimumab was discontinued in 31% of patients. Reasons for stopping adalimumab treatment were no response in 14%, loss of response 40%, intolerance 4%, pregnancy 6%, remission 3%, other 32%.

Conclusion : In a real life nationwide cohort of Crohn's patients treated with adalimumab dose escalation was needed in 34% and successful in 67%. Dose de-escalation was attempted in 52% and successful in 62%. Abnormal CRP at dose escalation predicted failure to dose de-escalation.

- D38 -

ASSOCIATION OF FAECALIBACTERIUM PRAUSNITZII AND DISEASE ACTIVITY IN ULCERATIVE COLITIS. K. Machiels, M. Joossens, V. De Preter, I. Arijis, V. Ballet, S. Organe, T. Coopmans, G. Van Assche, J. Verhaegen, P. Rutgeerts, S. Vermeire. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : In Crohn's disease, intestinal dysbiosis has been demonstrated. The most replicated species-specific finding so far has been the important reduction of *Faecalibacterium prausnitzii*, a commensal bacteria with anti-inflammatory properties. Also in ulcerative colitis (UC) patients, a decrease in intestinal biodiversity might be present. However, only limited data on the importance of *F. prausnitzii* in UC is available.

Aim : We hypothesized that *F. prausnitzii* is reduced in fecal samples of UC patients as compared to healthy subjects and furthermore studied the influence of disease activity on the amount of *F. prausnitzii*. We also evaluated the influence of fecal dry matter content on the bacterial count.

Methods : Fecal samples were collected from 83 UC patients (26 active (A-UC) and 57 in remission (R-UC)) and 43 healthy subjects (HS). Disease activity in UC was defined using the partial Mayo score. Written consent was given by all participants prior to collection of the samples. Participants were excluded if they had used antibiotics or probiotics in the last month. Bacterial DNA was extracted using a modification of the Pitcher et al method (*Letters in Applied Microbiology* 1989). Quantification of *F. prausnitzii* was performed by real-time PCR targeting the 16S rRNA gene in the V3 region. Bacterial counts were expressed as log₁₀ values per gram dry weight feces to prevent false low results in diarrhea. Dry weights were determined by lyophilizing a precisely weighed portion of the sample in duplicate. Results were analyzed with the Mann-Whitney U-test and the Spearman's correlation coefficient using SPSS 17.0 software. A p-value of < 0.05 was considered significant.

Results : The bacterial count of *F. prausnitzii* in UC patients was significantly lower (median, 11.5 [interquartile range (IQR), 10.3-12.3] log₁₀ colony-forming units (CFU)/g) as compared to HS (median, 12.1 [IQR, 11.8-12.6] log₁₀ CFU/g) ($p = 0.001$). We further more observed a correlation between the amount of *F. prausnitzii* and disease activity. Bacterial count was lowest in UC patients with severe disease (median, 10.3 [IQR, 9.2-11.5] log₁₀ CFU/g ; Mayo 3 ; $n = 10$) as compared to moderate disease (median, 11.2 [IQR, 9.3-12.2] log₁₀ CFU/g ; Mayo 2 ; $n = 16$) and quiescent disease (median, 11.7 [IQR, 10.6-12.4] log₁₀ CFU/g ; Mayo 0 + 1 ; $n = 57$) (Spearman's $r = -0.233$, $p = 0.034$).

Conclusion : A significant underrepresentation of *F. prausnitzii* was observed in UC patients when compared to healthy controls. We also observed an inverse correlation between the amount of *F. prausnitzii* and the disease activity. Our findings persisted, even after correcting for fecal water content. These results suggest that dysbiosis plays also a role in UC pathogenesis, and in particular points to the protective effects of *F. prausnitzii* against inflammation.

INCIDENCE AND CHARACTERISTICS OF POSTINFECTIOUS IBS (PI-IBS) : A MULTINATIONAL INTERNET SURVEY. R. Spiller (1), G.E. Boeckxstaens (2), J. Marshall (3), T. Card (1), F. Mearin (4), G. Barbara (5), A. Qasim (6), F. Azpiroz (4), P. Enck (7). (1) The University of Nottingham, Nottingham, United Kingdom ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) Mc Master University, Hamilton Ontario, Canada ; (4) Hospital General Universitario Valle Hebrón and Ciber-ehd del Instituto Carlos III, Barcelona, Spain ; (5) St. Orsola Hospital, Bologna, Italy ; (6) Barts and the London School of Medicine and Dentistry, London, United Kingdom ; (7) University Hospital Tübingen, Tübingen, Germany.

Aim : To define the characteristics of PI-IBS and any differences from IBS without an infectious onset in different countries.

Methods : IBS patients attending gastroenterology outpatient clinics were invited to complete an internet survey using a password. The website also allowed patient access without a password. The web questionnaire asked for demographics, bowel symptoms using the Rome bowel questionnaire, Hospital Anxiety and Depression scores (HADS) and PHQ12, a modification of the Personal Health Questionnaire 15 scale excluding the 3 GI items. It also asked about the mode of onset of symptoms and the characteristics of any episode of infectious diarrhoea which immediately preceded the onset of IBS. We defined PI-IBS as IBS with any 2 of the following characteristics : sudden onset, began while travelling, preceded by an illness with vomiting, fever, bloody diarrhoea or positive stool culture.

Results : Subjects were predominantly young, mean (SD) : 39(15 years), female (71%), well educated with 43% having a University degree, and were mostly living in a town or city. 1436 were European, 31 Asian, 93 South American and 37 North American. All had been diagnosed by a doctor as having IBS but only 71% met Rome III criteria. Overall 26% showed moderate/severe HADS depression scores, and 56% showed moderate/severe HADS anxiety scores. Abnormal somatisation was also common with 38% scoring moderate and 33% severe. 308 of 1640 patients (18%) met our definitions of PI-IBS. The illness preceding the development of PI-IBS was characterised as : sudden onset 58%, began while travelling 40%, preceded by an illness with vomiting 53%, fever 56%, bloody diarrhoea 21%, positive stool culture 29%. PI-IBS were more likely than other IBS to be female (Odd's ratio = 1.36, p = 0.03). The proportion of IBS that was PI-IBS was 21% in North Americans and Northern Europeans compared to the 14% for the rest of the world, Chi2 9.3, p = 0.002. Somatisation as assessed by PHQ12 increased the risk of PI-IBS, OR with mild elevation (score 5-8) being 1.3(0.84-1.90), moderate (9-12) 1.33(0.86-2.05) and severe 2.45(1.54-3.89), p for trend 0.0002.

Conclusion : This large internet survey suggests approximately 18% of all IBS worldwide is postinfectious. 40% begin during travel. PI-IBS is more common in females, North Americans and Northern Europeans and increases with increasing somatisation.

ENDOSCOPIC TREATMENT WITH A SOFT DIVERTICULOSCOPE FOR ZENKER'S DIVERTICULUM : LONG TERM FOLLOW UP. V. Huberty. ULB Erasme, Brussels, Belgium.

Introduction : Diverticulotomy, endoscopy together with surgery are standard treatment for Zenker's. The septum can be cut using a needle-knife incision, argon plasma coagulation (APC), or argon laser when used by ENT surgeons. Flexible endoscopy treatment may be performed "free-hand" or assisted with the use of an overtube.

Aim : We report our long term results of ZD treatment using a soft diverticuloscope and needle-knife.

Methods : A total of 141 (39 female, 102 male) patients with a ZD were treated from July 2002 to September 2010. The average age of the patients was 71.43 years (range 42 to 94). The procedure was performed using a soft diverticuloscope to expose the septum, which was then cut with a needle-knife. At the end of the procedure hemostatics clips were placed at the bottom of the diverticulum. Symptoms were compared before, after the procedure.

Results : The mean size of the Zenker's diverticulum was 3.43 cm (rang 1 to 8). The endoscopic incision was performed in one to three sessions. Five complications (3.54%) occurred (1 covered perforation, 3 subcutaneous emphysema and 1 pneumonia) and were managed clinically. The score of dysphagia (0 no dysphagia, 1 to solid, 2 to semi-solid, 3 to liquid, 4 aphagia) comes from 1.83 (N = 141) to 0.31 after treatment (N = 94) and 0.37 at long term (N = 96). There were recurrence of dysphagia in 26 patients of 96 (27.1%) for witch the follow up was available. Twenty one patients had a second session and one had a third. Their score of dysphagia comes from 2.09 (N = 22) before treatment to 1.5 (N = 16) before second treatment and 0.39 (N = 18) at long term. After retreatment 2 had a recurrence after 3 months, one after 15 months and one after 63 months. Recurrence comes after 14.7 months (range from 0 to 82). The time of follow up was 45.01 month (range from 1 to 91). Twenty three patients died during follow up for cause unrelated to the procedure.

Conclusion : Endoscopic incision of ZD with a soft diverticuloscope and hemostatics clips is a safe and efficient treatment.

THE MUCOSAL GENE EXPRESSION OF ENZYMES INVOLVED IN BUTYRATE UPTAKE AND OXIDATION IS DOWN REGULATED IN ULCERATIVE COLITIS. V. De Preter (1), M. Ramakers (1), I. Arijs (1), G. Vandermeulen (1), F. Schuit (2), P. Rutgeerts (1), K. Verbeke (1). (1) UZ Leuven Department of Gastroenterology, Leuven, Belgium ; (2) KUL, Leuven, Belgium.

Introduction : Butyrate, a colonic metabolite of carbohydrates, is considered as the major energy source for the colonic mucosa. An impaired butyrate metabolism in ulcerative colitis (UC) due to a defect in the butyrate oxidation pathway and/or transport has been reported.

Aim : In the present study, we correlated butyrate uptake and oxidation to the gene expression of the butyrate transporter MCT1 and the enzymes involved in butyrate oxidation (ACADS, ECHS1, HSD17B10 and ACAT2) in UC and controls.

Methods : Colonic mucosal biopsies were collected during endoscopy of 42 UC patients and of 15 control patients with normal colonoscopy. Disease activity was assessed using the endoscopic Mayo Score. Butyrate uptake and oxidation was measured by incubating biopsies with ¹⁴C-labeled Na-butyrate and measuring the released ¹⁴CO₂ by b-liquid scintillation counting. Results were corrected for protein content. For gene expression, total RNA was extracted from mucosal samples and used for quantitative RT-PCR.

Results : Both butyrate uptake and oxidation were significantly decreased in UC compared to controls (p < 0.001). The gene expression of MCT1, ECHS1 and ACAT2 was significantly lower in all UC patients as compared to controls (Table 1). Subsequent division of the UC patients into disease activity subgroups showed a reduced gene expression for all evaluated genes in mild (Mayo 2) and active (Mayo 3) disease. MCT1 gene expression was also lower in quiescent disease (Mayo 0-1). Butyrate uptake was significantly correlated with MCT1 gene expression (p = 0.035, R = 0.318) and butyrate oxidation was significantly correlated with the gene expression of ACADS (p < 0.001, R = 0.678), ECHS1 (p < 0.001, R = 0.540), HSD17B10 (p = 0.002, R = 0.472) and ACAT2 (p = 0.029, R = 0.341).

Table 1 : Butyrate uptake, oxidation and relative gene expression normalized for β-actin in controls and UC

| | Controls | UC all | UC subgroups | active | mild | quiescent |
|--------------------|----------|---------------|----------------|--------------|--------------|----------------|
| Butyrate uptake | | 2.88 ± 0.81 | 1.84 ± 0.72* | 1.48 ± 0.49* | 1.83 ± 0.72* | 1.95 ± 0.76* |
| Butyrate oxidation | | 25.77 ± 15.50 | 11.94 ± 10.88* | 2.32 ± 1.21* | 3.89 ± 3.39* | 16.42 ± 10.71* |
| Gene expression | MCT1 | 5.60 ± 2.02 | 2.17 ± 1.54* | 0.78 ± 0.42* | 0.88 ± 0.64* | 2.77 ± 1.44* |
| | ACADS | 2.21 ± 1.24 | 1.73 ± 1.30 | 0.27 ± 0.14* | 0.72 ± 0.21* | 2.36 ± 1.13 |
| | ECHS1 | 2.03 ± 0.52 | 1.22 ± 0.56* | 0.58 ± 0.35* | 0.66 ± 0.19* | 1.53 ± 0.38 |
| | HSD17B10 | 1.15 ± 0.44 | 1.21 ± 0.50 | 0.68 ± 0.26* | 0.81 ± 0.33* | 1.45 ± 0.40 |
| | ACAT2 | 1.31 ± 0.53 | 0.98 ± 0.42* | 0.62 ± 0.44* | 0.79 ± 0.34* | 1.11 ± 0.37 |

(*significantly different from control samples)

Conclusion : Our results demonstrated for the first time that the impaired butyrate uptake and oxidation in UC is due to a down regulation of the gene expression of the butyrate transporter MCT1 and the enzymes involved in the butyrate oxidation pathway.

RESPONSE OF CHRONIC CONSTIPATION SYMPTOMS TO PRUCALOPRIDE TREATMENT AND RELATIONSHIP WITH PATIENT SATISFACTION. L. Vandeplassche. Movetis, Turnhout, Belgium.

Introduction : Prucalopride (PRU) is a selective 5-HT₄ agonist, effective and approved in EU for treatment of chronic constipation (CC) in females in whom laxatives do not provide adequate relief.

Aim : The aim of this study was to assess the meaningfulness of changes in constipation symptoms and patient satisfaction after 4 weeks of treatment with placebo, PRU 2 mg or 4 mg. In addition, the relationships between changes in symptom scores and patient satisfaction were explored.

Methods : Symptoms of CC were assessed in 1552 female subjects of 3 identical pivotal trials. Subjects were selected if treated for at least 21 days and with data at baseline (BL) and 4 weeks of treatment. Symptom severity was evaluated by the Patient Assessment of Constipation Symptoms (PAC-SYM) questionnaire, a 12-item self-report instrument with abdominal (4 items), rectal (3 items) and stool (5 items) symptom subscales. Patient satisfaction with bowel habit and treatment was evaluated by the 5-item subscale of the Patient Assessment of Constipation Quality of Life questionnaire (PAC-QOL).

The meaningfulness of changes in the PAC-SYM items and patient satisfaction was assessed by the effect size (ES), i.e. mean change from BL divided by the standard deviation of the BL value. The relationship between changes after treatment with PRU in PAC-SYM items and patient satisfaction was evaluated using partial least squares path modeling (PLSPM).

Results : At BL the mean symptom severity score was “moderate” for abdominal symptoms, “severe” for stool symptoms, and “mild” for rectal symptoms. Treatment with PRU 2-4 mg resulted in a substantial relief of all symptoms with ES varying from moderate (ES : 0.5-0.8) to large (ES > 0.8) and with the largest ES for the abdominal bloating and discomfort symptoms for both doses of PRU. Analysis of the 3 subscales showed that the ES of PRU were large (> 0.8) for both the abdominal and stool symptoms. Comparison between placebo and PRU of the cumulative distributions of the changes from baseline showed that PRU provides a consistent benefit among patients. PRU treatment also resulted in large ES of patient satisfaction, with regularity of bowel movement frequency as the most responsive item. PLSPM showed that improvement in patient satisfaction can largely be attributed to relief of abdominal and stool related symptoms ($r = 0.6$).

Conclusion : Prucalopride is highly effective in relief of abdominal and stool related symptoms. Relief of these symptoms is associated with a substantial improvement in patient satisfaction with bowel habit and treatment.

EARLY TROUGH LEVELS AND ANTIBODIES PREDICT SAFETY AND SUCCESS OF RESTARTING INFLIXIMAB AFTER LONG DRUG HOLIDAY. F. Baert (1), D. Drobne (1), V. Ballet (1), I. Cleynen (1), G. Compennolle (2), G. Van Assche (1), P. Rutgeerts (1), A. Gils (2), S. Vermeire (1). (1) UZ Leuven Department of Gastroenterology, Leuven, Belgium ; (2) University of Leuven, Leuven, Belgium.

Introduction : Infliximab (IFX) maintenance therapy is effective in Crohn’s disease and ulcerative colitis. Long drug holidays have been discouraged for fear of immunogenicity and acute or delayed infusion reactions. When IFX is stopped for remission or loss of response and patients (pts) need to restart after a drug holiday, physicians often preemptively switch to a second anti-TNF.

Aim : To look for clinical and biologic predicting factors (including antibodies to infliximab (ATI) and IFX trough levels (TL)) for success of restarting IFX after a long drug holiday.

Methods : We identified a consecutive cohort of 39 pts (32 CD, 9 UC) in whom IFX was restarted after a drug holiday of > 6 months after an initial period of maintenance therapy. Serial TR and ATI were determined using an in house developed TR Elisa with TNF ± coated plates and a monospecific polyclonal rabbit antibody as a standard and an ATI bridging Elisa in prospectively collected serum samples during first IFX maintenance therapy (t-1), at (t0) and early after restarting (t1). Main outcome was treatment success (defined as clinical (and biological) response through w14) and safety (defined as absence of infusion reactions). Outcome was correlated to treatment modalities and TL and ATI at t-1, t0 and t1.

Results : IFX was restarted after a median drug holiday of 18 months (range 6-101) and was successful in 35/39 pts (90%). Predictors for success were : reason for stopping first IFX course (remission (successful in 26/27) vs stopping for loss of response after the first course (9/12) $p = 0.043$) ; a week 0-2-6 induction at start of first course (success in 20/20 compared to 10/13 pts without induction regimen $p = 0.024$) and concomitant immunomodulators (IMM) at restart (20/20 with IMM vs. 15/19 without IMM $p = 0.011$). Undetectable IFX TL at t1 was highly predictive of no response. Median t1 TL level in responders was 10.5 µg/ml compared to < 0.25 µg/ml in non responders ($p = 0.027$). The duration of the drug holiday and of the first IFX course did not correlate with clinical or biologic response. An infusion reaction occurred in 5/39 pts (12.8%) with the appearance of very high ATI titer at t1 in 3/4 pts. None of the pts retreated with the combination of IV steroids, a week 0-2-6 induction regimen and concomitant IMM therapy had an

infusion reaction. Remarkably, 9/12 refractory pts (75%) in whom IFX was stopped for complete loss of response regained good clinical and biological response after restarting.

Conclusion : Restarting infliximab after a long drug holiday is associated with a high success rate, including in pts in whom IFX was discontinued previously for complete loss of response. Having undetectable IFX TL and ATI present early after restarting is highly predictive of no response and/or an infusion reaction and should be determined when considering restarting IFX.

- D44 -

CORRELATION BETWEEN THE ENDOSCOPIC AND HISTOLOGICAL SCORE IN ASSESSING THE ACTIVITY OF ULCERATIVE COLITIS. B. Lemmens (1), I. Arijs (2), G. Van Assche (2), P. Rutgeerts (2), K. Geboes (1), S. Vermeire (2), G. De Hertogh (1). (1) UZ Leuven Department of Pathology, Leuven, Belgium ; (2) UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Colonoscopy with biopsies is regarded as the most accurate assessment of disease extent and activity in ulcerative colitis (UC). However, the degree of correlation between endoscopic activity and mucosal inflammation is controversial.

Aim : In this study, we investigated the level of agreement between the Mayo endoscopic subscore and 2 histological UC activity grading systems : a modified Riley scale (Gut, 1991) and the Geboes scale (Gut, 2000).

Methods : 125 randomly selected colonoscopic biopsy sets from 95 UC patients (47 men, mean age 46 yrs) were reviewed by 1 experienced GI pathologist with specific interest in IBD. Inflammation was scored according to the Riley and the Geboes scale. Scores were then converted to a 4-tiered grading system (gr 0 : inactive / gr 1 : mild / gr 2 : moderate / gr 3 : severe) to allow comparison with the Mayo endoscopic subscore. For the Geboes scale, 9 different conversions based on various upper and lower limits for grade 2 were constructed (Table). For each scale conversion, the number of misclassifications with regard to the Mayo score was calculated. Conversions were ranked for adaptability to the Mayo score based on this number and on the Spearman rank correlation coefficients.

Results : There was a statistically significant positive correlation between the Riley scale and the Mayo endoscopic subscore (Spearman's $\rho = 0.826$, $P < 0.0001$). The best correspondence was for Mayo grade 0 (normal / inactive disease), with only 1/24 misclassifications (4%). The number of misclassifications for the other Mayo grades were : grade 1 : 17/22 = 77% (with an equal distribution over histological grades 0 and 2), grade 2 : 6/32 = 19%, grade 3 : 16/47 = 34%. With Geboes's scale conversions nr 3 and 6, results very similar to the Riley scale were obtained (Spearman's $\rho = 0.809$, $P < 0.0001$). The single best alternative classification was Geboes's conversion nr 9, with a slightly better match for grade 1 (14/22 misclassifications, Spearman's $\rho = 0.826$, $P < 0.0001$). All other conversions were less optimal.

Conclusion : The histological scores by Geboes and Riley correlated well with colonoscopic UC activity. The best correspondence was observed for the Riley scale and for Geboes's scale conversions nrs 3, 6 and 9. With these grading systems, a Mayo endoscopic subscore 0 corresponded almost always with histologically normal biopsies or inactive

chronic colitis. On the other hand, about one-third of the Mayo's grade 3 are underrated by histology, and one-sixth of the Mayo's grade 2 are overrated. Tissue sampling and biopsy technication probably explain a large part of the discrepancy. Histology is also able to discern subcategories in Mayo grade 1. This is an added benefit, since detection of large inconsistencies in the setting of mildly active UC may trigger more aggressive treatment, possibly with other therapy modalities.

Table

| Mayo subscore | Riley scale | Geboes scale | Geboes conversions |
|----------------------------|-----------------------|-----------------------------|---|
| 0 | 0 (grade 0) | (0.0_0.3) | Grade 0 : (0.0-2B.0) |
| Normal/inactive | Normal/inactive | Normal/architectural change | Grade 1 : (2B.1-X) Grade 2 : (X-Y) Nr 1 = (2B.2-5.0) Nr 2 = (2B.2_5.1) Nr 3 = (2B.2_5.2) Nr 4 = (3.1-5.0) Nr 5 = (3.1-5.1) Nr 6 = (3.1-5.2) Nr 7 = (4.1-5.0) Nr 8 = (4.1-5.1) Nr 9 = (4.1-5.2) Grade 3 : (Y-5.4) |
| (1.0_1.3) | | | |
| Mononuclear cells only | | | |
| (2A.0_2A.3) | | | |
| Lamina propria eosinophils | | | |
| 1 | 1-3 (grade 1) | (2B.0_2B.3) | |
| Mild | LP neutrophils | LP neutrophils | |
| 2 | 4-6 (grade 2) | (3.0_3.3) | |
| Moderate | Neutrophils in crypts | Neutrophils in epithelium | |
| (4.0_4.3) | | | |
| Crypt destruction | | | |
| 3 | 7 (grade 3) | (5.0_5.4) | |
| Severe | Erosion/ulceration | Erosion/ulceration | |

EPIDURAL ANALGESIA AFFECTS SURVIVAL AFTER ESOPHAGECTOMY IN NODE POSITIVE ESOPHAGEAL CANCER PATIENTS. V. Ochieng, W. Ceelen, L. De Baerdemaeker, P. Pattyn. UZ, Gent, Belgium.

Introduction : Epidural anesthesia may theoretically affect survival after esophageal cancer surgery due to changes in visceral blood flow and immune function. Specifically, recent evidence suggests that tumor cells express both opioid receptors and their ligands, the opioid peptides, suggesting that opioids may affect tumor growth. Reducing the amount of opioids in the perioperative period by using epidural supplementation may therefore be a viable option to reduce cancer recurrence.

Aim : We analyzed the effect of general anesthesia with or without epidural supplementation (ES) in patients who underwent esophagectomy for locoregionally advanced cancer of the esophagus.

Methods : Data were extracted from a prospective database of locally advanced esophageal cancer patients treated with Ivor Lewis esophagectomy. A multivariate Cox model was used to assess the prognostic value of ES. Other independent variables entered into the model were tumor histology, size and margins, vascular invasion, chemo and/or radiotherapy, surgical technique, and blood transfusion. The analysis was performed separately in node negative (stage IIA) and node positive (stage IIB/III) patients. The dependent variable was disease free survival (DFS).

Results : One hundred and seventy-four patients (109 node-positive and 65 node-negative) were available for analysis. In node positive patients, ES was associated with improved DFS (median survival 30.5 vs 12.2 months ; $P = 0.048$) in univariate analysis. Multivariate analysis confirmed ES as an independent prognosticator of DFS (Hazard Ratio 0.34-0.97 ; $P = 0.04$). In node negative patients, ES did not impact DFS.

Conclusion : In node positive esophageal cancer patients, use of epidural supplementation during surgery is associated with improved DFS. Prospective trials are warranted to confirm this finding and to explore possible mechanisms.

RESEQUENCING OF POSITIONAL CANDIDATES IDENTIFIES LOW FREQUENCY IL23R CODING VARIANTS PROTECTING AGAINST INFLAMMATORY BOWEL DISEASE. Y. Momozawa (1), M. Mni (1), K. Nakamura (1), W. Coppieters (1), L. Amininejad (2), I. Cleynen (3), P. De Rijk (4), O. Dewit (5), D. Goossens (4), D. Laukens (6), C. Libiouille (1), C. Reenaers (7), P. Rutgeerts (3), D. Zelenika (8), M. Lathrop (8), J. Del-Favero (4), J.P. Hugot (9), M. De Vos (6), D. Franchimont (2), S. Vermeire (3), E. Louis (7), M. Georges (1). (1) University of Liège, Liège, Belgium ; (2) ULB Erasme, Brussels, Belgium ; (3) UZ Leuven Department of Gastroenterology, Leuven, Belgium ; (4) University of Antwerp, Antwerp, Belgium ; (5) UCL Saint-Luc, Brussels, Belgium ; (6) UZ, Gent, Belgium ; (7) CHU de Liège, Liège, Belgium ; (8) Centre National de Génotypage, Evry, France ; (9) Hôpital Robert Debré, Paris, France.

Introduction : Genome wide association studies (GWAS) have identified more than 70 and 30 loci associated with inherited predisposition to Crohn's disease (CD) and ulcerative colitis (UC), but they account for 75% «missing heritability». The possible contribution of highly penetrant, low frequency and rare variants not adequately tagged in GWAS is one of the favoured hypotheses.

Aim : The objective of this study was to use state-of-the-art high-throughput sequencing technology to search for rare risk variants in positional candidates from GWAS.

Methods : We developed a highly specific (> 98%) and sensitive (> 83%) protocol to detect rare variants by massively parallel resequencing (Roche 454 instrument) of the open reading frames (ORF) of 70 positional candidate genes amplified from equimolar pools of genomic DNA from 32 cases or controls. We applied a stage design in which all candidate genes would first be sequenced on 112 cases and 112 controls (stage I), followed by the sequencing of the most promising genes in up to 928 and 1,216 additional cases and controls (stage II). Individual genotyping of low frequency variants was done using Taqman assays.

Results : Although none of the analyzed genes showed a significant (Bonferonni corrected) excess load of rare NS variants neither in cases nor controls in stage I, we pursued the analysis of the 12 top genes (including NOD2 considered as positive control) in stage II. For one of these (IL23R) we confirmed a reduced genetic load in CD cases, reflecting selection against three previously unknown low frequency NS variants. The depletion of these presumably disruptive and therefore protective variants was confirmed by individual genotyping in 1,565 additional CD cases, as well as in

1,251 UC cases when compared to 2,000 additional controls. Because of their low frequency, the identified variants explain.

Conclusion : In this study we have identified three novel low frequency IL23R NS variants that presumably protect against IBD by dampening IL23 signalling. We conclude that low frequency and rare coding variants in positional candidates don't make a major contribution to inherited predisposition to IBD. However, with some adjustments, high-throughput resequencing may assist in the needed identification of causative genes amongst positional candidates.

- D47 -

EVOLUTION OF THE PATIENT ACCESS PROGRAM FOR CISAPRIDE IN BELGIUM BETWEEN 2002 AND 2010. J. Tack (1), H. Piessevaux (2), Y. Vandenplas (3), E. Present (4), P. Neels (5). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) Cliniques Universitaires Saint-Luc, Brussels, Belgium ; (3) UZ Brussels, Brussels, Belgium ; (4) Janssen-Cilag NV, Beerse, Belgium ; (5) Federal Agency for Medicinal and Health Products (FAHMP) and CHMP/EMA, Brussels/London, Belgium.

Introduction : Cisapride became commercially available in Belgium in 1990 and was widely used, but commercial distribution was discontinued worldwide after 2002, because of a substantial risk of QT interval prolongation and severe and sometimes fatal ventricular arrhythmias. The European Committee for Medicinal products for human use (CHMP) concluded in 2002 that the possible benefits of cisapride treatment did not outweigh the risks. In children, ESPHAN and NASPGHAN guidelines state that prokinetics are not recommended for treating GORD because adverse events outweigh potential benefits. A Prepulsid Patient Acces Program (PASREGG, followed by PPAP from January 2005) was started by Janssen-Cilag, making the remaining stock of cisapride available in agreement with the Belgian authorities. The modalities of this PPAP were published in a Royal Decree dated 27/12/2004 admitting the use of cisapride in :

1. Treatment of proven pathological gastro-oesophageal reflux, after failure of other treatments in children up to the age of 36 months.
2. Treatment of acute and severe symptoms of chronic gastro-parese of proven idiopathic or diabetic origin, after failure of other treatments in adults.

Similar patient access programs are available in Portugal (currently 4 patients registered) and France (currently 455 children and 47 adults registered). The end of the remaining cisapride stock is expected in the near future, leading to a discontinuation of the PPAP after 4/2011.

Aim : To analyse the usage of cisapride in the PPAP registry over time.

Results : At the start of PPAP in 2005, 574 children and 2790 adults were registered. The number of active patients (= data updated during the last 6 months) remained stable during the 6 years of PPAP (3364 in 2005, 2392 in 2006, 2760 in 2007, 2907 in 2008, 2525 in 2009 and 2541 in 2010 (528 children and 2013 adults)). Contrary to its objective, no gradual decline in the overall number of registered patients occurred, but numbers of registered patients have continued to rise to reach 3835 children and 13141 adults in the first half of 2010, indicating inclusion of new patients over the

years. The number of registered prescribing physicians rose from 580 at the start to 680 in 2010 (40% gastroenterologists, 35% paediatricians and 25% endocrinologists). The main indications for cisapride use in the PPAP were GORD in children (95.4%) and gastroparesis in adults (93.3%). Children were mainly initiated between the age of 0 and 1 year ; and the mean age of initiation for adults was 52.5 years (range 18 to 102 years). Sex distribution was not monitored in the PPAP.

Conclusion : Contrary to its initial objective, cisapride usage in the 6 years of PPAP registry remained high, suggesting potential over-use and/or a lack of therapeutic alternatives. In agreement with CHMP conclusion of a negative risk/benefit profile, and with the Belgian Committee for Medicinal Products for Human Use's statement that "there are no arguments for continuing or starting cisapride therapy at present", the PPAP program will come to an end in 4/2011.

- D48 -

TOLERABILITY OF SHORTENED INFLIXIMAB INFUSION TIMES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES : A SINGLE CENTER COHORT STUDY. C. Breynaert (1), M. Ferrante (1), H. Fidder (2), K. Van Steen (3), M. Noman (1), V. Ballet (1), S. Vermeire (1), P. Rutgeerts (1), G. Van Assche (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) UMC, Utrecht, Netherlands ; (3) University of Liège, Liège, Belgium.

Introduction : Scheduled maintenance therapy with infliximab decreases the risk of infusion reactions. Many centers have accelerated infusion times to 1 h in selected patients who tolerate 5 mg/kg infliximab infusions.

Aim : To compare the tolerability of 1-h and 2-h infliximab infusions in patients with IBD in a large single center cohort. The primary analysis concerned the proportion of 1-h infusions with infusion reactions compared to the proportion of 2-h infusions with infusion reaction. In addition, we sought to identify predictors of infusion reactions.

Methods : A retrospective chart analysis of all IBD patients treated with infliximab was performed. Infusions in scheduled maintenance for at least 6 months from December 1994 until March 2009 were included. All patients were treated at the infusion unit or during hospitalization under standard operating procedures. Infusion parameters were prospectively recorded. From 2004, in patients tolerating at least four 2-h infusions, infusions were given over 1 h. Infusions at start up, after a drug holiday, after a documented infusion reaction and all infusions before 2004 were given over 2 h or longer. The study was approved by our institution's ethics committee.

Results : As of March 2009, 953 patients with IBD (77.6% CD, 22.4% UC) had been treated with infliximab. 474 patients met the criteria of scheduled maintenance therapy. In total, 9155 maintenance infusions were administered (4307 over 1 h). No severe infusion reactions were documented. Mild acute reactions occurred in 0.6% (27/4307) of 1-h and 1.7% (80/4848) of 2-h infusions ($p = 0.0034$). Delayed infusion reactions occurred in 0.2% of 1-h and 0.5% of 2-h infusions ($p = 0.277$). Loss of tolerability due to infusion reactions (1-h group 2.9% versus 2-h group 4.1%) was evenly distributed ($p = 0.34$). None of the prespecified variables (gender, disease subtype, disease duration, age at moment of first infusion, use of an induction scheme (0-2-6 weeks), maintenance therapy, concomitant immunosuppression and use of corticosteroids) were predictive of infusion reactions in a multivariate analysis.

Conclusion : In patients with IBD tolerating 2-h infusions of infliximab scheduled maintenance therapy, the infusion time can be shortened to 1 h with good tolerability. No severe reactions were observed and no predictors of infusion reactions were identified. Optimizing infusion procedures and accelerated infusion times with acceptable safety offer perspectives for a decreased impact of the treatment on activities of daily life.

Posters

- D49 -

SERUM APOLIPOPROTEIN CIII LEVELS DECLINE AFTER WEIGHT LOSS INDUCED IMPROVEMENT IN HEPATIC STEATOSIS. A. Verrijken (1), S. Francque (1), S. Caron (2), I. Mertens (1), G. Hubens (1), E. Van Marck (1), B. Staels (2), P. Michielsen (1), M.R. Taskinen (3), L. Van Gaal (1). (1) UZ, Antwerpen, Belgium ; (2) Université Lille Nord de France Inserm U1011 UDSL Institut Pasteur de Lille, Lille, France ; (3) Helsinki University Central Hospital and Biomedicum, Helsinki, Finland.

Introduction : Apolipoprotein CIII (apoCIII) is a regulator of hepatic and plasma triglyceride metabolism. Recent findings of apoCIII gene polymorphisms, in patients with hepatic steatosis and NASH, have elicited the interest to study its connection to obesity and weight loss.

Aim : To study the effect of weight loss on apoCIII levels in a cohort of obese patients with prospective assessment of the presence of NAFLD.

Methods : Patients presenting for a problem of overweight underwent a metabolic and liver assessment. If NAFLD was suspected, a liver biopsy was proposed (NASH CRN scoring system). 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.

Results : A series of 53 patients (69.8% female) were prospectively included. At baseline, mean age was 47 ± 12 years, mean BMI was 37.6 ± 6.3 kg/m². A liver biopsy was performed in 40 patients at baseline and in 29 at follow-up. The whole spectrum of NAFLD from normal liver to NASH-cirrhosis (NAS ranging 0-6, fibrosis 0-4) was present. After 12 months of treatment, patients achieved a significant ($p < 0.001$) weight reduction of 16.9 kg. ApoCIII levels and triglycerides also decreased significantly ($p < 0.001$), and apoCIII and triglyceride decrease were significantly correlated ($p < 0.001$). Decrease in apoCIII did, however, not correlate with reduction in weight. There was a significant decrease in degree of steatosis, which positively correlated with the decline in apoCIII levels ($p = 0.0259$). Weight loss ($p = 0.003$) and decrease in apoCIII ($p = 0.010$) were independently correlated to the reduction in steatosis.

Conclusion : Not the amount of body weight reduction, but the loss of hepatic fat seems to be independently correlated to apoCIII decline.

- D50 -

EVOLUTION OVER A 15 YEAR PERIOD OF THE EPIDEMIOLOGICAL PROFILE OF 2884 NEWLY DIAGNOSED HCV PATIENTS IN BELGIUM. J.P. Loly (1), C. Gérard (1), D. Vaira (1), B. Bastens (2), B. Servais (3), E. Wain (4), C. Bataille (5), J. Delwaide (1). (1) CHU Sart Tilman, Liège, Belgium ; (2) Centre Hospitalier Chrétien, Liège, Belgium ; (3) Centre Hospitalier Bois Abbaye, Liège, Belgium ; (4) CHPLT La Tourelle, Liège, Belgium ; (5) Centre Hospitalier Huy, Liège, Belgium.

Introduction : Evolution over a 10 year period (1992-2003) of the epidemiological profile of 1726 newly diagnosed HCV patients in Belgium has been previously published (J Med Virol 2005). We extended this analysis over 5 additional years (until 2008) on 2884 pts.

Methods : The yearly evolution of some important epidemiological parameters (such as modes of infection, infecting genotypes) was analyzed retrospectively in a cohort of 2884 pts living in the south part of Belgium, who were diagnosed as HCV carriers by PCR between 1992 and 2008.

Results : The epidemiological profile of HCV pts showed significant changes during this period. The number of new pts increased with time until 2003 (with a maximum of 300 newly presenting pts/year), but after 2003, this number decreased rapidly (150 in 2008). The rate of newly presenting pts infected by transfusion before 1990 decreased, but only by 1.9%/year (reaching always the high figure of 20% of newly presenting pts in 2008). The proportion of intravenous drug users increased by 2%/year (40% in 2008). Pts presenting undefined risks factors increased by 0.6%/year (25% in 2008), in relation with migration in Belgium of pts coming from countries with high prevalence of HCV infection. It appeared that the distribution of risk factors (transfusion, drug use, and more surprisingly, invasive medical procedures) significantly differed between different geographical areas in Belgium. Significant linear annual decrease of 2% in the frequency of genotype 1b, counterbalanced by a significant increase of genotype 1a (0.5%) and 4 (0.7%) was observed (reaching respectively, 40%, 11%, 16% of the infecting genotypes of newly presenting pts in 2008).

Conclusion : Such analyses are useful for evaluating the epidemiological changes of C virus infection and for anticipating the future economical costs of HCV treatment in the next few years.

COMPARATIVE PHARMACOKINETICS OF PRUCALOPRIDE IN HEALTHY YOUNG AND ELDERLY SUBJECTS. L. Vandeplassche. Movetis, Turnhout, Belgium.

Introduction : Prucalopride (PRU) is a highly selective 5-HT₄ receptor agonist with strong enterokinetic activity, developed for the treatment of chronic constipation.

Aim : The aim of the study was to compare the single dose and steady state pharmacokinetics of PRU in healthy young and elderly subjects

Methods : This was an open, parallel group trial in 12 healthy elderly (8 male/4 female, aged 65-81 years) and 12 healthy young subjects (8 male/4 female, 20-32 years old). All were given a single 1 mg tablet of PRU on day 1, followed by a 1-week treatment of 1 mg o.d. on days 5 to 11. All doses were administered in the morning, 30 min before breakfast. Blood samples were taken up to 96 h after dosing on days 1 and 11, and pre-dose on days 9, 10 and 11. The complete urinary output of a 24-h dosing interval was collected on day 11. Plasma and urine concentrations of PRU were measured using radioimmunoassay, with a lower limit of quantification of 0.10 ng/ml in plasma and 20 ng/ml in urine. *In vitro* plasma protein binding of PRU was determined in the day 1 pre-dose samples by equilibrium dialysis. Pharmacokinetic parameters were calculated using standard non-compartmental methods.

Results After single dosing, maximum plasma concentration (C_{max}) was comparable in elderly (2.17 ± 0.67 ng/ml) and young subjects (2.24 ± 0.79 ng/ml), but area under the curve (AUC) was 19% higher in the elderly (69.6 ± 9.3 vs. 58.3 ± 14.7 ng.h/ml). Steady state was attained after 4 days of treatment. Steady state plasma concentrations were higher in the elderly than in the young subjects with the AUC increased by 28% (72.2 ± 12.5 ng.h/ml vs. 56.2 ± 16.5 ng.h/ml). The effect was larger on minimum plasma concentration (C_{min}) (+ 41%) than on C_{max} (+ 19%). Urinary excretion at steady state accounted for 61% (young) to 66% (elderly) of the dose. Both the apparent oral clearance and renal clearance were lower in the elderly than in the young subjects. The mean creatinine clearance was 132 ml/min in young and 78.6 ml/min in elderly subjects. Plasma protein binding of PRU was similar in elderly (32.8%) and young subjects (33.3%).

Conclusion : Urinary excretion is a major elimination pathway for PRU. As renal function decreases with age, the somewhat lower renal clearance and consequently higher exposure of PRU in elderly subjects is not unexpected. Results of clinical trials indicated that a lower dose is needed in elderly subjects (1 mg o.d.) than in young and middle-aged adult subjects (2 mg o.d.). However, there is no pharmacokinetic basis for this finding, as the increase in exposure by approximately 30% is too low to justify halving the dose.

CYTOTOXICITY OF FECAL WATER IS ASSOCIATED TO INCREASED CONCENTRATIONS OF ALCOHOLS.

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Introduction : Colonic fermentation of carbohydrates and proteins leads to the production of short chain fatty acids, whereas protein fermentation also results in branched chain fatty acids, phenols, sulphides and amines. Some of these metabolites are potentially toxic.

Aim : In the present study, we modified the degree of protein fermentation by changing protein intake and investigated whether fecal metabolites associated with cytotoxicity originate from protein fermentation.

Methods : After a 1-week run-in period with normal protein (NP) intake, 20 healthy volunteers followed an isocaloric high protein (HP, 30%) and a low protein (LP, 9%) diet for 2 weeks in a randomized cross-over study. Fibre and fat intake were kept constant. During the run-in period and during the second week of each diet period the volunteers completed a dietary journal and collected urine for 48h and feces for 72h. Dietary composition was analyzed. Colonic protein fermentation was estimated from the urinary concentration of p-cresol. Profiles of volatile organic compounds (VOC) were analysed in fecal samples using GC-MS. Metabolite profiles were compared using cluster analysis. Fecal water cytotoxicity was determined using the WST-1 assay and expressed as fold dilution at which 50% of the cells survived (fold dilution, FD50). Cytotoxicity was related to the metabolite profiles. Results are expressed as average and standard deviation.

Results : Protein intake accounted for 27 ± 4,15% of energy intake during the HP diet, 15 ± 2,19% during the NP and 12 ± 1,76% during the LP diet. Urinary p-cresol excretion was significantly correlated with protein intake (r = 0,314 ; p = 0,015). Fecal water cytotoxicity was not correlated with protein intake (p > 0.05). The average cytotoxic dilution of the NP samples was 52,3 ± 24,9 compared to 39,3 ± 22,2 of the HP samples and 45,5 ± 25,8 of the LP samples. Cluster analysis of metabolite patterns in fecal samples according to cytotoxicity revealed a separation between the high toxicity (FD50 > 75) samples and the low toxicity (FD50 < 50) samples. This separation was mainly due to the presence of alcohols and the sulfur-containing compounds, allylisocyanate and 2,4-dithiapentane, in the high toxicity samples and the presence of cycloalkanes and cycloalkenes in the low toxicity samples. SCFA did not contribute to the clustering of the samples.

Conclusion : Higher protein intake is associated with a higher degree of protein fermentation but not with a higher fecal water cytotoxicity. Metabolites associated with higher cytotoxicity are not exclusively derived from protein fermentation. Alcohols result from carbohydrate fermentation and cycloalkanes and alkenes originate from plant materials. Other types of toxicity, including genotoxicity, need to be assessed.

- D53 -

BASELINE ESOPHAGEAL IMPEDANCE : COMPARABLE FOR DIFFERENT TIME INTERVALS. D. Dario Ummarino (1), H. Bruno (2), A. Staiano (1), Y. Vandenplas (2). (1) University of Naples Federico II, Naples, Italy ; (2) UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

Aim : Oesophagitis is reported to decrease baseline impedance. Therefore, it is relevant to define the optimal duration of the time interval to calculate the baseline impedance (BImp).

Methods : We evaluated 30 multichannel intraluminal impedance/pH metries (MII/pH) recordings performed in children (mean age \pm SD : 96 ± 61 months ; 45-202 months) to assess during different fractions of time the variation (difference in BImp for the different time periods studied) and variability (difference in BImp in one interval). We calculated the mean esophageal BImp during different time intervals (1, 5, 15 and 30 min) of the tracing without reflux (acid, non-acid and gas) episodes in channels 1, 2, 5 and 6.

Results : We postulated that the BImp obtained during a 1 minute period equalled 100%. The BImp during the other time intervals were expressed as a difference in % to the 1 minute value of 100%. The variation was minimal. Compared to the 100% value at 1 min, the BImp calculated during a period of 5 min differed 0.52 % (range -2.06%-3.07%) ; during 15 min : 0.99% (range -2.84%-6.15%) ; during 30 min : 0.46 % (range -4.1%-6.55%). Also the variability was very low. If postulated to be 100% during 1 minutes, the variability during 5 min was 0.52%, during 15 min 0.99% and during 30 min 0.46%. Variation and variability was equal for all channels.

Conclusion : The evaluation of the BImp over a short period of 1 min provides identical information as measurements over longer periods (5, 15, 30 minutes).

PRESUMED HEALTHY DONOR ORGANS WITH HETEROZYGOUS ALPHA1-ANTITRYPSIN Z ALLELE MUTATION : IMPLICATIONS FOR LIVER TRANSPLANT RECIPIENTS. P. Roelandt (1), P. Dobbels (1), G. Matthijs (2), T. Roskams (3), R. Aerts (4), D. Monbaliu (4), J. Pirenne (4), L. Libbrecht (1), W. Laleman (1), C. Verslype (1), W. Van Steenberghe (1), F. Nevens (1), D. Cassiman (1). (1) UZ Leuven Department of Hepatology, Leuven, Belgium ; (2) UZ Leuven Department of Human Genetics, Leuven, Belgium ; (3) UZ Leuven Department of Pathology, Leuven, Belgium ; (4) UZ Leuven Department of Abdominal Transplantation Surgery, Leuven, Belgium.

Introduction : α_1 -antitrypsin (AAT) deficiency is an autosomal recessive disorder occurring in 1:1800 live births and is in general the most common genetic cause of chronic liver disease in children. Despite increasing efforts to identify AATD, only 0.35% of patients with AATD are being diagnosed. The Z allele (Glu342Lys) is a combined deficiency and dysfunctional allele and occurs in 4% of the population in northern Europe. Since most of the carriers of Z allele do not have overt liver disease, it is likely that Z allele containing livers previously have been used as presumed healthy donor organs for liver transplantation.

Aim : In this study, we analyzed the incidence, epidemiology and clinical features of AAT accumulation after liver transplantation.

Methods : All biopsies of liver transplantation patients between 1995 and 2006 were analyzed with period acid Schiff (PAS) staining, and if possible with specific monoclonal antibody against mutated AAT_Z protein. Genotyping of both receptor and donor was performed in case of positive staining.

Results : Out of 486 liver transplantation patients 6 patients (1.2%) with mutated AAT_Z accumulation in the transplanted liver were identified. Average time between transplantation and diagnosis was 27.4 months. In all 6 biopsies a cyto-keratin 7-positive (Krt7⁺) ductular reaction was noted, while in only 1 biopsy signs of cholestasis were present. Genetic sequencing confirmed the presence of Z allele. The average serum levels of AAT were within the normal range and therefore did not contribute to the diagnosis. However, there was a clear correlation between the result of the isoelectrical focusing of the receptor AAT after transplantation and the genotype of the donor.

Conclusion : As to be expected by the epidemiological data in the general population, presumed healthy donor organs containing Z allele were used for transplantation. The knowledge of presence or absence of Z allele may have more implications than just providing the diagnosis of AAT deficiency. Since the presence of Z allele is an independent risk factor for chronic liver disease, this knowledge can provide important information for the post-transplantation period and potentially change the choice of immunosuppressive therapy in the future.

HIPEC FOR PERITONEAL CARCINOMATOSIS : DOES AN ASSOCIATED UROLOGIC PROCEDURE INCREASE MORBIDITY ? C. Honoré, A. Souadka, D. Goéré, F. Dumont, F. Deschamps, D. Elias. Institut Gustave Roussy, Villejuif, France

Aim : The purpose of this study was to report the incidence, the morbidity rate, the specific complications and the mortality rate of urinary-tract procedures associated with a complete cytoreductive surgery (CCRS) plus Hyperthermic Intraperitoneal Chemotherapy (HIPEC) or Early Postoperative Intraperitoneal Chemotherapy (EPIC).

Methods : From a prospective database of patients with malignant peritoneal disease, all types of tumors included, treated with CCRS plus intraperitoneal chemotherapy (HIPEC or EPIC) ; patients who underwent a resection or a suture of the bladder, ureter or kidney were retrospectively studied.

Results : Between 1994 and 2010, 48 patients underwent a resection or a suture on the urinary tract among the 598 treated with CCRS plus intraperitoneal chemotherapy (8%). There were 4 nephrectomies, 19 partial cystectomies, 8 perforations of the bladder and 18 ureteral resections. The overall postoperative morbidity (grade > 2) was 41% (20/48). The mortality was 4% (2/48). Specific complication included 6 urinary fistulas (12%) : two among the 27 bladder sutures (7%) and four among the 18 ureteral sutures (22%) (pNS). In uni- and multivariate analysis, risk factors of urinary fistula were a severe preoperative denutrition (p = 0.05, RR = 7.3) and an extended peritoneal disease (PCI > 20, p = 0.05, RR = 8.3). Conservative treatment of urinary fistulas was successful in 83% of cases (5/6).

Conclusion : Associated urinary-tract procedures occur in 8% of the all CCRS plus intraperitoneal chemotherapy. They do not significantly increase postoperative morbidity and mortality. Therefore, urinary tract involvement by a carcinosis should not contraindicate a curative treatment, CCRS plus intraperitoneal chemotherapy. Severe preoperative denutrition and extended peritoneal disease increase the risk of postoperative urinary fistulas.

SELF-MANAGEMENT EDUCATION NEEDS IN ADOLESCENT LIVER TRANSPLANT RECIPIENTS : A QUALITATIVE RESTROSPECTIVE STUDY. I. Aujoulat (1), M. Janssen (2), F. Libion (1), C. Struyf (2), A.S. Charles (2), E. Sokal (2), R. Reding (2). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Introduction : Learning to self-manage one's condition is a major challenge for all chronically ill patients. Difficulties in self-management often result in poor adherence, which in turn may lead to organ rejection in solid organ transplant recipients.

Aim : The aim of our study was to better understand the factors which impact on self-management in adolescent liver transplant recipients, from the perspective of the patients.

Methods : We conducted a retrospective qualitative study through in-depth interviews, and inquired about (i) the experience of growing up with a liver transplant, and (ii) the process which leads to self-management of the condition during the transition from childhood to adulthood. All interviews were transcribed verbatim, and thematic content analysis was performed by two independent researchers, in order to identify common themes across the various reported experiences. The level of self-reported adherence was assessed using the Basel Assessment of Adherence with Immunosuppressive Medication Scale (BAASIS®).

Results : We included 15 young patients with a mean age of 21 years (16-30). Independently of the patients' level of self-reported adherence at the time of interview, a certain number of common questions, challenges or difficulties emerged during our process of analysis :

- To be able to name and explain the disease which had induced the need for transplantation,
- To understand how immunosuppression works, in particular when fluctuations in blood tests require an adaptation of the level of immunosuppression,
- To find the right balance between parental supervision and self-managed care at different ages, including during medical follow-up visits,
- To have a clear indication of what a risk-behaviour is in terms of both general health behaviours and non-adherence to treatment,
- To anticipate non-intentional non-adherence by inquiring about what to do when a dose is missed,
- To have a clear understanding of the possible impact of the treatment on fertility, and of a possible risk to transmit one's condition to descendants,
- To come to terms with the idea of being a survivor,
- To come to terms with feelings of guilt or obligation toward one's family or the donor,
- To maintain hope in the event of other concomitant adverse health circumstances.

Conclusion : Our study suggests several avenues for setting up a comprehensive self-management education programme. It provides for an illustration of the importance to discuss not only behavioural aspects of adherence, but also general health education needs, reproductive health issues, transition tasks within the family, the patient's condition prior to transplantation, and the donation process.

BASELINE ESOPHAGEAL IMPEDANCE IN DIFFERENT AGE IN CHILDREN. D. Dario Ummarino (1), R.P. Nunzia (1), A. Staiano (1), H. Bruno (2), Y. Vandenplas (2). (1) University of Naples Federico II, Naples, Italy ; (2) UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

Introduction : Several studies evaluate the baseline impedance (BImp) in the distal esophagus. If the BImp of different studies are combined, infants have a smaller BImp than adults (1750 Ω (range : 1500-2050 Ω) vs 4342 Ω (range : 3838-4889 Ω)).

Aim : Therefore, we measured the BImp in the proximal and distal esophagus in children according to age.

Methods : We evaluated 81 multichannel intraluminal impedance/pH metries (MII/pH) recordings performed in children (mean age ± SD : 48.2 ± 53.9 months ; range 1-203 months) to assess the variation of the BImp during the 24 h recording and according to age (3 groups : group 1 : 1-12 months ; group 2 : 13-84 months ; group 3 : > 85 months). We calculated the mean esophageal BImp every 4 hours during the 24h tracing during 1 min without reflux (acid, non-acid) and gas episodes, in channels 1, 2, 5 and 6. The results were evaluated with one-way ANOVA test and p < 0.05 was considered statistically significant.

Results : The results show an increase of BImp in all the channels in relation to age : the older the children, the higher the BImp (table 1).

| Group | Channel 1 | | Channel 2 | | Channel 5 | | Channel 6 | |
|-------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| 1 | 1913 | 1758-2068 | 1808 | 1718-1973 | 1945 | 1782-2287 | 2044 | 1711-2338 |
| 2 | 2609 | 2300-2863 | 2373 | 2243-2464 | 2233 | 1962-2412 | 2440 | 1955-2614 |
| 3 | 3081 | 2893-3361 | 2541 | 2362-2717 | 2689 | 2366-3198 | 2941 | 2428-3396 |

We calculated the 24 hours mean BImp of proximal and distal esophagus. All data were evaluated with one-way ANOVA test (p < 0,0001 according to the age). No circadian rhythm was found in mean BImp in proximal and distal esophagus.

Conclusion : The evaluation of the BImp shows an increase according to age. This result could be explained by the fact that the esophagus diameter increases with increasing age, allowing more air around the probe in the older children, considering that the MII/pH probes have the same diameter in infants, in children and in adults.

PER-CUTANEOUS GASTROSTOMY (PEG) IN CHILDREN USING A PUSH INTRODUCER GASTROPEXY TECHNIQUE. P. Bontems, L. Muyshont, A. Salame, M. Scaillon. Queen Fabiola Children's University Hospital, Brussels, Belgium.

Introduction : Children who have insufficient voluntary oral nutrient intake, who have swallowing disorders or special needs such as nutrition in metabolic diseases require supplementary or exclusive enteral feeding. A naso-gastric tube feeding should not be used more than two months to avoid certain complications. When a gastrostomy is indicated, the classical pull technique is not always possible (ie in small infants or when there are oesophageal disorders such as stenosis).

Aim : Evaluation of a push PEG technique in children.

Methods : We tested the last 4 years a new introducer PEG-gastropexy kit designed by Fresinius Kabi AG, Bad Homburg, Germany (Freka Pexact-15ch introducer PEG kit) to avoid surgical placement when a classical pull technique placement was not possible. After antibiotic prophylactic injection and under general anesthesia, the gastric wall was non-surgically sutured to the anterior abdominal wall using a dedicated device and a gastroscope for visualisation. Then the gastrostomy tube was placed using an introducer. This is similar to performing surgical gastropexy but under endoscopic control. Enteral feeding was started after an overnight fasting.

Results : This technique was applied in 18 children, 10M/8F (median age 0,9 y – range 0.5-19). Their median weight was 8.5 y (range 6-39). In one patient, the placement was not possible due to lack of trans-illumination (the gastrostomy was then placed by the surgeon). Few complications occur during the 5 first days (one gastric haemorrhage treated endoscopically, no infection, no peritonitis). In two patients we experience balloon deflation during the first 3 week but the tube was easily replaced without spillage of stomach contents in the peritoneal cavity and without infection. Late complications such as wound infection more than 4 weeks after placement or granulation tissue on the wound orifice were frequent but no more than with a gastrostomy placed by the classical pull technique or by surgical procedure.

Conclusion : This “push” introducer PEG placement technique is easily feasible and the rate of complications was similar than with other techniques and avoids surgery.

SEROPREVALENCE OF CELIAC DISEASE IN BELGIAN CHILDREN AND ADOLESCENTS. S. Vijgen, P. Gillis, A. Mewis, P. Declercq, P. Alliet. Jessa ziekenhuis, Hasselt, Belgium.

Introduction : Celiac Disease (CD) has a prevalence of 1:100-1:250 in the industrialized world.

Aim : Since no data are available on the prevalence in Belgium, we aimed to assess the seroprevalence of CD in children and adolescents in Belgium.

Methods : In 2006, serum samples from 1159 apparently healthy children and adolescents aged between 1-19 years, were collected by 15 Belgian diagnostic laboratories. In September 2009, all samples were analyzed for human tissue transglutaminase IgA antibodies (IgA tTG) and total IgA levels by a commercial ELISA and immunonephelometry resp. Positive sera were assessed by immunofluorescence for the presence of IgA and IgG antiendomysium antibodies (IgA/IgG EMA). In patients with IgA deficiency IgG antibodies against deamidated gliadin peptides (IgG DGP) and IgG antiendomysium antibodies (IgG EMA) were determined.

Results : Ten of the 1159 individuals (0.86%) tested positive for IgA tTG. A further 0.86% showed borderline IgA tTG results. In almost two percent (1.98%) of the analyzed samples total IgA levels below the lower limit of normal were observed. Four out of eight (50%) positive IgA tTG samples tested positive for IgA EMA. All samples with borderline IgA tTG results were negative for IgA EMA. Twenty-six percent (6/23) of the IgA deficient samples showed positive IgG DGP antibodies, but none of these tested positive for IgG EMA.

Conclusion : The seroprevalence of positive IgA tTG in the non-IgA deficient population (n = 1136) in Belgium is 1:114. The combined seroprevalence of positive IgA tTG and IgA EMA in that same population is 1:284. These seroprevalences are similar to those found in the neighbouring countries. In order to assess the real prevalence of CD, duodenal biopsies should be performed.

ABNORMAL PRIMARY CILIA IN CHOLANGIOCYTES CAUSE CHOLESTATIC LIVER DISEASE IN HNF1B DELETION IN HUMANS. P. Roelandt (1), A. Antoniou (2), W. Laleman (1), C. Verslype (1), W. Van Steenberghe (1), F. Nevens (1), R. De Vos (3), F. Lemaigre (2), D. Cassiman (1). (1) UZ Leuven Department of Hepatology, Leuven, Belgium ; (2) Université Catholique de Louvain, Brussels, Belgium ; (3) UZ Leuven Department of Pathology, Leuven, Belgium.

Introduction : HNF1² is a crucial transcription factor in the embryogenesis of the liver, pancreas and urogenital system. Heterozygous deletion or mutation results in pancreatic atrophy, diabetes, renal and urogenital abnormalities (previously known as maturity-onset diabetes of the young (MODY) type 5). HNF1² forms a complex network with HNF6 and SOX9, necessary for correct embryonic bile duct formation.

Aim : In mice, HNF1² deletion in liver leads to ductopenia, leading to elevated liver tests and fibrosis while the overall liver function remains normal. We recently diagnosed 2 patients with heterozygous HNF1² deletion in which the human liver pathology was studied extensively.

Methods : Liver biopsies of 2 patients with proven heterozygous HNF1² deletion and chronic cholestatic liver disease were examined thoroughly by using specific immunohistochemistry (SOX9, acetylated tubulin) and transmission electron microscopy to unravel the underlying structural defect.

Results : Both patients were found to have impaired bile duct formation, similar to the findings in mice. On routine pathology and immunohistochemistry, the bile duct cells appeared normal, however electron microscopy revealed complete absence of normal primary cilia. The few remaining cilia did not contain the normal structure with 9 + 1 microtubuli.

Conclusion : Heterozygous HNF1² deleted human cholangiocytes do not form normal primary cilia, which can be associated with cholestasis.

THE SUBJECTIVE IMPACT OF SWITCHING FROM PROGRAF TO ADVAGRAF IN ADOLESCENT AND YOUNG ADULTS : A PILOT STUDY. I. Aujoulat (1), M. Janssen (2), R. Reding (2). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Introduction : Forgetfulness is a major factor of non-adherence during the transition from paediatric to adult care. Although there is some evidence that patients do better taking their medication once a day over twice a day, there was until recently no alternative to twice-daily dosing for immunosuppressive therapies in solid organ transplant recipients.

Aim : The aim of our study was to assess the subjective impact of switching from Prograf to Advagraf in paediatric patients aged 16 and over.

Methods : We conducted semi-directed interviews 6 months post-switch, and inquired about cognitive, behavioural and emotional factors in relation with their medication. The level of self-reported adherence with Advagraf was assessed using the Basel Assessment of Adherence with Immunosuppressive Medication Scale (BAASIS®).

Results : 9 patients were included, with a mean age of 20 [16-27]. Taking of medication : 1 patient reported to have missed one dose in the previous 4 weeks, and rated his general level of adherence at 85% of the prescribed medication. The other 8 patients reported to not have missed a dose and rated their adherence at 100%. Timing of medication : 4 patients reported to take their medication two hours late or more, at least once a week, but did not think it was a problem. One patient had taken it unintentionally late once, and had experienced a high level of stress as a consequence. Cognitive and psychosocial aspects : all were able to name their medication, but only 4 were fully aware that they were still receiving tacrolimus at the same level than before. All considered that Advagraf was easier to take than Prograf, and said they would be disappointed if they ever had to switch back to Prograf. However, 3 patients reported to have experienced some fears associated with the switch during the first weeks. 3 patients mentioned that they had fewer headaches since the switch to Advagraf. All appreciated the increased opportunities for dialogue with the healthcare team due to the frequent visits to monitor the medical parameters after the switch. All said that the switch to Advagraf had strengthened their sense of self-efficacy in relation with taking their medication, and 2 stressed that they were receiving fewer reminders from relatives and friends. One patient mentioned that the switch had reinforced a feeling of self-determination and responsibility because for the first time in her medical history she had been presented with the possibility to make a choice regarding the management of her condition.

Conclusion : Our results suggest that there is a positive impact of the switch from Prograf to Advagraf on the quality of life and sense of responsibility in paediatric patients over 16 who are otherwise medically stable. We view the switch as a good opportunity to open a dialogue around self-management issues, especially in the transition period from paediatric to adult care.

RECOVERY OF GRAFT STEATOSIS AND PROTEIN-LOSING ENTEROPATHY AFTER BILIARY DIVERSION IN A PFIC 1 LIVER TRANSPLANTED CHILD. E. Nicastro, X. Stephenne, F. Smets, F. Fusaro, C. De Magnée, R. Reding, E. Sokal. UCL Saint-Luc, Brussels, Belgium.

Introduction : Progressive familial intrahepatic cholestasis type 1 (PFIC 1) is a genetic disorder characterized by hepatic and gastrointestinal disease, often requiring liver transplantation during childhood. Extrahepatic symptoms, such as diarrhoea and malabsorption, do not improve or may be aggravated after liver transplantation, as well as graft steatosis or steatohepatitis through graft loss may occur, probably as consequences of the interaction between transplanted liver and native bowel. Partial biliary diversion is employed in children without hepatic fibrosis in order to improve pruritus by interrupting the enterohepatic circulation of bile salts and decreasing the bile acid pool.

Aim : To evaluate the effectiveness of the external biliary diversion to improve post-transplantation diarrhoea and avoid liver graft dysfunction.

Methods : The patient presented with cholestasis in infancy. Mutations in double heterozygosis in the ATP8B1 gene (one of which novel, p.D734A, in exon 19) allowed diagnosis of PFIC 1. The child underwent living donor liver transplantation at 3.6 years of age, and he early developed severe refractory diarrhoea, secondary malabsorption with protein-losing enteropathy, and an early fatty liver disease through graft steatohepatitis with elevated aminotransferases was documented. The diagnosis of protein-losing enteropathy was confirmed at ¹¹¹Indium-transferrin scintigraphy. As the response to cholestyramine was unsatisfactory, we decided to perform an external biliary diversion by using the jejunal loop used for the cholangiojejunostomy.

Results : Diarrhoea resolved rapidly after surgery. He remained well after six months following biliary diversion, with normal stool output and no protein loss. ¹¹¹Indium-transferrin scintigraphy returned to normal. We documented a dramatic improvement of graft steatosis at histology as well as normalization of liver function test.

Conclusion : External biliary diversion can be considered a valuable treatment option to avoid organ dysfunction and loss in PFIC 1 transplanted patients who develop graft steatohepatitis. Furthermore, indications to liver transplantation should be discussed with caution in this subset of patients with familial cholestasis.

RADIOFREQUENCY ABLATION OF UNRESECTABLE PANCREATIC CANCER. PERSONAL EXPERIENCE. M. Citone, M. La Torre, M. Rossi, M. Cavallini, D. Cavaniglia, A. Rebonato, V. David, V. Ziparo. Sapienza University, Rome, Italy.

Introduction : Treatment options for unresectable pancreatic cancer are not well defined in standard clinical practice. Radiofrequency ablation (RFA) has been suggested as a new palliative option.

Aim : Authors present their experience of intraoperative pancreatic RFA in order to evaluate the feasibility and safety of this technique in locally advanced, unresectable, non metastatic pancreatic cancer.

Methods : Five patients (1 woman) with a mean age of 73 years with histologically proven unresectable pancreatic lesions underwent intraoperative RFA. RFA was performed for tumors located in the pancreatic head (3 patients), in the isthmus (1 patient) and in the body (1 patient). Medium lesion diameter at preoperative CT-scan was 3.7 cm (range 2.6-4.8). In all patients a cluster needle with 3 cm exposed cool tip (Radionics) was employed. RFA was performed under direct ultrasound guidance with a cooling saline flushing provided during procedure. In three patients a bilio-enteric diversion was associated. In one patient RFA was performed also on celiac plexus in order to treat a severe, chronic and life style-limiting pain.

Results : RFA was feasible in all cases, achieving a complete necrosis of the lesion. A CT-scan performed on post-operative day-7 showed hypodense non-enhancing areas in the target treatment sites. All patients developed a post-operative complication. A self-limiting pancreatic fistula occurred in two patients. Of these one patient is alive at the 17 months follow-up and the other one died 8 months after the procedure. The 30-day mortality rate was 60% and it wasn't related to tumor location ; two patients died for a massive gastro-enteric bleeding and one patient died for a severe acute necrotico-hemorrhagic pancreatitis.

Conclusion : A change of standard cool tip as well as a modification of current parameters of the RF system is necessary in order to improve the rate of postoperative complications and mortality of pancreatic RFA and maintain the encouraging outcomes in terms of palliation and quality of life.

AN UNUSUAL CASE OF TUBULAR NEUROENDOCRINE CARCINOMA OF THE SMALL BOWEL. S. Duquenne (1), A. Thiry (1), D. Dresse (2), J. Delflandre (2), B. Delhougne (2), P. Delvenne (1). (1) University of Liège, Liège, Belgium ; (2) CHR Citadelle, Liège, Belgium.

Neuroendocrine tumors (carcinoid tumors) are common encountered benign and malignant neoplastic lesions found in the small intestine.

Malignant gastro-intestinal tumors demonstrating mixed neuroendocrine and glandular differentiation (malignant adenocarcinoid tumors) are uncommon and associated with problems in pathological classification and prognosis prediction.

A morphological variant of the "classical" neuroendocrine carcinoma, exhibiting pseudo-glandular structures, is described as tubular neuroendocrine carcinoma (malignant tubular carcinoid tumor). The knowledge of this entity is important since its distinction with malignant adenocarcinoid tumor can be somewhat difficult and the prognosis of these entities is different.

We describe here the case of a 46 year-old man with a history of anemia, appendectomy, and bilateral hernia repair, who was admitted at the emergency services for a brutal and diffuse abdominal pain, without any other associated symptom.

Abdominal scanography demonstrated a heterogenous mass in the mesentery of the small bowel, without other lesions. An abdominal MRI also detected a suspicious 2,8 cm lesion in the segment 4 of the liver.

A surgical resection of the tumor, with lymphadenectomy and hepatic metastasectomy was performed. The pathologic examination revealed a well differentiated 2 cm neuroendocrine carcinoma with cells mimicking glandular morphology, invading intestinal wall up to the subserosa. One lymph node (4,7 cm) was involved on four lymph nodes examined, with capsular effraction and multiple lymphatic invasions. The hepatic metastasis (3,2 cm) was distant from resection margins.

A first diagnosis of "malignant adenocarcinoid tumor" was suggested, but the absence of mucin secretion highlighted by staining methods and the positivity of pseudoglandular cells for neuroendocrine markers by immunohistochemistry oriented the diagnosis to a tubular neuroendocrine carcinoma.

No recurrences were found after 6 months follow up.

BeSPGHAN

- E01 -

PREVALENCE OF HELICOBACTER PYLORI INFECTION IN BELGIUM. F. Mana, S. Vandebosch, D. Urbain. UZ Brussel, Brussels, Belgium.

Introduction : *Helicobacter pylori* (*H. pylori*) infection is a common pathogen with about 50% of the world population infected. The infection has been associated with peptic ulcer disease, dyspepsia, ITP, investigated iron deficiency anaemia, MALT lymphoma and non-cardiac gastric cancer. Gastric cancer is the most deadly and therefore, the most feared complication. Trials from China have shown that gastric cancer can be prevented by eradicating *H. pylori* infection before premalignant lesions are present. We also know that infection is primarily acquired before the age of 10 years and, once acquired, leads to a lifelong infection. In most people this infections will stay asymptomatic but in about 5% of people premalignant lesions will evolve to cancer. However, it is not know which premalignant lesions will evolve to cancer and when the premalignant lesions have reached the point of no return, hereby meaning that eradication will not change the course of the disease anymore. This means that eradication, in the prevention of gastric cancer, should best be done in young age. However, the prevalence of *H. pylori* infection is decreasing all over the world.

Aim : We conducted a study to estimate the prevalence of *H. pylori* infection in young people in Belgium.

Methods : Informed consent was signed and a questionnaire was filled out by the children and the parents about the origin, place of birth, familial situation, place were they grew up and schooling of parents.

Results : Thirty five children tested positive (9,0%), 5 results were in the grey zone (1,3%). Place of birth was not known in 34 children (8.7%), Belgium in 328 (84.7%), 19 in countries with high prevalence of *H. pylori* infection (4.9%) and 6 in Western Europe (France, Netherlands, Norway) (1.5%).

Conclusions : Prevalence of helicobacter pylori infection is decreasing all over the world. In Belgium the prevalence in a population between 12 and 22 years old is 9%.

Invited lecture

- E02 -

IS HELICOBACTER PYLORI INFECTION THE MAIN RISK FACTOR OF ULCER DISEASE IN PEDIATRICS.
P. Bontems. Pediatric Gastroenterology Unit, HUDERF, Brussels.

RISK FACTORS FOR ANTIMICROBIAL RESISTANCE OF HELICOBACTER PYLORI STRAINS IN BELGIUM : EVOLUTION DURING THE LAST 20 YEARS. V.Y. Miendje Deyi (1), P. Bontems (2), J. Vanderpas (3), E. De Koster (1), R. Ntounda (4), M. Scaillon (2), C. Van Den borre (1), S. Cadranel (2), A. Burette (5). (1) CHU Brugmann, Brussels, Belgium ; (2) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (3) Scientific Institute of Public Health, Brussels, Belgium ; (4) CHU Saint-Pierre, Brussels, Belgium ; (5) CHIREC – Basilique, Brussels, Belgium.

Introduction : Antimicrobial resistance is a major factor jeopardizing *Helicobacter pylori* eradication therapy that needs epidemiologic surveillance.

Aim : We analysed the rate of *H. pylori* resistance to currently used drugs and identified risk factors associated with resistance.

Methods : Gastric biopsies were collected between January 1990 and December 2009 from several digestive diseases centres in the region of Brussels. We routinely performed antimicrobial susceptibility test using disk diffusion for clarithromycin (CLA), metronidazole (MET), ciprofloxacin (CIP), amoxicillin (AMO) and tetracycline (TET). Both Univariate and Multivariate analysis were performed : antibiotic susceptibility was introduced as dependent variable ; covariates were gender, age groups, time periods, previous eradication therapy and ethnic background of the patients. Finally resistance rates were compared to antimicrobials use in Belgium.

Results : Over the 2 decades, 10825 isolates were collected but antimicrobial susceptibility testing failed in 155 cases. Among the 10670 evaluable results, 9430 strains were isolated from patients who were not previously treated for *H. pylori* (1527 from children and 7903 from adults), 1371 strains were isolated from patients who were unsuccessfully treated for *H. pylori* infection (162 from children and 1209 from adults). For 24 strains we had no information concerning previous eradication attempt. *H. pylori* strains were susceptible to all tested antimicrobials in 62.1% and 23.3%, resistant to one drug in 31.6% and 57.5% and to multiple drugs in 4.7% and 18.5% of cases, before and after treatment respectively ($p < 0.05$). No resistance was observed for AMO and TET-resistance was very rare (2/10670). Primary MET resistance (METr) remains stable over the year with significant lower rates among children isolates. CIP-resistance (CIPr) remained rare in children, while it increased significantly over the years in adults. We recently observed a significant decrease in primary CLA-resistance (CLAr) : 9.9% in 2009 compared to a peak of 19.2% in 2003 that correlates with lesser macrolides use. For both CLA, MET and CIP : female gender was a significant independent risk factor of resistance ; patients aged from 40 to 64 years exhibited higher resistance rates ; resistance rates increased significantly with the number of previous eradication attempts and we observed significant increase of resistance rates over the years for patients which were previously treated. A significant difference in CLAr was found between North European patients and Middle East group. Higher level of METr and CIPr were observed in sub-Saharan African patients.

Conclusion : Female gender, age groups, ethnical background, previous unsuccessful eradication attempt and time periods are independent risk factors of resistance to CLA, MET and CIP. This study highlights the need to update local epidemiological data of *H. pylori* antimicrobials resistance to optimize efficacy of eradication strategies. Originally, after an alarming period up to 2003, we found a significant decrease in primary CLAr in *H. pylori* strains that seems correlated with the decrease of macrolides use.

POST-TREATMENT HELICOBACTER PYLORI INFECTION. R. Ntounda (1), A. Burette (2), M. Nkuize (1), T. Serste (1), V. Muls (1), J.P. Mulkey (1), Y. Nang Atto (1), M. Van Gossum (1), M. Buset (1). (1) CHU Saint-Pierre, Brussels, Belgium ; (2) CHIREC - Basilique, Brussels, Belgium.

Introduction : Guidelines identifying which patients, infected with *Helicobacter pylori* (Hp), should be given eradication treatment having been published (1). The proportion of patients with first-line Hp therapy failure may be higher in clinical practice and it may increase thanks to diffusion of Hp treatment. The treatment's efficiency is limited by antibiotic resistance and by poor patient's compliance due to its side effects, number of tablets per day, and long duration of treatment (2). None of the treatments recommended for the eradication of Hp to date is 100%. One major factor predisposing to poor patient compliance is lack of proper counselling on the part of medical and paramedical staff (3). The four questions most often asked are : Why should we control the eradication of Hp infection ? When and how to control eradication of the infection ? Which tests to choose ? What are the results or findings in real life ?

The interest in eradicating the infection is to confirm the healing, to reassure the patient, to reduce significantly ulcer recurrence in long term, and to prevent gastric neoplasia. Screening and treatment for Hp infection is potentially cost-effective in the prevention of gastric cancer, particularly in high-risk populations (4). The eradication's control must be mandatory. Several tests are used but three of them are highly recommended : non-invasive tests such as Urea breath test (UBT) and *Helicobacter pylori* stool antigen (HpSA) test, and invasive test such as histology.

Results : *UBT* : detects current infection. It is the most accurate pre and post treatment test (sensitivity and specificity 95%). The sensitivity is reduced by PPIs, h₂-receptor antagonists, antibiotics and bismuth-containing compounds (do not perform UBT and HpSA within 2 weeks of PPI or 4 weeks of antibiotics). UBT remains available as non invasive test by children but with higher false positive rates by infants and children younger than six years compared with school-age children and adolescents. C14 UBT uses radioactive ingredient and requires special handling. C13 UBT does not require special handling and cleared FDA to test for cure.

HpSA : sensitivity reduced by PPIs, h₂-receptor antagonists, antibiotics, and bismuth-containing compounds. It's easy to perform it and the age does not matter : possible alternative to UBT.

Discrepancies between UBT negative / HpSA positive at 1 month of post-treatment, both virtually became HpSA negative after the 3d month of follow-up. The first month following the treatment may be too early to detect diminished stool antigen.

Histology (endoscopy) : invasive test, time consuming and uncomfortable, it tests small areas of the stomach. It's indicated if endoscopic monitoring is necessary : gastric ulcer, MALT lymphoma, neoplastic or pre-neoplastic lesions.

Culture : its interest is to obtain the possibility to adapt the second- line treatment. In practice it is not commonly used except for studies and centers which are used to (5).

Serology is not approved to test for cure. The sensitivity and specificity are low. It should primary be used when UBT may have false negative results (e.g. current bleeding ulcer or Hp suppressing drugs).

Discrepancies between clinical practice and guidelines on the management of Hp : Many studies revealed that these guidelines have a little impact on clinical practice particularly with general practitioners. One study showed that uncertainty seems to persist regarding indications for Hp treatment, the use of diagnostic testing, and patient's follow-up (6). In others studies, there is a significant difference between the routine clinical practice and counselling enhanced treatment, and highlight the urgent need for structured patient counselling to be introduced into routine clinical practice.

Conclusion : The UBT and the HpSA represent the recommended tests in those situations where post-treatment testing is required. Serology is not useful in this situation as antibody levels commonly remain elevated for months to years after successful treatment. The histology is indicated if endoscopic monitoring is necessary: gastric ulcer, MALT lymphoma, neoplastic or pre-neoplastic lesions. The time to perform the test is at least 4 weeks, even if a period of 6-8 weeks seems to be better. In term of gaps observed between guidelines on the management of hp and clinical practice, more teaching programs and nonstop medical education activities for primary care physicians are necessary and should be given by gastroenterologists, public and private sector academic institutions (6).

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HOW IMPROVING THE COMPLIANCE FOR HELICOBACTER PYLORI TREATMENT ? V. Lamy. CHU, Charleroi, Belgium.

Introduction : Eradication of Helicobacter pylori (Hp) infection should be well known due to National and European Guidelines regularly updated.

Like several infections (Mycobacterium tuberculosis, HIV) the cure of Helicobacter pylori infection could be achieved with a combine complex therapy of PPI and some antibiotics.

Aim : To explain the numerous factors who contribute in achieving this eradication.

Methods : This is a real multifactorial process challenging both the physician and the patient. Between the complexity and the accessibility to the treatment, the patient information and the potential sides effects, the increasing resistance to antibiotics, the most important factor is the compliance with the therapy.

Results : Today some prefer to use the term of adherence to the treatment. The possible improvement to the compliance/adherence to achieve such a plan for eradication of Helicobacter pylori will be reviewed in details as a partnership between the physician and the patient.

Conclusion : Compliance with the therapy is the most important factor contributing today with the Helicobacter pylori eradication. This is much more than to swallow pills at regular intervals.

PROFILE OF BELGIAN PEDIATRIC CROHN DISEASE (CD) PATIENTS : PRESENTATION AND DIAGNOSTIC FEATURES. E. De Greef (1), I. Hoffman (2), F. Smets (3), S. Van Biervliet (4), M. Scaillon (5), B. Hauser (1), I. Paquot (6), P. Alliet (7), W. Arts (8), O. Dewit (3), H. Peeters (4), F. Baert (9), G. D Haens (10), J.F. Rahier (11), I. Etienne (12), O. Bauraind (13), A. Van Gossum (14), S. Vermeire (2), F. Fontaine (15), V. Muls (16), E. Louis (17), F. Van De mierop (18), J.C. Coche (13), J. Mahachie (19), K. Van Steen (19), G. Veereman (1). (1) UZ Brussel Vrije Universiteit, Brussels, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) UCL Saint-Luc, Brussels, Belgium ; (4) UZ, Gent, Belgium ; (5) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (6) CHC Clinique de l'Esperance, Liège, Belgium ; (7) Virga Jesse Hospital, Hasselt, Belgium ; (8) Ziekenhuis Oost-Limburg, Genk, Belgium ; (9) Heilig Hartziekenhuis, Roeselare, Belgium ; (10) Imeldaziekenhuis, Bonheiden, Belgium ; (11) UCL, Mont-Godinne, Belgium ; (12) CHC de la Citadelle, Liège, Belgium ; (13) Clinique St. Pierre, Ottignies, Belgium ; (14) ULB Erasme, Brussels, Belgium ; (15) St Joseph Hospital, Liège, Belgium ; (16) ULB Saint-Pierre, Brussels, Belgium ; (17) CHU Sart Tilman, Liège, Belgium ; (18) Sint Augustinus Ziekenhuis, Antwerpen, Belgium ; (19) Systems and Modeling Unit Montefiore Institute and Bioinformatics and Modeling GIGA-R University of Liège Liège Belgium, Liège, Belgium.

Aim : A Belgian Registry for pediatric Crohn's Disease (BELCRO) was created in order to estimate incidence and describe disease presentation and phenotype.

Methods : Previously and newly diagnosed CD patients under 18 yrs were recruited over a 2y period (2008-2010) from 22 Belgian pediatric and adult centers after obtaining informed consent. Demographic and descriptive data were recorded.

Results : Of the 256 included patients, 68 were newly diagnosed : estimated incidence $1.4/10^5$ children < 18 y/year, diagnosis by a pediatric gastroenterologist in 70% and in a university center in 52%. Subjects' characteristics : 55% boys, 95% Caucasian, median age at diagnosis 12.5 y (1.6-18). Neonatal data : median birth weight 3.3 kg (1.4-4.6), median gestational age 40 wks (28-42), caesarian section in 12%, breast feeding in 78% for a median duration of 7 wks (0-140). In the 3 mths prior to diagnosis, 23% had a course of antibiotics, 23% suffered an infectious episode and 23% experienced a major stressful event. Past surgery was present in 52% ; 1% were active, 16% passive smokers. Food allergy was present in 16% with 17% on diet restrictions. Median duration of symptoms before diagnosis was 3 m (1-

12). Patients presented with diarrhea (72%), abdominal pain (84%), weight loss (72%) and growth retardation (51%) (median z-score for height : -1.04 (-6.74 to 2.07). Furthermore, extra intestinal manifestations were present in 25%, perianal disease in 28%, concomitant conditions in 28% and family history was positive for AI disease in 36%, for CD in 22% and for UC in 5%.

Montreal classification for disease location and PCDAI :

| Disease location | L1 | L2 | L3 | L4 |
|------------------------|----|----|-----|-----|
| Total Cohort (n = 256) | 33 | 62 | 156 | 179 |
| Not evaluated | 12 | 0 | 0 | 5 |
| < 6y (n = 10) | 3 | 4 | 3 | 8 |
| 6-11 y (n = 91) | 7 | 24 | 58 | 66 |
| 12-18 y (n = 154) | 23 | 34 | 94 | 104 |

| PCDAI | < = 10 | > 10- < = 30 | > 30 | missing |
|------------------------|--------|--------------|------|---------|
| Total Cohort (n = 256) | 10 | 121 | 116 | 10 |
| < 6 y (n = 10) | 0 | 7 | 3 | 0 |
| 6-11 y (n = 91) | 2 | 42 | 46 | 1 |
| 12-18 y (n = 155) | 7 | 72 | 67 | 9 |

Conclusion : Pediatric CD patients present with extensive disease and frequent upper GI involvement. Medical history prior to diagnosis is unremarkable but family history is frequently positive for auto-immune disease or IBD.

- E06 -

IBD-ASSOCIATED ANAEMIA IN CHILDREN AT DIAGNOSIS AND AFTER 3 MONTHS. S. Van Biervliet, S. Vande Velde, M. Van Winckel. UZ, Gent, Belgium.

Introduction : Fatigue is a frequently encountered problem in children with inflammatory bowel disease (IBD). However, the subnormal haemoglobin (Hb) concentrations, which can affect quality of life, are often tolerated. A recent Dutch cohort study revealed a high incidence (78%) of anaemia at diagnosis and after induction therapy (80%) with expectant management (1).

Aim : This study evaluates anaemia in IBD children at our centre.

Methods : Medical histories of all IBD children diagnosed and followed at our centre between September 2005 and September 2010 were reviewed. Haemoglobin, MCV, transferrin saturation, ferritin, as well as PCDAI at diagnosis and after 3 months were recorded. Anaemia is defined as mild when Hb was < -2 SD for age and moderate-severe when < -4 SD. Iron deficiency anaemia (IDA) was diagnosed when 2 of following were present : mean corpuscular volume < 77 fL, transferrin saturation < 15% and ferritin < 30 µg/L, if not they were classified as anaemia of chronic disease (ACD).

Results : A total of 43 patients were diagnosed at our centre with a median age of 12.25 yrs (6-15.5) of which 23 were male (9 ulcerative colitis (6-13.6 yrs), 34 Crohn's disease (6-15.5 yrs). Using the paediatric disease activity scores 28 (65%) had moderate-severe and 15 (35%) had mild disease. Anaemia was present in 26 (60%) patients at diagnosis of whom 14 had moderate to severe anaemia. After induction therapy 16 (37%) patients had persistent anaemia of which 3 had moderate-severe anaemia.

Conclusion : Although anaemia is less frequent in our population compared to the Dutch study and seems to improve in the majority of patients, still 16 patients have persisting anaemia after 3 months of treatment. Depending on the type of anaemia, iron therapy as well as disease control should be looked at when anaemia is persisting.

PROFILE OF BELGIAN PEDIATRIC CROHN'S DISEASE (CD) PATIENTS : ASSOCIATIONS BETWEEN VARIABLES AT DIAGNOSIS. E. De Greef (1), I. Hoffman (2), F. Smets (3), S. Van Biervliet (4), M. Scaillon (5), B. Hauser (1), I. Paquot (6), P. Alliet (7), W. Arts (8), O. Dewit (3), H. Peeters (4), F. Baert (9), G. D Haens (10), J.F. Rahier (11), I. Etienne (12), O. Bauraind (13), A. Van Gossum (14), S. Vermeire (2), F. Fontaine (15), V. Muls (16), E. Louis (17), F. Van De mierop (18), J.C. Coche (13), J. Mahachie (19), K. Van Steen (19), G. Veereman (1). (1) UZ Brussel Vrije Universiteit, Brussels, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) UCL Saint-Luc, Brussels, Belgium ; (4) UZ, Gent, Belgium ; (5) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (6) CHC Clinique de l'Esperance, Liège, Belgium ; (7) Virga Jesse Hospital, Hasselt, Belgium ; (8) Ziekenhuis Oost-Limburg, Genk, Belgium ; (9) Heilig Hartziekenhuis, Roeselare, Belgium ; (10) Imeldaziekenhuis, Bonheiden, Belgium ; (11) UCL, Mont-Godinne, Belgium ; (12) CHC de la Citadelle, Liège, Belgium ; (13) Clinique St. Pierre, Ottignies, Belgium ; (14) ULB Erasme, Brussels, Belgium ; (15) St Joseph Hospital, Liège, Belgium ; (16) ULB Saint-Pierre, Brussels, Belgium ; (17) CHU Sart Tilman, Liège, Belgium ; (18) Sint Augustinus Ziekenhuis, Antwerpen, Belgium ; (19) Systems and Modeling Unit Montefiore Institute and Bioinformatics and Modeling GIGA-R University of Liège Liège Belgium, Liège, Belgium.

Aim : In addition to describing disease phenotype, the aim of the Belgian Registry for pediatric Crohn's Disease (BELCRO) was to determine associations between variables at diagnosis.

Methods : Previously and newly diagnosed CD patients under 18 yrs were recruited over a 2y period (May 2008- April 2010) from 22 Belgian pediatric and adult centers after obtaining informed consent as well as assents. Demographic and descriptive data were recorded. Non-parametric association tests were used to investigate relationships between variables of interest at diagnosis.

Results : Neonatal parameters of birth weight, gestational age and mode of delivery reveal no associations with age, disease location or disease severity at diagnosis. However, a positive association between duration of breastfeeding – age at diagnosis ($p = 0.0003$) and duration of breastfeeding – disease location (L2) ($p = 0.015$) is established. No correlations are found between medical history (antibiotic use, major stressful events 3 months prior to diagnosis, surgery) and disease onset, location or severity at diagnosis. Younger age at diagnosis is positively correlated with an infectious episode 3 months prior to diagnosis ($p = 0.013$) and with familial IBD ($p = 0.03$) but disease severity, disease location and age at diagnosis are not associated with a family history of other auto-immune diseases. Growth (z-scores for height) and PCDAI are associated ($p = 0.004$). PCDAI is significantly higher in ileal disease (L1) ($p = 0.013$) and ileocolonic disease (L3) ($p = 0,011$) while no association between disease location and age at diagnosis is present.

Conclusion : Data from the BELCRO indicate that the younger patients are more likely to have a positive familial history for IBD, to have been breastfed and to have experienced an infectious episode prior to diagnosis. Patients with higher PCDAI scores at diagnosis are more likely to present with ileal or ileo-colonic disease and growth delay.

MR ENTEROGRAPHY IN CHILDREN WITH CROHN'S DISEASE : RESULTS FROM THE FROM THE BELGIAN PEDIATRIC CROHN'S REGISTRY (BELCRO). P. Alliet (1), B. Hauser (2), E. Janssens (1), E. De Greef (2), F. Smets (3), I. Paquot (4), G. Veereman (2). (1) Virga Jesse Hospital, Hasselt, Belgium ; (2) UZ Brussel, Brussels, Belgium ; (3) UCL Saint-Luc, Brussels, Belgium ; (4) CHC Clinique de l'Esperance, Liège, Belgium.

Introduction : Magnetic Resonance enterography (MRE) is an interesting non-invasive imaging modality avoiding ionizing radiation and the discomfort associated with enteroclysis.

Aim : Results of MRE at diagnosis in the patients of the Belgian Pediatric Crohn's Registry (Belcro) are compared to endoscopic and histologic results.

Methods : Patients in whom MRE was done at diagnosis were selected. Reports of MRE, endoscopy and histology were retrospectively analysed. MRE abnormalities were assigned to one of the following segments : upper GI tract, jejunum, ileum, ascending colon, transverse colon, descending colon and rectosigmoid. All patients underwent upper and lower endoscopy with biopsies.

Results : 22/256 patients included in Belcro had MRE at diagnostic work-up. The results of endoscopy, histology and MRE were concordant (either all negative or all positive) in the rectosigmoid, descending colon, transverse colon and ascending colon in resp 9, 8, 8 and 8 of 22 patients. In the cases with negative MRE in the colon but positive endoscopy and/or histology, subtle endoscopic lesions such as erosions were described. Findings in the ileum were concordant in 16/17 patients and MRE describes the length of ileal involvement. In 5 cases the ileocaecal valve could not be intubated. In all those patients MRE findings were abnormal. MRE detected ileal stenosis with prestenotic dilatation in 4/22 patients. The jejunum was affected in 3/22 patients and fistula were described in 2/22 children.

Conclusion : In this cohort, MRE fails to detect subtle endoscopic and histologic colonic lesions. In contrast, good concordance was seen at the level of the ileum. MRE describes the extent of ileal involvement, the (non)occurrence of ileal stenosis and is of additional diagnostic use in case of failure of intubation of the ileocaecal valve. It also describes jejunal involvement and the occurrence of fistula, enabling a better classification according to the Montreal criteria. MRE was not widely used in Belcro at diagnosis but is now increasingly in the prospective part of the registry.

IN PEDIATRIC PATIENTS WITH CROHN'S DISEASE, DEPENDENCY OR LOSS OF RESPONSE ARE COMMONLY OBSERVED IN LONG TERM INFLIXIMAB TREATMENT. F. Smets, C. Wanty, X. Stephenne, E. Sokal. Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Introduction : Efficacy of infliximab (IFX) to induce and maintain remission in pediatric Crohn's disease is currently well documented. However, optimal strategy for the treatment beyond one year is not yet established, nor is the possibility to stop IFX.

Aim : The aim of our study was to describe the long term outcome of pediatric Crohn's disease patients with IFX therapy, and to evaluate the clinical response to the therapy and the effect on the growth.

Methods : A retrospective chart review was performed. Clinical response to the therapy, effect on the linear growth and long-term outcome were examined. These parameters were analyzed according to the age of the patients, duration and localization of the disease as well as various associated therapies.

Results : We identified 52 Crohn's disease children younger than 16 years at the time of diagnosis. Among those, 20 patients (38%) received a biologic therapy at a mean age of 13,9 +/- 2 years. Fifteen patients received IFX therapy and 13 (86%) were in clinical remission 10 weeks after the first infusion. Among the responders, 82% were always in remission after one-year therapy and 66% after 2 years. Among patients receiving IFX for more than one year, we observed IFX dependency in 89%. Thirty-eight% of patients with initial IFX response showed a loss of response after a median of 30 months (from 3 to 42 months). Increasing the doses or shortening the time between injections did not restore the efficacy. After 2 years of treatment, median Z score for height among patients with presumed growth potential is improved slightly from -0,7 to -0,55 SD. No serious adverse events were observed.

Conclusion : This retrospective mono-centric study shows high dependency (89%) and not exceptional loss of response (38%) to long term IFX treatment in pediatric patients with Crohn's disease. A slight beneficial effect on the growth was observed after 2 years of treatment.

Posters

- E10 -

ACHIEVING ANAL PSEUDO-CONTINENCE BY COLONIC IRRIGATIONS IN PATIENTS WITH SPINA BIFIDA.
S. Vande Velde, S. Van Biervliet, M. Van Winckel, H. Verhelst. UZ, Gent, Belgium.

Aim : Descriptive study of the results of colonic irrigations in the treatment of anal incontinence in spina bifida (SB) patients in a single institution.

Methods : All SB patients followed in UZG using colonic irrigations since at least 6 months in the treatment of anal incontinence, are studied prospectively by a standardised questionnaire. Age at start, type of irrigation, volume used, evacuation time, continence status, independency and pain during administration of the enema are recorded. Results are anonymized.

Results : 40 SB patients (25 children and 15 adults) on a total of 140 SB patients are included. Retrograde colon enema (RCE) (18/25) are more frequently used than antegrade colon enema (ACE) in children. Adults use more frequently ACE (11/15). Median volume administered is 1 L (range 0.5-2 L) in SB children and 1.5 L in adults (range 0.75-3 L). Almost all use plain lukewarm tap water. Median time spent on the toilet during one irrigation is 30 minutes (range 15-60 min) in children and 60 min in adults (range 30-120 min). SB children have 3.5 (range 3-7) enemas a week, adults 3 (range 2.3-4).

Faecal continence without soiling episodes is achieved in 19/25 of the children. Social continence, defined as soiling less than one episode a month is reached in 22/25. The faecal continence rate in adults is lower, 9/15 are completely continent and 10/15 is social continent.

One in five SB children is able to perform the enemas without help (ACE : 2/7, RCE :3/18) whereas about half of the adults are self reliant.

Pain during the enema administration is reported by half of the SB patients, irrespective of using RCE or ACE.

Conclusion : Although not always successful, colonic irrigations are a valuable treatment option in SB patients with faecal incontinence.

- E11 -

N-3 PUFA IN IN-HOSPITAL AND SCHOOL CATERING : FROM MENUS TO RECOMMENDATIONS.
O. Molendi-Coste, V. Legry, I. Leclercq. UCL Saint-Luc, Brussels, Belgium.

Introduction : The prevalence of obesity, metabolic syndrome and NAFLD, which are systemic inflammatory diseases, increased during the last decades contemporary to changes in human nutrition characterized by increased consumption of fat and of vegetable oils rich in saturated and n-6 polyunsaturated fatty acids (PUFA) together with decrease in n-3 PUFA-rich foods, resulting in a n-6/n-3 ratio of 10-20/1 in Western diet (for a ratio around 1/1 in the diet of our ancestors). As literature provides compelling evidence for the health benefits of n-3 PUFA consumption and low n-6/n-3 ratio on inflammation and metabolic syndrome prevention and treatment, recommendations for n-3 PUFA supplies in the general population were reviewed upwards.

Aim : The aim of our study was to evaluate fatty acids profile in collective catering nutrition in relation to those recommendations.

Methods : We obtained composition of lunches provided to children and adults by the Township of Lille (France), and of "standard", "low-fat" and "for diabetics" menus from the university hospital St Luc (Brussels, Belgium) catering service. The average proportions of fish, meat, oils, and dairy were used to estimate total, saturated, monounsaturated and polyunsaturated (n-6 and n-3) fatty acids contents using the official tables of foodstuffs composition from the "French Agency for Food Safety", the project "Nutritional Composition of Aquatic Products", the French Institute for Nutrition, and the USDA National Nutrient Database for Standard Reference. French guidelines were taken as reference for daily recommended intakes.

Results : n-3 PUFA content in lunches provided by municipal catering and in in-hospital menus were slightly below recommended intakes. In hospital menus, n-3 PUFA enriched margarine contributed for 50% to daily intakes. However, the n-3/n-6 ratio was excessive (13-8 in hospital and 20 in municipal catering) and related to excessive n-6 PUFA supply. Following this evaluation, the dietician staff considered replacement of safflower oil (rich in n-6 PUFA) by colza oil (rich in n-3 PUFA) in 50% of the dressings. Such implementation would fulfil maximum n-3 PUFA requirements.

Conclusion : We point out that meeting n-3 PUFA nutritional recommendations remains challenging for collective catering. A detailed analysis of provided menus represents a powerful tool to increase awareness and foster improvement in practice. A larger study performed in major cities in France is underway.

PROSPECTIVE EUROPEAN MULTI-CENTRE EPIDEMIOLOGIC STUDY ON RISK FACTORS OF GASTRIC AND DUODENAL ULCERS OR EROSIONS IN CHILDREN : A CASE-CONTROL STUDY. P. Bontems (1), N. Kalach (2), B. Iwanczak (3), T. Casswall (4), S. Koletzko (5), I. Oderda (6), M.J. Martinez-Gomez (7), P. Urruzuno (8), A. Kindermann (9), J. Sykora (10), G. Veres (11), E. Roma-Giannikou (12), E. Pehlivanoglu (13), F. Megraud (14), S. Cadranel (1). (1) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (2) Clinique de Pédiatrie St Antoine, Lille, France ; (3) Medical University of Wroclaw, Wroclaw, Poland ; (4) Karolinska University Hospital, Stockholm, Sweden ; (5) Dr von Haunersches Kinderspital of the Ludwig-Maximilians University, Munich, Germany ; (6) University of Piemonte Orientale, Novara, Italy ; (7) Hospital Universitario Nino Jesus, Madrid, Spain ; (8) Hospital 12 de Octubre, Madrid, Spain ; (9) Emma Children's Hospital at the Academic Medical Centre, Amsterdam, Netherlands ; (10) Charles University in Prague, Prague, Czech Republic ; (11) Semmelweis University, Budapest, Hungary ; (12) Athens University Medical School, Athens, Greece ; (13) Marama University, Istanbul, Turkey ; (14) University Hospital Pellegrin, Bordeaux, France.

Introduction : A previous short term uncontrolled study showed a frequency of 8.1% of ulcers and/or erosions in children, occurring mainly in the second decade of life. *H. pylori* infection (27%) and gastrototoxic medications (23%) were less frequently implicated than expected.

Aim : To analyse risk factors associated with gastric and duodenal ulcers and/or erosions in children referred for upper GI endoscopy in a case control study.

Methods : Open, Prospective, multi-centre study. Data were anonymously reported for patients presenting gastric or duodenal ulcers and/or erosions and 2 controls that immediately follow the index case, cross-matched for age groups (1-11 m, 1-5 y, 6-11 y, 12-17 y). The study was carried out during 24 months between January 2008 and Decembre 2009) in 12 centres among 11 European countries.

Results : 244 patients (52 with ulcer(s) alone, 39 with ulcers and erosions, 153 with erosions alone) and 488 controls were included in the study. Median age of patients vs controls was (11.2 y – range 1 m- 17.8y vs 11,1 y – range 2 m to 17.9 y). *H. pylori* infection was significantly higher in patients vs controls (63/244 (27%) vs 81/244 (17%) OR 1.9 – $p < 0.001$). In fact *H. pylori* infection was significantly associated with duodenal ulcer vs controls (20/40 – OR 5.0 – $p < 0.0001$) and with duodenal erosions vs controls (14/45 – OR 2.3 – $p = 0.02$), whereas there was no significant association between *H. pylori* and gastric lesions. Children with ulcers and/or erosions vs controls were significantly older than 10y (95/244 vs 149/244 – OR 2.5, $p < 0.0001$). Other significant risk factors were also detected : male (57.7% vs 49.6%, OR 1.4 - $p = 0.04$), hematemesis (10.7% vs 1.0%, OR 11.5, $p < 0.0001$), melena (9.8% vs 2.8%, OR 2.8, $p < 0.001$), use of non steroidal anti-inflammatory drugs (NSAIDs – 14.3% vs 9.6%, OR 1.6, $p = 0.05$), alcohol consumption (7.8% vs 4.3%, OR 1.9, $p = 0.05$) and tobacco use (5.7% vs 0.8%, OR 7.4, $p < 0.0001$). On the contrary Heartburn (11.1% vs 16.6%, OR 0.6, $p = 0.05$), chronic cough (0.8% vs 3.5%, OR 0.2, $p = 0.04$), lung disease (3.7% vs 7.2%, OR 0.5, $p = 0.05$), and celiac disease (2.5% vs 7.8%, OR 0.3, $p < 0.01$) were more frequent in controls than in patients. Other symptoms or chronic diseases, socio-economic and lifestyle factors, the use of steroids, immune-suppressive drugs, antibiotics, antacids, H2-blockers and PPIs children were equally distributed between patients and controls. No risk factors were observed in 141/244 (57.8%) patients.

Conclusion : This study confirmed that *H. pylori* infection, age (older than 10 y), male gender, hematemesis, melena, NSAID use, alcohol and tobacco use were independent risk factors of gastric or duodenal ulcers and/or erosions in children. However, *H. pylori* is not a risk factors for gastric ulcer/erosions these children and *H. pylori* infection is only found in half of patients with duodenal ulcer/erosions. This study confirmed then the unexpected high frequency of primary gastric or duodenal ulcers and/or erosions.

STABLE PHENOTYPE AND OCCURENCE OF ANEUPLOIDY WITHOUT TRANSFORMATION IN LONG-TERM CULTURE ADULT HUMAN DERIVED LIVER PROGENITOR CELLS. I. Scheers, C. Maerckx, M. Paganelli, M. Najimi, E. Sokal. Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Introduction : Liver cell transplantation is a promising treatment for human liver inborn errors of metabolism diseases. During large scale ex vivo cell expansion, cell characteristics may be altered and spontaneous transformation can occur.

Aim : In this study we investigate in vitro and in vivo phenotype and genotype stability of adult derived human liver progenitor cells (ADHLPC) after long-term culture.

Methods : ADHLPC were isolated from twelve different healthy adult cadaveric donors. We followed the growth, cell morphology and anchorage dependance at each passage. The phenotype stability was demonstrated by measuring cell cytoplasmic/surface markers expression using flow cytometry and immunofluorescence. Hepatic differentiation potential was assessed by measuring three key hepatic metabolic functions. Genotype stability was investigated up to senescence by performing karyotype, expression of telomere maintenance mechanisms and gene expression related to tumorigenesis. In vivo tumorigenicity was assessed after subcutaneous injection in Nude mice.

Results : Cell cultures showed a variable proliferative capacity. All cell populations maintain stable phenotype features during long-term expansion. ADHLPC could acquire mature hepatic metabolic functions up to cell senescence. The cells that grew fast (mean doubling time 6,35 days) were cytogenetically stable and reached senescence after a culture period of 142 ± 47 days. Four cell cultures demonstrated early growth slowdown (mean doubling time 28,6 days) correlated to premature senescence demonstrated by positive senescence associated beta-galactosidase staining. In those, random karyotype instability was detected at the sixth culture passage. Cytogenetic anomalies were different for all cell populations excluding a localised chromosomal fragility. The cells didn't express telomerase activity or alternative telomere lengthening mechanisms. Human telomerase reverse transcriptase expression was not detected. Function and/or expression of tumor related genes p53, p16, pRb and p21 were normal. We injected ADHLPC subcutaneously into Nude mice and did not observe local tumorigenesis.

Conclusion : ADHLPC can be expanded in vitro up to senescence while maintaining a stable phenotype and differentiation capacity. Some ADHLPC cultures enter early in a senescent phase and show cytogenetic instability. However, all ADHLPC cultures progressively display growth arrest without evidence of in vitro or in vivo transformation.

PHARMACOKINETICS AND TOLERABILITY OF RABEPRAZOLE IN CHILDREN WITH GASTROESOPHAGEAL REFLUX DISEASE : AN OPEN-LABEL, SINGLE- AND MULTIPLE-DOSE STUDY. E. Sokal (1), F. Smets (1), A. Vertruyen (2), G. Leitz (3), D. Doose (3), P. Zannikos (3), S. Rusch (4), M. Gonzalez (4), B. Solanki (3), A.E. Mulberg (3). (1) Cliniques Universitaires Saint-Luc, Brussels, Belgium ; (2) Sint-Vincentiusziekenhuis, Antwerp, Belgium ; (3) Johnson & Johnson Pharmaceutical Research and Development, Titusville, United States ; (4) Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium.

Introduction : Rabeprazole (RBZ) pharmacokinetics (PK) has been characterized in adults and adolescents previously.

Aim : The aim was to obtain same data in children.

Methods : This phase I, open-label study evaluated the PK and safety of RBZ after a single oral dose and daily administration for 5 days in children 1 to 11 years of age with gastroesophageal reflux disease (GERD). Subjective evaluations of GERD severity, RBZ effectiveness, palatability, and safety were also evaluated. In part I, 8 patients received RBZ 0.14 mg/kg ; in part II, 20 patients were randomized to receive 0.5 mg/kg or 1 mg/kg. PK parameters of RBZ and the thioether metabolite (formed via a non-P450-dependent pathway) were calculated using noncompartmental methods.

Results : RBZ concentrations increased in a dose-dependent manner, with little or no accumulation after once-daily administration. Plasma AUC values of RBZ and the metabolite were poorly correlated with individual age and body weight and oral RBZ clearance values (unadjusted for weight) were similar to historical adult data. These results imply RBZ dose-adjustment based on age or total body weight in this age group is not warranted. Weight-adjusted values were higher for the pediatric patients ; approximately 2- to 3-times the mg/kg dose of RBZ in these children was necessary to achieve comparable concentrations in adults. Improvement of GERD symptoms was observed in most patients. Palatability of the formulation was reported to be good or excellent. RBZ was well tolerated, with no notable differences in safety among the dose groups.

Conclusion : RBZ was safe and the PK parameters in children were comparable to those in adults. RBZ is currently being evaluated during an ongoing phase III study in children 1-11 years old with GERD.

IBD RESEARCH GROUP

Invited lecture

- I01 -

EPITHELIAL-MESENCHYMAL INTERACTIONS IN IBD, ARE THEY DRIVING INFLAMMATION ? G. Van Den brink. Academic Medical Center, Amsterdam, Netherlands.

Differentiated cells in rapidly renewing tissues such as epithelia of the gastrointestinal tract are in a dynamic equilibrium with precursor cells in order to balance the rate of proliferation with cell loss at the epithelial surface. The balance between input and output in homeostatic dynamic equilibria depends on the presence of negative feedback loops. In recent years we have learned much about the way Wnt signaling specifies the fate and proliferation of intestinal epithelial precursor cells. Much less is known about the mechanisms in place to control Wnt signaling. We found that Indian Hedgehog (Ihh) secreted by differentiated epithelial cells at the luminal surface of the intestine. Conditional activation of Hedgehog signaling results in loss of Wnt signaling with depletion of intestinal precursor cells which undergo premature differentiation to the enterocyte lineage. Conversely, conditional loss of Ihh resulted in increased Wnt signaling and accumulation of precursor cells with lengthening and multiplication of the intestinal crypts. Loss of Ihh not only resulted in epithelial changes that are characteristic of an intestinal wound healing response but additionally in the recruitment of macrophages and fibroblasts two other typical features of wound healing. Prolonged loss of Ihh resulted in progressive leukocyte infiltration of the crypt area, the development of mucosal damage and intestinal fibrosis. Our data show that Ihh is a negative feedback regulator of intestinal precursor cell fate and that its loss results in the activation of multiple aspects of a wound healing response which ultimately results in the development of chronic inflammation and fibrosis. Thus Ihh is a signal derived from the superficial epithelial cells that may act as a critical indicator of epithelial integrity.

- I02 -

THE HYPOXIA ADAPTIVE RESPONSE REGULATES METALLOTHIONEIN EXPRESSION IN INTESTINAL EPITHELIAL CELLS. L. Devisscher, P. Hindryckx, H. Peeters, M. De Vos, D. Laukens. UZ, Gent, Belgium.

Introduction : In active intestinal inflammation, particularly in the gut epithelium, the expression of metallothioneins (MTs), a family of acute phase proteins, is down-regulated. Hypoxia-inducible factor 1 \pm (HIF-1A) is up-regulated during intestinal inflammation-dependent hypoxia. The combined role of HIF-1A and MTs has been investigated in different inflammatory diseases and both proteins have independently been proposed in the pathogenesis of Inflammatory Bowel Disease (IBD).

Aim : In this study, we investigated the interdependent role of HIF1A and MTs in colonic epithelial cells.

Methods : Dimethylxalylglycine (DMOG) was used to subject colonocytes to hydroxylase inhibition and HIF1A stabilization in three experimental models (*in vitro*, *in vivo* and *ex vivo*). Small interfering RNA targeting HIF1A (SiRNA-HIF1A) and MT (SiRNA-MT) and zinc mediated MT induction was used in HT29 cells to study the interaction of HIF1A and MT. MT expression and HIF1A levels were measured using quantitative real-time PCR and ELISA respectively.

Results : Hydroxylase inhibition down-regulated MT expression in cultured HT29 cells, in freshly isolated human colonocytes and in colonocytes isolated from mice treated with DMOG. SiRNA-HIF1A treated cells displayed significant higher basal MT levels ($p < 0.05$) and an attenuated MT down-regulation after DMOG treatment. In turn, HIF1A stabilization was significantly lower in zinc treated control cells, displaying high levels of MT, compared to SiRNA-MT cells treated with DMOG ($p < 0.05$).

Conclusion : We, for the first time, demonstrated a HIF1A-mediated down-regulation of acute stress genes called metallothioneins in colonocytes. In turn, MTs were able to attenuate HIF1A stabilization. The observed reciprocity needs to be further explored for its role in intestinal inflammatory processes. Where HIF1A is over-expressed in IBD patients with protective properties in murine models of colitis, the low MT profile in IBD patients may point to a hypoxia-driven adaptive response in the course of gut inflammation.

MUCOSAL GENE EXPRESSION PROFILING DIFFERENTIATES EARLY FROM LATE ILEAL CROHN'S DISEASE. I. Arijis (1), L. Van Lommel (2), G. De Hertogh (3), K. Machiels (1), K. Lemaire (2), K. Van Steen (4), G. Van Assche (1), S. Vermeire (1), F. Schuit (2), P. Rutgeerts (1). (1) Department of Gastroenterology, University Hospital Leuven, KUL, Leuven, Belgium ; (2) KUL, Leuven, Belgium ; (3) University Hospital Gasthuisberg, Leuven, Belgium ; (4) Systems and Modeling Unit, Montefiore Institute and Bioinformatics and Modeling, GIGA-R, University of Liège, Liège, Belgium, Liège, Belgium.

Introduction : Early Crohn's disease (CD) is characterized clinically by an inflammatory pattern, whereas with increasing duration of the disease the majority of the patients develop complications including strictures or abscesses/fistulas. Moreover, early CD seems to respond better than late CD to immunosuppressive therapy and anti-TNF treatment.

Aim : In this study, ileal mucosal gene expression profiles of early and late ileal CD and controls were compared.

Methods : Ileal mucosal biopsies were obtained during ileo-colonoscopy from 8 patients with < 1 year diagnosis of CD (= new CD), 6 patients with recurrent CD within 1 year after ileo-colonic resection (= recurrent CD), 8 anti-TNF naïve CD patients refractory to standard treatment with > 5 year diagnosis of CD and/or with > 5 year ileo-colonic anastomosis (= late CD) and 9 control individuals. Total RNA isolated from biopsies was used to analyze gene expression via Affymetrix GeneChip® Human Gene 1.0ST arrays. Data was analyzed using Bioconductor software.

Results : Unsupervised hierarchical clustering of the top 50 probe sets with highest variation across 31 arrays showed 2 distinct clusters. Cluster I includes 7/8 late CD, 2/6 recurrence CD and 1/8 new CD patients. Cluster II comprises 2 sub-clusters : IIa which includes 7/9 controls and 1/8 new CD patients, and IIb which includes 6/8 new CD, 4/6 recurrence CD, 1/8 late CD patients and 2/9 controls. Pair-wise comparisons of ileal mucosal gene expression profiles were performed between controls, new CD, recurrent CD and late CD (significant probe sets/genes : false discovery rate < 5% and > 2-fold change). No significant gene expression differences were found between new and recurrent CD, and both disease groups are further named as early CD. As compared to controls, more significant gene expression differences were found in late CD (672 probe sets) than in early CD (25 probe sets). All significant dysregulations in early CD were also significantly dysregulated in late CD. The significant genes in early CD were mainly involved in innate immunity (e.g. C2, CFI, DUOX2 and LCN2) or related to epithelial barrier function (e.g. MUC1 and MUC4). The significant genes in late CD were predominantly involved in immune/inflammatory response and chemotaxis.

Conclusion : Our data show differences in mucosal gene expression profiles in early ileal CD and late ileal CD when compared to controls, with less dysregulation in early CD than in late CD. The differentially expressed genes in early CD remained abnormal in late CD and included genes related to innate immunity/barrier function. The other dysregulations in late (not in early) CD included a great number of genes involved in overall immune response. Our study gives more insight in the changes of immune processes during the disease course, and therefore may provide new therapeutic and prognostic targets for ileal CD.

FIBROPLASTS IN IBD, THE GOOD OR THE BAD. G. Rogler, Zurich.

2D-DIGE PROTEOMIC STUDY FOR INFlixIMAB RESPONSE PREDICTION IN INFLAMMATORY BOWEL DISEASES. M.A. Meuwis (1), S. Vermeire (2), M. Fillet (3), C. Reenaers (4), M. Malaise (5), J. Belaiche (4), P. Rutgeerts (6), M.P. Merville (3), E. Louis (4). (1) GIGA Proteomic Platform, ULg, Liège, Belgium ; (2) Department of Gastroenterology, University hospital Leuven, KUL, Leuven, Belgium ; (3) Medical chemistry, GIGA-R, ULg, Liège, Belgium ; (4) Gastroenterology, hepatology and digestive oncology, GIGA R, CHU Liège., Liège, Belgium ; (5) Rhumatology, GIGA R, CHU Liège, Liège, Belgium ; (6) University of Leuven, Leuven, Belgium.

Introduction : Despite infliximab (IFX) use for at least one decade in Inflammatory Bowel Disease (IBD) treatment, response prediction is still difficult.

Aim : To highlight potential serological biomarkers linked to IFX response in Ulcerative Colitis (UC) and Crohn's Disease (CD).

Methods : From a large cohort of IBD patients treated for the first time with infliximab, we selected one subgroup of patients with complete biological and clinical response (R) and one without any clinical or biological response (NR). Biological response was assessed by CRP and clinical response by CDAI in CD and Mayo Score in UC, 4 weeks after first IFX infusion in luminal CD and 10 weeks in fistulizing CD and UC. The sera from 48 patients were pooled in several groups according to the pathology (UC : 18, CD : 30) and to IFX response (50% R and 50% NR). These pools were pretreated using the Proteominer[®] kit (BioRad) and analyzed by 2D-DIGE (GE). Relevant comparisons including R vs NR in CD, UC and both IBD phenotypes were addressed. Protein spots found significantly differentially distributed were selected for identification after trypsin digestion using mass spectrometry (nanoHPLC coupled with an Ion trap Mass Spectrometer, Amazon, Bruker) and database Mascot Search (Matrix Sciences, vs 2.2.2).

Results : The serum proteins that could be indicators of IFX success in UC include a fragment of Thrombin, Apolipoproteins A1, E, A4 and Vitronectin (VTCN). While for CD patients, Vitamin D binding protein, Inter-alpha-trypsin inhibitor heavy chain H4, Alpha-1-antitrypsin and VTCN were highlighted. Their distributions were significantly different between R and NR. Intriguingly, VTCN appears inversely distributed in CD compared to UC.

Conclusion : Response to IFX in IBD appears to correlate with some systemic proteins partly different in UC and CD and present before treatment. These might be predictive biomarkers of IFX treatment response which should be further confirmed.

IL-13 DELAYS RECOVERY AFTER DEXTRAN SODIUM SULFATE INDUCED ACUTE COLITIS. C. Perrier (1), C. Breynaert (1), J. Ceuppens (1), P. Rutgeerts (2), G. Van Assche (2). (1) KU, Leuven, Belgium ; (2) UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Although IL-13 has been implicated only in ulcerative colitis inflammation so far, this cytokine might be implicated in fibrotic events of Crohn's disease patients, since IL-13 signaling has recently been associated with deposition of collagen and fibrosis in a chronic model of colitis.

Aim : To determine the influence of IL-13 deficiency in dextran sodium sulfate (DSS)-induced acute colitis and to evaluate the induction of the fibrosis during the recovery phase.

Methods : Groups of IL-13 knock-out (KO) and wild type (wt) mice (n = 25) were subjected to 2% DSS in drinking water for 7 days and were followed for 7 additional days during the recovery. Weight was measured every day, and colon tissue samples were harvested for histology. Martius scarlet blue staining was performed on colon cross sections to evaluate the deposition of collagen. Student t test was used to perform statistics.

Results : IL-13 KO mice were less affected by DSS-induced colitis than their wt littermates. The difference was most visible during the recovery phase, where their relative weight was less decreased than wt mice (mean weight, IL-13KO : 97.3 ± 9.1 , wt : 91.9 ± 6.8 p = 0.022). The macroscopic score of inflammation was also increased in IL-13KO mice (mean score : IL-13KO : 3.9 ± 1.7 , wt : 5.5 ± 1.6 p = 0.032). The colon length was not significantly different at day 9 in the two groups. Preliminary results of quantification of deposition of collagen suggest that IL-13 deficiency does not impair the initiation of fibrotic events in experimental DSS induced colitis, since both group of mice display the same amount of blue staining on colonic cross sections.

Conclusion : IL-13 delays recovery after DSS-induced acute colitis, but does not seem to be critical for the induction of fibrotic events.

T2 RELAXOMETRY DISTINGUISHES ACUTE MURINE DSS COLITIS FROM CHRONIC RELAPSING COLITIS. A NOVEL TOOL TO ASSESS TRANSMURAL FIBROTIC LESIONS ? C. Breynaert (1), T. Dresselaers (2), C. Perrier (1), S. Vermeire (3), P. Rutgeerts (3), J. Ceuppens (4), U. Himmelreich (2), G. Van Assche (1). (1) Department of gastroenterology/experimental immunology Catholic University Leuven, Leuven, Belgium ; (2) Biomedical NMR unit - MoSAIC Catholic University Leuven, Leuven, Belgium ; (3) Department of gastroenterology Catholic University Leuven, Leuven, Belgium ; (4) Department of experimental immunology Catholic University Leuven, Leuven, Belgium.

Introduction : In human Crohn's disease (CD), the value of MRI and CT enterography as a non invasive assessment tool of transmural inflammation and extraluminal complications is increasingly recognized. However, data on MR imaging as a tool to study murine colitis are very limited.

Aim : The aim of this study was to study whether micro(μ)MR imaging using in vivo T2 relaxometry is able to distinguish inflammatory and fibrotic lesions in DSS induced acute and chronic colitis.

Methods : DSS colitis was induced in 6 week-old C57BL/6/J mice. Three groups were compared : control mice (n = 6) had normal drinking water, acute colitis mice (n = 8) 2% DSS in the drinking water 7 days prior to scanning and chronic colitis mice (n = 12) 2 cycles of 7 days of DSS followed by 2 weeks of normal drinking water. Two mice per condition were scanned. The other mice were sacrificed for scoring and FACS analysis of blood and mesenteric lymph nodes (MLN). T2 weighted images and T2 maps of the distal colon were recorded on a 9.4T μ MRI system (Bruker). Regions of interest delineating the colon wall were identified on T2 weighted images. Histograms were created from T2 maps in 4 slices per animal. After scanning the distal colon was harvested for histology. Collagen deposition was quantified with Martius-Scarlet-Blue staining and measured using ImageJ in 4 cross-sections per mouse. Statistical analysis was performed using student t-test or ANOVA. This study was approved by the Institutional Animal Care Commission and Ethics Committee.

Results : The disease activity score normalized in the chronic colitis group compared to the acute colitis group. However, the macroscopic score of the colon (p = 0,035) and the colon weight (p = 0,004) were significantly higher in the chronic model compared to the acute model. CD4 + foxp3 + cells in peripheral blood decreased in the acute model but normalized in the chronic phase (p = 0.009). IL17 + cells in MLN were significantly higher in the chronic model

compared to the acute model ($p = 0.026$). IFN γ + cells in MLN had the same trend. The histograms of the colon T2 values showed a clear shift towards higher values for the acute colitis mice but lower values for the chronic colitis versus the control condition. Histology showed an increased collagen deposition and more \pm SMA + cells in the submucosa of the chronic versus the acute colitis mice, indicating more fibrosis and mesenchymal hyperplasia in chronic colitis compared to acute colitis and normal mice.

Conclusion : The immune profile of a chronic DSS model with induction of relapse and remission is clearly different from the acute DSS model. Moreover, these μ MRI data suggest that *in vivo* MRI T2 relaxometry could distinguish between inflammatory and fibrotic lesions in a murine model of IBD, and should be explored in patients with CD.

- I08 -

INTRARECTAL ADMINISTRATION OF OXYGENATED PERFLUORODECALIN PROMOTES HEALING OF MURINE COLITIS BY TARGETING INFLAMMATORY HYPOXIA. P. Hindryckx, L. Devisscher, D. Laukens, H. Peeters, M. De Vos. UZ, Gent, Belgium.

Introduction : Intestinal inflammation is associated with enhanced mucosal hypoxia, which contributes to the ongoing inflammatory process and hampers appropriate mucosal healing.

Aim : We questioned whether local treatment with an oxygen-carrying and -releasing molecule (oxygenated perfluorodecalin, O₂-PFD) could positively influence the course of experimental colitis.

Methods : The impact of intrarectal treatment with O₂-PFD was tested using the murine dextran sodium sulfate (DSS)-induced model of distal colitis, both in preventive and therapeutic settings. Colonic mucosal hypoxia was visualized by pimonidazole-staining. Colonic permeability was evaluated with FITC-dextran.

Results : In the preventive study, mice treated with O₂-PFD were protected against DSS colitis compared to saline-treated mice, as demonstrated by reduced shortening of colon length, reduced colonic TNF- α levels and a lower histological inflammation score ($P < 0.05$ for all parameters). In the therapeutic study, administration of O₂-PFD resulted in accelerated recovery of colitis compared to saline-treated littermates, and this was reflected by a better weight evolution, lower myeloperoxidase activity and a lower histological inflammation score ($P < 0.05$ for all parameters).

It was found that O₂-PFD established its therapeutic effects through (i) intrinsic anti-inflammatory effects of the PFD molecule and (ii) O₂-induced preservation and healing of the intestinal epithelial surface. Further *in vitro* and *in vivo* studies showed that the barrier-protective activity of O₂-PFD was obtained through prevention of colonocyte apoptosis and stimulation of colonocyte proliferation during inflammatory hypoxia.

Conclusion : These data show that intrarectal treatment with oxygenated PFD promotes colitis healing by the combined actions of direct anti-inflammatory effects and O₂-induced restitution of the epithelial barrier. As such, O₂-PFD enemas could be an attractive treatment option for patients with distal IBD.

FIBROSIS IN CROHN'S DISEASE, THE OVERLOOKED VILLAIN. C. Fiocchi. Cleveland Clinic, Cleveland, United States.

Introduction : Fibrosis is an almost universal consequence of tissue injury caused by a variety of insults, such as infection, trauma and cellular malfunction. Although fibrosis has the physiological purpose of promoting tissue repair, it may represent a serious pathological outcome depending on its location, intensity and duration. This scenario is very well exemplified by Crohn's disease (CD), where fibrosis is a common and potentially serious complication responsible for important clinical symptoms, significant morbidity and need for surgical intervention. The natural history of CD has shown that this condition evolves along inflammatory, fistulizing and stenosing pathways, each alone or in combination with the others. CD progresses over time, and the cumulative probability of remaining free of each behavior gradually diminishes, with fibrotic complications occurring in up to 80% of patients at the end of a two decade follow up period. This does not necessarily imply that all CD patients with intestinal fibrosis will become significantly impaired or will necessitate an operation, but a considerable number of them will display clinical manifestations related to intestinal fibrosis.

Aim : The aims include assessing the response of the intestine to inflammation, discussing inflammation-driven intestinal fibrosis, and proposing new research pathways in intestinal fibrosis.

Methods : Critical review of key issues relevant to fibrosis in CD based on currently available information.

Results : The mechanisms of fibrosis in CD are incompletely understood. The practical observation that inflammation precedes and eventually progresses to luminal stenosis makes the transmural inflammation typical of this condition the primary suspect responsible for the secondary fibrotic response. In addition to local fibroblast and muscle cell proliferation, it is becoming increasingly clear that intestinal fibrosis in CD (and likely in most form of tissue fibrosis) is induced by multiple cell types and processes. Under the influence of cytokines, chemokines and growth factors as well as products derived from the gut microbiota, local stellate cells, circulating fibrocytes and bone marrow-derived stem cells transform into activated myofibroblasts, the main cell type responsible for deposition of collagen and other extracellular matrix proteins ; under the same influences, epithelial and endothelial cells and pericytes can apparently transdifferentiate into activated myofibroblasts and contribute to the fibrotic response of CD. An intriguing question is whether the mesenteric adipose tissue, known to be expanded in CD and representing an additional source of proinflammatory cytokines and adipokines, may also contribute to intestinal fibrosis.

Conclusions : Thus, fibrosis in CD is a complex multifactorial process, and the same factors that condition the primary disease, e.g. environment, genes, gut flora and immunity, may also be responsible for the secondary fibrotic response. This obviously complicates the therapeutic perspectives for prevention or cure of this complication. In fact, during the last three decades, a period of time in which the management of intestinal inflammation in CD has witnessed major advances, surgical rates for fibrotic complications of CD have remained essentially unchanged, a painful demonstration of our ignorance on the pathophysiology of intestinal fibrosis and our inability to prevent or control this response. At present we have no means to eliminate or suppress the triggers of fibrosis ; we can suppress inflammation or reduce tissue damage, but this has limited beneficial effects on fibrosis ; finally, we still do not know how to inhibit myofibroblasts activation or prevent extracellular matrix deposition. The last but probably critical factor to be considered is the timing of intervention in the management of fibrosis in CD. Recent animal models show that early removal of inflammatory stimuli can reduce fibrosis, but once fibrosis has become established it continues to propagate even when the triggering inflammatory insult is eliminated.

SEROREACTIVITY TO ELONGATION FACTOR EF-TS AND TO TRPR BINDING PROTEIN FROM KLEBSIELLA PNEUMONIAE IN CROHN'S DISEASE AND ULCERATIVE COLITIS PATIENTS. K. Op De beéck (1), S. Vermeire (2), K. Claes (2), V. Ballet (2), R. Derua (1), E. Waelkens (1), P. Rutgeerts (2), X. Bossuyt (2). (1) KUL, Leuven, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Crohn's disease (CD) and ulcerative colitis (UC) are considered to originate from an aberrant immune response towards bacteria in the gut in genetically predisposed patients. Patients with inflammatory bowel disease (IBD) have been found to exhibit an enhanced immune response to various bacterial agents including *Klebsiella pneumoniae*. *Klebsiella* may be regarded as an opportunistic pathogen found in mammalian mucosal surfaces.

Aim : We aimed to identify the major target antigens of *K. pneumoniae* in IBD patients.

Methods : We separated a total protein extract of *K. pneumoniae* by 2D gel electrophoresis and performed Western blotting with sera obtained from well-characterized IBD patients and controls. Protein spots to which there was differential reactivity were excised and identified by MALDI-TOF/TOF. These proteins were then recombinantly produced using the Gateway technology (Invitrogen). ELISA was performed for evaluation of serum reactivity to these proteins.

Results : Western blotting revealed seroreactivity against several proteins of *K. pneumoniae* in CD patients and healthy controls (HC). Clear differences were observed between CD patients (n = 12) and HC (n = 13) for antibodies to Elongation Factor Ts (EF-Ts) (46.2% and 8.3% respectively) and Tryptophan Repressor (TrpR) binding protein (61.5% and 16.7% respectively). To consolidate these findings, 133 CD, 122 UC, 92 gastro-intestinal controls (GiCo) and 115 HC were screened for antibodies to EF-TS and TrpR binding protein by ELISA. Using a cutoff that corresponded to a specificity of 95% in HC, anti-EF-Ts antibodies were found in 30% of CD (p < 0.0001), 10% of UC (p = 0.27), and 16.3% of GiCo (p = 0.016). Moreover, the antibody titer in CD patients was significantly higher than in all other groups (CD vs UC : p < 0.0001 ; CD vs GiCo : p = 0.025). At a specificity of 95% in HC, anti-TrpR binding protein antibodies were found in 69.2% of CD, 60% of UC, and 66.3% of GiCo (p < 0.0001 for all groups). No significant differences were found between these groups. Increased antibody titers to both proteins were found in 39/133 CD (29.3%), in 11/122 UC (9%), in 14/92 GiCo (15.2%) and in 2/115 HC (1.7%).

Conclusion : We observed increased seroreactivity to *Klebsiella*, namely to EF-Ts and TrpR binding protein in CD and, to a lesser extent, in UC. EF-Ts has a function in chain elongation during polypeptide synthesis at the ribosome. TrpR binding protein interacts selectively and non-covalently with flavin mononucleotide, the coenzyme or the prosthetic group of various flavoprotein oxidoreductase enzymes. Both proteins are also present in other bacteria (e.g. *Lactobacillus*, *Escherichia coli*, *Yersinia*, *Shigella* and *Salmonella*). Antibodies to these proteins may rise as a consequence of an increased immune response caused by the invasion of bacteria in the bowel mucosa due to a defective mucosal barrier.

TOWARDS IN VIVO CHARACTERIZATION OF THE TRANSCRIPTIONAL EFFECTS OF GENETIC RISK VARIANTS FOR CROHN'S AND OTHER INFLAMMATORY DISEASES. V. Deffontaine (1), B. Charlotiaux (1), C. Reenaers (1), P. Gast (2), C. Van Kemseke (2), P. Leclercq (2), M. Mni (1), F. Crins (1), Y. Momozawa (1), E. Théâtre (1), E. Louis (2), M. Georges (1). (1) University of Liège, Liège, Belgium ; (2) CHU de Liège, Liège, Belgium.

Introduction : Genome wide association studies have led to the identification of tens of loci associated with predisposition to common complex inflammatory diseases. However, for the vast majority of these, neither the causative genes and mutations, nor the functional consequences are known. Expression quantitative trait loci (eQTL) studies may reveal transcriptional effects of risk variants thereby incriminating the regulated genes in disease pathogenesis. However, and so far, human eQTL information has primarily been restricted to lymphoblastoid cell lines thereby limiting their scope. **Aim** : The aim of this study is to generate genome-wide eQTL data for 9 human primary tissues that are of direct relevance to inflammatory diseases including Crohn's.

Methods : We collected intestinal biopsies and venous blood from > 200 healthy Caucasians. Leukocytes were fractionated in 5 cell populations by positive antibody-mediated selection. RNA was extracted from all cell types using standard procedures. Each individual was genotyped for > 700 000 SNPs and genome-wide transcriptome analysis is being conducted for all tissues interrogating > 47 000 transcriptional units. After extensive QC of both genotype and transcriptome data, eQTL analysis will be conducted using standard procedures : gene-specific expression levels will be analyzed using a mixed model including fixed effect (*e.g.* sex, age) and random locus-specific (either SNP or haplotype effect), polygenic and residual effects.

Results : By the end of 2010, (i) tissue samples have been collected and processed for > 200 individuals, (ii) 200 individuals have been genotyped with the 700K SNP array, and (iii) transcriptome analysis have been conducted for 4/9 tissues, (iv) data QC and eQTL analysis have been initiated. Latest results will be presented.

Conclusion : Our study will identify eQTL effects for variants that are associated with predisposition to Crohn's and/or other inflammatory diseases, thereby yielding novel insights in pathogenesis.

CHARACTERIZATION OF BACTERIA IN BIOPSIES OF COLON AND STOOLS BY HIGH THROUGHPUT SEQUENCING OF THE V2 REGION OF BACTERIAL 16S RRNA GENE IN HUMAN. Y. Momozawa (1), V. Deffontaine (1), E. Louis (2), J. Medrano (3), M. Georges (1). (1) University of Liège, Liège, Belgium ; (2) CHU de Liège, Liège, Belgium ; (3) University of California-Davis, Davis, United States.

Introduction : The characterization of the human intestinal microflora and their interactions with the host have been identified as key components in the study of intestinal disorders such as inflammatory bowel disease. High-throughput sequencing has enabled deep, culture-independent analysis of bacteria in the gut.

Aim : This study examined (1) the number of reads needed to ascertain differences between samples, (2) the effect of DNA extraction procedures and PCR reproducibility, and (3) differences between biopsies and stools in order to design a large scale systematic analysis of gut microbes.

Methods : The microflora obtained from seven anatomic regions of the human colon and from two types of stool specimens was analyzed by pyrosequencing the V2 region of the bacterial 16S ribosomal RNA (rRNA) gene on a 454 Roche instrument. Reads were assigned to specific operational taxonomic units using BLAST. We applied both weighted and unweighted UniFrac distances to estimate difference between sample sources.

Results : It was shown (1) that sequence coverage lower than 1,000 reads influenced quantitative and qualitative differences between samples measured by UniFrac distances. Distances between samples became stable after 1,000 reads. (2) Differences between individuals were much higher than any other differences caused by experimental design. (3) Quantitative and qualitative difference in bacterial composition from ileum to rectum were not observed, but there was a significant positive trend between distances within colon and quantitative differences. Between sample type, biopsies or stools, quantitative and qualitative differences were observed.

Conclusion : This experimental design can stably grasp individual differences but stool samples are not good representatives for the human intestinal microflora.

ALTERED COLONIC METABOLITE PROFILE IN ACTIVE VERSUS QUIESCENT ULCERATIVE COLITIS. V. De Preter, G. Vandermeulen, K. Windey, S. Vermeire, P. Rutgeerts, K. Verbeke. UZ Leuven Department of Gastroenterology, Leuven, Belgium.

Introduction : Ulcerative colitis (UC) is a chronic relapsing disorder characterized by inflammation of the large intestine. Although the aetiology is incompletely understood, the intestinal microbiota is generally accepted to play a key role. At present, little is known about the metabolic activity of the microbiota of UC patients and whether metabolites could be related to the pathogenesis of the disease.

Aim : The aim of this study was to characterize the faecal volatile compounds (VOC) fingerprint of UC patients according to disease activity.

Methods : Faecal samples were obtained from 11 patients with mild/active UC (endoscopic Mayo score 2 and 3) and 11 patients with quiescent UC (endoscopic Mayo score 0 and 1). A purge-and-trap sample preparation system, coupled on line to a GC-MS (time-of-flight) was applied to analyse the VOC. AMDIS software was applied to extract purified mass spectra from overlapping components. Partial least square analysis was used to compare the metabolic profiles.

Results : A total of 127 different VOCs were identified in the faecal samples with an average of 50 ± 7 per person. Sixteen VOCs were found in all analyzed samples and twenty-eight were subject specific. VOC fingerprints clearly clustered in 2 groups, discriminating between active and quiescent UC. Samples from patients with active disease were characterized by higher levels of S-containing compounds such as carbon disulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, as compared to samples from patients with quiescent disease. Relative concentrations of other VOCs did not contribute to the clustering.

Conclusion : We report a detailed analysis of the pattern of fermentation metabolites in UC. S-containing compounds were found as discriminating metabolites between active and quiescent UC. Previously, increased levels of especially hydrogen sulphide and higher viable counts of sulphate-reducing bacteria have been demonstrated in faeces of UC patients.

NUTRITION GROUP

Invited lecture

- N01 -

UPDATE ON NUTRITION DAY 2010. J.C. Preiser

- N02 -

MALNUTRITION ON ADMISSION TO HOSPITAL IN ELDERLY BEFORE SURGERY AND STAFF ATTITUDES IN NUTRITIONAL NURSING CARE. B. Geurden (1), A. Bécuwe (2), B. Meere (3), D. Ysebaert (3). (1) Antwerp University, Antwerp, Belgium ; (2) Sint Augustinus Ziekenhuis, Antwerpen, Belgium ; (3) Antwerp University Hospital, Antwerp, Belgium.

Introduction : Malnutrition on admission to hospital is not a new problem but it is often underestimated. Malnutrition is a risk factor in patients undergoing surgery and the degree of preoperative malnutrition is predictive of postoperative morbidity and mortality.

Aim : The aim of this study is to determine the prevalence of malnutrition in free living elderly on admission to hospital for surgery. At the same time we determined attitudes in nutritional care by nurses and nurse aids responsible for these surgical patients.

Methods : In a cross sectional multi centre study we used the NRS2002 as recommended by ESPEN to determine the prevalence of malnutrition in free living elderly (65 y and over) who required surgery with routine hospitalization for at least 3 days. Malnutrition was defined as NRS-score 3 or more. At the same time, using the SANN-scale, we measured attitudes in nutritional care in the nurses and nurse aids responsible for these patients hospitalized in 8 surgical wards in 3 hospitals.

Results : 208 patients were included and 51,5% had a NRS-score of 3 or more at admission and before surgery. In the malnourished group (n = 107) there were more men (56,1%) and the mean age (76,8 y SD +/- 5,8 Range 65-90) was higher (p < 0.001) than in the well nourished group (48,5% men ; 72,7 y SD +/- 6,9 Range 65-90). When NRS-score 3 or more, the mean BMI was 24,8 (SD +/- 4,5 Range 14,9-38,5) and if NRS-score < 3 the mean BMI was 26,0 (SD +/- 3,9 Range 18,8-38,2) (p = 0,031). Most common surgical procedures were cardio vascular surgery (29%) and gastrointestinal surgery (35,5%). According to the SANN only 7,5% of the nurses and nurse aids (n = 200) responsible for these surgical patients had a positive attitude, 73% a moderate and 19,5% a bad attitude in nutritional nursing care.

Conclusion : According to the NRS2002 51,5% of the free living elderly (65 y and over) are malnourished on admission to hospital and before surgery. A majority (73%) of the nurses and nurse aids responsible for these patients showed a moderate attitude and only 7,5% showed a positive attitude in nutritional nursing care. The high prevalence of pre-operative malnutrition on admission in elderly and its consequences are urging for adjustments in attitudes of nurses and nurse aids in nutritional care.

NUTRITIONAL RISK SCREENING (NRS 2002) IN HOSPITALIZED PATIENTS : FOCUS ON FOOD INTAKE DECREASE. M. Arvanitakis, S. Vereecken, A. Lintermans, M. Raedemaeker, A. Coudray, A. Van Gossum. ULB Erasme, Brussels, Belgium.

Introduction : Screening tools such as the Nutritional Risk Screening (NRS 2002) are recommended for screening hospitalized patients.

Aim : The aim of this study was to assess the ability of the NRS 2002 in predicting outcome, as well as to focus on the group of patients presenting with decreased food intake.

Methods : Patients admitted in the Gastroenterology Medico-surgical department between 1/11/2009 and 24/12/2009 (7 weeks) were prospectively screened with the NRS 2002. Patient presenting with food intake decrease filled up an additional questionnaire focusing on dietary habits, lifestyle and socio-economic background.

Results : 137 patients were included. The majority (100/137, 73%) were admitted on a non-emergency basis and 78.1% (107/137) were less than 70 years old. The NRS 2002 was positive in 102 patients (74.5%) and 61/102 had a total score of ≥ 3 . A positive NRS 2002 was associated with a longer length of stay (8.6 vs 4.8 days, $p = 0.02$) and decreased autonomy after discharge. A decrease of food intake was observed in 63 (46%) patients and 59 filled in the additional questionnaire. 16/59 (27.1%) were ≥ 70 years old and 22/59 (37.3%) lived alone. The majority (38/59, 64.4%) prepared their meals themselves. The most common cause of decreased food intake was loss of appetite (43/59, 72.9%), followed by swallowing difficulties (9/59, 15.2%) and dental problems (4/59, 6.7%). Most patients experienced fatigue (49/59, 83.1%), as well as difficulty coping with everyday life (38/59, 64.4%). In this subgroup of patients presenting with decrease food intake, older age (≥ 70 years) appears to be associated with the fact of living (62.5% vs 27.9%, $p = 0.017$) and eating alone (75% vs 30.2%, $p = 0.003$).

Conclusion : The majority of hospitalized patients are at risk for undernutrition as detected by the NRS 2002. Furthermore, in patients with decreased food intake, other socio-psychological factors might play in role in appetite loss, especially in older patients.

IN PATIENTS ON LONG-TERM HOME PARENTERAL NUTRITION (HPN), TRANSIENT ELASTOGRAPHY (FIBROSCAN®) CORRELATES WITH CHOLESTASIS BUT NOT WITH LIVER FIBROSIS. A. Van Gossum (1), L. Pironi (2), C. Moreno (1), P. Demetter (1), F. Joly (3). (1) ULB Erasme, Brussels, Belgium ; (2) University, Bologna, Italy ; (3) INSERM Bichat-Beaujon, Paris, France.

Introduction : Impending or overt HPN-associated liver failure is an indication for a life-saving liver and/or small bowel transplantation in patients with chronic intestinal failure. The decision about the timing and the type of transplantation depends on the degree of liver fibrosis, whose evaluation requires liver histology. The non-invasive transient elastography (Fibroscan) is likely to assess the degree of hepatic fibrosis in various liver disorders. The goal of this study was to assess whether Fibroscan® is correlated to the histologic score of liver fibrosis in HPN patients.

Methods : A multicenter prospective study was carried out, enrolling adult patients with benign intestinal failure who were on HPN for at least 3 months and who were receiving at least 3 nutritional bags/week. Inclusion criteria were : severe alteration of hepatic enzymes (mainly cholestasis) and/or candidate for transplantation.

The following were evaluated in each patient : characteristic of HPN, underlying disease, gut anatomy and presence of a stoma ; clinical assessment ; biochemical work-up ; liver biopsy ; Fibroscan® assessment (score of liver stiffness). Liver histologic fibrosis was scored according the Brunt classification (grade : 0 to 2 ; stage : 0 to 4). Histologic liver cholestasis was graded from 0 to 3.

Results : Thirty patients were enrolled (11 females / 19 males, mean age : 42 y.).

Reasons for liver biopsy were severe altered hepatic enzymes ($n = 17$) or candidate for transplantation ($n = 13$). The mean duration of HPN was 75 months (3 to 348). The number of bags was 7/week in 24 patients. Fourteen had a stoma. Liver histology showed severe fibrosis (Brunt stage > 2) in 18 patients (60%) and severe cholestasis (grade 2-3) in 9 patients (30%). Abnormal Fibroscan score was observed in 21 patients (70%).

The Fibroscan® score was positively correlated with the histologic grade of cholestasis ($r = 0.684$; $p = 0.0001$) as well with the presence of a stoma ($r = 0.484$; $p = 0.007$). No correlation was found between the Fibroscan® score and the Brunt score.

Conclusion : In adult patients on HPN for intestinal failure, with impending or overt HPN-associated liver failure, Fibroscan® assessment reflects the histologic degree of liver cholestasis rather than the degree of liver fibrosis.

NUTRITIONAL COMPLICATIONS OF BARIATRIC SURGERY – A CASE APPROACH. J.P. Thissen, Cliniques St Luc, Brussels.

HOW TO APPLY NUTRITION GUIDELINES AT THE BEDSIDE ? V. Fraipont. CHR Citadelle, Liège, Belgium.

Introduction : Guidelines are usually considered by caregivers as a set of recommendations for patient care based on the best clinical available data. International American and European guidelines (respectively from the SCCM/ASPEN society and the ESPEN society) were recently released. Evidence-Based clinical practice guidelines were also regularly updated (<http://www.criticalcarenutrition.com>).

Aim : Barriers and enablers of the implementation of the guidelines at the bedside are discussed. A proposal of a didactic tool is made by the SBNC (Belgian French-speaking Society of Clinical Nutrition).

Methods : Literature research and working group.

Results : Large multicenter cluster studies demonstrate that when guidelines are actively disseminated in ICUs, the patients are fed earlier and receive more kilocalories from enteral nutrition. Yet factors such as the heterogeneity of patients, previous experience of the caregivers in nutritional aspects or the difficulty to reach the caloric goal by the enteral route may partly account for individual differences in the clinical outcomes, regardless of the use of guidelines. Others studies suggest wide variations in the application of the guidelines, regarding the use of motility agents, small bowel feeding or pharmaconutrition. Some barriers to the implementation of the guidelines relate to the guidelines themselves (readiness, availability, complexity...) while others barriers are more user-related (resistance to change, process...). The clinical condition of the patient is also a challenge for the provision of enteral nutrition. As these guidelines mostly refer to evidence-based medicine, some areas of uncertainty are left and important issues remain therefore unresolved. According to behavioural psychology, a change of practice should be achieved by restructuring information and rephrasing choices provided in a clinical pathway. A modification of heuristics (intuitive procedures) is much more difficult to achieve and implies some practice task-specific process and audit-feedback mechanisms. Protocols, bedside reminders, one-to-one discussions, benchmarking, feedback and the input of local opinion leaders in a multifaceted approach, could improve the use of the guidelines. It is necessary to analyse local situations if to identify gaps, assess barriers and propose tailored interventions.

Conclusion : A group of multiprofessional caregivers involved in the nutrition field, propose a text that follows a clinical step-by-step process, on behalf of the SBNC. In accordance with the current literature and adapted to the Belgian market, it provides some suggestions for issues not discussed in the guidelines and could serve as a starting point for a local implementation.

ENTERAL NUTRITION SHOULD BE USED TO INDUCE REMISSION IN CHILDHOOD CROHN'S DISEASE. S. Van Biervliet, S. Vande Velde, M. Van Winckel. UZ, Gent, Belgium.

Introduction : Exclusive enteral nutrition (EEN) has been proven as efficient as steroids to induce remission in children with acute Crohn's disease. Despite the reported efficacy, the lack of side effects and the advantages on growth, EEN is still not widely taken up as first-line therapy.

Aim : At our centre the policy of leaving the patient a choice and using a nasogastric tube only after initial oral intake failure was changed into starting with a nasogastric tube (placed during anaesthesia for gastroduodeno- and colonoscopy) with removal of the tube when patients were able to drink at least 2,5 litre Modulen[®], 3 days in a row.

Results : 3 boys aged (12, 13 & 15 years) were started recently on this treatment after the diagnosis of terminal ileum Crohn's disease. Symptoms were present during 4, 6 and 8 months respectively before diagnosis. Only one had diarrhoea. They complained of abdominal pain, fatigue, weight loss (3, 3 and 6 kg) and episodes of fever. Their BMI z-score was -3.2, -2.8 and -2.7 and length z-score was -2.6, -2.2 and -0.8. Their bone age was 10, 12 and 15 years. They had anaemia (Hb 10.4, 9.4, 10 mg/dL) and increased inflammatory parameters (CRP 1.4, 5.6 & 13 mg/dL). The youngest also displayed hypoalbuminaemia. The paediatric Crohn's disease activity index (PCDAI) was 62.5, 60 and 47.5, indicating moderate to severe disease in all 3. Tube feeding was gradually increased starting with 2 litre continuous drip. As they wanted they could drink extra Modulen[®]. After 4 days they gained 700 g, felt better and inflammation decreased (CRP 0.3, 1.7, 3.9 mg/dL). The tube was removed after 7 days. After 2 weeks of EEN therapy a weight-gain of 6.7, 3.5 and 4.3 kg was observed, anaemia improved and inflammation disappeared. They drank 2.5-3.5 litres/day. Azathioprin was proposed as maintenance therapy. Two months after diagnosis there was a PCDAI of 0, their weight gain was 6.7, 6.9 and 10 kg respectively without inflammation.

Conclusion : If well explained nutritional therapy is acceptable for adolescents with Crohn's disease. It is, however, mandatory to use the tube to improve the clinical symptoms before expecting the patient to be able to drink. The big advantage of this therapy is the fast reversal of growth stagnation at this critical age for growth.

TOLERANCE AND COMPLIANCE WITH PREOPERATIVE ORAL IMMUNONUTRITION WITH ORAL IMPACT[®] BEFORE MAJOR ABDOMINAL SURGERY. M. Robert, C. Malherbe, N. Perin, A.M. Verbrugge, J. Joris. CHU de Liège, Liège, Belgium.

Introduction : Oral Impact[®] from Nestlé Nutrition available in Belgium since March 2009 is a nutrition enriched in immunonutrients : omega-3 free fatty acid, arginine, and nucleotides. This immunonutrition is recommended by the ESPEN and SFGCD before cancer surgery of the gastrointestinal tract. It reduces the risk of postoperative infectious complications and the duration of hospitalisation in malnourished as well in non-malnourished patients. Patient tolerance and compliance with this immunonutrition have however never been studied.

Aim : We therefore investigated the tolerance of oral Impact[®] in 57 consecutive patients before major surgery of the GI tract using a questionnaire.

Methods : Fifty-seven patients scheduled for major abdominal surgery were included in this study. Three bags of oral Impact[®] per day (900 Kcal) were prescribed to these patients, malnourished or not, for the 7 days preceding surgical procedure. This treatment costs the patient 80 €. Patients were given the same preoperative information concerning how to use and prepare it as well as potential benefits of the immunonutrition by an anesthesiologist and a member of the nutritional team of our institution at the time of the pre-anesthetic visit. During the hospitalisation, patients were asked to fill a questionnaire concerning tolerance and side effects during the preoperative preparation with oral Impact[®].

Results : Patients (M/F : 25/32) aged 63 ± 14 yo had a BMI of $22,7 \pm 4,6$ kg/m². Malnutrition (following ESPEN criteria) was detected in 58%. 67% underwent colorectal surgery. 75% ingested all the prescribed doses and 86% at least 15 bags. Three patients tolerated only 2 bags : 2 for mechanical problems (likely subocclusion) and one for taste dislike. Four patients reported nausea, only one described unpleasant fish taste. With regards to nutrition tolerance, patients reported : difficulties to take 3 bags every day (28%), a reduction in usual appetite (19%), postprandial epigastric discomfort (16%), disturbance of intestinal transit (7%). Coffee taste was preferred by 70% of the patients. 88% of the patients found easy the preparation of the mixture.

Conclusion : To conclude, we consider that patients tolerance of oral Impact[®] and their compliance with the prescription are very good, particularly since this nutrition is proposed to cancer patients frequently anorexic secondary to their cancer or their chemo- or/and radiotherapy. Beside qualities of this nutritional preparation the good compliance may be partly explained by the expected major benefits of the immunonutrition before surgical procedures potentially leading to severe complications and by the fact patients have to pay.

THE USE OF NASOJEJUNAL FEEDING TUBES AS AN ALTERNATIVE FOR PARENTERAL NUTRITION, EXPERIENCES IN A REGIONAL HOSPITAL. N. Hiltrop, N. Bailleul, G. Nackaerts, M. Cool, G. Deboever, G. Lambrecht. AZ Damiaan, Oostende, Belgium.

Introduction : Postpyloric feeding is an important and promising alternative to parenteral nutrition. Indications for this kind of feeding are increasing and include a variety of clinical conditions, such as gastroparesis, acute pancreatitis, gastric outlet stenosis, hyperemesis (including gravida), recurrent aspiration, tracheoesophageal fistula and stenosis in gastroenterostomy.

Aim : To prospectively evaluate the success rate of endoscopic guided nasojejunal feeding tube placement, the complications and efficacy and the completion of nutritional goals with the nasojejunal feeding tube and nutritional guidelines used in our hospital.

Methods : During an 8-month period, 51 patients eligible for nasojejunal feeding were included in the registry. Informed consent was obtained from the patient or his family. An endoscopic guided nasojejunal feeding tube (Easy-Inn, Fresenius) was placed by an experienced gastroenterologist and insertion time was recorded. Nursing staff was trained to adjust feeding volumes according to nutritional protocols available in the hospital. A different protocol was used for conscious and unconscious patients. Complications of the feeding tube and completion of nutritional goals was evaluated daily by intensive care physicians, gastroenterologists and the nutrition support team including a nurse and a dietician. After (accidental) dislodgment of the tube, it was repositioned when evaluated as being useful. We evaluated for success of tube placement, rate of (accidental) dislodgment and replacement, completion of nutritional goals, feeding tube related complications and new endoscopically diagnosed pathology during tube placement.

Results : Mean time for tube placement was 8,25 minutes. In 25 out of 51 patients (49%), there was (accidental) tube dislodgment causing repositioning of the tube. In 6 patients, the tube was repositioned several times. Nutritional objectives were not complete in 10 patients (19%) and in only 24 patients (47%), these goals were reached within the first three days. In 27 patients (53%), the endoscopy guided tube placement revealed previously unknown pathology.

Conclusion : Nasojejunal feeding tubes are an important and promising alternative to parenteral nutrition. Placement of a nasojejunal feeding tube by an experienced physician generally does not take more than ten minutes. The rate of tube dislodgment and replacement was higher than expected. The use of endoscopy during tube placement might reveal unknown relevant pathology and allows immediate control of tube positioning. Nursing staff and on duty physicians are unfamiliar with specific handling of the tube causing unnecessary feeding tube complications. Specific training and the use of a nutrition support team can be useful to optimise results of nasojejunal feeding in a regional hospital setting.

DIRECT PERCUTANEOUS ENDOSCOPIC JEJUNOSTOMY : A RETROSPECTIVE ANALYSIS OF INDICATIONS, SUCCESS AND OUTCOME. M. Arvanitakis, A. Ballarin, O. Le Moine, A. Van Gossum. ULB Erasme, Brussels, Belgium.

Introduction : Percutaneous access to the jejunum can provide enteral feeding if the gastric route is not possible.

Aim : The aim of this study was to evaluate indications, success rate, short- and long-term complications and outcome in patients in whom a percutaneous endoscopic jejunostomy (PEJ) was performed.

Methods : Clinical data concerning patients in whom a PEJ was scheduled between 1/2006 and 1/2010 were retrospectively collected and analyzed.

Results : Twenty-two patients were included. In 4 patients, the procedure was unsuccessful, due to lack of transillumination ; therefore the success rate was 81%. In 3 of these patients, a percutaneous endoscopic gastrostomy with a jejunal extension was placed and one patient underwent surgery. The remaining 18 patients (10 men, 8 women) who underwent successful PEJ placement had a median age of 57 years (22-79) and a body mass index of 18,5 (13-33). Regarding underlying disease, 5 patients (27.8%) had previous lung transplantation and 7 (38.9%) had gastrectomy. Indications for jejunal access were as follows : gastroparesis (n = 5, 27.8%), gastrectomy (n = 7, 38.9%) and severe reflux (n = 6, 33.3%). Eight patients had previous nasogastric (n = 2) or nasojejunal (n = 6) tube. An enteroscope was used in 11 patients, whereas a gastroscope was used in 6 and a pediatric colonoscope in 1 patient. Eighteen french tubes with an internal bumper were used in all patients. No patient presented with short-term complications. Six patients had long-term complications (tube migration and local infection). Seven patients required a second procedure during follow-up. Enteral feeding was well tolerated in 13 patients (72.2%) and weight gain was observed in 6 (33.3%). Total median length of jejunal feeding was 6 months (1-36).

Conclusion : PEJ is a useful endoscopic alternative for providing enteral nutrition when gastrostomy is not possible. Frequent indications in our series include previous gastrectomy and gastroparesis following lung transplantation.

Invited lecture

-N11-

SUMMARY OF RECOMMENDATIONS FOR PERI OPERATIVE ARTIFICIAL NUTRITION IN ELECTIVE SURGERY. J. Berre, Hopital Erasme, ULB, Brussels.

BGDO

-P01-

CYTOREDUCTIVE SURGERY PLUS HYPERTHERMIC INTRAOPERATIVE PERITONEAL CHEMOTHERAPY IN UPPER GASTROINTESTINAL CANCER : THE HIPCUPP TRIAL. G. Sergeant, A. Van Den Broeck, R. Aerts, C. Verslype, E. Van Cutsem, W. Laleman, H. Prenen, S. Tejpar, J. Vannoote, W. Van Steenberghe, V. Vandecaveye, T. Roskams, B. Topal. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : A large portion of patients with upper gastrointestinal cancer, such as gastric, biliary, or pancreatic carcinoma, either present with peritoneal metastatic disease or develop peritoneal recurrence after previous treatment with curative intent. Peritoneal carcinomatosis (PC) results in very poor survival, irrespective of the type of treatment modality. Cytoreductive surgery (CRS) plus hyperthermic intraoperative peritoneal chemotherapy (HIPC) has been proposed as feasible with acceptable perioperative morbidity and mortality resulting in improved survival compared to routine intravenous chemotherapy in PC from colorectal carcinoma, appendiceal carcinoma and pseudomyxoma peritonei.

Aim : The aim of the current monocentric phase II study is to evaluate in these patients the effectiveness of CRS + HIPC with cisplatin.

Methods : From August 2010, all patients between 18 and 75 years of age presenting with primary or recurrent gastric, biliary and pancreatic carcinoma confined to the abdominal compartment underwent laparoscopic exploration. Patients with PCI not more than 20 were included in the trial after obtaining written informed consent. Any systemic chemotherapy +/- biological is allowed before +/- after CRS + HIPC. The study was designed to have at least 80% power to detect a 40% increase in 1-year overall survival common to all strata (gastric-biliary-pancreas) after CRS + HIPC versus conventional palliative chemotherapy. Over an anticipated period of 2 years, 60 patients will undergo CRS followed by HIPC. An open coliseum technique is used, with perfusion of cisplatin at a concentration of 100 mg/m² for 60 minutes at intracavitary temperatures ranging between 40 and 41°C. Updated data will be presented at the BWG.

Results : Until November 29, eight patients (4 male, 4 female) underwent CRS + HIPC (5 gastric and 3 pancreatic adenocarcinoma). Two patients were included for peritoneal recurrence after total gastrectomy. All 6 other patients were primary cancers with isolated peritoneal disease. Median (range) Peritoneal Cancer Index was 10 (4-18). Complete cytoreduction (CCR-0) was obtained in all patients. Median (range) intra-operative blood loss was 500 (100-3000) ml. Median operating time was 360 min. No postoperative mortality was registered. Median (range) ICU stay was 3 (1-11) days. Postoperative pancreatic fistula (POPF) was observed in 5/8 patients (1 grade A and 4 grade B). The severity of postoperative complications ranged between TOSGS grade 1 and 4a.

Conclusion : Interval evaluation of our early experience shows acceptable morbidity and no mortality after cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy with cisplatin to treat primary or recurrent peritoneal carcinomatosis from upper gastrointestinal cancer. (NCT01116791)

IS LAPAROSCOPIC RESECTION OF COLORECTAL LIVER METASTASES AN ADEQUATE ONCOLOGICAL APPROACH ? A SINGLE CENTER MID-TERM RESULTS. M. Natale, C.M. Scandavini, D. Raglione, B. Van Den bossche, M. Sainz-Barriga, F. Berrevoet, X. Rogiers, K. Geboes, S. Laurent, R. Troisi. University Hospital, Ghent, Belgium.

Introduction : The indication of laparoscopic surgery for liver cancer has been delayed by the fear of less oncological procedures and by the difficulty to identify adequate margin to remove the tumor.

Aim : Aim of this study is to evaluate overall mid-term results of laparoscopic liver surgery for colorectal liver metastases (CRLM).

Methods : Between January 2005 and September 2009 a total 533 liver resections were performed in our institution. During this period laparoscopic liver resection of CRLM was performed in 40 cases. Endpoints were : overall morbidity, R0 resection rates, recurrence rates, hospital stay, overall survival and DFS.

Results : The mean age was of 64 ± 11.5 y. After a median follow-up of 19 month (minimum of 15 m), the recurrence rate was of 30% (12/40). Conversion to laparotomy was required in 4 (10%) out of 40 laparoscopic cases due to uncontrolled bleeding in two and after a repeat laparoscopic resection in the third for oncological reasons. Major hepatectomies were performed in 20% of patients. Six out of 12 patients with recurrent disease were resected again with the laparoscopic approach. The overall morbidity was 10% (4/40). R0 resection rate was 35/40 (87.5%) and the hospital stay was of 7.7 ± 2.7 days. The three-year overall and disease-free survival was of 85% and 66% respectively

Conclusion : According to this experience, laparoscopic resection of CRLM is safe and displays good oncological outcomes. Relapse of CRLM can eventually be treated with the same technique.

EVALUATION OF THE 7TH UICC TNM CLASSIFICATION FOR ESOPHAGEAL CANCER USING A SINGLE CENTER DATABASE. P. Naftoux, J. Moons, P. Deleyn, W. Coosemans, H. Decaluwé, G. Decker, D. Van Raemdonck, T. Lerut. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : The 7th edition on Cancer tumor-nodes-metastasis (UICC TNM) staging system was published recently.

Aim : The aim of this study is to evaluate the performance of the 6th versus the 7th editions of the UICC TNM staging system in esophageal cancer and to compare these results with the recently published nomogram by Lagarde for the group of Adenocarcinoma (AC).

Methods : 1334 Patients receiving primary surgical R₀-resection between 1990 and 2009 were included. Patients were staged using the 6th and 7th UICC edition staging systems and according the nomogram according Lagarde. To obtain a homogeneous group, patients with gastro-esophageal (GEJ) tumor location were restaged as esophageal tumors instead of gastric tumors for the 6th UICC edition. Survival analysis was performed with a Cox regression model. The homogeneity, discriminatory ability, and monotonicity of gradients of all staging systems were compared using linear trend chi², likelihood ratio chi² statistics, and Akaike information criterion (AIC) calculation.

Results : Eight hundred eighty-eight (66%) patients presented with AC, 456 (34%) with Squamous Cell Carcinoma (SCC). The overall five-year cancer specific survival rate for the entire cohort (exclusive mortality) was 58.6% (57.8% for AC and 60.0% for SCC). The 7th edition showed a significantly better differentiation for early stages (introducing stages IA and IB) and stratification of survival according to the number of positive lymph nodes (introducing pN 1, 2 and 3). The UICC 7th edition has a higher linear trend chi² and likelihood ratio chi² scores and a lower AIC score compared to the 6th edition, thus indicating better homogeneity, discriminatory ability, and monotonicity of gradients. However the nomogram for AC shows a still better homogeneity and monotonicity over the 7th edition, indicating that other factors (e.g. extracapsular lymph node involvement) have an additional prognostic influence.

| | Homogeneity | | Disciminatory ability | | Monotonicity | |
|----------|-----------------------------------|--------------|---|---------------|---------------------------|---------------|
| | <i>Cox LR Chi² (*)</i> | | <i>Linear trend chi² (*)</i> | | <i>AICc (**)</i> | |
| | AC | SCC | AC | SCC | AC | SCC |
| TNM 6th | 78,85 | 44,61 | 314,79 | 93,15 | 3395,2 | 1847,8 |
| TNM 7th | 99,32 | <u>57,31</u> | <u>343,79</u> | <u>110,06</u> | 3370,2 | <u>1832,2</u> |
| Nomogram | <u>107,94</u> | | 305,69 | | <u>3369,5</u> | |
| | (*) higher score = better | | | | (**) lower score = better | |

Conclusion : The 7th edition of the UICC TNM staging system performs better than the 6th edition, with better differentiation of early disease and better stratification of survival according to number of positive lymph nodes. This may have implications on the choice of treatment and thus indicating that accurate pre-treatment staging is of the utmost importance.

- P04 -

COLORECTAL CANCER SCREENING PROGRAM OF THE FRENCH COMMUNITY OF BELGIUM : PRELIMINARY RESULTS AFTER ONE YEAR OF ACTIVITY. ON BEHALF OF THE CENTRE COMMUNAUTAIRE DE RÉFÉRENCE POUR LE DÉPISTAGE DES CANCERS (CCR), CENTRE DE GESTION DU PROGRAMME DE DÉPISTAGE DU CANCER COLORECTAL, BELGIAN GROUP OF DIGESTIVE ONCOLOGY (BGDO), SOCIÉTÉ BELGE D'ENDOSCOPIE DIGESTIVE (SBED), SOCIÉTÉ ROYALE BELGE DE GASTRO-ENTÉROLOGIE (SRBGE). M. Polus, M. Candeur, C. Bourdon, S. Carboneille, A. Vandenbroucke. Centre Communautaire de Référence pour le dépistage des cancers, Mont-Saint-Guibert, Belgium.

Introduction : Colorectal screening program has been launched in March 2009 in the French Community. During the first round of this program, a population of 1 200 000 people from fifty to seventy-four years old are welcome to participate on a period of two years. We present preliminary results of the first year of the screening program.

Aim : To analyse and to improve a public health program for colorectal cancer screening in French Community of Belgium.

Methods : The program proposes to perform a guaiac faecal occult blood testing (Hemoccult®) for moderate risk subjects from the general population followed by a colonoscopy if positive (Hemoccult® group). An immediate colonoscopy is proposed to high or very high risk subjects (colonoscopy group). The general practitioner is on the core of the program and orientates patients according to their risk level.

Results : A total of 535 926 people were invited between 1 March 2009 and 31 January 2010.

Between 1 March 2009 and 28 February 2010, 42 928 people actively participated, which means an initial participation rate of 8%.

The number of active GPs is actually encouraging with 4604 participants.

The Hemoccult® group concerns 40 866 people and the colonoscopy group 2062 people.

In the Hemoccult® group, the rate of positivity of the test is 3,13%.

Out of 1280 positive tests, 85 cancers (PPV : 6,6%) and 253 advanced adenomas were identified as the worst lesions.

In the colonoscopy group, we found 22 cancers and 102 advanced adenomas.

From a global number of 3342 planned colonoscopies, the final results at the time of analysis were obtained for 2412 exams. The complication rate of colonoscopy is 0,80, including 2 perforations (0,090) et 12 hemorrhages (0,50).

Conclusion : The French Community program gives first results in agreement with the expected results from a mass colorectal cancer screening program, which is encouraging. The participation rate of the target population is encouraging for a beginning but has to be improved.

- P05 -

CONFOCAL LASER ENDOMICROSCOPY IN BARRETT'S ESOPHAGUS : INTEROBSERVER AGREEMENT AND ACCURACY AMONG AN INTERNATIONAL GROUP OF GASTROENTEROLOGISTS AND PATHOLOGISTS. C. Trovato (1), R. Bisschops (2), G. De Hertogh (3), X. Sagaert (3), M. Goetz (4), A. Sonzogni (1), M. Vieth (5), C. Crosta (1). (1) Instituto Europea di Oncologia, Milan, Italy ; (2) UZ Leuven Department of Gastroenterology, Leuven, Belgium ; (3) UZ Leuven Department of Pathology, Leuven, Belgium ; (4) University of Mainz, Mainz, Germany ; (5) Bayreuth Hospital, Bayreuth, Germany.

Introduction : Endoscope-based confocal laser endomicroscopy (CLE) is a new technology for in vivo imaging of the intestinal mucosa.

Aim : Our aim was to assess the preliminary interobserver agreement and accuracy among an international group of gastroenterologists and pathologists in the diagnosis of neoplasia in Barrett's esophagus (BE) using CLE.

Methods : 3 experienced CLE endoscopists, one experienced CLE pathologist and 2 novice CLE pathologists from 3 different European University Centers, blinded to histopathologic and endoscopic findings, reviewed CLE images of 79 biopsy matched sites of BE for the diagnosis of neoplastic vs. non-neoplastic confocal pattern. Confocal images were classified according to the Mainz Confocal Barrett Classification. In order to standardize image interpretation a training set of 20 images with known histology was first reviewed, followed by blinded review of 20 unknown images. Quality of image (scored as good, average or poor) was scored for each set of images. Observers had to indicate if the images were neoplastic or not.

Accuracy, sensitivity and specificity were calculated for endoscopists and pathologist separately, as well as interobserver agreement.

Results : In the validation set (n = 79), 16 cases showed neoplasia (dysplasia or carcinoma) at histology. All set of images was judged good or excellent for quality. Interobserver coefficient agreement among endoscopists and among pathologists were 0.82 (95%CI 0.72-0.92) and 0.70 (95%CI 0.58-0.82), respectively.

The accuracy, sensitivity and specificity for the diagnosis of neoplastic confocal pattern were of 98.7%, 93.7% and 100% for gastroenterologists ; 93.7%, 75.1%, 98.4% for all pathologists ; 94.9%, 81.25% and 98.4% for the expert pathologist, respectively.

Conclusion : CLE for the diagnosis of neoplasia in BE has reliability and high accuracy among an international collaboration group of expert and non-expert in this technique. Our data suggest that confirmation of CLE images by an expert pathologist trained in CLE interpretation may substitute classical biopsies. This will be further assessed in an ongoing multicenter trial.

- P06 -

ROLE OF RESPONSE PREDICTION WITH PET IN COLORECTAL CANCER. P. Flamen. Bordet, Brussels.

- P07 -

ROLE OF EUS IN RESPONSE PREDICTION. P. Deprez. UCL, Brussels.

Invited Lecture

- P08 -

RESPONSE PREDICTION IN UPPER GASTRIC CANCER : UPDATE OF THE MUNICON STUDY. F. Lordick.
Braunschweig – Germany.

Invited Lecture

-P09-

COLORECTAL CANCER : CAN HISTOPATHOLOGY PREDICT OUTCOME ? C. Langne. Graz, Austria.

-P10-

PROCARE, A MULTIDISCIPLINARY PROJECT ON RECTAL CANCER IN BELGIUM. T. Vandendael, K. Beirens, L. Van Eycken. Belgian Cancer Registry on behalf of PROCARE a multidisciplinary Belgian Project on Cancer of the Rectum, Brussels, Belgium.

Introduction : The main objective of PROCARE, a multidisciplinary project on rectal cancer, is to improve outcome in patients with rectal cancer by reducing diagnostic and therapeutic variability.

Methods : Multidisciplinary guidelines and quality indicators on rectal cancer care were elaborated and published. The quality of care program is based on online prospective registration on pre-treatment data, surgery, pathology, radiotherapy, chemotherapy and follow-up of rectal cancers. In addition, a continuous program of quality of care assessment was set up. Feedback is given regularly to each center.

Results : Actually, 83 hospitals are participating, resulting in more than 3400 registrations over five years. PROCARE encompasses projects in different disciplines. The Total Mesorectal Excision (TME)-evaluation program generates an anonymous quality control of resected specimens, pathology and surgery data. Expert pathologists assess the quality of the resected specimen on evaluable cases. Afterwards, surgeons perform a second review resulting in a final decision on the quality of the resected specimen and on adherence to guidelines. The first phase resulted in 25 PROCARE TME-trainers, allowing them to assist surgeons at TME-resections. The second phase consists of an at random quality control of all registered TME-cases. So far, 400 cases were selected ; 100 were considered evaluable by the expert pathologists. The PROCARE radiology (RX) and radiotherapy (RT) reviewing projects were launched during spring 2010. PROCARE RX aims to provide an anonymous second opinion within 24 hours on cTN and cCRM based on CT and MRI images. Participation should strongly be encouraged to launch this project. PROCARE RT performs a central review based on the provided planning CT images (target volume) : 18 hospitals are participating, 200 cases have been reviewed.

Conclusion : So far, the multidisciplinary PROCARE project has led to valuable results. A prospective registration system was set up with yearly feedback. Seventy-five percent of the Belgian hospitals have registered cases for PROCARE. Furthermore, projects in the different disciplines have enabled continuous evaluation and reviewing of the critical determinants in rectal cancer care. Participation to this promising project is therefore strongly encouraged.

- P11 -

BILIOPANCREATIC CANCER. I. Borbath.

- P12 -

COLORECTAL CANCER. E. Van Cutsem.

- P13 -

OESOPHAGASTRIC CANCER. M. Peeters.

- P14 -

THE EOSINOPHILIC GASTROINTESTINAL DISORDERS. A. Driessens, Maastricht.

SYSTEMIC EOSINOPHILIC DISORDERS AND THE GASTROINTESTINAL TRACT. A. Hoorens. UZ Brussel, Brussels, Belgium.

Secondary eosinophilic disorders always have to be excluded before making the diagnosis of primary eosinophilic oesophagitis, eosinophilic gastroenteritis or eosinophilic colitis, particularly in case of peripheral blood eosinophilia. Secondary eosinophilic disorders include infectious, hypersensitivity, inflammatory, or neoplastic illnesses. Parasitic infections are well-known to present with eosinophilia of the gastrointestinal mucosa. A drug-induced aetiology should also always be taken into consideration. Gastrointestinal eosinophils may be a feature of connective-tissue disease, especially scleroderma, and can accompany vasculitis in case of polyarteritis nodosa and Churg-Strauss syndrome. Neoplasia-associated eosinophilia is relatively common in association with gastric or colonic adenocarcinomas. In case of very pronounced peripheral eosinophilia hypereosinophilic syndrome (HES) with gastrointestinal involvement, clonal eosinophilia and lymphocytic variant hypereosinophilia should be considered. The HES is defined as eosinophilia ($e 1.5 \times 10^9/L$) persisting for at least 6 months, no known cause of eosinophilia, with signs or symptoms of organ involvement. The GI tract is affected in up to 25% of cases. When only the digestive tract is involved, it may prove difficult to distinguish between HES and primary eosinophilic gastroenteritis. During its long-term course, extra-intestinal manifestations may appear and facilitate the diagnosis. Eosinophilic infiltration of the GI tract in HES should be distinguished from eosinophilic infiltration of the GI tract in lymphocytic variant hypereosinophilia, where eosinophilia is associated with phenotypically abnormal and/or clonal T lymphocytes. Clonal eosinophilia is characterized by neoplastic proliferation of eosinophils as part of an underlying myeloid malignancy and can accompany any one of the myeloid malignancies. Two distinct subcategories of clonal eosinophilia are however recognized, chronic eosinophilic leukaemia- NOS and myeloid/lymphoid neoplasms involving PDGFRA, PDGFRB or FGFR1. Final diagnosis of secondary eosinophilic disorders requires correlation of endoscopic and biopsy findings together with careful clinical examination.

A DISEASE WITH DIFFERENT FACES. S. Corvers, M. Abdul-Hamid, P. Van Paassen, A. Driessen. University Hospitals, Maastricht, Netherlands.

Introduction : A 38-year old male was referred to our hospital because of longstanding abdominal complaints with severe weight loss. Because of persistent abnormal liver function tests, pointing towards cholestasis, a liver biopsy was performed. This biopsy revealed features, compatible with a primary sclerosing cholangitis (PSC). In contrast to the classical picture of a PSC our patient presented with other unusual symptoms. Besides liver problems this patient had a skin rash, which on biopsy was due to an urticarial vasculitis. After months the patient had also a generalized lymphadenopathy, which in association with severe weight loss raised the suspicion of a malignant lymphoma. Besides these organs salivary glands were also affected.

A common feature in all the specimens examined was the presence of IgG4-positive plasma cells, suggesting multi-organ involvement due to an IgG4-related sclerosing disease.

Conclusion : IgG4-related sclerosing disease is a syndrome of unknown etiology, occurring in middle-aged and elderly, predominantly male. Symptoms are related to one or more involved organs. Practically any organ-site can be affected, most commonly pancreas, hepatobiliary tract, salivary gland, orbit or lymph node.

Awareness of this IgG4-related sclerosing disease is important as it may mimic a malignancy, resulting in a wrong treatment. The multi-organ involvement may hamper the diagnosis, although the diagnosis can easily be confirmed by serology, revealing an increase of IgG4. The increase of this type of immunoglobulin is the clue for the diagnosis of this multi-system disease.

ISOLATED ERYTHEMA MULTIFORME OF THE OESOPHAGUS TWO YEARS AFTER CUTANEOUS INVOLVEMENT. L. Verset, J. Deviere, P. Demetter. ULB Erasme, Brussels, Belgium.

A 64-year old woman consulted the Gastroenterology Department of the Erasme Hospital in July 2009 because of atypical symptoms of gastroesophageal reflux. In her medical history, we noticed erythema multiforme in 2007 with an erythematous pruriginous maculopapular rash (“target lesion”) on the trunk and the limbs and an ulcerated lesion of the oral cavity after taking tetrazepam. Moreover, she had a history of hypertension treated by losartan and by an association of hydrochlorothiazide and triamterene. Diabetes was treated by metformine.

A grade I oesophagitis, a Chatzki ring, a hiatal hernia and a gastroparesia attributed to a type 2 diabetes were highlighted. Reflux improved slightly by iterative dilatation of the Chatzki ring and by medical treatment made up of cisapride and a proton pump inhibitor.

In August 2009, a new oesogastroduodenoscopy was performed and revealed multiple ulcers and mucosal shedding throughout the oesophagus. Histopathological examination showed an ulcerated mucosa, an epithelium with individual apoptotic cells and a predominantly lymphocytic inflammatory infiltrate, suggesting erythema multiforme of the oesophagus. The patient received oral corticoids; this treatment resulted in improvement of the oesophageal lesions.

Erythema multiforme is considered a hypersensitivity response to certain infections and drugs. Oesophageal involvement is probably underestimated; isolated involvement of the oesophagus has, however, never been described

CHARACTERIZATION OF A CELL CULTURE MODEL FOR AGGRESSIVE HEPATOCELLULAR CARCINOMA INDUCED BY CHRONIC HYPOXIA AND PATHWAY ANALYSIS; A CENTRAL ROLE FOR TGF-BETA AND PPAR-ALPHA. H. Van Malenstein (1), C. Verslype (1), R. Van Eijnsden (2), P. Windmolders (1), L. Libbrecht (3), F. Nevens (1), J. Van Pelt (1). (1) University Hospital Gasthuisberg, Leuven, Belgium; (2) VIB, Leuven, Belgium; (3) UZ, Gent, Belgium.

Introduction : Hepatocellular carcinoma (HCC) is an aggressive cancer and the microenvironment plays an important role in the behavior of HCC. Recently, we demonstrated with an in vitro model the prognostic role of chronic hypoxia. **Aim** : We wanted to characterize our in vitro model more in detail and study affected signaling pathways. Pathway analysis can be a useful tool to identify therapeutic targets.

Methods : We used the hepatoblastoma cell line HepG2. To define a model for chronic hypoxia we assessed the effect of different oxygen concentrations (1%, 2% and 5% O₂) at various time points (24 hrs, 48 hrs and 72 hrs). We studied both gene expression as well as protein levels in our in vitro model using microarray, real-time PCR and immunocytochemistry. In a second analysis we used different pathway analysis and gene – and protein interaction software (Ingenuity Pathway Analysis, GenMapp and STRING) to identify relevant affected signaling pathways by chronic hypoxia.

Results : We identified 2% O₂ during 72 hours as the best settings to study chronic hypoxia in our in vitro model of HCC. At this time point the HepG2 cells have adapted with the onset of a new equilibrium, which is very different from the acute response. Well known hypoxia related genes and pathways, such as Hif-1 α and VEGF are less prominent after 72 hours. On pathway level we saw both up- and downregulation within the different signaling cascades. The most prominent affected signaling centered on TGF- β 1 signaling and PPAR α /RXR α signaling. Furthermore, we saw a downregulation of liver specific detoxification pathways including cytochrome P450's and glutathion-S-transferases.

Conclusion : Chronic hypoxia differs from acute hypoxia and 2% O₂ during 72 hours can serve as a representative model for chronicity. Chronic hypoxia has an effect on several cellular processes, such as proliferation, differentiation, metabolism, cell-to-cell signaling and detoxification. The most striking finding is the identification of both up- and downregulated genes in a single pathway, which support the onset of a new balance during prolonged exposure to hypoxia. The prominent role of TGF- β 1- and PPAR α /RXR α signaling warrants their further investigation as therapeutic targets.

SCIRRHOUS VARIANT OF HEPATOCELLULAR CARCINOMA : A CASE-REPORT. D. Wendum, O. Scatton, B. Bessoud, O. Rosmorduc, J.F. Fléjou. Hôpital Saint Antoine, Paris, France.

Aim : We report a case of a rare variant of hepatocellular carcinoma (HCC) developed in a non-cirrhotic liver in a 64 year-old patient.

Results : A 64 y.o. patient was admitted because of abdominal pain associated to a liver mass. CT-scan showed large hypovascular nodules in the right liver lobe. Alpha-fetoprotein levels were very high (58000 ng/ml). There was no sign suggesting chronic liver disease. No primary carcinoma was found (in particular no gastric or ovarian carcinoma). After transarterial chemoembolization and portal embolization, a right hepatectomy was performed. Histopathological analysis of the liver specimen showed a moderately differentiated HCC with fibrous stroma with no diagnostic criteria for fibrolamellar HCC (Scirrhous HCC). The tumour cells expressed Heppar-1, glypican-3, AFP and also cytokeratin 19. The nontumoral liver showed no fibrosis, no steatosis or iron deposition.

Conclusion : The diagnosis of primary liver carcinomas with fibrous stroma, the diagnostic criterias of fibrolamellar HCC and the significance of cytokeratin 19 expression in HCC will be discussed.

MESENTERIC LESIONS : ANATOMO-RADIOLOGICAL CORRELATIONS. E. Danse, C. Sempoux, R. Detry, A. Mourin. UCL Saint-Luc, Brussels, Belgium.

Aim : The authors report two cases of mesenteric lesions. The first one concerns a 60-year-old woman for whom a calcified mesenteric mass was detected on abdominal CT required for a non specific pelvic pain. Small bowel enema was performed before surgery. The mass was totally removed. Pathology concluded to a calcifying fibrous tumor of the mesentery. The second case concerns a 83-year-old man complaining of abdominal pain and weight loss. A ill-defined solid mesenteric mass was found together with ascites and enlarged lymph nodes. Surgical exploration was performed and macrobiopsies were taken. The final diagnosis was sclerosing mesenteritis. Imaging findings are presented with close correlation with macroscopic and microscopic illustrations of the lesions.

Conclusion : This presentation is the opportunity to review the imaging findings suggestive of mesenteric diseases and to correlate these findings with pathological data.

ROLE OF FUNCTIONAL MRI IN RESPONSE PREDICTION OF ABDOMINAL CANCER. V. Vandecaveye.

ACCUMULATION OF LARGE AMOUNTS OF INTRAPERITONEAL SOFT TISSUE MATERIAL, MIMICKING PSEUDOMYXOMA PERITONEI. K. Buccauw, R. Vanslembrouck, K. Op De beeck, S. Haegeman, M. Thijs, D. Vanbeckevoort. UZ Leuven Departement of Radiology, Leuven, Belgium.

A 37-year-old man, with no relevant issues in his medical history, presented with low abdominal discomfort, weight loss and red blood loss per anum. The patient underwent both an ultrasound and a CT scan which showed an accumulation of large amounts of intraperitoneal soft tissue material with small amount of ascites. Subsequently, an exploratory laparotomy was performed with resection of this extensive peritoneal soft tissue mass, omentectomy, segmental enterectomy and appendectomy. Rather surprisingly, this lesion was histologically consistent with a high-risk gastrointestinal stromal tumor (GIST). The patient is further treated with systemic chemotherapy.

AN UNCOMMON CAUSE OF PROXIMAL INTESTINAL SUBOBSTRUCTION. E. Halet, H. Peeters, D. Van De putte, L. Delrue, M. De Vos. UZ, Gent, Belgium.

Introduction : A 23-year old Caucasian man with a history of a hernia operation presented at the emergency room because of longstanding complaints of weight loss, vomiting and abdominal pain worsening over time. At time of presentation he was complaining of diffuse abdominal pain and vomiting. On physical examination there was abdominal distention with percussion pain. Routine laboratory tests were normal. A Ct-scan was performed showing duodenal dilation and retraction of the ileum, suggestive for a subobstruction due to adhesions. A laparoscopy was performed but could not show any adhesions nor internal herniations. Because the complaints persisted a transit of the small bowel (Rx SMD) was performed showing a severely slowed down gastrointestinal transit with pendular movements at the proximal duodenum. Decompression of the duodenum was associated with a temporary higher motility. These findings are highly suggestive for a Wilkie syndrome also known as Superior mesenteric artery syndrome. It is most commonly caused by loss of the mesenteric fat pad resulting in a compression of the third portion of the duodenum by the abdominal aorta and the overlying superior mesenteric artery. A conservative treatment with high caloric drinks, small meals and prokinetics was started in order to gain weight and reverse the precipitating factor. Over the following months the patient gained weight and the symptoms resolved.

Conclusion : In conclusion, we present a rare case of subobstruction due to compression of the duodenum by the superior mesenteric artery also called Wilkie syndrome.

IMAGING FEATURES OF CAROLI DISEASE AND SYNDROME : A RESTROPECTIVE ANALYSIS IN 8 PATIENTS. M. Lefere, F. Claus, M. Thijs, G. De Hertogh, C. Verslype, W. Laleman, D. Vanbeckevoort, W. Van Steenberghe. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Segmental, nonobstructive dilatation of intrahepatic bile ducts is referred to as Caroli disease (CD). When associated with congenital hepatic fibrosis, the term Caroli syndrome (CS) is used. These disease entities share a common pathogenetic mechanism called ductal plate malformation.

Aim : To present an overview of the imaging features of hepatobiliary anomalies in CD and CS.

Methods : The clinical and radiological records of 8 patients admitted between 1992 and 2009 with pathologically proven CD or CS were reviewed. Available imaging studies were retrospectively scored for the presence of central dot signs*, the degree of intra- and extrahepatic bile duct dilatation, intrahepatic calculi and secondary signs of portal hypertension such as splenomegaly and oesophageal varices. (*The 'central dot sign' is defined in Caroli patients in the literature as a bundle or dot of strong contrast enhancement within a dilated intrahepatic bile duct).

Results : Two patients had the 'pure form' Caroli disease, whereas in the other six cases the diagnosis of Caroli syndrome was made. Age at diagnosis ranged from 3 to 74 years. Presenting signs and symptoms : (hepto)splenomegaly (n = 4), hematemesis and/or melena (n = 3), cholangitis (n = 3). There was no evidence for malignancy in any of these patients. 7 out of 8 patients showed central dot signs on various imaging modalities. In the remaining case, where no contrast-enhanced studies were available, hepatic lesions very closely resembling central dots ('dot-like' sign) were seen. Right hepatic lobe predominant disease was present in 6 cases. Intrahepatic bile calculi were found in three patients. Secondary signs of portal hypertension were observed in all but one patient.

Conclusion : The central dot(-like) sign can be considered a hallmark sign of Caroli disease and Caroli syndrome. It was reliably detected by current imaging techniques in all patients.

FALSE POSITIVE DIFFUSION WEIGHTED IMAGING FINDINGS IN AN ONCOLOGICAL SETTING. B. Op De beeck, A. Snoeckx, M.J. Spinhoven, V. Van Marck, T. Moreels, D. Ysebaert, M. Peeters. Antwerp University, Antwerp, Belgium.

Introduction : Diffusion Weighted Imaging DWI is a new powerful technique for detecting metastases showing higher sensitivity values than the classical MR sequences for subcentimetric liver metastases. Little is known about false positive findings.

Aim : We like to present the selective imaging findings correlated with pathology of two patients with an oncological history and false positive DWI findings.

Results : The first patient had a history of a Ewing sarcoma treated with chemo-radiation and surgery who also had an associated hemochromatosis. The second patient had a cholangiocarcinoma which was preoperatively treated with radiotherapy. In the first patient, multiple hypervascular nodules without iron overload mimicking metastases were found in the liver. The false positive DWI was created by the iron overload in the surrounding "normal" liver parenchyma. The hypothesis for these lesions are sideronecrotic nodules with nodular regenerative hyperplasia. The second patient showed acute radiation induced hepatitis in the radiation field with inside two DWI false positive hypovascular pseudolesions, probably due to vascular changes creating hypoperfusion with hypoxia and nutritional ischemia.

Conclusion : DWI is a very powerful technique for detecting liver metastases but treatment-induced microvascular changes may induce false positive findings.

ATYPICAL FOCAL HEPATIC MASS IN A YOUNG WOMAN. E. Vanhoutte, J. Soens, E. Willems, C. Verslype, D. Vanbeckevoort, D. Bielen. UZ Leuven Departement of Radiology, Leuven, Belgium.

Aim : To describe the distinct clinical, radiologic and histologic findings of fibrolamellar hepatocellular carcinoma starting from a case report and to differentiate fibrolamellar from other liver tumors.

Discussion : We report the case of a young woman of 22 years who presented with vague abdominal complaints and a palpable epigastric mass. Her medical history revealed a deep venous thrombosis in the leg, treated with a vena cava filter and marcoumar. The initial CT scan in portovenous phase demonstrated a large mass in the left liver lobe with a central scar, thrombosis of the left portal vein and mediastinal adenopathy. Additional triple-phase CT study of the liver showed a large heterogeneous mass predominantly hyperdense and with a central hypodense stellate scar with inlying calcifications. The central scar did not show any significant enhancement. An explorative laparoscopy was performed and biopsies were taken. Histopathologic examination confirmed the diagnosis of fibrolamellar hepatocellular carcinoma (FLC). These distinct imaging features can help to distinguish FLC from other benign and malignant lesions such as FNH, giant hemangioma, conventional HCC and malignant degeneration of hepatocellular adenoma. Diagnosing FLC is important, because in contrast to the benign lesions, FLC preferentially is treated by surgical resection. Even an advanced disease of FLC with lymphadenopathy, invasion of adjacent organs or limited metastasis does not preclude curative resection. In this particular case, medical treatment was chosen because of the presence of the mediastinal adenopathy.

Conclusion : Diagnosing FLC is important because of the different prognosis and therapy. Therefore, it should be included in the differential diagnoses of a large hepatic mass with central scar and inlying calcifications in a young patient with no underlying liver disease.

RETROPERITONEAL FAT-CONTAINING TUMOURS. F. De Munck, E. Vanderlinden, F. Vandenbroucke, A. Hoorens. UZ Brussel Vrije Universiteit, Brussels, Belgium.

Introduction : A 30-year-old and 51-year-old woman presented at the emergency department with respectively impression of a left flank mass and unrelenting abdominal pain. In both patients ultrasonography showed a large heterogeneous mass involving almost the entire left flank.

Aim : We want to give an overview of the diagnostic possibilities for such a tumours.

Methods : On abdominal computed tomography (CT), the masses appeared well-circumscribed and contained fat. There was a heterogeneous contrast captation. The first patient underwent resection of the mass. In the second patient a CT-guided biopsy was performed. Pathologic examination of both lesions revealed fat-containing tumours.

Results : The first patiënt had an angiomyolipoma of the kidney and the second patiënt had a retroperitoneal dedifferentiated liposarcoma. Angiomyolipoma is a tumour composed of varying admixtures of blood vessels, smooth muscle cells and adipose tissue ; any one or two of these elements may predominate. Liposarcoma is one of the most common soft tissue sarcomas of adult life. Together with an adrenal myelolipoma, they represent the three most common fat-containing masses in the retroperitoneal region. The latter is a benign tumour composed of mature fat and interspersed hematopoietic elements that resemble normal bone marrow.

Conclusion : Final diagnosis of these retroperitoneal fat-containing tumours is usually not possible based on imaging characteristics alone as these lesions have overlapping features. Demographic and clinical data, however, will allow refining the diagnostic options and will help to determine treatment.

Invited lecture

“NEW CLASSIFICATION OF LIVER ADENOMAS”. T. Roskam.

EFFICACY OF THE COMBINED USE OF BEVACIZUMAB AND IRINOTECAN IN A HUMAN COLORECTAL CANCER XENOGRAFT MODEL ANALYSED BY SPECT IMAGING. C. Vangestel (1), C. Van De wiele (1), N. Van Damme (1), S. Staelens (2), K. Geboes (1), S. Laurent (1), M. Peeters (1). (1) UZ, Gent, Belgium ; (2) UZ, Antwerpen, Belgium.

Introduction : Colorectal tumors are dependent on angiogenesis for growth and VEGF is a key mediator of tumor angiogenesis. Antiangiogenic drugs can induce a transient normalization of the tumor vasculature and can thus potentiate the activity of co-administered chemotherapy.

Aim : The efficacy of anti-human VEGF antibody (bevacizumab) with or without irinotecan was evaluated in a human colorectal cancer xenograft model.

Methods : Colo205-bearing mice were treated with a single dose (ip) of bevacizumab (5 mg/kg) 2, 4 or 6 days prior to administration of a single dose of irinotecan (100 mg/kg) or 0.9% NaCl. Microvessel density (MVD), collagen covered tumor vessels (Masson's Trichrome staining), pericyt coverage (α -smooth muscle actin immunostaining) and tumor hypoxic fraction (Pimonidazole staining) was determined at the three different time points following treatment of bevacizumab. To investigate the possible synergistic effects of the combination therapy, the apoptosis-detecting radiotracer ^{99m}Tc His-ann A5 was injected iv (0.5 mCi) in mice 12, 24 and 48 hours after start of the irinotecan treatment and also to control mice (n = 3 in each time group). MicroSPECT imaging was subsequently performed 3.5 hours after injection of the radiotracer. The results were correlated to histological analysis for apoptosis (caspase-3 activation).

Results : MVD decreased significantly, α -smooth muscle actin and collagen covered vessels were increased compared to control tumors, 4 days after bevacizumab treatment, suggesting normalization of the tumor vasculature. Hypoxic fraction was slightly reduced 4 days after treatment with bevacizumab. SPECT analyses demonstrated a significant increase in tumoral ^{99m}Tc His-ann A5 uptake 4 days after bevacizumab treatment and 24 h after irinotecan administration ($180 \pm 37\%$ injected dose/ tumor volume, $p < 0.05$) compared to each monotherapy demonstrating a synergistic effect of both therapies. Quantitative ^{99m}Tc -ann A5 tumor uptake correlated well with the number of apoptotic cells as determined by caspase-3 immunostaining ($R^2 = 0.81$, $p = 0.04$).

Conclusion : Four days after administration, VEGF inhibition with bevacizumab normalizes the tumor vasculature in the Colo205 model, leading to an improved cytotoxic effect of irinotecan.

STUDY ON PLASMA OF PATIENTS WITH COLORECTAL CANCER, ADENOMA AND OF HEALTHY CONTROLS BY LABEL FREE DIFFERENTIAL QUANTITATIVE PROTEOMIC APPROACH. M.A. Meuwis (1), G. Mazzucchelli (2), P. Leclercq (3), M.P. Merville (4), M. Polus (3), D. Van Daele (3), E. Depauw (2), M. Malaise (5), E. Louis (3), M. Fillet (4). (1) GIGA Proteomic Platform, ULg, Liège, Belgium ; (2) Laboratory of mass spectrometry GIGA-R, CART, ULg, Liège, Belgium ; (3) Gastroenterology, hepatology and digestive oncology, GIGA R, CHU Liège., Liège, Belgium ; (4) Medical chemistry, GIGA-R, ULg, Liège, Belgium ; (5) Rheumatology, GIGA R, CHU Liège, Liège, Belgium.

Introduction : Discrimination of early stage of colorectal carcinoma (CRC) and Adenoma (A) is still not possible based on a single biomarker. Hence we investigated their discrimination using plasma taken from patients suffering from Adenoma, CRC and Healthy controls. Label free differential quantitative analysis on nanoUPLC-SynaptTM HDMSTM G1 allows identification and relative quantitation of proteins present in complex mixtures, even at low abundance (lower limit of quantification being 25 fmoles).

Aim : To select potential biomarkers for discrimination of CRC, A and HC.

Methods : We pooled equimolar fractions of every subject's plasma for each group studied (CRC : 17, A : 16 and HC : 17). The 3 pools were spiked with 2 proteic internal standards and further depleted from the 14 most abundant proteins found in blood using the depletion Seppro[®] IgY14 spin columns kit (Sigma). The complex protein mix obtained after depletion, were digested using trypsin and analyzed in triplicate on nanoUPLC-SynaptTM HDMSTM G1 and differential analysis was performed with the ProteinLynx Global server vs 2.3 (Waters) on the Uniprot human database.

Results : We obtained a list of identified and quantified proteins, some being significantly differentially abundant between pools and could represent possible systemic indicators of disease. Tetranectin for example was detected in HC and not detected in A and CRC pools in these experimental conditions. Carboxypeptidase N catalytic chain was only detected in CRC. APO AI and AII were significantly decreased in CRC compared to A and to HC (HC > A > CRC).

Conclusion : Using a label free proteomic approach, we could identify proteins with significant different abundance distribution in healthy controls, adenoma and CRC plasma pools. These results should be confirmed by other proteomic and non proteomic approaches, on individual patients' samples to address specificity, sensitivity and accuracy of these potential biomarkers, which might be the bases for new diagnostic tools development and be investigated for researches on CRC etiology.

METASTATIC PANCREATIC CANCER : COMMUNITY HOSPITAL EXPERIENCE WITH PROPHYLACTIC LMWH (ENOXAPARIN). D. Masfrancx, J. Dekoninck, F. Geurs, S. Ponette, J. Ponette, K. Bulté. Sint Maria Ziekenhuis, Halle, Belgium.

Introduction : The symptomatic management of metastatic pancreatic cancer has much improved in the last decade, mainly by improved quality of life with gemcitabin (1-3).

Aim : Whether the global survival, seen in large phase III trials, can be confirmed in community hospital setting (with an elderly and less fit population) remains unclear (2). The prophylactic use of lmwh's like enoxaparin might also be associated with an increase in survival (3) but this initial publication was also not corroborated in clinical practice.

Methods : We reviewed the files of all 30 patients (from 1/11/2005-1/11/2010) who presented with metastases of pancreatic cancer. All these patients had little bowel complaints and no signs of obstruction. Median age was 78 y (46-87 y), Male 15, Female 15. They were treated with standard CT regimens : namely gemcitabin. All patients received prophylactic enoxaparin with the start of chemotherapy.

Results : Six patients died less than six weeks after starting chemotherapy. Response rate was 12% in the remaining 24 patients, median Time To Progression was 3.5 months (1-14 months) median survival was 7,4 months (1-26 months). One patients had subsequent second line treatment with oxaliplatin and FU.

Conclusion : The actual median survival of our patients presenting with st IV pancreatic cancer is actually similar to the most recently published data : seven months median, although median age is 15 years older in our population than the median of 63 years which was recently published (2) There was no increase in response rate nor an increase in survival associated with the prophylactic use of enoxaparin, but trombosis of the portal vein and paraneoplastic tromboembolic phenomena were no longer seen once this attitude was adopted.

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ALTERNATIVE TREATMENT OPTIONS IN COLORECTAL CANCER PATIENTS WITH 5-FLUOROURACIL OR CAPECITABINE INDUCED CARDIOTOXICITY. N. Hiltrop, J. Hoste, M. Cool, G. Lambrecht, G. Deboever. AZ Damiaan, Oostende, Belgium.

Most chemotherapy regimens in colorectal cancer treatment are 5-fluorouracil/leucovorin.

(5-FU/LV) or capecitabine based. Cardiotoxicity is a less common but potentially lethal complication of 5-FU or capecitabine treatment, and most physicians are probably unfamiliar with treatment alternatives. Rechallenging patients induces the same cardiotoxicity in 45-90% of retreated patients and should be avoided. We present 2 cases of 5-FU induced cardiotoxicity and discuss therapeutic alternatives. The circadian rhythm of activity of dihydropyrimidine dehydrogenase (DPD), the first and rate-limiting enzyme in 5-FU catabolism, has been held responsible for variable 5-FU plasma concentrations during constant infusion, with a peak drug concentration observed in the first half of the night. Although the relationship between 5-FU induced cardiotoxicity and DPD deficiency remains unclear. Raltitrexed is a specific thymidylate synthase inhibitor whose metabolism is independent of DPD. In many European digestive oncology units, raltitrexed is probably the treatment of choice in patients with previous 5-FU or capecitabine induced cardiotoxicity, especially in those with documented DPD deficiency. Response rate and median survival obtained with raltitrexed are equivalent to IV 5-FU/LV bolus or infusional regimens, but disease free survival, progression free survival, toxicity and quality-of-life score are worse. UFT and S-1 are combinations of an other 5-FU prodrug and a DPD inhibitor, but experience with these products in this field is limited to case reports, and excess severe diarrhoea grade 3/4 can be expected in Western patients.

HIPEC FOR PERITONEAL CARCINOMATOSIS : DOES AN ASSOCIATED UROLOGIC PROCEDURE INCREASE MORBIDITY ? C. Honoré, A. Souadka, D. Goéré, F. Dumont, F. Deschamps, D. Elias. Institut Gustave Roussy, Villejuif, France.

Aim : The purpose of this study was to report the incidence, the morbidity rate, the specific complications and the mortality rate of urinary-tract procedures associated with a complete cytoreductive surgery (CCRS) plus Hyperthermic Intraperitoneal Chemotherapy (HIPEC) or Early Postoperative Intraperitoneal Chemotherapy (EPIC).

Methods : From a prospective database of patients with malignant peritoneal disease, all types of tumors included, treated with CCRS plus intraperitoneal chemotherapy (HIPEC or EPIC) ; patients who underwent a resection or a suture of the bladder, ureter or kidney were retrospectively studied.

Results : Between 1994 and 2010, 48 patients underwent a resection or a suture on the urinary tract among the 598 treated with CCRS plus intraperitoneal chemotherapy (8%). There were 4 nephrectomies, 19 partial cystectomies, 8 perforations of the bladder and 18 ureteral resections. The overall postoperative morbidity (grade > 2) was 41% (20/48). The mortality was 4% (2/48). Specific complication included 6 urinary fistulas (12%) : two among the 27 bladder sutures (7%) and four among the 18 ureteral sutures (22%) (pNS). In uni- and multivariate analysis, risk factors of urinary fistula were a severe preoperative denutrition (p = 0.05, RR = 7.3) and an extended peritoneal disease (PCI <U> > </U> 20, p = 0.05, RR = 8.3). Conservative treatment of urinary fistulas was successful in 83% of case (5/6).

Conclusions : Associated urinary-tract procedures occur in 8% of the all CCRS plus intraperitoneal chemotherapy. They do not significantly increase postoperative morbidity and mortality. Therefore, urinary tract involvement by a carcinoma should not contraindicate a curative treatment, CCRS plus intraperitoneal chemotherapy. Severe preoperative denutrition and extended peritoneal disease increase the risk of postoperative urinary fistulas.

RADIOFREQUENCY ABLATION OF UNRESECTABLE PANCREATIC CANCER. PERSONAL EXPERIENCE. M. Citone, M. La Torre, M. Rossi, M. Cavallini, D. Cavaniglia, A. Rebonato, V. David, V. Ziparo. Sapienza University, Rome, Italy.

Introduction : Treatment options for unresectable pancreatic cancer are not well defined in standard clinical practice. Radiofrequency ablation (RFA) has been suggested as a new palliative option.

Aim : Authors present their experience of intraoperative pancreatic RFA in order to evaluate the feasibility and safety of this technique in locally advanced, unresectable, non metastatic pancreatic cancer.

Methods : Five patients (1 woman) with a mean age of 73 years with histologically proven unresectable pancreatic lesions underwent intraoperative RFA. RFA was performed for tumors located in the pancreatic head (3 patients), in the isthmus (1 patient) and in the body (1 patient). Medium lesion diameter at preoperative CT-scan was 3.7 cm (range 2.6-4.8). In all patients a cluster needle with 3 cm exposed cool tip (Radionics) was employed. RFA was performed under direct ultrasound guidance with a cooling saline flushing provided during procedure. In three patients a bilio-enteric diversion was associated. In one patient RFA was performed also on celiac plexus in order to treat a severe, chronic and life style-limiting pain.

Results : RFA was feasible in all cases, achieving a complete necrosis of the lesion. A CT-scan performed on post-operative day-7 showed hypodense non-enhancing areas in the target treatment sites. All patients developed a post-operative complication. A self-limiting pancreatic fistula occurred in two patients. Of these one patient is alive at the 17 months follow-up and the other one died 8 months after the procedure. The 30-day mortality rate was 60% and it wasn't related to tumor location ; two patients died for a massive gastro-enteric bleeding and one patient died for a severe acute necrotico-hemorrhagic pancreatitis.

Conclusions : A change of standard cool tip as well as a modification of current parameters of the RF system is necessary in order to improve the rate of postoperative complications and mortality of pancreatic RFA and maintain the encouraging outcomes in terms of palliation and quality of life.

AN UNUSUAL CASE OF TUBULAR NEUROENDOCRINE CARCINOMA OF THE SMALL BOWEL. S. Duquenne (1), A. Thiry (1), D. Dresse (2), J. Delflandre (2), B. Delhougne (2), P. Delvenne (1). (1) University of Liège, Liège ; (2) CHR Citadelle, Liège.

Neuroendocrine tumors (carcinoid tumors) are common encountered benign and malignant neoplastic lesions found in the small intestine.

Malignant gastro-intestinal tumors demonstrating mixed neuroendocrine and glandular differentiation (malignant adenocarcinoid tumors) are uncommon and associated with problems in pathological classification and prognosis prediction.

A morphological variant of the “classical” neuroendocrine carcinoma, exhibiting pseudo-glandular structures, is described as tubular neuroendocrine carcinoma (malignant tubular carcinoid tumor). The knowledge of this entity is important since its distinction with malignant adenocarcinoid tumor can be somewhat difficult and the prognosis of these entities is different.

We describe here the case of a 46 year-old man with a history of anemia, appendicectomy, and bilateral hernia repair, who was admitted at the emergency services for a brutal and diffuse abdominal pain, without any other associated symptom.

Abdominal scanography demonstrated a heterogenous mass in the mesentery of the small bowel, without other lesions. An abdominal MRI also detected a suspicious 2,8 cm lesion in the segment 4 of the liver.

A surgical resection of the tumor, with lymphadenectomy and hepatic metastasectomy was performed. The pathologic examination revealed a well differentiated 2 cm neuroendocrine carcinoma with cells mimicking glandular morphology, invading intestinal wall up to the subserosa. One lymph node (4,7 cm) was involved on four lymph nodes examined, with capsular effraction and multiple lymphatic invasions. The hepatic metastasis (3,2 cm) was distant from resection margins.

A first diagnosis of “malignant adenocarcinoid tumor” was suggested, but the absence of mucin secretion highlighted by staining methods and the positivity of pseudoglandular cells for neuroendocrine markers by immunohistochemistry oriented the diagnosis to a tubular neuroendocrine carcinoma.

No recurrences were found after 6 months follow up.

**7 SOCIETIES SYMPOSIUM :
CLINICAL CONTROVERSIES IN GE, HEPATO-PANCREATOLOGY AND GI ONCOLOGY**

- S01 -

GERD : LAPAROSCOPIC ANTIREFLUX SURGERY VS LONG TERM PPI ? J. Tack. KUL.

- S02 -

SEDATION BY THE GASTROENTEROLOGIST : MYTH OR NEEDED REALITY ? O. Lemoine. ULB < BSGIE.

DIVERTICULITIS : WHAT INDICATION AND TIMING FOR SURGERY ? A. Kartheuser-M. Thomas. UCL, < RBSS.

Invited lecture

CAN WE STOP ANTI-TNF TREATMENT IN CROHN'S DISEASE ? E. Louis. CHU Liège, Liège, Belgium.

Long-term anti-TNF treatment is associated with prominent benefits for the patients with moderate to severe Crohn's disease. Sustained remission, mucosal healing, decrease in hospitalisations and surgeries have been confirmed. Long-term safety has also proven to be reasonably good with only a very small increase in opportunistic and severe infections and a possible small increase in the risk of lymphoma. Globally long-term efficacy appears superior to the one of immunosuppressant and long term-safety, probably at least as good. Nevertheless because of long term costs and some specific circumstances, such as wish of pregnancy or personal preferences, anti-TNF withdrawal may be contemplated. Short term anti-TNF treatment as a bridge to immunosuppressant has been evaluated in a controlled study and has proven to be an unsatisfactory strategy. Retrospective analysis of mono-center experiences as well as uncontrolled retrospective studies suggest the possibility to discontinue anti-TNF after a prolonged period of scheduled treatment in a substantial subgroup of patients. The GETAID embarked a prospective cohort study to confirm the level of this risk of relapse and to try and disclose demographic, clinical, endoscopic or biological factors associated with the time-to-relapse. This study showed a 50% risk of relapse over two years after infliximab discontinuation in a cohort of patients in stable steroid-free remission under combined therapy with an immunosuppressant for more than one year. Furthermore a subgroup of patients representing $\frac{1}{4}$ to $\frac{1}{3}$ of the study population and having a very low risk of relapse could be identified through simple clinical and biological markers. In relapsers, as previously suggested in smaller retrospective series as well as in other IMIDs, remission could be recovered with infliximab in almost all the patients. In conclusion, after a prolonged period of remission under a combined therapy with anti-TNF and immunosuppressant, if necessary, the withdrawal of the anti-TNF can be contemplated in a selected subgroup of patients characterized with a full normalisation of clinical, biological and endoscopic parameters.

FOOD ALLERGY : IS IT CLINICALLY RELEVANT ? S. Bisschop. Stuttgart, Germany.

Invited lecture

- S06 -

SB VIDEO CAPSULE AFTER 10 YEARS : DID IT CHANGE THE OUTCOME OF OUR PATIENTS ? M. Penazzio, It.

Capsule endoscopy (CE) is a major advance in the investigation of small bowel diseases. This technology allows non-invasive visualization of the small bowel. The indications for CE are constantly expanding and evolving and currently include, among others, obscure gastrointestinal bleeding, Crohn's disease, complicated celiac disease, abnormal small bowel radiology, and polyposis syndromes. The initial studies of CE have focused on diagnostic yield, in which respect CE has been shown to be superior to other small bowel imaging techniques. Diagnostic yield, although important, can be misleading, given the detection of potentially nonspecific findings. In addition, the real impact of CE findings on the clinical management of patients is still undefined and the available information mainly relates those with obscure bleeding which represent the majority of patients subjected to CE. Although published non-randomized studies showed that 25% to 60% of patients with obscure bleeding received specific therapeutic interventions or changes in management based on a finding from CE, however two recent randomised prospective trials showed that the significant improvement in diagnostic yield with CE does not translate into improved outcomes for the patients (1,2). As was learned from the earlier studies with upper GI bleeding, an improved diagnostic ability can only affect outcome if the improved diagnostic ability is coupled with a therapeutic intervention that has a beneficial effect. For new technologies, such as deep enteroscopy which offers new therapeutic options, preliminary data suggest that endoscopic therapy appears to be effective in changing outcomes in terms of transfusion requirements or the proportion of patients able to maintain a normal hematocrit (3,4). Therefore, large prospective controlled studies, with outcome data for the different etiologies and clinical presentations of obscure bleeding, a predefined and standardized therapeutic protocol for findings at CE or after deep enteroscopy diagnosis, and adequate follow-up are needed to determine whether the use of these modern technologies leads to improved patients' long-term outcomes and affects the natural history of small-bowel disorders.

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- S07 -

CONTROVERSY IN VIRAL HEPATITIS THERAPY. H. Reynaert. BASL - VUB.

- S08 -

CONTROVERSY IN TRANSPLANTATION INDICATIONS FOR HCC. J. Pirenne. KUL.

- S09 -

ACUTE PANCREATITIS : HOW TO *EARLY* STAGE ? M. Delhay. ULB, < BPC.

- S10 -

CONTROVERSY IN MANAGEMENT OF *PANCREATIC* CYSTIC LESIONS : SHOULD WE OPERATE ALL ?
D. Ysebaert. UZA, < RBSS, BGDO.

CONTROVERSY IN MANAGEMENT OF PANCREATIC CYSTIC LESIONS : SHOULD WE DOCUMENT AND FOLLOW ? P. Smeets. UZ, Gent, Belgium.

Pancreatic cysts are frequently incidentally detected by CT (+/- 2% of abdominal studies) or MRI (+/- 20%).

Most incidental cysts are detected on routine abdominal studies. However, if a cyst needs to be characterized, it is recommended that a diagnosis of a specific cyst type is not made unless the patient undergoes a dedicated "pancreas study". Dedicated MRI is the imaging procedure of choice to characterize a pancreatic cyst. Mucinous cystic masses, namely IPMNs and MCNs, have a well-established malignant potential. Hence, it has become increasingly important and difficult for radiologists to frame the report to help guide appropriate management.

Asymptomatic cystic pancreatic tumors are most often benign or indolent, low-grade neoplasms (17% serous cystadenomas, 28% mucinous cystic neoplasms (MCNs), 27% intraductal papillary mucinous neoplasms (IPMNs), 2.5% cystic ductal adenocarcinomas, and pseudocysts 3.8%).

A useful imaging-based classification system differentiates pancreatic cystic masses into 4 morphologic types :

- (1) Unilocular (pseudocysts, mucinous cystic neoplasms, lymphoepithelial cysts, small IPMNs, and small serous tumors),
- (2) Microcystic (serous cystadenomas and lymphoepithelial cysts),
- (3) Macrocytic (mucinous cystic neoplasms, oligocystic serous tumors, and IPMNs) and
- (4) Cysts with solid components (solid appearing serous tumors, solid pseudopapillary neoplasms, and cystic islet cell tumors).

The "typical" most frequently detected asymptomatic cyst (particularly on MRI) has a diameter of +/- 1 cm.

Imaging is not able to characterize these lesions.

Many studies support the nonsurgical management of pancreatic cysts < 3 cm which don't display "alarming features" (larger size, mural nodules, dilation of the common bile duct, involvement of the main pancreatic duct, lymphadenopathy).

Malignancy or premalignancy does not correlate with cyst size alone.

Establishing a cyst as mucinous is important because mucinous lesions of any size are premalignant. Morphologic features that aid in diagnosis of a MCN include

- (1) the presence or absence of septa (MCNs generally are multilocular, with large cysts),
- (2) the position of calcification (MCNs typically have peripheral calcification, whereas serous tumors have central calcification),
- (3) location within the pancreas (head, tail,...), and
- (4) the presence of main pancreatic duct involvement.

An MCN can be suspected when a cyst is present in the tail of the pancreas in a perimenopausal woman. The presence or absence of direct communication with the main pancreatic duct must be established to distinguish a mucinous cystic tumor from a branch duct IPMN. Three-dimensional imaging with MRI (or CT) can address this question.

Serous cystadenoma characteristically displays variably dense radial septa in a honeycombed or spongiform pattern and central calcification (central scar). The more peripheral cysts are larger than the more central cysts. Serous cystadenoma is a benign lesion. However, these lesions may grow. Therefore, some recommend resecting serous cystadenomas > 4 cm regardless of the presence of symptoms, or in symptomatic patients regardless of size.

Solid pseudopapillary epithelial neoplasm is a low-grade malignancy that can present with cystic-appearing components. The majority are found in young women. They frequently contain peripheral calcification and variable content (most characteristically hemorrhages) within the cysts. Solid pseudopapillary epithelial neoplasm lesions should undergo resection.

The Incidental Findings Committee of the American College of Radiology recommends the following for managing incidental pancreatic cysts :

Surgery should be considered for patients with cysts > 3 cm. If the lesion is a serous cystadenoma, surgery is deferred until the cyst is > 4 cm. Solid pseudopapillary epithelial neoplasm tumors should be resected. Patient factors ultimately determine the appropriateness of surgical treatment. Patients with simple (not containing any solid elements) cysts < 3 cm can be followed. Attempts should be made to characterize all cysts > 2 cm at the time of detection. Magnetic resonance imaging is the imaging procedure of choice. Cyst aspiration is strongly advised before any surgery is undertaken in a patient with a cyst of this size. Cysts < 2 cm can be followed less frequently than those between 2 and 3 cm. Avoid characterizing cysts < 1.5 to 2 cm unless absolutely characteristic. The presence of symptoms is a critical factor in deciding appropriate therapy. The frequency of malignancy in small cysts is significantly higher in symptomatic patients.

A follow-up examination must clearly establish the stability of a cyst. Therefore, patients should be advised to undergo

serial imaging at facilities with protocols for dedicated pancreatic imaging. Although there is no clear consensus regarding the optimal imaging test for follow-up of pancreatic cysts, a limited MRI examination relying exclusively on T2-weighted unenhanced acquisitions has been proposed as a practical follow-up strategy.

There is no universally accepted follow-up protocol. Most proposed programs are based on the Sendai criteria : Cysts < 1 cm are followed yearly, cysts between 1 and 3 cm are sent for further imaging (endoscopic ultrasound or MRI) looking for septa and mural nodules, and simple cysts are followed at 6-month intervals for 2 years and then yearly. If they grow above 3 cm or develop any worrisome features, patients are considered candidates for resection.

The ACR recommendations are :

(1) Cysts < 2 cm may be followed at 1-year intervals, and if there is no growth, follow-up ceases if the patient remains asymptomatic.

(2) A cyst that is > 3 cm is considered a surgical lesion unless it is a serous cystadenoma or if patient comorbidities preclude benefit from resection.

(3) A cyst between 2 and 3 cm may be characterized and followed semi-annually if mucinous, yearly if uncharacterized, and every 2 years if it is a serous cystadenoma.

Belgian Pancreatic Club

Invited lecture

- T01 -

PREVENTING POST-ERCP PANCREATITIS : ESGE GUIDELINES. J.-M. Dumonceau (1), A. Andriulli (2), J. Deviere (3), A. Mariani (4), J. Rigaux (3), T.H. Baron (5), P. A. Testoni (4). (1) Service of Gastroenterology and Hepatology, Geneva University Hospitals, Geneva, Switzerland ; (2) Division of Gastroenterology, Casa Sollievo Sofferemza Hospital, IRCCS, San Giovanni Rotondo, Italy ; (3) Departments of Gastroenterology and Hepato-Pancreatology, Erasme University Hospital, Brussels, Belgium ; (4) Division of Gastroenterology and Gastrointestinal Endoscopy, Vita-Salute San Raffaele University, IRCCS San Raffaele Hospital, Milan, Italy ; (5) Department of Medicine, Division of Gastroenterology and Hepatology, Mayo Medical Center, Rochester, Minnesota, USA.

Learning objectives :

- Identify patients and procedure at increased risk for post-ERCP pancreatitis
- Learn which measures have been shown to effectively prevent post-ERCP pancreatitis

Summary of key messages :

- For high-risk ERCPs, including ampullectomy, pancreatic sphincterotomy, precut biliary sphincterotomy, known or suspected sphincter of Oddi dysfunction, pancreatic guidewire-assisted biliary cannulation and endoscopic balloon sphincteroplasty, prophylactic pancreatic stent placement should be considered.
- For low-risk ERCPs, preprocedure rectal administration of NSAIDs is recommended.

Introduction : Guidelines about the prophylaxis of post-ERCP pancreatitis were commissioned by the European Society of Gastrointestinal Endoscopy (ESGE). The final draft was presented at the ESGE in November 2009. After review by ESGE members, by the Editorial Board of the journal *Endoscopy* as well as international peer reviewers, guidelines were endorsed by the ESGE and published in the journal *Endoscopy*.¹ The full version is freely available from the ESGE website (<http://www.esge.com/esge-guidelines.html>). A summary including evidence statements and recommendations is reproduced below. Evidence levels and grades of recommendation were those recommended by the Scottish Intercollegiate Guidelines Network.²

Summary of the ESGE guidelines : Pancreatitis is the most frequent complication after ERCP with an incidence of 3.5% in unselected patients ; it is of mild or moderate severity in approximately 90% of cases. Independent patient-related and procedure-related risk factors for PEP are listed in Table 1. Risk factors synergistically increase the risk of PEP (Evidence level 1 +).

There is no evidence that hospital volume has an influence on the incidence of PEP ; data about a potential relationship between PEP incidence and endoscopist volume are conflicting. Failed ERCP is more frequently seen when performed by endoscopists and in centers that perform a low annual number of procedures (Evidence level 2 +).

Serum amylase values < 1.5 times the ULN obtained at 2-4 hours post-ERCP virtually exclude PEP ; values > 3 or 5 times the ULN at 4-6 hours post-ERCP have increasing positive predictive values for PEP (Evidence level 2 +). It is recommended to measure serum amylase value in patients to be discharged on the day of ERCP ; patients with amylase values < 1.5 times ULN can be discharged without concern about risk of PEP. (Recommendation grade B).

Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the incidence of PEP ; effective PEP prophylaxis has only been demonstrated using 100 mg of diclofenac or indomethacin administered rectally (Evidence grade 1 + +). Routine rectal administration of 100 mg of diclofenac or indomethacin immediately before or after ERCP is recommended (Recommendation grade A).

Nitroglycerin reduces the incidence of PEP ; however, when administered transdermally, it is ineffective (Evidence grade 1 + +). Side effects such as transient hypotension and headache may occur. We do not recommend the routine use of nitroglycerin for prophylaxis of PEP (Recommendation grade A).

Cephtazidime reduced the incidence of PEP in a single study (Evidence grade 1-). Further data are needed before recommending cephtazidime for the prophylaxis of PEP (Recommendation grade C).

Based on an ad-hoc meta-analysis of results from 10 high quality RCTs, somatostatin proved to be ineffective in preventing PEP (Evidence 1 + +). We do not recommend universal administration of prophylactic somatostatin in average risk patients undergoing ERCP (Recommendation grade A). Administration of somatostatin might be more efficacious using specific dose schedules, but caution is needed when interpreting the results of subgroup analyses as they often exaggerate differences between treatments in RCTs.

Octreotide administration did not affect the overall incidence of PEP when data from 8 high quality trials were pooled (Evidence 1 + +). Prophylaxis with octreotide is not recommended (Recommendation grade A). In future studies the efficacy of prophylactic administration of octreotide should be evaluated using a dose ≥ 0.5 mg.

Prophylaxis with gabexate or ulinastatin does not reduce the incidence of PEP (Evidence 1 + +). Neither drug is recommended for prophylaxis of PEP (Recommendation grade A).

There is no evidence that glucocorticoids, drugs reducing sphincter of Oddi pressure (other than nitroglycerin), antioxidants, heparin, interleukin-10, or some anti-inflammatory drugs (other than diclofenac and indomethacin), such as pentoxifylline, semapimod and the recombinant platelet activating factor acetylhydrolase reduce the incidence of PEP (Evidence grades from 1-to 1 + +). None of these drugs is recommended for PEP prophylaxis (Recommendation grade A).

There is no evidence that the incidence of PEP is influenced by patient position during ERCP (Evidence level 2 + +). Therefore, no recommendation is made regarding patient position.

Trauma resulting from repeated attempts at biliary cannulation has been proven to be a risk factor for the development of PEP (Evidence level 2 + +). The number of cannulation attempts should be minimized. (Recommendation grade A).

Injection of contrast medium into the pancreatic duct is an independent predictor of PEP (Evidence level 1 +). If pancreatic duct injection occurs incidentally or is required, the number of injections and volume of contrast medium injected into the pancreatic duct should be kept as low as possible (Recommendation grade B).

Compared to traditional, high-osmolality contrast agents, low-osmolality contrast agents are costlier but without reduction in the rates of PEP (Evidence level 1-). The routine use of these agents for ERCP is not recommended (Recommendation grade B).

Use of CO₂ as a replacement of air for luminal insufflation during ERCP does not influence the incidence of PEP but decreases the incidence and severity of post-procedure abdominal pain (Evidence level 1 +). CO₂ is recommended for insufflation, and might be particularly useful for outpatient ERCs to reduce pain and to avoid confusion with PEP (Recommendation grade B).

For deep biliary cannulation, the wire guided technique reduces the risk of PEP and increases the success rate of primary cannulation when compared with the standard contrast-assisted method (Evidence level 1 + +). The wire guided technique is recommended for deep biliary cannulation (Recommendation grade A).

The incidence of post-sphincterotomy pancreatitis is not influenced by the type of electrosurgical current used (whether pure-cut or blended) (Evidence level 1 +). Blended current is recommended for biliary sphincterotomy, particularly in patients at high risk of bleeding (Recommendation grade A).

Data about the usefulness and safety of pancreatic guidewire placement to facilitate biliary cannulation in difficult cases are conflicting. Prophylactic pancreatic stent placement decreases the incidence of PEP with this technique (Evidence level 2 +). Pancreatic guidewire-assisted biliary cannulation may facilitate biliary cannulation mostly in case of inadvertent but repeated cannulation of the pancreatic duct ; if this method is used, prophylactic pancreatic stent placement should be performed. (Recommendation grade B).

Various techniques of precut biliary sphincterotomy have been described ; the fistulotomy technique may present a lower incidence of PEP than standard needle knife sphincterotomy but further RCTs are required to determine which technique is safer and more effective, based upon the papillary anatomy. There is no evidence that the success and complication rates of biliary precut are affected with the level of endoscopist experience in this technique but published data only report on the experience of one endoscopist (Evidence level 2-). Prolonged cannulation attempts using standard techniques may impart a risk for PEP greater than the precut sphincterotomy itself (Evidence level 2 +). Precut sphincterotomy should be performed by endoscopists with expertise in standard cannulation techniques (Recommendation grade D). The decision to perform precut biliary sphincterotomy, the timing, and the technique are based on anatomic findings, endoscopist preference and procedural indication (Recommendation Grade C).

Compared to endoscopic sphincterotomy, endoscopic papillary balloon dilation (EPBD) using small caliber balloons (≤ 10 mm) is associated with a significantly higher incidence of PEP and significantly less bleeding (Evidence level 1 + +). EPBD is not recommended as an alternative to sphincterotomy in routine ERCP but may be useful in patients with coagulopathy and altered anatomy (e.g., Billroth II). (Recommendation grade A). If balloon dilation is performed in young patients the placement of a prophylactic pancreatic stent should be strongly considered (Evidence level 4, Recommendation grade D).

Potential advantages of performing large balloon dilation in addition to endoscopic sphincterotomy for extraction of difficult biliary stones remain unclear (Evidence level 3). Endoscopic sphincterotomy plus large balloon dilation does not seem to increase the risk of PEP and can avoid the need for mechanical lithotripsy in selected patients but not enough data are available to recommend routine use over biliary sphincterotomy alone in conjunction to lithotripsy techniques (Recommendation grade D).

In patients undergoing pancreatic sphincter of Oddi manometry, the standard perfusion catheter, without an aspiration port has been shown to increase the risk of PEP compared to modified water perfusion catheters (Evidence level 2 + +). Pancreatic sphincter of Oddi manometry should be performed with a modified triple lumen perfusion catheter with simultaneous aspiration or a microtransducer catheter (non-water perfused) (Recommendation grade B).

Prophylactic pancreatic stent placement is recommended to prevent PEP in patients who are at high-risk for development of PEP. Short, 5-French diameter, plastic pancreatic stents with no internal flanges are currently recommended. Passage of the stent from the pancreatic duct should be evaluated within 5 to 10 days of placement and retained stents should be promptly removed endoscopically (Evidence level 1 + ; Recommendation grade A.)

Conclusion : For high-risk ERCPs, including ampullectomy, pancreatic sphincterotomy, precut biliary sphincterotomy, known or suspected sphincter of Oddi dysfunction, pancreatic guidewire-assisted biliary cannulation and endoscopic balloon sphincteroplasty, prophylactic pancreatic stent placement should be considered. For low-risk ERCPs, periprocedure rectal administration of NSAIDs is recommended.

References

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Table 1. — **Independent risk factors for PEP***

| | Adjusted odds ratios (95% CI in parentheses except where indicated otherwise) | Pooled incidence of PEP in patients with vs. those without risk factor |
|---------------------------------------|--|---|
| Patient-related risk factors | | |
| – Definite risk factors | | |
| • Suspected SOD | 4.09 (3.37-4.96) | 10.3% vs. 3.9% |
| • Female gender | 2.23 (1.75-2.84) | 4.0% vs. 2.1% |
| • Previous pancreatitis | 2.46 (1.93-3.12) | 6.7% vs. 3.8% |
| – Likely risk factors | | |
| • Younger age | 1.09-2.87 (range, 1.09-6.68) | 6.1% vs. 2.4% |
| • Non-dilated extrahepatic bile ducts | NR | 6.5% vs. 6.7% |
| • Absence of chronic pancreatitis | 1.87 (1.00-3.48) | 4.0% vs. 3.1% |
| • Normal serum bilirubin | 1.89 (1.22-2.93) | 10.0% vs. 4.2% |
| Procedure-related risk factors | | |
| – Definite risk factors | | |
| • Precut sphincterotomy | 2.71 (2.02-3.63) | 5.3% vs. 3.1% |
| • Pancreatic injection | 2.2 (1.60-3.01) | 3.3% vs. 1.7% |
| – Likely risk factors | | |
| • High n° of cannulation attempts** | 2.40-3.41 (range, 1.07-5.67) | 3.7% vs. 2.3% |
| • Pancreatic sphincterotomy | 3.07 (1.64-5.75) | 2.6% vs. 2.3% |
| • Biliary balloon sphincter dilation | 4.51 (1.51-13.46) | 9.3% vs. 1.9% |
| • Failure to clear bile duct stones | 3.35 (1.33-9.10) | 1.7% vs. 1.6% |

CI, confidence interval ; NR, not reported ; PEP, post-ERCP pancreatitis.

*For definite risk factors, adjusted odds ratios and pooled incidences of PEP are reproduced from Masci et al ; for likely risk factors, adjusted odds ratios are reproduced from included studies that identified the characteristic as an independent risk factor while pooled incidences were calculated using figures available in all of included studies that provided sufficient data for calculation (see text for details about included studies).

**High (vs. low) n° of cannulation attempts was defined as a number of attempts before final cannulation of the desired duct > 5 or > 1, depending on the studies.

SPONTANEOUS DISLODGE MENT RATE AND CLINICAL IMPLICATIONS OF PROPHYLACTIC PANCREATIC STENTS. J. Rigaux, M. Arvanitakis, M. Delhaye, J. Devière. ULB Erasme, Brussels, Belgium.

Introduction : Prophylactic placement of pancreatic duct (PD) stent reduces the risk of post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) in high-risk patients.

Aim : The aim of this study was to prospectively evaluate the spontaneous dislodgement rate and the clinical implications of prophylactic PD stents in a tertiary centre.

Methods : All patients receiving a prophylactic PD stent between January 2009 and July 2010 were contacted after the ERCP to be included in the study. All stent were 5 French, straight, and unflanged. Clinical features, stent length and stent indications were recorded. Abdominal radiographs were performed 48 hours, 4 days and 6 days following stent placement to assess spontaneous stent dislodgement. If the stent was still present 6 days after placement, it was removed by endoscopy. The rate of PEP was recorded. The incidence of PD stent-induced ductal injury was evaluated for the patients having undergone a MRCP previously to the ERCP.

Results : On about 1600 ERCP performed during this period, 97 procedures with indication of prophylactic stent were identified. Sixty patients received a prophylactic PD stent and 45 of them were included (62.2% female, mean age 55.1 years). The most common indications for prophylactic stent placement were pancreatic sphincterotomy, multiple PD injections, ampullectomy, and recurrent acute pancreatitis. Prophylactic stent length varied from 2.5 cm to 7 cm. PEP occurred in 8 patients (17.7%), with most cases (5/8) being mild and none severe. The overall spontaneous dislodgment rate observed after 6 days was 71.1% (32/45), with 31.3% (10/32) of the stents migrated within 2 days, 34.3% (11/32) within 4 days and 34.3% (11/32) within 6 days. There was no significant association between PEP occurrence and spontaneous stent dislodgement. However, spontaneous dislodgement was significantly more frequently observed with shorter ($=$ or $<$ 4 cm) stents ($p = 0.04$). MRCP was available in 28 patients previously to the ERCP ; 11 of these patients had a MRCP performed (mean interval, 44,1 days) after the ERCP. In 91% (10/11) of the patients, the calibre of the PD was unchanged, while one patient developed a complete PD obstruction in the body not present before the placement of the prophylactic stent.

Conclusion : Indications for prophylactic PD stents remain uncommon (97/1600, 6.06%) and its placement is performed in two thirds of these cases. PEP rate remains high but most episodes observed were mild. The spontaneous stent dislodgment rate was lower than previously described but was more frequently observed with shorter stents. Stent-induced ductal injury observed after such a short interval seems uncommon but caution should be used due to the small sample size of the study.

CYSTIC MASSES OF THE PANCREAS : HOW USEFUL IS CYST FLUID ANALYSIS IN THE DIAGNOSIS ?

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Introduction : Evaluation of the cystic masses of the pancreas is a challenge in the routine daily practice. Pancreatic cyst fluid analysis seems to have an added value in the differential diagnosis, but the utility in the daily practice is questioned.

Aim : The objective of this study was to investigate the accuracy and value of cyst fluid analysis in the differentiation of benign from (pre-)malignant masses of the pancreas in our center.

Methods : A prospectively maintained registry of patients who underwent EUS FNA with cyst fluid aspiration between 2007 and 2010 was reviewed. Data collected included clinical history with imaging, FNA cytology and definitive pathology if available. CEA, amylase, lipase and CA19.9 in the cyst fluid was determined, if enough fluid could be aspirated. CEA > 192 ng/ml was considered as a premalignant or malignant cystic lesion. Amylase > 5000 U/L with low CEA was diagnostic for a pseudocyst.

Results : We performed a total cystic fluid analysis in 54 patients. There were an equal number of male and female patients with a mean age of 61,2 yrs. Mean cyst diameter was 3,5 cm (range : 0,9 cm-9 cm). In 20 patients we had a definitive pathological diagnosis : benign pathology in 7 patients, potential malign in 2 patient and malign pathology in 11 patients. Cyst fluid analysis predicted benign pathology in 6/7 patients and non-benign pathology in 11/13 patients (sens : 84%, spec : 85%, accuracy : 85%) . Pseudocyst was correctly diagnosed in all 4 patients. In patients with cystic neoplasms the cyst fluid analysis could differentiate mucinous from non-mucinous lesions in 3/4 patients. In patients with cystic malignant lesions we found high CEA levels with a mean of 3472 ng/ml (range : 446-10652). 7/11 patients had a CEA > 1000 ng/ml. 2/11 patients in this group had a normal CEA. In 15 additional patients without a definitive surgical pathology, cystic pancreatic lesions could be considered as a pseudocyst due to a typical clinical presentation, imaging results and follow up. In 17/19 pseudocysts (4 operated and 15 typical cases), the cyst fluid analysis confirmed the diagnosis.

Conclusion : Cyst fluid analysis helps to differentiate benign (pseudocyst, serous cystadenoma) from non-benign pancreatic masses (mucinous cystadenoma, IPMN, cystic adenocarcinoma and cystic neuroendocrine tumors) in our

center. Results of cyst fluid analysis are very accurate in differentiating pseudocysts from other cystic pancreatic lesions. In possible malignant cysts, a high CEA value in the cyst fluid can be helpful in the diagnosis if cytology and imaging are inconclusive.

- T04 -

EARLY AND LATE COMPLICATIONS AFTER CORPOROCAUDAL AND DISTAL PANCREATECTOMY WITH OR WITHOUT SPLENIC VESSEL PRESERVATION. M. Van Lieshout, F. Berrevoet, A. Vanlander, I. Adriaensens, L. De Caluwé, O. Uyttebroek, K. Geboes, X. Rogiers, R. Troisi. UZ, Gent, Belgium.

Introduction : During corporocaudal - and distal pancreatectomy (CCDP) it is possible to preserve the spleen with preservation of the splenic vessels or to perform splenectomy as well. The theoretical advantages for spleen preservation both in benign and malignant disease are clear.

Aim : the aim of this study is to review our experience with spleen preserving CCDP.

Methods : Sixty-four patients who underwent CCDP with/without splenectomy were enrolled as study group. Thirty patients were operated for malignancy, while 34 patients underwent a CCDP operation for benign or pre-malignant disease. All patients underwent CT scan or MRI of the abdomen pre-operatively. Patient characteristics, surgical morbidity, quality of life and survival were evaluated.

Results : All thirty patients treated for adenocarcinoma had an associated splenectomy with resection of the splenic vessels for oncological reasons. In the benign or pre-malignant group splenic preservation was attempted in all cases. Three patients were operated laparoscopically. In the postoperative course only one patient developed a splenic infarction and had to be re-operated having a splenectomy. Both in open and laparoscopic surgery the morbidity rate was low with acceptable quality of life in the non-malignant group of patients

Conclusions : In our experience, spleen preserving CCDP is a safe and feasible operation, but spleen preservation per se represents a condition possibly leading to specific early and late complications.

According to the literature, spleen preserving CCDP with sacrifice of the splenic vessels doubles the complication rate in the long term follow-up so that this technique should not be applied as routine procedure.

PANCREATIC SURGERY : A 10-YEAR EVALUATION IN A TERTIARY REFERRAL CENTER. J. Bontinck, F. Berrevoet, M. Van Lieshout, K. Van Den heede, M. Beysens, S. Laurent, X. Rogiers, R. Troisi. UZ, Gent, Belgium.

Introduction : Advances in imaging, minimally invasive techniques, and regionalization have changed pancreatic surgery. Therefore, the aims of this report are to determine what the expectations for referring doctors and their patients might be regarding the outcome of pancreatic operations for carcinoma in a high-volume center.

Aim : To evaluate the morbidity, mortality and quality of life for patients with pancreatic resections for malignancy.

Methods : From April 2000 through September 2010, 377 pancreatic resections for malignant disease were performed at the University of Ghent Hospital. Besides patient characteristics, the type of surgical procedures, pathology reports regarding TNM and R status of resection, surgical and overall morbidity, the use of adjuvant therapy and short-term outcome and quality of life were evaluated.

Results : In the 5-year period 2005-2010, more operations were performed (n = 133 vs n = 240), while the mean age did not change (66 years \pm 11 y). Most operations performed consisted of a pancreaticoduodenectomy in both time era's and the number of pylorus-preserving procedure did not change (56% vs. 58%). However, during recent years more venous reconstructions (17% vs 11%) and less surgical re-interventions were performed. Superior mesenteric artery involvement is up to today still considered a contra-indication for surgical resection. The treatment of complications by interventional radiology increased over the years. In-hospital mortality improved to less than 0.8% in 2009.

Conclusions : At a high-volume pancreatic surgery center, the number of treated patients, the characteristics of the tumor, the percentage of resectability, even with vascular involvement, all have increased, whereas the outcome continued to improve.

POSTOPERATIVE OUTCOME IN PATIENTS WITH INTRADUCTAL PAPILLARY MUCINOUS NEOPLASIA OF THE PANCREAS AND PANCREATIC NEUROENDOCRINE TUMORS. F. Puleo (1), N. Van Damme (2), M. Arvanitakis (1), J. Closset (1), P. Demetter (1), F. Berrevoet (2), K. Geboes (2), E. Cesmeli (2), J.L. Van Laethem (1), M. Delhaye (1). (1) ULB Erasme, Brussels, Belgium ; (2) University Hospital, Ghent, Belgium.

Introduction : Intrapapillary mucinous neoplasia (IPMN) of the pancreas and pancreatic neuroendocrine tumors (PNET) are two rare forms of pancreatic lesions which, compared to pancreatic adenocarcinoma, have a low malignant potential and a relative indolent progression.

Aim : The aim of the study is to describe clinicopathological features and outcomes after surgery in both operated IPMN and PNET.

Methods : The files of 110 patients (34 IPMN and 76 PNET) operated between 2/1999 and 6/2010 in two tertiary academic hospitals were reviewed retrospectively. Data collected included general demographic data, type of surgery, presence of malignancy, postoperative complications and mortality.

Results : The study group included 64 (58.2%) men and 46 (41.8%) women, with a median age of 57 years (28-81) ; 61 years for IPMN and 56 for PNET (p = 0.033). In the whole series, 78% of patients presented with symptoms (IPMN 97%, vs. PNET 67% ; p = 0.012). The median interval between symptoms and surgery was 19 (1-372) months for IPMN and 30 (1-52) months for PNET (p = 0.02).

Type of surgery for IPMN and PNET was respectively : pancreaticoduodenectomy 62% (n = 21) and 24% (n = 18), segmental pancreatectomy 18% (n = 6) and 31% (n = 24), distal pancreatectomy 20% (n = 7) and 18% (n = 14), enucleation 0% and 17% (n = 13), total pancreatectomy 0% and 2% (n = 2).

The pathological analysis revealed 9 cases (26%) of malignant IPMN (6 in situ carcinoma and 3 invasive carcinoma). All the operated IPMN patients had a R0 type resection. In PNET, nodal and liver metastasis were detected in 19% of cases and 69 cases had a R0 resection (90%).

Postoperative complications occurred globally in 53% of the cases (IPMN 69% vs. PNET 45%, p = 0.016). Local complications for IPMN and PNET were respectively : collections 22% (n = 7) and 20% (n = 14), fistula 9% (n = 3) and 2% (n = 2), bleeding 3% (n = 1) and 4% (n = 3), wound infection 0% and 1% (n = 1). Globally, systemic complications were sepsis 2.9% (n = 3) and pneumonia 4.9% (n = 5). Eighteen patients needed reintervention (IPMN 23% ; PNET

13%, $p = \text{NS}$); type of reintervention was endoscopy (7 patients, 6.7%), radiology (3 patients, 2.9%) and surgery (8 patients, 7.7%). Length of stay was 15 days (8-111), without any significance difference between the two groups. Late complications were new diabetes 10.5%, new exocrine failure 8%, gastroparesis 1.5% and other 5.3%. We didn't register any death in the perioperative period and at the 30 postoperative day. During a median follow up of 25 months obtained for 83 patients, mortality during FU was 8.4% ($n = 7/83$) (IPMN 4% vs. PNET 10%, $p = \text{NS}$).

Conclusions : Surgery is proposed for the management of IPMN and is also recommended for all PNET, because of their potential malignancy. In this retrospective series, pathological analysis revealed more than 70% of benign disease. However, the early surgical morbidity has to be taken into consideration for each individual patient.

- T07 -

MOLECULAR CHANGES IN PANCREATIC CANCER : IMPLICATIONS FOR MOLECULAR TARGETING THERAPY. P. Demetter, R. Maréchal, J.L. Van Laethem. ULB Erasme, Brussels, Belgium.

Pancreatic ductal adenocarcinoma (PDAC) is by far the most frequent type of pancreatic cancer accounting for approximately 85% of all pancreatic cancers. Despite curative surgery, which concerns only 10% to 15% of patients at the time of diagnosis, the 5-year overall survival is less than 5%. New strategies and therapies are urgently needed whereas we also need to identify new prognostic and predictive biomarkers.

Chemokines and their receptors are implicated in the development of different types of cancer. Recent research has shown that CXCR4 and CXCR7 could be attractive targets for PDAC therapy. Small molecule CXCR4 antagonists are currently being tested in phase I/II clinical trials and could be attractive therapeutic candidates to combine with gemcitabine, a 2'2'-difluoro-2-deoxycytidine analogue that inhibits DNA replication and repair. Human equilibrative nucleoside transporter 1 (hENT-1) and human concentrative nucleoside transporter (hCNT) 1 and 3 are the major transporters responsible for gemcitabine uptake into cells. Emerging results suggest the prognostic value of hENT1 and hCNT3 protein expression in resected pancreatic cancer patients treated with gemcitabine monotherapy followed by concomitant radiotherapy and gemcitabine. These translational data suggest that the combination of hENT1 and hCNT3 expression in tumour cells plays an important role in patients' clinical outcome after gemcitabine-based adjuvant therapy.

In the absence of more effective drugs, one possible strategy to improve PDAC patient's outcome would be to overcome the resistance to gemcitabine. Cellular resistance to gemcitabine treatment may be an initial property (intrinsic resistance), but can also be acquired during gemcitabine treatment. For this aim, detailed knowledge on the regulation of hENT1, hCNT3 and other metabolizing enzymes is clearly mandatory. On the other hand, modifications of gemcitabine not rendering it dependent on nucleoside transporters may be a successful future mode of chemotherapy treatment.

LYMPHOEPITHELIAL CYST OF THE PANCREAS : A RARE MIMICKER OF OTHER FOCAL PANCREATIC LESIONS. L. Verset, M. Arvanitakis, M.A. Bali, C. Matos, P. Loi, J. Closset, M. Delhay, P. Demetter. ULB Erasme, Brussels, Belgium.

A 49-years old man came to the Erasme Hospital because of renal colics. In his medical history, we noted hyperlipidemia and viral hepatitis. He did not use alcohol. Laboratory data were normal except for CA19.9 reaching 291 U. Abdominal tomography showed a pancreatic cystic lesion. An echoendoscopy was performed with fine needle aspiration of the cyst fluid ; analysis revealed a CEA level of 29100 ng/ml but no neoplastic cells. A duodenopancreatectomy was realised and macroscopic examination of the Whipple specimen showed a cystic lesion filled with keratin. Microscopic analysis confirmed the diagnosis of lymphoepithelial cyst.

A 36-years old woman was admitted to the Erasme Hospital in April 2005 for a first episode of acute pancreatitis which was complicated by a caudal pseudocyst that resolved spontaneously. Magnetic resonance cholangiopancreatography showed, however, a retrogastric cystic lesion estimated to 36 mm in diameter. The CA19.9 level was normal. Echoendoscopic guided fine needle aspiration cytology was in favour of a pseudocyst. Two other episodes of acute pancreatitis were observed in April 2006 and in February 2010. The cause of the pancreatitis remained indetermined. In April 2010, abdominal tomography highlighted a calcification estimated to 11 mm of diameter, and persistence of the retrogastric cystic lesion which seemed to have increased slightly in size. Thus, a caudal pancreatectomy was realized and the pathologic assessment revealed also a lymphoepithelial cyst.

Lymphoepithelial cysts of the pancreas are benign and extremely rare. As illustrated here, these cysts can mimick other focal pancreatic lesions and different clinical presentations are possible ; this makes a preoperative diagnosis difficult. Lymphoepithelial cysts should, however, be considered in the differential diagnoses of cystic lesions, even in the presence of high intracystic CEA levels.

RARE AND MORE COMMON CAUSES OF ACUTE PANCREATITIS : A DIAGNOSTIC ROLE FOR EUS AND ERCP. P. Deprez. Saint-Luc University Hospital, Brussels, Belgium.

DIABETES, OBSTRUCTIVE JAUNDICE AND PANCREATIC TUMOR IN A 76 YEAR-OLD WOMAN. M. Delhaye, M. Arvanitakis, M.A. Bali, C. Matos, L. Verset, P. Demetter, J.L. Van Laethem, E. Cogan, J. Devière. ULB Erasme, Brussels, Belgium.

Diagnosis of focal pancreatic lesions in the pancreas is sometimes difficult even in the era of advanced imaging procedures.

We report the case of a 76 year-old woman who presented with epigastric and right upper quadrant pain for 2 months. She developed jaundice, pruritus and slight fever. She had lost 11 kg in 2 y but her BMI was still 30 kg/m².

In her previous history, she reported diabetes type II, dyslipidemia, hypertension, discoid lupus treated by Chloroquine during 20 years and then stopped because of a drug-induced macular degeneration. She had stopped smoking 10 y ago and she did not drink alcohol.

Laboratory data showed increase of serum bilirubin (8.8 mg/dl), alkaline phosphatase (at 10 × N), gGT (at 20 × N), AST (at 8 × N) and ALT (at 12 × N). CRP was 10 mg/dl. Pancreatic enzymes and tumoral markers CEA and CA 19.9 were normal.

Imaging procedures (CT/EUS) displayed a mass in the head of the pancreas, and peripancreatic lymph nodes. EUS-guided FNA was not contributive. During ERCP, a stricture in the distal CBD was demonstrated and a biliary stent was inserted.

A duodenopancreatectomy was proposed but the patient left the hospital against medical advice.

Two years later, she developed hemiparesis and pain on the right side of the body diagnosed as pseudothalamic pain syndrome. Her BMI was at 27 kg/m².

Laboratory data showed normal bilirubin and liver tests, and normal pancreatic enzymes.

Imaging procedures (CT/MRI) revealed severe atrophy of the pancreatic parenchyma without any mass identified in the head.

A diagnostic procedure was performed.

BSGIE / SMALL BOWEL GROUP

Invited lecture

- V01 -

ENDOSCOPY AND ANTIPLATELET AGENTS : THE ESGE 2011 GUIDELINE. C. Boustière. Saint Joseph Hospital, Marseille, France.

- V02 -

VALUE OF PREOPERATIVE ESOPHAGOGASTRODUODENOSCOPY IN MORBIDLY OBESE PATIENTS UNDERGOING LAPAROSCOPIC ROUX-EN-Y GASTRIC BYPASS. M. Dhondt, M. Steverlynck, B. Deylgat, H. Pottel, D. Devriendt, F. Van Rooy, F. Vansteenkiste. AZ Groeninge, Kortrijk, Belgium.

Introduction : Morbid obesity is associated with different concomitant diseases, such as arterial hypertension, diabetes mellitus, coronary heart disease, sleep apnea syndrome and pathological conditions in the upper gastrointestinal tract. Therefore, all patients undergo esophagogastroduodenoscopy (EGD) prior to bariatric surgery in our Hospital.

Aim : The main outcome of this study was to determine the prevalence of clinically significant preoperative endoscopic findings in morbidly obese patients undergoing primary laparoscopic Roux-en-Y gastric bypass (pLRYGB) and to determine the proportion of patients who had a change in surgical management.

Methods : We evaluated records of patients undergoing EGD prior to pLRYGB between August 2003 and November 2009 at the Groeninge Hospital, Kortrijk. The prevalence of endoscopic findings of clinical significance was determined.

Results : Six hundred and fifty two patients (462 females and 190 males) patients underwent pLRYGB at the Groeninge Hospital. The mean age was 39.5 + 11.3 years and mean body mass index was 42.8 + 5.0 kg/m². Findings at EGD were hiatal hernia 24.3% (n = 159), esophagitis 30.8% (n = 201), Barret esophagus 0.8% (n = 5), gastritis 36.2% (n = 236), gastric or duodenal ulcers 7.5% (n = 49), gastric cancer was found in 2 patients. The prevalence of H. pylori infection was 17.6%. In 23.0% of patients bariatric surgery was postponed or surgical management was changed as a consequence of endoscopy.

Conclusion : Routine preoperative EGD detects different abnormalities which need specific approach prior to bariatric surgery. EGD should be included in the preoperative workup prior to pLRYGB since surgery was postponed or surgical management was changed in almost a quarter of our patients.

LOWER GASTROINTESTINAL ENDOSCOPY AMONG SYMPTOMATIC HIV-INFECTED PATIENTS IN THE HAART ERA. M. Nkuize, R. Ntounda, V. Muls, S. De Wite, Y. Nang, J. Mulkay, R. Saidi, T. Sersté, M. Vangossum, H. Tibary, M. Buset. CHU Saint-Pierre, Brussels, Belgium.

Introduction : Since the advent of HAART in 1996, survival of HIV-infected patients has significantly increased. Since that time, many studies have reported an increased prevalence of non-AIDS cancer. However, no Belgian trial focusing on colorectal or anal disease has been carried out in HIV-infected patients.

Aim : Thus, the aim of our study was to describe colorectal and anal diseases among symptomatic HIV-infected patients undergoing lower gastrointestinal endoscopy (LGIE) and/or proctologic examination (PE).

Methods : This was an observational, longitudinal study performed from January 2004 to March 2010 (All HIV-infected patients with a complaint who underwent LGIE and PE were included. Parameters studied were : demographics, HIV risk behavior, CD4 count, HIV viral load, symptoms, and endoscopic, histological, and microbiological diagnoses. Patients were stratified according to CD4 counts (> 200) and data were compared.

Results : Among the 183 patients, 127 underwent LGIE (24 rectoscopies, 10 left colonoscopies, 93 ileo-colonoscopies) and 203 underwent PE. Main characteristics included : mean age 34 years, 123 male, mean BMI 23,7, heterosexuality or homosexuality and IV drug use as HIV risk behavior, median viral load 200 cells/ μ L main diagnoses were normal colonoscopy, hemorrhoidal disease, proctitis, ulcerated recto-colitis, and anal cancer.

Conclusion : In the HAART era, LGIE and PE show varied diagnoses. The worst diagnoses were ulcerated recto-colitis, and anal cancer in patients with a better immunity. The most frequent diagnoses were normal colonoscopy, benign polyps, hemorrhoidal disease, infectious proctitis and anal condyloma. Proctologic complaints and examinations were frequent in our patients.

ENDOSCOPIC MUCOSAL RESECTION IN THE ESOPHAGUS : HOW LONG IS THE LEARNING CURVE ? R. Bisschops (1), J. Bergman (2), H. Willekens (1), G. Coremans (1), P. Rutgeerts (1). (1) UZ Leuven Department of Gastroenterology, Leuven, Belgium ; (2) Academic Medical Center, Amsterdam, Netherlands.

Introduction : Endoscopic resection (ER) is the first choice treatment for early mucosal esophageal neoplasia. Little is known however on the learning curve for this procedure.

Aim : The aim of this study was to assess the technical learning curve for esophageal ER in the setting of a dedicated training for endoscopists and nurses.

Methods : Cap-ER or multiband mucosectomies were performed by one endoscopist and a single nurse who received training in a structured dedicated training program (www.endosurgery.nl) starting in January 2007. Previous experience in ER was limited to 20 procedures. The training programme consisted of 4 days theoretical background and hands-on training on animal models. Individual hands-on training on patients was provided during 4 days. The first 20 ERs were recorded for written feedback by the trainer (JB). All procedures were prospectively recorded for procedural time, complications and oncological outcome. ERs were divided into time-blocks of 30 or 20 procedures for comparison. Total endoscopy time, total ER time, total number of pieces, number of complications and time needed per resected specimen were calculated. Data were compared using ANOVA and Turkey-Kramer (TK) tests for multiple comparisons.

Results : Between March 2007 and September 2010, 124 esophageal ERs were performed by a single endoscopist and a single nurse, after entering a dedicated training program. 6 of these procedures had to be abandoned because of non-lifting sign. Total procedure time, total ER time and the mean number of resected pieces differed not significantly between the different 30 or 20 procedure time-blocks. However, mean time needed per resected specimen was significantly increased during the first 30 procedures (19.04 ± 2.1 min vs 11.41 ± 1 ; 11.18 ± 0.88 and 13.10 ± 0.9 min for the second, third and fourth block of 30 procedures (One way Anova $p = 0.002$; TK test $p < 0.001$; $p < 0.001$ and $p < 0.05$ respectively). There was no significant difference for this parameter between procedure 31-60, 61-90, and 91-118. The number of complications in the different groups was comparable (13,11,11 and 9 respectively) and were mainly minor bleedings. Even after correction for a perforation that occurred during the first part of the learning curve the difference remained statistically significant. A similar calculation using blocks of 20 ERs showed a significant decrease in mean time per resected specimen after 40 ERs (One-way Anova $p = 0.0015$).

Conclusion : According to the time needed per resected specimen, we conclude that it takes 30-40 procedures to reach the plateau on the learning curve for esophageal ER.

SUCCESS RATE OF CECAL INTUBATION WITH THE SINGLE-BALLOON ENTEROSCOPE AFTER INCOMPLETE CONVENTIONAL COLONOSCOPY. E. Macken, P. Pelckmans, T. Moreels. Antwerp University Hospital, Antwerp, Belgium.

Introduction : Single-balloon enteroscopy (SBE) enables deep endoscopic intubation of the gastrointestinal tract, even resulting in complete visualisation of the small bowel. This concept of push-and-pull endoscopy may also enable visualisation of the entire colon after former incomplete conventional colonoscopy.

Aim : We performed push-and-pull endoscopy of the colon using the Olympus SIF Q180 SBE after unsuccessful conventional colonoscopy.

Methods : 10 consecutive patients with former incomplete conventional colonoscopy (using 130 cm long colonoscope) due to redundant colon, sigmoid loop formation or diverticula, underwent SBE colonoscopy. Success rate, time and length to reach the cecum and dose of sedation were recorded.

Results : The male female ratio was 3/7 with a mean age of 61 ± 5 years. Cecal intubation was achieved in 8 patients (80%) in a mean time of 15 ± 3 minutes. The mean working length of the endoscope to reach the cecum was 114 ± 4 cm. Two SBE procedures failed to reach the cecum due to pain in one patient and sigmoid diverticula in the other. All procedures were performed under conscious sedation using midazolam with or without fentanyl. Comparison of the dose of midazolam and fentanyl in patients who underwent both conventional and SBE colonoscopy procedures under conscious sedation, showed that SBE colonoscopy does not require more sedation than conventional colonoscopy. SBE colonoscopy was able to diagnose colonic cancer and polyps in the right colon and Crohn's disease in the terminal ileum. Additional interventions like polypectomy and mucosal biopsy were performed in 6/10 patients (60%).

Conclusion : SBE colonoscopy is a valuable alternative to successfully perform complete colonoscopy after former failure to intubate the cecum due to long redundant colon or loop formation. We showed that this is not because of the longer length of the SBE enteroscope or because of deeper conscious sedation. It is the concept of push-and-pull endoscopy with a balloon- and overtube-loaded endoscope that allows to overcome the difficulties due to redundant colon and loop formation. In 60% of the patients additional endoscopic interventions were performed during SBE colonoscopy.

Invited lecture

RADIOFREQUENCY ABLATION FOR BARRETT'S ESOPHAGUS. THE BELGIAN SITUATION. R. Bisschops, KULeuven.

Invited lecture

-V07-

COMPARATIVE OVERVIEW OF SINGLE-BALLOON, DOUBLE-BALLOON AND SPIRAL ENTEROSCOPY.
H. Aktas. Erasmus Medical Center, Rotterdam, Netherlands.

During the past 10 years several techniques are introduced for the diagnostic and therapeutic approach of the small bowel, including balloon-assisted enteroscopy (BAE) using 2 balloons (double-balloon enteroscopy [DBE]) or 1 balloon (single-balloon enteroscopy [SBE]) and spiral enteroscopy (SE). Although DBE has widely been introduced, some issues hamper its daily clinical use. Preparation and handling of the DB endoscope are complex and cumbersome, involving attachment of a balloon to the tip of the endoscope as well as in- and deflation of a two-balloon system. In 2008, single-balloon enteroscopy (SBE) was introduced as an alternative balloon-assisted enteroscopy system for deep small-bowel exploration. The advantage of this system is its simplified design, making it easier to use and less time consuming in preparation compared with the DBE system. However, SBE may be less efficient for deep intubation of the small bowel as compared to the DBE system, and may cause adverse effects due to the hooking technique during straightening of the SB endoscope. SE is the latest deep small-bowel enteroscopy system to be introduced. In contrast to the BAE techniques, which follow the push-and-pull principle, this new enteroscopic technique pleats the small bowel by rotating. SE represents to be a promising method. The first studies have shown that SE is effective and safe method for deep small-bowel enteroscopy. In the future, larger prospective studies comparing the 3 systems (DBE, SBE, and SE) can be useful for making of conclusive assessments.

- V08 -

LOWER RECURRENCE RATE WITH ESD VS. EMR FOR THE CURATIVE TREATMENT OF ESOPHAGEAL SUPERFICIAL SQUAMOUS CELL CARCINOMA. P. Deprez, R. Altwegg, A. Jouret-Mourin, C. Sempoux, R. Yeung, T. Aouattah, H. Piessevaux. Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Introduction : The role of endoscopic submucosal dissection (ESD) in squamous cell carcinoma is still discussed in Europe with many centres proposing piecemeal EMR, although many studies from Japan showed the importance of R0 that can only be achieved by en-bloc resection with free deep and lateral margins.

Aim : The aim of our study was to analyse our experience with ESD and to compare these with historical results of piecemeal EMR.

Methods : EMR and ESD have been performed for superficial squamous cell cancer since Dec 1999 and Jan 2007, respectively. Follow-up was done at 2, 6, 12 months after resection, then annually. Results are expressed as median and range and statistical analysis done by Mann Whitney U-test and chi square.

Results : 60 patients (30 pts by EMR since Dec 1999 and 30 pts by ESD since Jan 2007). No significant difference could be seen in terms of age, sex, Paris classification, tumor localisation, and R0 resection (table 1). Complications rate was similar : no perforation, no early or delayed bleeding, 17-20% strictures, with 4 pts with severe fibrosis (type 3) due to previous EMR, eosinophilic esophagitis, radiotherapy or surgery. In the ESD group, 6 lesions were HGIN, 3 pT1m2, 11 pT1m3, 8 pT1sm1, and 2 pT1sm2. In the EMR group, 16 lesions were HGIN, 4 pT1m2, 5 pT1m3, 3 pT1sm1, 1 pT1sm2 and 1 T2. Recurrence rate was significantly lower with ESD (1 case [3%] vs. 8 [26%] with EMR, $p = 0,01$), after a mediantime of 11,5 months [3-53]. Eight pts and 7 received adjuvant therapy, in the ESD and EMR groups respectively.

| | ESD | EMR | P |
|------------------------|---------------|--------------|--------|
| Age | 59y [40-83] | 59y [49-79] | NS |
| Sex | 25M/5W | 19M/11W | NS |
| En-block | 100% | 27% | 0.0001 |
| R0 | 80% | 63% | NS |
| Height of lesions (mm) | 30 [8-90] | 15 [5-50] | 0.004 |
| Surface resected | 775 [96-4160] | 285 [54-900] | 0.0008 |
| Duration (min) | 102 [17-243] | 51 [20-85] | 0.001 |
| Median follow-up (m) | 13.5 | 43.5 | 0.001 |
| Recurrence rate | 3% | 26% | 0.001 |

Conclusion : ESD can be safely performed for the curative treatment of mucosal esophageal squamous cancer, with low rates of bleeding and perforation, higher rates of en-block resection, fewer recurrences compared to piece-meal EMR. We therefore suggest referring those patients to centres with ESD expertise.

- V09 -

COMPARISON OF DOUBLE-BALLOON AND SINGLE-BALLOON ENTEROSCOPE FOR ENDOSCOPIC RETROGRADE CHOLANGIOGRAPHY AFTER ROUX-EN-Y SMALL BOWEL SURGERY. T. Moreels, E. Macken, P. Michiels, G. Hubens, D. Ysebaert, B. Op De beek, P. Pelckmans. Antwerp University Hospital, Antwerp, Belgium.

Introduction : Roux-en-Y reconstructive surgery of the small bowel excludes the afferent limb and the biliopancreatic system from conventional endoscopic access. Therefore, postoperative problems in the biliopancreatic system are often dealt with surgically. Balloon-assisted enteroscopy allows therapeutic endoscopic retrograde cholangiopancreatography (ERCP) in these patients, avoiding rescue surgery.

Aim : We retrospectively compared the use of double-balloon (DBE) and single-balloon enteroscopy (SBE) to perform ERC after Roux-en-Y reconstructive surgery.

Methods : 52 procedures were performed in 37 patients with suspected pathology of the biliary system after Roux-en-Y reconstructive surgery. Both the Fujinon EN-450T5 DBE (since 2005) and Olympus SIF Q180 SBE (since 2008) were used. All procedures were performed under general anaesthesia and fluoroscopic control. Only procedures resulting in therapeutic ERC were considered successful. Dedicated accessory material was used to perform ERC with DBE and SBE.

Results : 31 (60%) procedures were performed with DBE and 21 (40%) with SBE. Overall ERC success rate was 74% for DBE and 71% for SBE ($P > 0.05$ Chi square). Failure was due to inability to reach or cannulate the papilla or bilioenteric anastomosis. Repeated procedures were all performed in patients with stenotic bilioenteric anastomosis. In 4 patients repeated procedures were successfully performed using both DBE and SBE. Success rate was higher (80% vs. 59%, $P < 0.05$ Chi square) when ERC was performed at the bilioenteric anastomosis ($n = 28/35$) as compared to ERC of the native papilla ($n = 10/17$). Therapeutic procedures performed included conventional papillotomy, precut papillotomy, dilation of the papilla or the bilioenteric anastomosis, common bile duct stone extraction using extraction balloon or basket and multiple plastic stent placement and removal. Complication rates were 10% for both DBE and SBE and were all dealt with conservatively : small bowel perforation at the level of the papilla, cholangitis, post-papillotomy bleeding and defect of the endoscope.

Conclusion : ERC after Roux-en-Y reconstructive surgery of the small bowel is feasible and safe using both DBE and SBE. Both techniques show good and comparable success rates (74%-71%) with an acceptable complication rate (10%). ERC at the level of the native papilla is less successful as compared to the biliodigestive anastomosis (59%-80%). Dedicated accessory material is mandatory to perform ERC with DBE and SBE.

- V10 -

GUIDE WIRE ASSISTED AND BALLOON-ANCHORED DIRECT PERORAL CHOLANGIOSCOPY WITH ULTRA SLIM GASTROSCOPE IN HUMANS : PROOF OF PRINCIPAL. P. Bossuyt, P. Cuyle, V. Moons, G. Dhaens, A. Van Olmen, P. Christiaens. Imelda GI Clinical Research Centre, Bonheiden, Belgium.

Introduction : Recent technical advances in endoscopic devices make direct endoscopy of the biliary tract feasible. "Mother and baby" devices give rapid access to the biliary tract. But these systems are limited by the fact that two operators are needed and rather poor image quality is obtained. In contrast direct endoscopy of the biliary tract has the advantage of a single operator platform, equal image quality to standard endoscopy and separate water and air channels. Getting access to the biliary tract by direct endoscopy can be challenging. We tested two guiding systems to overcome this challenge.

Aim : The aim of this study is a proof of principal in humans.

Methods : Direct cholangioscopy was performed in 4 patients. Procedures were performed by the oral route with an ultra slim nasal gastroscope (GIF-XP180N, Olympus). This gastroscope has a diameter of 5.5 mm, a working length of 110 cm and a 2 mm working channel. The image quality and NBI function is equal to the normal GIF-Q180 gastroscope. Initially standard ERCP was performed and cholangiogram was obtained in all patients. Primary goal was successful access to the biliary system. Two guiding systems were tested : a directly inserted very stiff guide wire (THSF-35-480, Cook Medical) and an in-house developed balloon-anchoring system (FS-8.5-12-15-A, Cook Medical) both placed under fluoroscopic control. The balloon was inflated and anchored in the intrahepatic biliary tree. The inflation channel was obstructed with fast acting glue. This made transection of the proximal part possible leaving the balloon inflated. After withdrawal of the duodenoscope the ultra slim gastroscope was inserted over the balloon catheter.

Results : The procedure was successful in four patients. Three patients with bile duct stones, one patient with malignant obstruction due to pancreatic. Biliary access was obtained by a 12 mm sphincteroplasty in one patient and a wide (> 10 mm) sphincterotomy in the other patients. Main obstacle for introduction was the biliary-duodenal angulation of > 120°. Selective cannulation of left and right intrahepatic system and visualization of common bile duct, cystic duct and bifurcation was achieved in three patients (cfr video). In one patient introduction was only possible up to the malignant stenosis. Intraductal biopsies were taken in one patient. Image quality was excellent. There were no complications.

Conclusion : Our results proof the principal that direct peroral cholangioscopy in humans is possible with a ultra slim gastroscope over different simple guiding systems. This extends the working field of the endoscopist for both diagnostic and therapeutic interventions.

ENDOSCOPIC REMOVAL OF A TRANSGASTRIC ERODED LAPAROSCOPIC ADJUSTABLE SILICONE GASTRIC BAND (LASGB). P. Christiaens, P. Bossuyt, V. Moons, G. Van Olmen. Imelda GI Clinical Research Centre, Bonheiden, Belgium.

Introduction : Laparoscopic adjustable gastric banding (LASGB) is a frequently used procedure for bariatric surgery. Trans- or intragastric migration of gastric banding or the erosion of the band through the stomach wall into the gastric lumen is a well known complication. The suggested primary etiologic factor is pressure applied to the gastric wall and the estimated risk is up to 1%.

Aim : A patient, with the suspicion of a band migration due to unexpected weight gain, underwent an upper endoscopy. This showed a partial transgastric migration of the LASGB. Instead of reoperation we considered the endoscopic removal of the partially migrated LASGB.

Methods : Under local anesthesia the injection port and the silicone connecting tube were resected. A standard ERCP guide wire of 4m was inserted between the partially migrated LASGB and the stomach wall and then picked up at the other side of the LASGB with a polypectomy snare and pulled out, creating a loose around the band. The metal spiral sheath of a mechanical ERCP-lithotriptor was passed over both ends of the guide wire, these were winded up until the LASGB was cut. The two pieces of the band were retrieved endoscopically one after the other by using a polypectomy snare. Endoscopic control revealed no complications

Results : Successful endoscopic gastric band retrieval without complications.

Cfr VIDEO.

Conclusion : A minimally invasive technique for band removal after adjustable gastric band migration is described, offering the patient a low-risk procedure. With this minimal invasive procedure a laparoscopic operation can be avoided.