

ABSTRACTS

XXIVth Belgian Week of Gastroenterology 2012

ABSTRACTS

- A01 — A45 Belgian Association for the Study of the Liver (BASL)/Belgian Liver Intestine Committee (BLIC)
- B01 — B20 Research Group “Gastrointestinal Regulatory Mechanisms (OG-FWO)”
- D01 — D14 Plenary Session Belgian Week of Gastroenterology
- E01 — E19 Belgian Group of Pediatric Gastroenterology, Hepatology and Nutrition (BeSPGHAN)
- G01 — G13 Belgian Society for Gastrointestinal Endoscopy (BSGIE) and Small Bowel Group
- I01 — I24 IBD Research Group (BIRD)
- N01 — N14 Research Group of Clinical Nutrition and Metabolism (SBNC and VVKVM)
- P01 — P32 Radiology, Pathology and Nuclear Medicine
- S01 — S15 Seven Societies Postgraduate Course
- T01 — T12 Research Group “Belgian Pancreatic Club” (BPC)

IMPACT OF VITAMIN D DEFICIENCY ON SEVERITY, INFLAMMATION AND FIBROSIS IN ALCOHOLIC LIVER DISEASE. E. Trépo (1), R. Ouziel (1), P. Pradat (2), Y. Momozawa (3), E. Quertinmont (4), C. Gervy (1), T. Gustot (1), D. Degré (1), V. Vercruysse (4), P. Deltenre (5), B. Gulbis (1), D. Franchimont (1), J. Devière (1), A. Lemmers (1), C. Moreno (1). (1) Erasme Hospital, Brussels, Belgium ; (2) La Croix-Rousse Hospital, Lyon, France ; (3) University of Liege, Liège, Belgium ; (4) ULB Faculty of Medicine, Anderlecht, Belgium ; (5) Hôpital de Jolimont, Haine-Saint-Paul, Belgium.

Introduction : Vitamin D deficiency has been frequently reported in advanced liver disease. However, its influence on alcoholic liver disease (ALD) has been poorly elucidated.

Aim : Firstly, we investigated the association of vitamin D with clinical, biological and histological parameters and survival in ALD patients. In addition, using peripheral blood mononuclear cells (PBMCs) from ALD patients and human hepatic stellate cells (HSCs), we investigated the biological role of 1,25(OH)₂D upon inflammation and fibrosis in alcoholic cirrhotic (AC) patients *in vitro*.

Methods : Three-hundred-and-twenty-four Caucasian ALD patients with excessive alcohol intake (> 40 g/day) were tested for 25-hydroxyvitamin D [25(OH)D] serum levels. PBMCs from 8 consecutive AC patients were pre-treated with 1,25(OH)₂D for 1 h before stimulation. After 24 h, production of TNF-α was assessed by ELISA in culture supernatants. We evaluated the effect of 1,25(OH)₂D pre-treatment on human HSCs activation by analysis of α-smooth muscle actin (α-SMA) expression.

Results : 324 patients (median follow-up of 5 months) were included (65% males, mean age 54.3 ± 9.4 years, 54% with a BMI > 25 kg/m², 75% drank > 80g/day, 78% had cirrhosis, and 21% had alcoholic hepatitis (AH), median MELD score 12.0 [7.3-17.9], median Child-Pugh score 9 [6-11], mean hepatic venous pressure gradient (HPVG) 14 ± 7.3 mmHg). Severe deficiency in 25(OH)D (< 10 ng/ml) was significantly associated with higher aspartate aminotransferase levels (p = 1.00 × 10⁻³), increased HVP (p = 5.80 × 10⁻⁶), a higher rate of AH (p = 2.14 × 10⁻⁴), MELD (p = 2.50 × 10⁻⁴) and Child-Pugh scores (p = 8.50 × 10⁻⁷). Furthermore, a low 25(OH)D concentration was an independent risk factor for cirrhosis (OR = 2.13, 95% CI = 1.18-3.84, p = 0.013) and mortality (HR = 2.36, 95% CI = 1.14-4.87, p = 0.020). In addition, 1,25(OH)₂D pre-treatment decreased TNF-α production (p = 3.00 × 10⁻³) and α-SMA expression (p = 0.019) in stimulated PBMCs of AC patients and human HSCs, respectively, in a dose-dependent manner.

Conclusions : Low 25(OH)D levels are associated with increased liver damage and mortality in ALD patients. *In vitro* experiments suggest that 1,25(OH)₂D could improve the pro-inflammatory cytokine profile of AC patients and might downregulate activation of HSCs. Therefore, vitamin D may well represent both a biomarker of severity and prognosis in ALD, and a therapeutic target worth exploring in ALD.

KERATIN19 : A KEY ROLE PLAYER IN THE INVASION OF HUMAN HEPATOCELLULAR CARCINOMAS. O. Govaere (1), M. Komuta (1), B. Spee (1), A. Durnez (1), S. Vander Borgh (1), C. Verslype (1), R. Aerts (1), B. Topal (1), J. Pirenne (1), F. Nevens (1), V.J. Desmet (1), L.R. Roberts (2), S.S. Thorgeirsson (3), T. Roskams (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) Mayo Clinic, Rochester, United States ; (3) National Institutes Of Health (NIH), Bethesda, United States.

Introduction : The expression of keratin(K) 19 in hepatocellular carcinomas (HCCs) has been related with progenitor cell origin, higher recurrence and poor prognosis.

Aim : In his study we investigated the role of keratin 19 in the invasive potential of HCCs.

Methods : Keratin(KRT)19 associated genes were identified using microarray analysis on 139 human samples and validated through quantitative PCR and immunohistochemistry. Primary human HCCs, selected on the presence of keratin(K)19, were submitted to an *in vitro* invasion assay (n = 6). HUH7-12D cell line was transiently transfected with synthetic siRNAs against KRT19 (n = 3). The biological effect was quantified by means of qPCR and *in vitro* invasion assays.

Results : Besides the elevated expression of biliary/progenitor cell markers (e.g. S100A6, ANXA3, CD133, EPCAM, KRT7, NOTCH2) and reduced expression of hepatocytic markers (e.g. HNF4A, ALB), KRT19 positive HCCs show a high increase in cancer/invasion related genes (e.g. VASP, CTBP2, TACSTD2, FAM57A, EZR, RAB27B, PDGFRA). According to immunohistochemical results, several markers turned out to be novel biliary/ progenitor cell(PC) markers, indicating that similar pathways might be active in non-neoplastic PCs and K19 positive HCCs. Furthermore K19 positive HCCs show a strong interaction with their micro-environment by producing laminin, as seen in the non-neo-

plastic PCs. Submission of human primary dissociated HCCs to an ExtraCellular Matrix (ECM) invasion assay functionally demonstrated that K19 positive tumour cells possess an increased invasive potential. Impairing the expression of *KRT19* in the HUH7-12D cell line significantly reduced the invasiveness of the cell line. Furthermore the knockdown of *KRT19* results in a reduction of several biliary/progenitor cell markers (e.g. *CD133*, *S100A6*, *NOTCH2*) and invasion makers (e.g. *TACSTD2*, *VASP*, *CTBP2*, *PDGFRA*).

Conclusions : The expression of keratin 19 in HCCs is more than just a phenotype linked with poor prognosis ; keratin 19 plays an important role in the invasive potential of this subtype of tumours.

- A03 -

PROTECTIVE ROLE OF VITAMIN E ON CCL4-PENTOBARBITAL INDUCED LIVER DAMAGE IN RATS.

S. Wamutu (1), S. Francque (2), S. Chatterjee (3), G.W. Muyombya (1), E. Musisi (1), J. Weyler (3), E. Van Marck (3), G. Bimenya (1), P. Michielsen (2). (1) Makerere University, Kampala, Uganda ; (2) Antwerp University Hospital, Antwerpen, Belgium ; (3) University Of Antwerp, Antwerpen, Belgium.

Introduction : Vitamin E is reported to be a powerful antioxidant. However, its protective role against oxidative stress associated by liver damage is still poorly understood.

Aim : To investigate further the protective role of VitE against oxidative stress using CCl₄ rat model of liver damage.

Methods : Four groups (n = 10, each) of male albino rats were used.

Group 1 (control) received i.m. injections of saline daily. Group 2 received i.p. 0.5ml/kg CCl₄ (1 :1 olive oil) every 3 days plus pentobarbital in drinking water for 3 weeks. Group 3 was pre-treated with daily i.m. injections of 100 mg/kg/day a-tocopherol acetate for 5 days before co-administration with CCl₄ in doses similar to group 2. Group 4 received similar doses of a-tocopherol acetate only for 4 weeks. At the end of week 4, blood and liver samples were collected for examination. Biochemical data were analysed using Student's t test and scores (based on Brunt & Metavir systems) for liver histology by Pearson Chi Square. P < 0.05 was considered significant.

Results : CCl₄ significantly increased ALT (189.50 ± 6.23 U/L vs. 134.38 ± 11.12 U/L, p < 0.01) ; AST (258.13 ± 12.04 U/L vs. 212.50 ± 13.91 U/L, p < 0.05) ; GGT (34.5 ± 1.3 U/L vs. 19.5 ± 0.9 U/L, p < 0.0001) and malondialdehyde (nmol/mg tissue) (1.88 ± 0.08 vs 1.29 ± 0.07, p < 0.001). It also significantly decreased glutathione (nmol/mg tissue) (44.19 ± 2.68 vs 59.19 ± 2.11, p < 0.01) and total bilirubin (7.21 ± 0.44mmol/L vs.8.50 ± 0.40mmol/L, p = 0.047). In the VitE pre-treated group, ALT (162.50 ± 10.51, p = 0.044) ; GGT (28.1 ± 2.4, p = 0.036) and malondialdehyde (1.65 ± 0.07nmol/mg tissue, p = 0.035) were significantly lower compared to CCl₄ group. AST remained insignificant. Pre-treatment with VitE significantly (p = 0.031) preserved glutathione (51.55 ± 1.48 nmol/mg tissue) but not total bilirubin (p > 0.05). Histology scores for lobular and portal inflammation and fibrosis in the pre-treated rats significantly decreased (respectively, p = 0.006, 0.019 and 0.022) but remained insignificant (p > 0.05) for apoptosis, ballooning and steatosis compared to CCl₄ group. No side effects due VitE alone were observed.

Conclusions : Vitamin E significantly restrained most of the biochemical changes of CCl₄ induced liver damage, including oxidative stress and seemed to protect the liver against fibrosis but not steatosis, suggesting its moderate histology benefit in rats.

- A04 -

HISTOPATHOLOGICAL PARAMETERS ARE ASSOCIATED WITH BOTH SEVERITY AND SURVIVAL IN ALCOHOLIC HEPATITIS.

L. Verset, E. Trepo, N. Nagy, D. Degre, T. Gustot, J. Deviere, P. Demetter, C. Moreno. Erasme Hospital, Brussels, Belgium.

Introduction : Alcoholic hepatitis (AH) which includes both histopathological features and a clinical syndrome is associated with increased morbidity/mortality. To date, no well-established histopathological criteria are linked to AH natural history.

Aim : This study aims to evaluate whether histopathological parameters are associated with disease severity and survival in AH .

Methods : 170 patients (mean age 53 years, 62% males, mean MELD 15, median Maddrey [mDF] 32) with excessive alcohol intake (> 40g/day) and biopsy proven AH were included. Histological parameters were blindly assessed by one pathologist using semiquantitative scores : steatosis (0 = none, 1 = < 5%, 2 = 5-33%, 3 = 33-66%, 4 = > 66%) ; fibrosis (0 = none, 1 = perisinusoidal or periportal fibrosis, 2 = perisinusoidal and periportal fibrosis, 3 = bridging fibrosis, 4 = cirrhosis) ; perisinusoidal fibrosis (0 = none/mild, 1 = moderate, 2 = severe), lobular inflammation (1 = < 2 foci per 200x field, 2 = 2-4 foci per 200x field, 3 = > 4 foci per 200x field), portal inflammation (0 = none to minimal, 1 = greater than minimal), polymorphonuclear leukocytes infiltration (0 = none to moderate, 1 = severe), cholestasis (0 =

none, 1 = mild, 2 = moderate to severe), satellitosis (0 = none, 1 = a few, 2 = many), ballooning hepatocytes (0 = none or a few, 1 = many) and Mallory bodies (0 = none or a few, 1 = many). Univariate and multivariable analyses were performed to identify histopathological parameters associated with MELD score, presence of severe AH (compatible histology, serum bilirubin > 5 mg/dl and mDF > 32) and with 6-month survival.

Results : In univariate analysis, patients with increased polymorphonuclear leukocytes infiltration ($p = 0.012$), cholestasis ($p < 0.001$), satellitosis ($p = 0.005$) and Mallory bodies ($p = 0.008$) had a significantly higher MELD score. In addition, perisinusoidal fibrosis ($p < 0.001$), lobular inflammation ($p = 0.018$), cholestasis ($p < 0.001$), polymorphonuclear leukocytes infiltration ($p = 0.009$), satellitosis ($p < 0.001$), ballooning hepatocytes ($p = 0.008$) and Mallory bodies ($p < 0.001$) were associated with severe AH (mDF > 32). Furthermore, increased perisinusoidal fibrosis ($p < 0.001$), polymorphonuclear leukocytes infiltration ($p = 0.022$), satellitosis ($p = 0.004$) and cholestasis ($p < 0.001$) were associated with a greater 6-month mortality. In multivariable analysis, only cholestasis was independently associated with MELD score ($p < 0.001$). Moreover, cholestasis and perisinusoidal fibrosis remained associated with severe AH ($p < 0.001$ and $p = 0.030$) just as with 6-month mortality ($p = 0.011$ and $p = 0.046$).

Conclusions : Typical histopathological features are associated with both severity and survival in AH. These histopathological parameters may contribute to identify patients with worse form of AH and poor prognosis.

- A05 -

CHRONIC ALCOHOL CONSUMPTION INDUCES HEPATIC INSULIN RESISTANCE UNRELATED TO KUPFFER CELL ACTIVATION. V. Lebrun (1), O. Molendi-Coste (2), P. Starkel (1), I. Leclercq (1). (1) Université Catholique De Louvain, Brussels, Belgium ; (2) Université Catholique De Louvain, Brussels, Belgium.

Introduction : Alcohol consumption is one of the most common causes of chronic liver disease. Besides reactive oxygen species and aldehydes resulting from alcohol metabolism, endotoxin-mediated activation of Kupffer cells has been postulated to play a crucial role in the pathogenesis of alcohol-induced liver injury in rodents as KC inhibition protects from alcohol-induced liver injury. Obesity and insulin resistance are associated with histological changes, collectively called non-alcoholic fatty liver diseases (NAFLD), similar to those induced by alcohol. We previously demonstrated that in this context, KC activation is key in the development of insulin resistance.

Aim : The aims of the present study are (1) to assess whether chronic alcohol consumption induces hepatic insulin resistance and (2) to evaluate the role of KC activation in this process.

Methods : Female C57B16 mice, aged 8 weeks, were fed for 20 days a modified Lieber-DeCarli diet in which alcohol concentration was gradually increased up to 35% of daily caloric intake ; controls receiving a isocaloric alcohol-free liquid diet (pair-feeding). Insulin sensitivity was assessed in vivo using the euglycemic-hyperinsulinemic clamp technique and activation of the insulin signaling pathway ex vivo by western blotting. KC were depleted by repeated injections of liposome-encapsulated clodronate (i.v, once weekly), and compared with animals receiving PBS liposomes.

Results : Compared to pair-fed controls, chronic alcohol administration induced hepatomegaly and steatosis with a 2-fold increase in total liver lipid content. Small inflammatory infiltrates were present in the liver parenchyma. Enlarged F4/80+ Kupffer cells, increased hepatic expression of CD68, CD11c, CD11b, CD14, IL-6 and TNF- α transcripts, and increased serum LPS were highly suggestive of KC activation in this model. Upon insulin stimulation, phosphorylation of the insulin receptor and of down-stream signaling intermediates Akt and ERK was attenuated in the liver but not in skeletal muscles of OH-fed mice compared to controls. Consistently, in vivo clamps studies confirmed decrease insulin sensitivity and, in particular, hepatic insulin resistance in OH-fed mice.

Clodronate liposomes substantially depleted KC in OH-fed mice. KC depletion to OH-fed mice failed to improve significantly hepatic steatosis, insulin sensitivity as assessed by clamp.

Conclusions : Alcohol-induced steatohepatitis is associated with hepatic insulin resistance. Unlike in NAFLD, KC activation and hepatic inflammation only marginally participate to hepatic insulin resistance in this rodent model of chronic alcohol feeding suggesting that metabolic rather than inflammatory changes induced by alcohol alter insulin sensitivity.

- A06 -

ECHOCARDIOGRAPHY FOR DETECTION OF PORTOPULMONARY HYPERTENSION IN LIVER TRANSPLANT CANDIDATES. S. Raevens, K. Reyntjens, M. Depauw, A. Geerts, R. Troisi, F. Berrevoet, X. Rogiers, H. Van Vlierberghe, I. Colle. Ghent University Hospital, Ghent, Belgium.

Introduction : Portopulmonary hypertension (PPHT), a rare complication of liver cirrhosis, may be a contraindication to transplantation because of the elevated risk of peri- and post-transplantation morbidity and mortality. Because PPHT is frequently asymptomatic, systematic screening is strongly recommended. Doppler echocardiography, performed dur-

ing pre-transplantation evaluation, is a useful noninvasive tool to document or exclude PPHT. Pulmonary hypertension on right heart catheterization is defined as a mean pulmonary artery pressure above 25 mmHg and a wedge pressure below 15 mmHg.

Aim : To evaluate the current cut-off of 30 mmHg estimated systolic pulmonary artery pressure (sPAP) and to determine the 'ideal' cut-off at which patients should be referred for right-heart catheterization. To assess the accuracy of Doppler echocardiography for the diagnosis of PPHT in patients with cirrhosis, candidates for liver transplantation, at the time of pre-transplantation evaluation, as compared with preoperative and/or peroperative catheterization data, using different cut-offs : 30, 35 and 38 mmHg.

Methods : One hundred fifty-two patients who were evaluated for liver transplantation between January 2005 and December 2010 had Doppler echocardiography at evaluation. These results, specifically the estimated sPAP, were compared with pulmonary artery pressures measured during pre-transplantation work out or at the beginning of the transplantation procedure. Receiver Operating Curve analysis was used to determine the 'ideal' cut-off with maximal sensitivity and maximal specificity. The proportion true positives, true negatives, false positives and false negatives were calculated and sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood and accuracy of Doppler echocardiography as screening tool were determined at different cut-offs.

Results : The prevalence of PPHT in our cohort of 152 patients listed for liver transplantation is 4,6%. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood and accuracy of echocardiography to predict PPHT for different cut off values of sPAP is given in the following table.

sPAP	30 mmHg	35 mmHg	38 mmHg
Sensitivity	100%	100%	100%
Specificity	54%	70%	82%
Positive predictive value	10%	14%	22%
Negative predictive value	100%	100%	100%
Positive likelihood	2,164	3,295	5,8
Accuracy	56%	71%	84%
Prevalence	4,6%	4,6%	4,6%

Receiver Operating Curve analysis demonstrated an 'ideal' cut-off of 38 mmHg which carries maximal negative predictive value, sensitivity and specificity. The area under the curve is 0,974, representing the high accuracy of Doppler echocardiography.

Conclusions : Portopulmonary hypertension (PPHT) occurs in 4,6% of patients worked out for liver transplantation. Doppler echocardiography is a highly sensitive screening test for detecting PPHT at pre-liver transplantation evaluation. Based on the results of our study, we can advise that liver transplantation candidates with an sPAP > 35 mmHg or even 38 mmHg should be referred for right-heart catheterization to confirm or rule out the diagnosis of PPHT. By increasing the cut-off estimated sPAP measured during echocardiography from 30 mmHg to 35 or even 38 mmHg, the amount of patients who should undergo an invasive right-heart catheterization at evaluation could be safely reduced.

A ROLE FOR AUTOPHAGY DURING HEPATIC STELLATE CELL ACTIVATION. L.F. Thoen (1), E.L. Guimarães (1), L. Dollé (1), I. Mannaerts (1), M. Najimi (2), E. Sokal (2), L.A. Van Grunsven (1). (1) Vrije Universiteit Brussel, Jette, Belgium ; (2) Université Catholique De Louvain, Brussels, Belgium.

Introduction : Autophagy is a metabolic process by which cells degrade and metabolize own constituents, *e.g.* intracellular organelles and proteins, with many connections to human disease and physiology.

Aim : We studied the role of autophagy in hepatic stellate cell (HSC) activation, a key event in liver fibrogenesis.

Methods : Analysis of the autophagic flux during *in vitro* activation of primary mouse HSCs was performed using a DsRed-GFP-LC3B encoding plasmid. The effect of autophagy inhibition by bafilomycin A1 on the *in vitro* activation process of human and mouse HSCs was examined by measuring proliferation, presence of activation markers by RT-qPCR, immunofluorescence, and western blotting. Analysis of lipid droplet and microtubule-associated protein light chain 3 beta (LC3B) colocalization in the presence of PDGF-BB was investigated by immunocytochemistry.

Results : A significant increased autophagic flux was observed during culture-induced mouse HSC activation. Treatment with autophagy inhibitor bafilomycin A1 results in a significant decreased proliferation and expression of activation markers in mouse and human HSCs. In addition, a significant increased amount of large lipid droplets was observed in bafilomycin A1-treated mouse HSCs. Besides this, lipid droplet and LC3B colocalization was increased after PDGF-BB treatment in quiescent HSCs.

Conclusions : During HSC activation, autophagic flux is increased. The demonstration of partly inhibition of *in vitro* HSC activation after treatment with an autophagy inhibitor unveils a potential new therapeutic strategy for liver fibrosis.

The observation of LC3B and lipid droplet colocalization in quiescent HSCs after PDGF-BB treatment and the increased amount of large lipid droplets after seven days of bafilomycin A1 treatment suggests a role for autophagy in lipid droplet metabolism. Additional experiments that address this role for autophagy in HSCs will be presented.

- A08 -

USE OF EARLY-TIPS FOR HIGH-RISK VARICEAL BLEEDING. RESULTS OF A POST-RCT SURVEILLANCE STUDY. W. Laleman (1), M. Di Pascoli (2), J.C. Garcia-Pagan (2), K. Caca (3), C. Bureau (4), B. Appenrodt (5), A. Luca (6), A. Zipprich (7), J. Abraldes (2), F. Nevens (1), J.P. Vinel (4), J. Bosch (2). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) Hospital Clinic, Barcelona, Spain ; (3) Klinikum Ludwigsburg, Ludwigsburg, Germany ; (4) Hôpital Purpan, Toulouse, France ; (5) University Hospital, Bonn, Germany ; (6) Istituto Mediterraneo Per I Trapianti E Therapie Ad Alta Specializzazione, Palermo, Italy ; (7) Martin Luther Universität Halle-Wittenberg Klinik, Halle, Germany.

Introduction : In a recent randomized international clinical trial (RCT) in high-risk cirrhotic patients with acute variceal bleeding, the early use of TIPS was associated with a marked and significant reduction in failure to control variceal bleeding and rebleeding and in mortality.

Aim : To assess whether these results are reproduced in clinical practice outside RCTs.

Methods : Retrospective review was performed of patients admitted after inclusion of the last patient in the RCT for acute variceal bleeding and high risk of treatment failure (Child C < 14 or Child B plus active bleeding) at the same centres of the original RCT study. All patients treated with early-TIPS were included (n = 45) ; patients receiving medical therapy with drugs+EBL were included until the moment when the hospital adopted the strategy of using Early-TIPS for admitted patients (n = 30).

Results : TIPS was performed within 24 hours in 28 patients ; 24-48 h in 11 and 48-72 h in 6. There were no differences in the baseline characteristics of the patients treated with TIPS (median age 56, male/female 34/11, Child B/C 18/27) or Drug+EBL (median age 55, male/female 18/12, Child B/C 10/20). Median follow-up was 11.2 months. Patients treated with early-TIPS had a much lower incidence of failure to control bleeding or rebleeding than patients receiving Drug+EBL (3 vs 15 ; p < 0.001). The 1-year actuarial probability of remaining free of this composite end point was 93% vs 53% (p < 0.001). The same was observed in mortality (1-year actuarial survival was 86% vs 70% respectively (p = 0.056). More important, actuarial curves of failure to control bleeding + rebleeding and of survival in this observational study were well within the confidence intervals of those observed in the RCT.

Conclusions : In daily practice, the application of the early use of TIPS in patients with cirrhosis and a high-risk variceal bleeding offers results similar to those previously observed in the RCT, supporting its use in clinical practice.

- A09 -

KUPFFER CELLS MODULATE THE DUCTULAR REACTION IN A MOUSE MODEL OF SCLEROSING CHOLANGITIS. J. Best (1), L. Dolle (2), N. Van Hul (3), L.P. Bechmann (1), S. Wing-Kin (4), F. Heindryckx (5), J.P. Sowa (1), L. Peeters (2), G. Kneiseler (1), I.A. Leclercq (6), I.O. Colle (5), A. Canbay (1), L.A. Van Grunsven (2). (1) University Hospital Essen, Essen, Germany ; (2) Free University (Vub), Brussels, Belgium ; (3) Université Catholique De Louvain, Brussels, Belgium ; (4) The Institute Of Hepatology, London, United Kingdom ; (5) Ghent University, Ghent, Belgium ; (6) Université Catholique De Louvain, Brussels, Belgium.

Introduction : Primary and secondary sclerosing cholangitis are progressive disease entities with increasing prevalence. Cholestatic liver diseases are characterized by massive periportal Kupffer cell (KC) accumulation, peribiliary extracellular matrix deposition (ECM), ductular reaction (DR) and neo-vascularisation. A persistent inflammatory response plays a key role in progression from a type-I DR observed during disease onset to a type-II DR, representative of advanced cholestatic liver injury.

Aim : This study aimed to elucidate the role of KC in the progression of chronic cholangiopathies through KC depletion in a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) mouse model.

Methods : C57BL/6J mice received a single i.p. injection of clodronate encapsulated liposomes (CLDLPS) before initiation of a 7d DDC treatment. Another group received one injection of CLDLPS at day 7 of a 14d DDC treatment. Control animals were co-treated with PBSLPS in a corresponding setup. Mice were sacrificed after 7 or 14 days of treatment for (immuno-)histochemical assessment of KC activation (F4/80), ECM deposition (Sirius Red, Laminin), DR (CK19) and neo-vascularisation (Endoglin).

Results : KC depletion during 14d DDC treatment resulted in a significant inhibition of ECM deposition and neo-vascularisation. Porto-lobular migration pattern of laminin-rich ECM and ductular structures were significantly attenuated

and a progression from a type-I to type-II DR was inhibited by KC depletion. Ductular proliferation was confined to portal regions, without amorphous cells clusters, as observed in a type-II DR during PBSLPS treatment.

Conclusions : KCs play a key role in progression from a type-I DR to a type-II DR. Depletion of KC by CLDLPS is associated with reduced ECM deposition and inhibited migration of CK19+ cells into the lobule.

- A10 -

SEVERE HYPONATREMIA IS THE BEST PREDICTOR OF MORTALITY IN CIRRHOSIS AND REFRACTORY ASCITES. T. Sersté (1), T. Gustot (2), P.E. Rautou (3), C. Francoz (3), H. Njimi (2), F. Durand (3), D. Valla (3), D. Lebrech (3), R. Moreau (3). (1) Ulb Saint-Pierre, Brussels, Belgium ; (2) Erasme Hospital, Brussels, Belgium ; (3) Hôpital Beaujon, Clichy, France.

Introduction : The MELDNa score was developed to improve the prognostic value of the MELD score in cirrhosis and was built for serum sodium concentrations numerically capped between 125 and 140 mmol/L. This model is not validated in a well-defined population of patients with cirrhosis and refractory ascites in whom severe hyponatremia (< 125 mmol/L) is frequent.

Aim : This study assessed the prognostic value of severe hyponatremia and the MELDNa score in these patients.

Methods : A consecutive, single-centre, observational, prospective study was performed in patients with cirrhosis and refractory ascites defined according to the International Ascites Club criteria. The prevalence of low serum sodium was assessed in this population. Predictive factors of mortality were analyzed and compared. The accuracy to predict mortality of MELD, MELDNa and Child-Pugh scores was assessed by the Area Under Receiver Operating Curves.

Results : One hundred seventy-four patients were included. Sixty-six (37.9%) had low serum sodium (< 130 mmol/L). Sixty-one (35.1%) had severe hyponatremia (< 125 mmol/L) leading to the discontinuation of diuretics. The median MELDNa score was 23 (10-33). The 1-year cumulative incidence of death was 55% (95%CI : 55 to 56%).

After a multivariate analysis, the best predictive factors of mortality were the following : severe hyponatremia (< 125 mmol/L) as an underlying cause of refractory ascites, Child-Pugh C, beta-blocker therapy, and frequency of large volume paracentesis.

MELD and MELDNa scores were not independently associated with death. The Child-Pugh score had a higher Area Under Receiver Operating Curve to predict mortality than MELDNa.

Conclusions : In patients with cirrhosis and refractory ascites, severe hyponatremia (< 125 mmol/L) leading to the discontinuation of diuretics is frequent. Severe hyponatremia and Child-Pugh score are better predictors of mortality than MELDNa in this situation.

- A11 -

ROLE OF INFLAMMATION AND INSULIN RESISTANCE IN A NEW MODEL OF NASH : THE FOZ/FOZ MICE. L. Poekes (1), N. Lanthier (1), G. Farrell (2), I. Leclercq (1), V. Legry (1). (1) Université Catholique De Louvain, Brussels, Belgium ; (2) Anu Medical School At The Canberra Hospital, Canberra, Australia.

Introduction : Non-alcoholic fatty liver disease (NAFLD) is a progressive disease associated with insulin resistance and obesity. The spectrum of NAFLD ranges from simple steatosis to NASH (non-alcoholic steatohepatitis) characterized by steatosis, inflammation and progressive fibrosis. The understanding of NASH pathogenesis has been limited by the lack of appropriate animal models. Recently, a new model was described : the *foz/foz* mice, an obese mouse strain harbouring a spontaneous mutation in the Alström gene (*Alms1*). Under a high-fat diet (HFD), *foz/foz* mice rapidly become obese and insulin resistant and develop a NASH with a progressive pericellular fibrosis after 24 weeks.

Aim : The aim of this study was to evaluate the hepatic and adipose inflammation in the early development of insulin resistance and NASH in *foz/foz* mice.

Methods : Male *foz/foz* and wild-type (WT) mice were fed with a HFD or a normal diet (ND) during 6 weeks. Hepatic steatosis was analyzed by histology and quantification of the total lipid content. To evaluate glucose homeostasis and insulin signalling, we performed intraperitoneal glucose tolerance test and western blots. Inflammation in the epididymal adipose tissue and in the liver was investigated by immunohistochemistry and RT-qPCR. In order to see the role of hepatic macrophages in the development of insulin resistance, liposome-encapsulated clodronate was injected intravenously to selectively deplete Kupffer cells without affecting macrophages of adipose tissue, during the last 10 days of the experiment.

Results : After 6 weeks of ND, *foz/foz* mice already showed hyperglycemia (156 ± 23 vs 116 ± 24 mg/dL, $p = 0.03$), an altered hepatic insulin signalling (decreased phosphorylation of the insulin receptor and AKT) and adipose inflammation (a 2-fold increase in TNFa, F4/80 and CD68 mRNA, $p < 0.05$). HFD induced a marked steatosis (2.5-fold increase

in hepatic lipid content, $p = 0.01$) in *foz/foz* but not in WT mice. Compared to WT mice in response to HFD, *foz/foz* mice had a more pronounced increase in body weight ($+25.0 \pm 3.7$ vs $+8.6 \pm 4.6$ g in WT, $p = 0.003$), glucose intolerance ($p = 0.001$), and a highly increased adipose inflammation (a 21-fold vs 5-fold increase in F4/80, TNF α , CD68 and CD11c expression, $p < 0.003$). Noteworthy, HFD induced similarly hepatic inflammatory gene expression (F4/80, TNF α , CD68, CD11c and MCP1) in WT and *foz/foz* mice. However, Kupffer cell depletion improved HFD-induced glucose intolerance ($p = 0.007$) in *foz/foz* mice but not in WT mice.

Conclusions : This new model mimics the metabolic context and progressive liver disease seen in human NASH. This early development towards NASH is characterized by steatosis, insulin resistance and adipose, more than hepatic inflammation.

- A12 -

NORMAL VERSUS ELEVATED ALT AND GGT IN AN OBESE POPULATION, ASSOCIATION WITH LIVER HISTOLOGY. A. Verrijken (1), S. Francque (1), I. Mertens (1), M. Ruppert (1), G. Hubens (1), E. Van Marck (2), P. Michielsens (1), L. Van Gaal (1). (1) Antwerp University Hospital, Antwerpen, Belgium ; (2) Antwerp University, Antwerpen, Belgium.

Introduction : Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide and is frequently associated with obesity and insulin resistance. Plasma liver enzymes are measured routinely in people with obesity. The true prognostic value of an abnormal result is, however, debatable.

Aim : To study the association of different ranges of alanine aminotransferase (ALT) (ULN 40 U/L) and gamma glutamyltransferase (GGT) (ULN 29 U/L) with metabolic and histological parameters of NAFLD in a cohort of prospectively included obese patients.

Methods : Patients presenting for a problem of overweight underwent a metabolic and liver assessment. If NAFLD was suspected, a liver biopsy was proposed. The biopsy was scored according to the NASH Clinical Research Network Scoring System.

Results : A series of 208 patients (70.2% female) was prospectively included. Mean age was 44 ± 12 years and mean body mass index (BMI) was 37.8 kg/m^2 (IQR 34.0-41.9). Patients with ALT > 40 U/L have a significantly higher waist ($p = 0.023$), more visceral fat ($p = 0.002$), higher fasting glucose ($p < 0.001$), higher c-peptide ($p < 0.001$), higher HbA1C ($p = 0.001$) and are more insulin resistant ($p = 0.004$) compared to patients with ALT $= < 40$ U/L, BMI did not differ between groups ($p = 0.861$). Similar results are found between the groups with low versus high GGT. Patients with ALT > 40 U/L have more definite nonalcoholic steatohepatitis (NASH) ($p < 0.001$), higher mean NASH activity score (NAS) ($p < 0.001$) and more advanced fibrosis ($p = 0.001$). However, in patients with ALT $= < 40$ U/L, 22.1% can be diagnosed with NASH and 8.8% has a fibrosis stage = 2. Similar differences are found in patients with GGT $= < 29$ U/L. ALT correlates with NAS, but levels are systematically higher in men compared to women. After correction for gender, ALT is still significantly associated with NAS.

Conclusions : Patients with elevated ALT and GGT values have more severe metabolic and histological parameters of NAFLD. Even patients with ALT < 40 U/L or GGT < 29 U/L can show signs of nonalcoholic steatohepatitis and advanced fibrosis. Revision of gender specific normal limits for ALT level is advisable.

This work is part of the project "Hepatic and adipose tissue and functions in the metabolic syndrome" (HEPADIP), supported by the European Commission as an Integrated Project under the 6th Framework Program (Contract LSHM-CT-2005-018734).

Invited lecture : BASL-BLIC spring meeting lecture

- A13 -

LIVER TRANSPLANTATION IN BELGIUM SEEN FROM THE EUROTRANSPLANT VIEW POINT : IMPLICATIONS OF MELD IN BELGIUM. A. Rahmel (Eurotransplant, Leiden, The Netherlands).

- A14 -

EFFICACY OF EVEROLIMUS IN 719 LIVER TRANSPLANT RECIPIENTS : A RANDOMIZED, CONTROLLED STUDY. F. Nevens (1), P. De Simone (2), L. De Carlis (3), H.J. Metselaar (4), S. Beckebaum (5), F. Saliba (6), S. Jonas (7), D. Sudan (8), L. Fischer (9), J. Fung (10). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy ; (3) Niguarda Cà Granda Hospital, Milano, Italy ; (4) Erasmus Medical Center, Rotterdam, Netherlands ; (5) University Duisburg-Essen, Duisburg, Germany ; (6) Hôpital Paul Brousse, Villejuif, France ; (7) University Hospital, Leipzig, Germany ; (8) Duke University Medical Centre,

Durham, United States ; (9) Universitätsklinikum, Hamburg Eppendorf, Germany ; (10) Cleveland Clinic, Cleveland, United States.

Introduction : Early reduction or elimination of calcineurin inhibitors (CNI) after liver transplantation (LTx) may reduce CNI-associated deterioration of renal function.

Aim : The present study aims to evaluate this concept in *de novo* LTx recipients using immunosuppression based on the mTOR-inhibitor everolimus (EVR).

Methods : A 24-month (M), multicenter, open-label, randomized study in *de novo* LTx recipients to compare efficacy and safety of EVR (C0 3-8ng/mL) plus reduced-exposure tacrolimus (C0 3-5 ng/mL ; EVR+rTAC) or EVR (C0 6-10 ng/mL) with TAC withdrawal (TAC-WD) at 4 months to standard exposure TAC (C0 : 6-10ng/mL ; TAC-C) ; all arms include corticosteroids. Following LTx and a 30-day run-in period with TAC-based immunosuppression (+/- mycophenolate), patients were randomized 1:1:1 to the 3 groups. Primary endpoint at M12 was the composite efficacy failure rate of treated biopsy proven acute rejection (tBPAR), graft loss or death. Key secondary endpoint was evolution of renal function from randomization (RDN) to M12 using estimated glomerular filtration rate (eGFR) based on the four-variable Modification of Diet in Renal Disease (MDRD4) equation.

Results : Enrollment into the TAC-WD arm (N = 231) was prematurely stopped due to a higher incidence of BPAR, clustered around time of TAC elimination. At M12, the composite efficacy failure rate in the EVR+rTAC group (N = 245) was lower compared to TAC-C (N = 243) : 6.7% vs. 9.7% ; demonstrating non-inferiority (against the NI margin of 12%) with -3.0% [-8.7, 2.6] in favor of EVR+rTAC ($p < 0.001$). The incidence of BPAR was 19.9% for TAC-WD, but significantly lower for EVR+rTAC (4.1%) compared to TAC-C (10.7% ; $p = 0.005$). The severity of tBPAR (RAI score) was also lower with EVR+rTAC vs. TAC-C and in particular no moderate or severe rejections were observed with EVR+rTAC. The evolution of renal function from RDN to M12 was superior with EVR+rTAC (mean difference in eGFR changes for EVR+rTAC vs. TAC-C : $+8.50 \pm 2.12$ mL/min/1.73m² ; $p < 0.0001$). The overall safety profile was favorable and consistent with previous EVR studies in solid organ transplantation.

Conclusions : Early TAC elimination with EVR did not provide sufficient efficacy. However, early introduction of EVR at 1 month after LTx allowed substantial TAC reduction in *de novo* LTx recipients demonstrating fewer and less severe BPAR and superior renal function compared to standard exposure TAC at M12.

- A15 -

ROLE OF ANGIOGENESIS AND OXIDATIVE STRESS IN A DB/DB MOUSE MODEL FOR NON-ALCOHOLIC STEATOHEPATITIS. S. Coulon (1), N. Rohr-Udilova (2), F. Heindryckx (1), C. Vansteenkiste (1), L. Libbrecht (1), H. Vanvlierberghe (1), A. Geerts (1), M. Peck-Radosavljevic (2), I. Colle (1). (1) Universitair Ziekenhuis Gent, Gent, Belgium ; (2) Medical University Of Vienna, Wien, Austria

Introduction : Non-alcoholic steatohepatitis (NASH) is a progressive liver disease that is characterised by inflammation and accumulation of fat in the liver. Unfortunately, the exact mechanism of disease progression is not fully understood. Oxidative stress has been implicated in the transition from simple steatosis to NASH. VEGF is a potent angiogenic cytokine and is increased by conditions associated with the generation of ROS intermediates. In this manner, oxidative stress and angiogenesis are important elements in the pathogenesis of inflammatory diseases. However, this has never been investigated in the pathophysiology of NASH.

Aim : Elucidate the role of angiogenesis and oxidative stress in a db/db mouse model for NASH at different time-points.

Methods : Female db/db mice, aged 10 weeks, were fed a methionine choline deficient (MCD) diet (n = 8/group) or a control diet (n = 5/group). The progression from steatosis to NASH was evaluated by analyzing liver samples taken at 1, 2, 4, and 8 weeks. Sections were stained with H&E for routine histology. Hepatic neovascularization was investigated by immunohistochemical staining for endoglin. Quantitative expression of VEGF was analyzed with an ELISA. Oxidative stress was measured with a thiobarbituric acid reactive substances (TBARS) assay.

Results : Histology of the liver showed that db/db mice had NASH after 2, 4 and 8 weeks of MCD diet. VEGF protein concentration in the liver was significantly higher in db/db mice after 1,2,4 and 8 weeks of MCD diet compared to controls ($p < 0.05$). Immunohistochemical hepatic expression of endoglin, an endothelial cell marker which is up-regulated by angiogenic factors like VEGF, showed a significant increased expression after 2, 4 and 8 weeks of MCD fed db/db mice compared to controls ($p < 0.05$). Livers of db/db mice exhibited significantly elevated TBARS after 2, 4 and 8 weeks of MCD diet compared to controls ($p < 0.01$). At different time-points hepatic TBARS correlated with serum VEGF (2w, $r_2 = 0.88$, $p < 0.001$; 4w, $r_2 = 0.75$, $p = 0.007$ and 8w, $r_2 = 0.81$, $p = 0.004$).

Conclusions : These data show a significant increased expression of endoglin, VEGF and TBARS in the liver of db/db mice with NASH. Furthermore, we found a strong correlation between the VEGF and hepatic TBARS expression. Therefore, we hypothesised that angiogenesis and oxidative stress play a synergistic role in the disease progression of NASH.

ENHANCED SEPSIS-ASSOCIATED MORTALITY OF PATIENTS WITH CIRRHOSIS IN ICU : EPIC II STUDY. T. Gustot, H. Njimi, F. Taccone, C. Moreno, J.L. Vincent. Ulb Erasme, Brussels, Belgium.

Introduction : Infection is a leading cause of death and becomes the principal therapeutic challenge in advanced cirrhosis. However, little prospective multicenter information is available about worldwide epidemiology of such severe infections in intensive care units (ICUs).

Aim : We describe worldwide epidemiological and microbiological data of sepsis in a large cohort of cirrhotic patients in ICU.

Methods : We analyzed the data of cirrhotic patients in a 1-day, prospective, international, multicenter point prevalence study. 460 cirrhotic patients upon 13796 patients (3.3%) were present in one of the 1265 participating ICUs. Demographic, physiological, bacteriological and therapeutic data were collected from all patients present in participating ICUs on May 8, 2007. Follow-up data until hospital discharge or for 60 days, and ICU and in-hospital outcomes were recorded.

Results : In this international cohort, overall in-hospital mortality of cirrhotic patients was 40.9% compared to 23.6% in the non-cirrhotic population ($p < 0.001$). Prevalence of documented or suspected infections was higher in cirrhotic and non-cirrhotic patients (57.6 vs. 57.6%, $p < 0.01$). In the two groups, near 70% of infections are culture-positive. In the two groups, lungs were the most common site of infection, but cirrhotic patients had more frequently abdominal infections (29.8 vs. 19.2%, $p < 0.001$). Infected cirrhotic patients had more often gram-positive isolates (55.5 vs. 46.5%, $p < 0.05$) and less often gram-negative isolates (54.9 vs. 62.5%, $p < 0.05$). Moreover, Methicillin-resistant *Staphylococcus aureus* (MRSA) was more frequently found in cirrhotic than non-cirrhotic patients. In cirrhotic patients, infection was associated with higher number of organ failures underlying by higher SOFA scores. Infection-associated circulatory failure and renal failure were more often observed in cirrhotic than non-cirrhotic patients. In a parallel manner, infected cirrhotic patients required more frequently vasopressors or renal replacement therapy than infected non-cirrhotic patients. Severe sepsis and septic shock were associated with dramatic 60 days survival rates 43.2 and 72.9% in cirrhotic patients compared to 30.4% and 48.6% respectively in non-cirrhotic patients (43.2 vs. 61.1% $p < 0.04$, 20.2 vs. 42.3% $p < 0.001$).

Conclusions : Patients with cirrhosis are particularly susceptible to sepsis-associated organ failures and death compared to general population. ICU-hospitalized patients with cirrhosis are frequently infected by gram-positive cocci, in particular MRSA.

INCREASED INTRAHEPATIC RESISTANCE IN STEATOSIS BY ENDOTHELIAL DYSFUNCTION AND MICROVASCULAR CHANGES. S. Francque (1), W. Laleman (2), L. Verbeke (2), C. Van Steenkiste (3), C. Casteleyn (4), W. Kwanten (4), C. Van Dijck (4), M. D'hondt (4), A. Ramon (1), W. Vermeulen (4), B. De Winter (4), E. Van Marck (1), P. Pelckmans (1), P. Michielsen (1). (1) Antwerp University Hospital, Antwerpen, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) Ghent University Hospital, Ghent, Belgium ; (4) Antwerp University, Antwerpen, Belgium.

Introduction : Non-alcoholic fatty liver disease can progress to steatohepatitis and fibrosis and is also associated with impaired liver regeneration in case of surgery or transplantation. The pathophysiology remains elusive. We recently showed that severe steatosis is associated with an increase in portal pressure suggesting liver flow impairment.

Aim : To directly assess total intrahepatic resistance and its potential functional and structural determinants in a *in situ* perfusion model.

Methods : Male Wistar rats fed a control ($n = 30$) or a methionine-choline deficient (MCD) diet ($n = 30$) for 4 weeks were compared. Liver tissue and serum analysis, *in vivo* haemodynamic measurements, *in situ* perfusion experiments and vascular corrosion casts were performed.

Results : The MCD-group showed severe steatosis without inflammation or fibrosis at histology. Serum levels of interleukin-6, Tumor necrosis factor alpha, interleukin-1b and interferon-gamma and liver tissue levels of myeloperoxidase activity were comparable between groups, excluding significant inflammation. Flow-pressure curves were significantly different between groups for all flows (slope values : 0.1636 ± 0.0605 mm Hg/mL/min in controls vs. 0.7270 ± 0.0408 mm Hg/mL/min in MCD fed rats, $p < 0.001$) indicating an increased intrahepatic resistance, which was haemodynamically significant (portocaval pressure gradient 2.2 ± 1.1 vs. 8.2 ± 1.3 mm Hg in controls vs. MCD, $p < 0.001$). Dose-response curves to acetylcholine were significantly reduced in MCD fed rats ($p < 0.001$). Responsiveness to methoxamine was even so significantly reduced in MCD fed rats ($p < 0.001$). Vascular corrosion casts showed a replacement of the regular sinusoidal anatomy by a disorganised pattern with multiple interconnections and vascular extensions.

Conclusions : Severe steatosis induces a haemodynamically significant increase in intrahepatic resistance which precedes inflammation and fibrogenesis. Both functional (endothelial dysfunction) and structural factors are involved. This phenomenon might significantly contribute to steatosis-related disease.

- A18 -

EFFICACY OF BOCEPREVIR IN PATIENTS WITH NULL RESPONSES TO PEGINTERFERON/RIBAVIRIN : THE PROVIDE STUDY. Y. Horsmans (1), I. Jacobson (2), J. Vierling (3), S. Flamm (4), S. Gordon (5), E. Lawitz (6), J.P. Bronowicki (7), M. Davis (8), E. Yoshida (9), L. Pedicone (10), W. Deng (10), M. Treitel (10), C. Brass (10), J. Albrecht (10), F. Poordad (11). (1) Clinique Universitaire St Luc, Brussels, Belgium ; (2) Weill Cornell Medical College, New York, United States ; (3) Baylor College Of Medicine, Houston, United States ; (4) Northwestern Feinberg School Of Medicine, Chicago, United States ; (5) Henry Ford Hospital, Detroit, United States ; (6) Alamo Medical Research, San Antonio, United States ; (7) University Henri Poincare, Vandoeuvre-Lès-Nancy, France ; (8) South Florida Center Of Gastroenterology, Wellington, United States ; (9) University Of British Columbia And Vancouver General Hospital, Vancouver, Canada ; (10) Merck, Whitehouse Station, United States ; (11) Cedars-Sinai Medical Center, Los Angeles, United States.

Background : Combination therapy with boceprevir (BOC), an NS3 protease inhibitor, and peginterferon alfa-2b + ribavirin (PR) vs PR alone has been studied in adults chronically infected with hepatitis C virus (HCV) genotype 1 who were previously untreated (SPRINT-2) or had failed prior PR treatment (RESPOND-2). Patients in the PR control arms of these studies who did not achieve sustained virologic response (SVR) were eligible to enroll in the PROVIDE study and receive BOC + PR.

Aim : We examined the efficacy of BOC + PR in the subset of patients in PROVIDE who were PR null responders in the previous study (< 2 log₁₀ decline in HCV RNA at treatment week 12).

Methods : BOC (800 mg TID with food) was given with P 1.5 mg/kg/week subcutaneously and weight-based R (600-1400 mg/day) BID for up to 44 weeks. If > 2 weeks had elapsed since end of treatment in the previous study, PR was given for 4 weeks before starting BOC+PR. Undetectable HCV RNA was defined as below the limit of detection (LLD = 9.3 IU/mL ; Roche TaqMan).

Results : 48 prior null responders from SPRINT-2 or RESPOND-2 enrolled in the PROVIDE study : 65% were male, 69% were white, mean age was 51 years (range, 25-66 years), and mean body mass index was 26.8 kg/m². Baseline viral load was > 800,000 IU/mL in 88% (mean log₁₀ baseline viral load of 6.5) ; Metavir fibrosis scores were F3/4 in 8% of patients. 65% had subtype 1a and 35% had subtype 1b. 45/48 initiated BOC treatment and 42 completed follow-up ; 2 are still on treatment and 1 in early follow-up. The proportion of patients with undetectable HCV RNA at various time points is shown below. Following a null response to prior PR therapy, 38% achieved SVR (27% of black patients, 42% of non-black patients and 41% genotype 1a) with BOC + PR.

Patients With Undetectable HCV RNA % (n/N)	
Weeks of BOC	Total
6	25 (11/44)
12	50 (22/44)
24	47 (20/43)
44/EOT	47 (20/43)
End of follow-up	38 (16/42)

Conclusions : When retreated with BOC + PR, 38% of prior PR null responders achieved SVR. This SVR rate in prior null responders was comparable to the SVR rate (33%) observed in poorly IFN responsive patients (< 1 log₁₀ decline after 4-week PR lead-in) treated in the SPRINT-2 and RESPOND-2 studies, for the combined BOC groups.

- A19 -

RESOURCE USE AND COST OF HEPATITIS C RELATED CARE IN BELGIUM. F. Nevens (1), I. Colle (2), P. Michiels (3), G. Robaey (4), C. Moreno (5), K. Caelbergh (6), M. Lamotte (6), V. Wyffels (7). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) Ghent University Hospital, Gent, Belgium ; (3) Antwerp University Hospital, Antwerpen, Belgium ; (4) Zol, Genk, Belgium ; (5) Erasme Hospital, Brussels, Belgium ; (6) Ims Consulting Group, Vilvoorde, Belgium ; (7) Janssen-Cilag Nv, Beerse, Belgium.

Introduction : Chronic hepatitis C virus (CHC) is a significant health problem which can lead to decompensated cirrhosis, hepatocellular carcinoma (HCC) and eventually death, all of which are associated with significant healthcare costs.

Aim : To update the cost of care of CHC according to the different disease severity stages in a West European country such as Belgium.

Methods : Medical records of 157 patients, who were referred to the medical specialist at different disease stages were reviewed to identify medical costs over a follow-up period of 3 years or 2 years in the case of liver transplantation (LT). Six disease stages were defined based on histology (Metavir classification) and/or clinical data.

Results : In comparison with mild disease the cost increased 1.6 times in case of decompensated cirrhosis, 1.9 times in case of HCC and 3.4 in case of LT. The costs for medication, hospitalization and ambulatory care were respectively on the one hand for mild disease : 81%, 8% and 11% and on the other hand for LT : 18%, 79% and 3%. In case of sustained viral response (SVR) the cost of follow-up within 3 years decreased with 45% for patients with mild and moderate disease.

Conclusions : Antiviral treatment is the most important cost driver in mild and moderate disease but once complications of CHC occur hospitalization costs far exceed the cost of antiviral therapy. SVR decreased significantly the cost already during the first 3 years of follow-up. Treating patients with CHC in an early stage has the potential to be cost-effective.

- A20 -

SEARCH FOR AN OBJECTIVE PARAMETER OF THE SEVERITY OF POLYCYSTIC LIVER DISEASE.
F. Temmerman, R. Vanslebrouck, W. Coudyzer, B. Bammens, W. Laleman, D. Cassiman, C. Verslype, W. Van Steenberghe, J. Van Pelt, J. Pirenne, F. Nevens. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : The most common complication of polycystic liver disease (PCLD) is extensive hepatomegaly, which may lead to invalidating abdominal symptoms and malnutrition. Liver transplantation (LT) is the only curative option in those patients. Currently, a decreased mid-upper arm circumference (MUAC) in the non-dominant arm, – a parameter of severe malnutrition –, can be used to give those patients priority on the LT waiting list (Eurotransplant). However, this measurement is subjective. Moreover, this parameter has never been validated in this condition.

Aim : To investigate a more objective parameter to assess the extent of the disease.

Methods : 84 patients with PCLD (Gigot type II and III) were screened ; 47 were selected because of volume-related symptoms. The extent of the hepatomegaly (actual liver volume : ALV) was calculated by CT-scan volumetry software, validated in our previous placebo-controlled trial with lanreotide (interobserver variability by Pearson correlation : $r = 0.994$, $p < 0.01$). The estimated standard liver volume (ESLV) was obtained based on the body surface area (BSA) of the patient and Urata's equation, in order to calculate ALV/ESLV.

Results : There were 42 women (89%) and 5 men (11%) with a mean age of 52 ± 9 years : 37 (79%) suffered from ADPKD and 10 (21%) ADPLD. The mean ESLV was 1254 ± 127 ml ; the mean ALV 5542 ± 2390 ml ; and the mean ALV/ESLV 4.4 ± 1.8 . Between the ADPKD and ADPLD group, there was no significant difference in ALV and ALV/ESLV (t-test, P value resp. : 0.6 and 0.9). These data were compared with MUAC (mean 25.2 ± 3.0 cm).

Pearson's correlation showed that an increased ALV was correlated with a decreased MUAC (Correlation Coefficient : -0.351 ; $P < 0.05$). The correlation coefficient increased when ALV/ESLV was used (-0.473).

Finally, the study group was divided in patients who were considered for LT ($n = 20$) and those who did not ($n = 27$). This decision was based on clinical judgement. Patients in group LT had a significant lower MUAC vs group no LT (resp. 24.1 ± 2.7 cm and 25.9 ± 3.1 cm ; $p = 0.04$). No significant difference was observed in ALV between the two groups (Mann-Whitney Rank Sum test resp. : 5438ml and 4891ml ; $p = 0.07$). However, ALV/ESLV was higher in the group LT vs group no LT (Mann-Whitney Rank Sum test resp. : 4.8 and 4.2 ; $P = 0.025$). ALV/ESLV offered additional prognostic information to MUAC for the need of LT (difference in AUC : 0.130 ; $p = 0.01$).

Conclusions : The size of hepatomegaly in PCLD can accurately and in an objective way be measured by CT volumetry and reflects the severity of the liver disease. It is correlated with the mid-upper arm circumference and offers additional information on the extent of the liver disease.

OUTCOME AFTER LIVER TRANSPLANTATION USING DCD DONORS : A SINGLE-CENTER EXPERIENCE.
D. Monbaliu, J. Francois, S. Vandenbussche, R. Aerts, W. Laleman, C. Verslype, S. Vandermerwe, W. Van Steenberghe, D. Cassiman, F. Nevens, J. Pirenne. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Liver Transplantation (LTx) using Donation after Cardiac Death (DCD) donors are increasingly used to expand the donor pool but considered a risk factor for poor outcome. Also there are concerns regarding short & long-term outcome after DCD-LTx.

Aim : We therefore reviewed the results of DCD-LTx at our center.

Methods : Between 2003 and 2010, 30 DCD LTx were performed (6% of all LTx). Medical records of DCD donors and recipients were retrospectively reviewed. Donor demographics, LTx indications, post-LTx peak transaminase (AST), biliary complications, and graft rejection were analyzed. Patient/graft survival was analyzed and compared to outcome using Brain Dead Donors (DBD).

Results : Of 30 DCD-LTx, 25 livers were locally procured. Mean donor age was 47.3 yo (range :13-69). Mean warm ischemia time (stop ventilation to cold perfusion) was $23 \pm 11'$. Mean cold ischemia time was $415 \pm 104'$. Mean recipient age was 58 yo (range : 24-71). Mean labMELD score was 17 (range : 8-31). Indications for LTx were cirrhosis related to post-ethyl (13), HCV (4), NASH (3), unknown (4), PBC (1), PSC (1), acute liver failure (1), congenital disorder (1), or HCC without cirrhosis (2). Eleven recipients (37%) had hepatocellular carcinoma. Most recipients (22/30) were at home ; 7 were hospital-bound and 1 of them in ICU. Post-LTx AST peak was 1712 IU/L. Reasons for graft loss were : hepatic artery thrombosis (1), ductopenic rejection (1) and diffuse intrahepatic biliary strictures (1). 10 patients (35%) developed biliary complications (non-anastomotic in 9), requiring conservative treatment (2), endoscopic interventions (7) and re-Tx (1). Acute graft rejection occurred in 3 recipients : 2 responded to steroids but one developed refractory ductopenic rejection and awaits re-LTx. Follow-up ranged from 1 to 93 mths. Actuarial 1, 3, & 5-yr patient survival was 92, 83 and 83%, and 1, 3, & 5-yr graft survival was 89, 79, and 79%, respectively and not different from outcome using grafts from brain dead donors (actuarial 1, 3 & 5- yr patient survival of 90, 82 & 75%, $p = 0,846$ and graft survival of 88, 79 & 73%, $p = 0,707$ respectively).

Conclusions : Despite substantial ischemic injury (high peak AST & high rate of biliary complications) short- & long-term survival after DCD-LTx is comparable to DBD-LTx. Rapid donor surgery, careful donor and recipient selection, short warm and cold ischemia times are key factors to optimize outcome after DCD LTx. However, strategies to reduce ischemic injury and biliary complications are warranted.

Invited lecture : Marc Hautekeete Lecture

WHAT ADULT GASTROENTEROLOGISTS SHOULD KNOW ABOUT PAEDIATRIC LIVER DISEASE.
G. Mieli-Vergani. King's College Hospital, London, United Kingdom.

The interest in paediatric liver disease started in the late 1960s, early 1970s. It was considered a rare condition with poor prognosis. In the 1970s, the mortality of children with liver disease within two years from diagnosis was as high as 60%, only a minority of children growing into adult life with liver pathologies, mostly those with benign conditions that resolve spontaneously. It is now well established that liver disease in children is not so rare and, with better diagnostic and management tools, its survival is over 90%. Hence, most patients diagnosed with liver disease in childhood reach adulthood and become patients of adult gastroenterologists.

Over the past 40 years, the differential diagnosis of cholestatic liver disease in infancy has become very complex. Though biliary atresia remains the most common severe form of infantile liver disease, new conditions, mostly genetically determined, have been described, including Alagille syndrome and progressive intrahepatic cholestases (PFIC) type 1, 2 and 3. Children with biliary atresia surviving into adult life after Kasai portoenterostomy without the need for liver transplantation in childhood represent 25-45% of all surgically treated cases. They are generally well, but have chronic liver disease with portal hypertension, and are prone to the development of ascending cholangitis ; girls may be more prone to miscarriage ; but most importantly, as they have never experienced normality, their attitude to disease is quite different from that of patients who develop liver disease in adult life. Alagille syndrome and PFIC1 protein deficiency (PFIC type 1) are systemic disorders, in which liver disease is only one of several problems that need to be known by the attending clinician, as they are not cured by liver transplantation. PFIC type 2 and type 3 are associated with early development of hepatocellular carcinoma and surveillance is necessary.

Autoimmune liver disorders in childhood include autoimmune hepatitis and autoimmune sclerosing cholangitis. According to the type of circulating autoantibodies, autoimmune hepatitis is divided in type 1 (anti-nuclear and/or anti-smooth muscle antibody positive) and in type 2 (anti-liver kidney microsome antibody positive). Autoimmune hepatitis is particularly aggressive in children and progresses rapidly unless immunosuppressive treatment (steroids and

azathioprine) is started promptly, but with appropriate treatment 80% of patients achieve remission and survive into adult life. Autoimmune sclerosing cholangitis, which serologically is similar to autoimmune hepatitis type 1, responds to the same treatment used for autoimmune hepatitis in regards to parenchymal inflammation, but bile duct disease progresses in about 50% of cases, leading to a worse prognosis and higher transplantation requirement ; it has a high recurrence rate post-liver transplant.

Long term complications after liver transplantation performed in infancy are often different from those in patients transplanted in adult life : for example outflow problems due to remodelling of a reduced size liver are frequent and need to be dealt by an expert team of surgeons and physicians ; patients transplanted during childhood have significance learning difficulties often requiring special education.

One of the most important issues related to growing up with chronic liver problems, either chronic liver disease or liver transplant, is that the disease started when the patient could not understand the disease itself or the implications of its treatment. This leads to frequent lack of adherence to treatment : at least 20% of adolescents/young adults who have had liver disease from childhood experience severe morbidity or mortality as a consequence of non-adherence to treatment. A multi-disciplinary Transition Service (paediatric hepatologist, adult hepatologist, surgeon, psychologist and social worker), where all team members are knowledgeable about paediatric liver problems, is essential to care for these patients appropriately.

- A23 -

EXPRESSION OF INFLAMMATORY AND ANGIOGENIC FACTORS IN THE SERUM OF A PATIENTS WITH STEATOSIS AND NASH. S. Coulon (1), S. Francque (2), A. Geerts (1), A. Verrijken (2), B. Blomme (1), F. Heindryckx (1), H. Vanvlierberghe (1), L. Vangaal (2), I. Colle (1). (1) Universitair Ziekenhuis Gent, Gent, Belgium ; (2) Antwerp University Hospital, Antwerpen, Belgium.

Introduction : The liver is a major target of injury in obese patients. Non-alcoholic fatty liver disease (NAFLD) is present in 17 to 33% of obese Americans and can range from simple steatosis to the more severe non-alcoholic steatohepatitis (NASH). The onset of a chronic inflammatory reaction marks the progression from steatosis to NASH and the expansion of adipose tissue is strongly associated with angiogenesis. Therefore, we determined the expression of inflammatory (tumor necrosis factor alpha (TNF α) and interleukin 6 (IL6)) and angiogenic (vascular endothelial growth factor (VEGF), soluble VEGF receptor 1 (sVEGFR1) and sVEGFR1) cytokines in the serum of an obese population with simple steatosis and NASH compared to healthy controls.

Aim : The rationale of this study was to reveal the expression pattern of TNF α , IL6, VEGF, sVEGFR1 and sVEGFR2 in a population of control subjects and biopsy proven steatosis and NASH patients.

Methods : The expression of these cytokines was determined in the serum by commercial available ELISA kits (R&D systems, UK). The population consisted out of 30 obese patients, which were diagnosed with simple steatosis and 32 patients with NASH and compared to 30 age-and-sex matched healthy controls.

Results : Mean serum TNF α levels were elevated in the serum of steatosis and NASH patients compared to healthy controls, reaching significance in NASH patients ($p < 0.05$). IL6 was significantly increased in steatosis and NASH patients compared to the healthy controls ($p < 0.001$). VEGF levels were significantly elevated in patients with steatosis ($p < 0.05$) and borderline significantly elevated in NASH patients compared to the serum levels of healthy control subjects ($p = 0,062$). The expression of sVEGFR1 was significantly increased in steatosis and NASH patients compared to controls ($p < 0.001$). sVEGFR2 expression was not significantly different in the three groups ($p > 0.05$).

Conclusions : These data indicate the involvement of inflammatory (TNF α and IL6) and angiogenic (VEGF and sVEGFR1) cytokines in the pathophysiology of NASH. However, there is only very little data about the role of these factors in the pathogenesis of NAFLD in humans. Therefore, our data suggest that not only inflammatory factors such as TNF α and IL6 are related to the disease progression of NASH but that angiogenesis could also play an important role in the development of NASH.

- A24 -

RISK FACTORS OF BLEEDING AND CLINICAL IMPLICATIONS IN PATIENTS UNDERGOING LIVER TRANSPLANTATION. M. Esmat Gamil, F. Nevens, H. Van Malenstein, M. Verhaegen, B. Desschans, D. Monbaliu, R. Aerts, W. Laleman, D. Cassiman, C. Verslype, W. Van Steenberghe, J. Pirenne. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Liver disease is characterized by prolonged global coagulation tests such as the prothrombin time (PT). Relationship between the abnormality of this test and bleeding risk has been recently challenged.

Aim : In the present study we investigated the risk factors of bleeding and the clinical implications in patients who underwent Liver Transplantation (LT).

Methods : Patients who received LT between 2005 and 2010 were studied. Combined transplantations, a re-transplantation or LT due to acute liver failure were excluded. Pre-and post LT clinical and biochemical data were collected prospectively. Risk factors were assessed with linear regression models.

Results : 286 patients with a mean follow up of 32 months were studied. Pre LT INR, bilirubin, creatinine, ascites and portal vein thrombosis were correlated with number of blood units transfused in univariate analysis. In multivariate analysis, only INR ($p = 0.015$) and ascites ($p = 0.015$) were independently positive correlated with need of transfusion. The best cut off for the prediction of the need for blood transfusion was an INR value = 1.6. In the group of patients with INR < 1.6 vs. INR = 1.6 the appreciation of the surgeon during the intervention (wet or dry), the amount of blood transfusion, fresh frozen plasma, total stay in ICU and total stay in hospital were statistical significant different (< 0.001). The first year survival was worse in patients with active bleeding during surgery (82%) than those with no active bleeding (93%).

Conclusions : Bleeding during LT affects outcome. This risk factor is independently influenced by : 1) The presence of ascites (probably reflecting the degree portal hypertension) 2) INR = 1.6. Therapeutic interventions to reduce the need for blood transfusions during LT, in an attempt to improve the survival, should be further explored in this high risk population.

- A25 -

SIGNIFICANCE OF PRIMARY POOR FUNCTION (PPF) FOR THE PROGNOSIS AFTER LIVER TRANSPLANTATION. G. Uijterhaegen (1), I. Haentjens (2), R. Troisi (2), G. Vanmaele (1), H. Vanvlierberghe (2), X. Rogiers (2). (1) Universiteit Gent, Gent, Belgium ; (2) Universitair Ziekenhuis Gent, Gent, Belgium.

Introduction : Primary poor function (PPF) is a term used to describe temporary malfunction of the transplanted liver. Over 20 different definitions of PPF can be found in the literature and there is only a few cases in which its impact on survival is described. In this paper we try to define the impact of different definitions of PPF on medium term patient and graft survival.

Aim : Define primary poor function of the liver in a way that is relevant for the prognosis of graft and recipient

Methods : Data of 90 transplantations performed between July 2007 and October 2009 at the University Hospital of Ghent were analyzed retrospectively. The transplantations were classified by 7 definitions of PPF and correlated with patient and graft survival.

Results : The main indications for transplantation were alcoholic cirrhosis (40,0%) and HCV cirrhosis (10,0%). Mean age of transplantation was 53,6 years. Mean follow-up duration was 33,8 months (range 24-47 months) The rate of PPF differed from 10,0% to 55,6% depending on the definition used. Of the 7 tested definitions only 2 (Strasberg *et al.* and Cieslak *et al.*) showed a significant correlation with graft- and patient survival according to Kaplan-Meier tests. The definition of Strasberg *et al.* (The presence of at least one of the following : AST or ALT > 2000 IU/L on post-operative day 2, INR > 1.6 or serum bilirubin > 10.0 mg/dL on post-operative days 2-10) showed a very significant correlation (p -value < 0,001).

Conclusions : Our study shows that only two of the seven most used definitions of PPF show a significant correlation with short and medium term graft and patient survival. The definition of Strasberg *et al.* defines best the risk of the patient for graft loss and death and should therefore be used as the definition of choice.

- A26 -

NORMALISATION OF N-GLYCOSYLATION AFTER LIVER TRANSPLANTATION : A PROOF OF CONCEPT. X. Verhelst (1), B. Blomme (1), E. Padalko (1), I. Colle (1), A. Geerts (1), R. Troisi (1), X. Rogiers (1), N. Callewaert (2), H. Vanvlierberghe (1). (1) Universitair Ziekenhuis Gent, Gent, Belgium ; (2) Vib, Gent, Belgium.

Introduction : Changes in N-glycosylation profiles of serum proteins are present in patients with chronic liver diseases. Qualification and quantification of these profiles - also called *glycomics*-has proved to have a discriminative function in the assessment of fibrosis and cirrhosis (¹). Liver transplantation is the sole curative treatment for end stage liver disease. However, the evolution of N-glycosylation profiles after liver transplantation has never been studied.

Aim : The aim was to study the evolution of serum N-glycosylation profiles after liver transplantation.

Methods : In 6 liver transplant patients one pre-transplant serum sample and one post-transplant serum sample were retrospectively analysed for N-glycan profiles using DNA sequencer-assisted-fluorophore-assisted capillary electrophoresis (DSA-FACE). The timing after transplantation varied among the different patients (range : 5 days-20 months).

Results : A serum N-glycan profile of a patient consists of 13 different peaks. The height of every peak is a measure for the abundance of the corresponding glycan. Pre-transplant samples of all patients showed an important increase of peak 7, a biantennary bisecting GlcNAc N-glycan, and decrease of peak 8, a triantennary N-glycan, compatible with findings in cirrhotic patients. After transplantation we observed in all patients a collapse of peak 7 and an increase of peak 8 after transplantation, similar to findings in healthy volunteers. Peak 1, an agalacto biantennary N-glycan, which has been linked to hepatic inflammation eg. in NASH patients tends to lower in the months after transplantation. Twenty months after transplantation, one serum sample showed an increase of this glycan, however in a patient diagnosed with important steatohepatitis at that particular moment.

Conclusions : In this small patient sample a rapid and dramatic normalisation of serum N-glycan profiles was observed after liver transplantation consisting of a rapid disappearance of signs of cirrhosis (within days) and a slow regression of inflammation (within months). These limited but consistent data are the first implementation of *glycomics* in transplantation medicine. It would be interesting to validate these intriguing data in a large prospective cohort and explore their clinical significance.

Reference :

1. Callewaert N., Van Vlierberghe H., Van Hecke A., Laroy W., Delanghe J., Contreras R. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. *Nat. Med.*, 2004, 10 : 429-434.

- A27 -

HEPATITIS E SEROPREVALENCE IN BELGIUM. F. Vanhoecke, T. Vanmaerken, M. Debouille, A. Geerts, H. Vanvlierberghe, I. Colle, E. Padalko. Universitair Ziekenhuis Gent, Gent, Belgium.

Introduction : Hepatitis E virus (HEV) typically causes waterborne outbreaks of acute hepatitis in parts of the world with poor sanitation. However, HEV infection is now increasingly recognized as a cause of hepatitis in developed countries as well. This autochthonous hepatitis E is usually caused by genotype 3, which has been shown to have a high prevalence in pig populations worldwide and which may be transmitted zoonotically. Previous seroprevalence studies in Western Europe have yielded widely differing estimates, probably because of the variability in analytical performance of the assay used for detection of anti-HEV antibodies.

Aim : The goal of this study is to provide an estimate of the seroprevalence of HEV in Belgium, since data for our country are currently lacking.

Methods : One hundred patients presenting to our hospital without gastroenterological problems were randomly selected to be tested for anti-HEV IgG antibodies. This was done using a sensitive indirect ELISA (Biorex, Antrim, United Kingdom) and, in the case of a borderline result, a strip immunoassay (Mikrogen, Neuried, Germany) for confirmation.

Results : The anti-HEV IgG seroprevalence was found to be 14%.

We compared this figure to published seroprevalence rates of HEV in other Western European countries : 3.2% to 16.6% in France, 2.0% to 15.5% in Germany, 1.1% to 6.0% in the Netherlands, 5.3% to 16.2% in the UK and 0.8% to 7.3% in Spain. As pigs may serve as a reservoir for HEV infection in humans, we calculated pig/inhabitant ratios by dividing the number of pigs by the number of inhabitants for these countries (data from 2009). The pig/inhabitant ratio was 0.59 in Belgium, 0.23 in France, 0.33 in Germany, 0.73 in the Netherlands, 0.08 in the UK and 0.57 in Spain. It is clear from these data that a simple linear correlation between the seroprevalence of HEV and the pig/inhabitant ratio does not exist. However, it should be kept in mind that HEV seroprevalence rates from different studies are difficult to compare due to large differences in the analytical sensitivity of the available anti-HEV IgG assays.

Conclusions : The observed seroprevalence rate suggests that HEV infection is not an uncommon event in Belgium. Comparisons with published seroprevalence data of other Western European countries should be made with caution due to differences in the analytical performance of anti-HEV IgG assays.

Frederik Van Hoecke and Tom Van Maerken have equally contributed to this work.

- A28 -

THE VALUE OF MINIMALLY INVASIVE SURGERY FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA. H. Topal, J. Tiek, S. Fieuws, R. Aerts, C. Verslype, J. Pirenne, T. Roskams, F. Nevens, B. Topal. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Little is known about the oncologic outcome and prognostic indicators of patients with hepatocellular carcinoma (HCC) who undergo minimally invasive liver surgery (MILS).

Aim : To evaluate the long-term oncologic outcome of MILS in patients with HCC and determine prognostic indicators.

Methods : From January 2004 until April 2009 prospectively collected data of 119 consecutive patients who underwent surgical treatment for HCC were analyzed. MILS (resection 13 ; RFA 54 ; resection + RFA 5) was performed in 72 and open liver surgery (OLS ; resection 40 ; RFA 4 ; resection + RFA 3) in 47 patients. Postoperative outcome, overall (OS) and disease free survival (DFS) rates were studied. In addition, 18 potential predictive factors were assessed for their prognostic value.

Results : The severity of postoperative complications was higher in the OLS-group (36% vs. 7% ; $p = 0.0002$). The rate of surgical site complications was similar in both groups while more non-surgical site complications were observed in the OLS vs. MILS-group (25.5% vs. 2.8% ; $p = 0.0002$). Patients who underwent OLS had a longer hospital stay (median 10 days vs. 3 days after MILS ; $p < 0.0001$). Survival (DFS and OS) rates were comparable in the two groups (HR 0.73 (95% CI : 0.47-1.13) ; $p = 0.016$ and HR 0.76 (95% CI : 0.44-1.32 ; $p = 0.34$)). In cirrhotic patients with HCC, postoperative liver transplantation (LT) was found to be the only independent predictor of both OS and DFS.

Conclusions : MILS seems to result in better postoperative outcomes and similar survival rates as compared to OLS. Following surgery for HCC, liver transplantation seems to be the only independent factor having a beneficial impact on survival of cirrhotic patients with HCC.

- A29 -

SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA (HCC) AND GASTROESOPHAGEAL VARICES (GEV) BY BELGIAN GASTROENTEROLOGISTS. P. Michielsen (1), J. Delwaide (2), C. Degalocsy (3). (1) Antwerp University Hospital, Antwerpen, Belgium ; (2) ULg Sart Tilman, Liège, Belgium ; (3) Hôpitaux Iris Sud Bracops, Brussels, Belgium.

Introduction : Patients with cirrhosis are at risk of developing HCC and GEV bleeding. Guidelines on surveillance for these complications have been established based on medical evidence.

Aim : Evaluation of surveillance on HCC and GEV in cirrhosis by Belgian gastroenterologists based on a GLEM/LOK questionnaire.

Methods : A questionnaire on surveillance for HCC and GEV was constructed and distributed to the Belgian GLEM/LOK groups as one of the national themes.

Results : Surveillance of HCC : 113 answers were received. A large majority (93%) applies abdominal ultrasound and alfa fetoprotein determination for surveillance, in 76% at 6 monthly interval. When a nodule > 2 cm is detected, 77% will proceed to dynamic magnetic resonance imaging and 20% to dynamic computed tomography for further characterization. Imaging-directed fine-needle biopsy of a suspected focal lesion will be considered in 38% of the respondents, another 28% will proceed to biopsy when ablation of the needle tract can be performed, before potential curative treatment (resection, transplantation or ablation).

Surveillance of GEV : 111 answers were received. A large majority (95%) will perform screening esophagogastrosopy in a patient with diagnosis of cirrhosis. 90% uses a 3 grade system (small, medium, large). 68% will repeat endoscopy every 2-3 years in case of no varices at initial examination and 52% will repeat endoscopy at yearly intervals in case of initial small varices in compensated cirrhosis. In case of large varices (> 5 mm) 70% will use a nonselective betablocker as primary prophylaxis of bleeding, and endoscopic therapy when betablockers are contra-indicated or not tolerated.

Conclusions : The majority of Belgian gastroenterologists participating in the questionnaire adhere to the international guidelines concerning surveillance of HCC and GEV. A majority will proceed to liver biopsy, especially when ablation of the needle tract can be performed before a potential curative treatment.

Acknowledgement : thanks to B. Van Langenhove for his help in computing the data.

- A30 -

DIFFERENTIATION OF FOCAL CYSTIC LIVER LESIONS BASED ON RADIOLOGICAL IMAGING FINDINGS. J. Liem, F. Willemsen. Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : Focal cystic liver lesions can be caused by many pathological processes, which require different management strategies.

Aim : Can benign cystic liver lesions be differentiated from other cystic lesions, based on radiological imaging findings ?

Methods : Between 2000 and 2011, 35 patients were included who had pathologically proven cystic liver lesions and prior imaging by CT- or MRI-examinations. Mean age was 58.3 years (range 35-83y), 30 (86%) were women. Imaging features of 36 lesions were evaluated on either multiphase contrast enhanced CT (32 patients) and/or contrast enhanced MRI (13 patients). Size, shape, wall, internal structure and enhancement characteristics were noted.

Results : Pathological analysis revealed 14 (39%) benign (all simple cysts), 7 (19%) premalignant (all biliary cystadenomas), 5 (14%) malignant (e.g. cystadenocarcinoma, sarcoma) and 10 (28%) infectious/inflammatory lesions (e.g. echinococcal cyst, abscess). Mean diameter was 13.2 cm (range 1.3-28.7 cm). Thirteen out of 14 (93%) benign lesions showed a thin wall without enhancement or solid components. A small internal peripheral located septum was noted in 7 out of 14 (50%) benign lesions. Six out of 10 (60%) infectious/inflammatory lesions showed a thickened irregular wall with enhancement. Contrast enhancement of multiple internal septations (8 lesions) and/or solid components (6 lesions) was noted in all 7 premalignant and 5 malignant lesions.

Conclusions : Benign cystic liver lesions can accurately be differentiated from other cystic lesions. A solitary thin internal septum can be present in simple liver cysts. Malignant and premalignant lesions all show multiple septations with internal enhancement, solid components or both.

- A31 -

HISTOLOGICAL DIVERSITY IN CHOLANGIOCELLULAR CARCINOMA REFLECTS THE DIFFERENT CHOLANGIOCYTE PHENOTYPE. M. Komuta, O. Govaere, V. Vandecaveye, W. Van Steenberghe, C. Verslype, J. Pirenne, R. Aerts, F. Nevens, B. Topal, T. Roskams. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Cholangiocellular carcinoma (CC) originates from topographically heterogeneous cholangiocytes. The cylindrical mucin-producing cholangiocytes are located in large bile ducts and the cuboidal non-mucin producing cholangiocytes are located in ductules containing bipotential hepatic progenitor cells (HPCs).

Aim : We investigated the clinicopathological and molecular features of 85 resected CCs [14 hilar CCs (so-called Klatskin tumor), 71 intrahepatic CCs (ICCs) including 20 cholangiocellular carcinomas (CLCs) (thought to be originated from HPCs)], and compared these with the different cholangiocyte phenotypes, including HPCs.

Methods : Immunohistochemistry was performed with biliary/HPC and hepatocytic markers. Gene expression profiling was performed in different tumors, and compared with non-neoplastic different cholangiocyte phenotypes obtained by laser microdissection. Invasion and cell proliferation assay were assessed using different types of CC cell lines : KMC-1, KMCH-1, and KMCH-2.

Results : Among 51 ICCs, 31 (60.8%) contained only mucin-producing CC features (muc-ICCs), while 39.2% displayed histological diversity ; focal hepatocytic differentiation and ductular areas (mixed-ICCs). Clinicopathologically, muc-ICCs and hilar CCs showed a predominantly (peri-) hilar location, smaller tumor size, and more lymphatic and perineural invasion compared with mixed-ICCs and CLCs (predominantly peripheral location, larger tumor size, and less lymphatic and perineural invasion). Immunoreactivity was similar in muc-ICCs and hilar CCs, and in mixed-ICCs and CLCs. *S100P* and *MUC1* were significantly up-regulated in hilar CCs and muc-ICCs compared with mixed-ICC and CLC, while *NCAM1* and *ALB* tended to be up-regulated in mixed-ICCs and CLCs compared with other tumors. KMC-1 showed significantly higher invasiveness than the others.

Conclusions : Muc-ICCs had a similar clinicopathological, immunohistochemical, and molecular profile to hilar CCs (from mucin-producing cholangiocytes), while mixed-ICCs had a similar profile to CLCs (thought to be of HPC origin), possibly reflecting their respective cells of origin.

- A32 -

MELD SCORE AND THE NEED FOR LIVER TRANSPLANTATION IN PATIENTS WITH ACUTE SEVERE AUTOIMMUNE HEPATITIS. G. Mavrogenis (1), P. Warzee (2), C. Dabo (1), M.L. Woehl (1), B. Ellero (1), E. Sauleau (1), T. Baumert (1), C. Coltescu (1), M. Audet (1), S. Dritsas (1), P. Lutun (1), P. Wolf (1), M. Doffoel (1), F. Habersetzer (1). (1) Notre Dame, Charleroi, Belgium ; (2) Hôpitaux Universitaires, Strasbourg, France.

Introduction : Liver transplantation is indicated for patients with autoimmune hepatitis presenting with acute liver failure or decompensated cirrhosis with a MELD score > 15.

Aim : In this study we evaluated the predictive factors for liver transplantation in patients with acute severe autoimmune hepatitis.

Methods : Between 1995 and 2010, 17 patients (median age of 59 years, range : 15-73) presenting with acute severe autoimmune hepatitis were identified.

Results : Eight patients presented encephalopathy. Median values at admission for INR, bilirubin, ALT, creatinine and MELD score were 2.3 (range : 1.4-5.4), 428 $\mu\text{mol/L}$ (range : 136-797), 1063 IU/L (range : 69-2200), 80 $\mu\text{mol/L}$ (range : 55-394), 27 (range : 19.9-47.5), respectively. Nine patients underwent transplantation with a median delay of 15 days (range 2-35). Predictive factors of transplantation were the presence of encephalopathy (77.7% versus 12.5%, $p = 0.007$), a higher median MELD score (36.1, range 26.4-47.5 versus 24, range 19.9-29, $p = 0.001$), INR (3.4, range : 1.81-5.4, versus 1.79, range : 1.4-2.5, $p = 0.002$), bilirubin values (468.6 $\mu\text{mol/L}$, range : 154-797, versus 305.5, range :

136-500, $p = 0.046$) and a younger median age (52 yrs, range 15-63 versus 65.5, range : 50-73, $p = 0.036$). Totally, 15 patients received steroids with a median delay of 37 days (range : 14-140). Eight of them responded and presented a significant decrease of bilirubin levels and of the MELD score at day 3, of the transaminases at day 8 and of the INR at day 14.

Conclusions : A high MELD score at admission is a predictive factor for liver transplantation in patients with acute liver failure due to acute severe autoimmune hepatitis. Patients without encephalopathy and a within the first week improvement of bilirubin and MELD score may respond to steroid therapy and avoid transplantation.

- A33 -

PROSPECTIVE RANDOMIZED DOUBLE BLIND PLACEBO CONTROLLED STUDY ON SOMATOSTATIN IN LIVER TRANSPLANTATION. M. Sainz-Barriga (1), E. De Wolf (2), F. Berrevoet (2), X. Rogiers (2), E. Codarin (3), G. Tell (3), H. Reynaert (4), I. Colle (2), R. Troisi (2). (1) Ghent University, Ghent, Belgium ; (2) Ghent University Hospital, Ghent, Belgium ; (3) University Of Udine, Udine, Italy ; (4) University Hospital Brussel (Vub), Brussels, Belgium.

Introduction : Somatostatin (S) is a peptide hormone regulating the endocrine system, which is capable to decrease splanchnic flow in cirrhotic patients with variceal bleeding.

Aim : We set a study to confirm this property in patients with portal hypertension (PHT) undergoing liver transplantation and its role in I/R injury.

Methods : Between December 2010 and October 2011, the first 15 patients of this randomized study transplanted for end-stage liver disease and PHT were unblinded and analyzed according to the protocol. PHT was intraoperative measured recording the gradient between the portal vein and the caval vein. Only patients having a gradient > 10 mmHg were included in the study. M/F ratio was 1.5/1 and the mean age was of 57 ± 10 y. Indications for transplantation were : alcoholic ($n = 7$) ; HCV ($n = 5$) ; HCC ($n = 2$) PSC ($n = 1$). Liver biopsy specimens were taken at the back table and 1 h following liver reperfusion.

Results : The group placebo (P) ($n = 6$) presented a mean PVP of 19.5 mmHg and of 19.8 mmHg before and 2 min. after bolus infusion of 500 mg Somatostatin ($p = 0.4$). The group Somatostatin (S) ($n = 9$) presented a mean portal vein pressure (PVP) of 26.4 mmHg and of 19.5 mmHg respectively ($p = 0.04$). Responders in groups S were 5/9 (55%). Portal vein flow (PVF) in the P group was of 1188 ± 996 ml/min. before and 1260 ± 1124 ml/min. after infusion ($p = 0.4$). In the group S, PVF was of 976 ± 708 ml/min. and 649 ± 582 ml/min. respectively before and after infusion ($p = 0.18$). Peak AST/ALT in-group P was of 833 ± 524 and 508 ± 270 respectively. Peak AST/ALT in group S was of 1986 ± 1586 and 964 ± 1097 ($p = 0.12$ and 0.15 for group P vs. group S for AST and ALT respectively).

Conclusions : Somatostatin infusion in liver transplant recipients with PHT and a portal vein gradient above 10 mmHg significantly decrease the PVP in 55% of cases potentially avoiding the postreperfusion pressure in transplantation of partial grafts. I/R injury measured, as by postoperative AST/ALT peak did not differ. Proteomics analysis as expression of I/R injury in both groups is under evaluation.

- A34 -

HBSAG LEVELS IN NUCLEOS(T)IDE ANALOGUE SUPPRESSED HBEAG NEGATIVE PATIENTS AND HBSAG CARRIERS. E. Ho, S. Francque, M. Ieven, D. Ursi, V. Van Hoof, P. Michiels. Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Serology and HBV DNA quantification are used in the assessment and follow up of the treatment of HBV, either by nucleos(t)ide analogues (NA) or (pegylated) interferon.

Aim : However, these tests make no distinction between asymptomatic carriers and NA treated HBeAg-patients who achieve successful HBV suppression.

In this study, we compared these groups using the quantification of HBsAg (qHBsAg).

Methods : Exclusion criteria for asymptomatic carriers :

HBeAg+

HIV, HCV, HDV co-infection

Current treatment against HBV (antiviral therapy, interferon). Previous treatments must have ended at least 6 months before recruitment.

Liver fibrosis (Metavir $> F3$), cirrhosis, HCC

ALT $> 2N$

HBV DNA > 2000 IU/mL

We compared this group to HBeAg- treated patients : HBeAg-, HBV DNA < 2000 IU/mL, ALT < 2N, treatment with NA. Recruitment occurred retrospectively (until 6/30/2011)/prospectively (7/1/2011-9/30/2011) from samples taken from outpatient and inpatient clinics in the University Hospital of Antwerp.

HBsAg quantification was done with the Roche Elecsys assay.

Statistical comparison was done using the Mann-Whitney U test.

Results : In 21 months we recruited 28 asymptomatic carriers and 27 HBeAg- NA treated patients. When comparing these two groups, we found a statistical difference in qHBsAg (means : 6043 IU/mL \pm 2112 IU/mL vs. 11985 IU/mL \pm 3357 IU/mL, $p = 0.012$) but no difference in HBV DNA ($p = 0.243$) or ALT ($p = 0.572$). A negative correlation ($R^2 = 0.193$, Pearson coefficient = -0.439 , $p = 0.025$) was found between qHBsAg and the duration of (ongoing) NA therapy.

Conclusions : qHBsAg can differentiate between asymptomatic HBV carriers and successfully suppressed HBeAg-patients.

- A35 -

NEED AND TIMING OF KIDNEY IN RELATION TO LIVER TRANSPLANTATION IN POLYCYSTIC LIVER DISEASE. F. Temmerman, T. Darius, J. Pirenne, D. Monbaliu, R. Aerts, B. Bammens, W. Laleman, D. Cassiman, C. Verslype, W. Van Steenberg, D. Kuypers, F. Nevens. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : The most common complication of polycystic liver diseases (PCLD) is extensive hepatomegaly which may lead to invalidating abdominal symptoms. The majority of patients with PCLD are also suffering from ADPKD (autosomal dominant polycystic kidney disease. Liver (LT) and/or kidney transplantation (KT) are for some of these patients the only curative therapeutic options for.

Aim : To study the need/timing of KT in relation to LT in patients with PCLD.

Methods : All patients who underwent a LT for PCLD between 1995 and 2011 in our center were studied : ADPKD ($n = 44$) and ADPLD ($n = 4$) (autosomal dominant polycystic liver disease).

Results : In the ADPKD subgroup there were 36 women (82%) and 8 men (18%) ; in the ADPLD group all patients were women. In the ADPKD subgroup, 31 patients (70,5%) developed renal failure and underwent a KT at some time in their disease process : 18 patients (58%) received immediately a combined LT+KT (clearance of < 30 ml/min). 7 patients (23%) first received a KT and received then a combined LT+KT or a LT ; 6 patients (19%) first received a LT and subsequently a KT. None of the ADPLD patients required a KT. Post-transplant survival rates in PCLD were excellent (LT only : 91.6% ; combined LT+KT : 80%).

Conclusions : After a KT, a LT was needed in 16% on average after 15 years ; and this occurred especially in men and mostly because of liver volume related problems and recurrent liver cysts infections. After a LT, in 14% of the patients, especially women, a KT was needed on average after 8 years. Finally, combined LT+KT was necessary in 41% of the ADPKD patients.

- A36 -

PORTOPULMONARY HYPERTENSION : HAS THE PROGNOSIS IMPROVED ? J. Verbeek, D. Monbaliu, S. Van Der Merwe, D. Cassiman, W. Laleman, C. Verslype, W. Van Steenberg, R. Aerts, J. Pirenne, M. Delcroix, F. Nevens. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Portopulmonary hypertension (POPH) is a rare complication of liver disease with a 1 yr-survival of 46% and a 5 yr-survival of 14% (natural history) (Swanson *et al.*, *Am J Transplant*, 2008 ;8 : 2445-2453).

Aim : The present study evaluated whether long-term prognosis has improved after the introduction of specific medical therapy (MT) for pulmonary hypertension and liver transplantation (LT).

Methods : From the patients with end-stage liver disease evaluated for LT in our center between 2000-2010 ($n = 906$), we identified 27 (3%) with moderate or severe pulmonary hypertension on echocardiography. In 17 of these patients (1.9%) the diagnosis of POPH was confirmed by invasive measurement of pulmonary hemodynamics.

Results : Baseline characteristics (% of total group or mean \pm SD) : male 59%, age 55 ± 10 yrs, MELD 13 ± 4 , mean pulmonary artery pressure 48 ± 10 mmHg ($= < 25$), pulmonary vascular resistance 669 ± 296 dynes/s/cm⁵ ($= < 240$), pulmonary capillary wedge pressure 8 ± 3 mmHg (< 15), NYHA functional class III-IV 47% and 6-minute walk distance 342 ± 122 m. Cause of liver disease : alcohol (47%), HCV (23,5%), cryptogenic (12%), auto-immune (12%) and HBV (6%). The mean follow-up was 47 ± 43 months.

In 13/17 patients POPH was diagnosed during evaluation pre-LT and MT was started consequently ; only 2/13 of these ultimately underwent LT : 1 died 2 days after LT due to cardiorespiratory failure, the other received combined lung-heart-liver transplantation and is still alive after 11 yrs. The remainder (11/13) thus received MT solely of which only

1 patient is still alive ; causes of death were liver failure (n = 3), cardiorespiratory failure after non-liver related surgery (n = 2), intestinal ischemia (n = 1) and unknown (n = 4).

In 3/17 patients POPH was diagnosed at the start of LT (previous echocardiography negative) and MT was initiated in the peri-operative period ; all could be transplanted successfully.

One patient did not get MT nor LT.

Group survival (n = 17) was 88% at 1 yr, 74% at 2 yrs and 37% at 5 yrs. The 1-yr survival post-LT (n = 5) was 80%. Patients with alcoholic liver disease had a significantly poorer survival (P = 0.005), despite 6/8 stopped drinking.

Conclusions : Only a limited number of patients (15%) with POPH reach LT after MT. MT and LT seem to have improved overall survival.

- A37 -

INFECTION OF N-BUTYL-2-CYANOACRYLATE IN GASTRIC VARICES AS A CAUSE FOR RECURRENT SEPSIS. F. Ausloos (1), S. Hillaire (2), P. Bedossa (2), F. Bert (2), C. Moreno (3), A. Geubel (1), D. Valla (2), A. Plessier (2). (1) Ucl Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (2) Hôpital Beaujon, Clichy, France ; (3) Erasme Hospital, Brussels, Belgium.

Introduction : Injection of N-butyl-2-cyanoacrylate glue is now the recommended initial treatment for the prevention of recurrent bleeding from gastric varices. Bacterial infection after intravariceal glue injection has been mentioned but poorly characterized.

Aim : This work aimed to analyze the features and outcome of bacterial infection following intravariceal glue injection.

Methods : From December 1995 to September 2011, patients from 7 centers were considered for enrollment if they fulfilled all of the following criteria : (1) oesophageal or gastric varices related to portal hypertension ; (2) intravariceal injection of N-butyl-2-cyanoacrylate ; (3) evidence that sepsis was related to infection of the injected material (same strain isolated in resected material and blood cultures, or positive blood cultures in the absence of any other focus of infection) ; and (4) recurrent fever despite antibiotic therapy .

Results : Nine patients were identified from the files of the participating centers. Underlying disease was non cirrhotic portal hypertension in 5 patients, including 3 with portal vein thrombosis, and 1 with splenic vein thrombosis ; cryptogenic cirrhosis with partial portal vein thrombus in 1 ; cirrhosis without splanchnic vein thrombosis in 4. All patients but one had glue injected in gastric varices. Bacterial strains were of 3 different types : oropharyngeal flora in 4 patients, gut flora in 5, and *Pseudomonas aeruginosa* in 3. Multiple strains were isolated in 4 patients. Follow-up blood cultures showed development of resistance to antibiotics in 5 patients. The 3 patients infected with *P. Aeruginosa* died. All patients required antibiotic administration for a minimum of 2 months before infection was eradicated. The median duration of treatment was 3 months (range 2 to 18 months). In 6 out of 7 patients in whom a PET-CT was performed, an abnormal fixation co-localizing with the lipiodol-admixed glue was observed. A surgical portocaval shunt was constructed in 2 patients (one with thrombectomy and one with a gastrectomy), a mesentericocaval shunt in one, and a partial gastrectomy in one. Two of the 4 operated patients died. The three patients infected with *P. Aeruginosa* died. The 6 other patients are alive with a median follow up of 6 years (range 0,5 to 16 years).

Conclusions : Infection of injected glue in gastric varices is a rare but often fatal entity. It is associated with preexistent portal vein thrombosis and non-cirrhotic portal hypertension, suggesting a role for thrombus associated facilitation of bacterial infection. Bacterial seeding occurs through direct inoculation by the injecting system contaminated with oropharyngeal strains or through intestinal translocation. PET-CT allows for the identification of the infectious focus. Prolonged antibiotic therapy is needed. Antibacterial prophylaxis should be considered in patients with thrombosis in the portal venous system.

- A38 -

SIDE POPULATION IN HUMAN HEPATIC CARCINOMAS REFLECTS A POPULATION WITH PROGENITOR CELL FEATURES. O. Govaere (1), M. Komuta (1), K. Van Den Eynde (1), J. Wouters (1), A. Van Den Broeck (1), C. Empsen (2), L. Gremeaux (1), R. Aerts (1), L. Van Grunsven (2), B. Topal (1), H. Vankelecom (1), T. Roskams (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) University Hospital Brussel (Vub), Brussels, Belgium.

Introduction : Side Population (SP) analysis has been shown to be a useful technique to investigate cancer stem(-like) cells in different types of cancer, including hepatocellular carcinoma (HCC) cell lines. To our knowledge this study is the first to isolate and characterise SP in primary human hepatic carcinoma samples.

Methods : Tissue samples (n = 30) from patients diagnosed with either hepatocellular carcinoma (HCC) or mixed hepatocellular/cholangiocarcinoma (mixed-type), were obtained through informed consent immediately after surgery and stored in liquid nitrogen. Dissociated samples were stained with Hoechst 33342 and sorted by flow cytometry into

SP and non-SP cohorts. Sorted cells were processed for RNA extraction and analysed through quantitative PCR. Genes of interest were localised on protein level by means of immunohistochemistry.

Results : Flow cytometric analysis showed that the mean percentage SP proportion in hepatic carcinomas differs from 3,27% in keratin 19 negative HCCs to 8,68% in keratin 19 positive HCCs and 15,43% in the mixed-type ; indicating that the size of the SP inclines with possible progenitor cell origin and in turn reflects their more aggressive behaviour. Gene expression analysis of all the samples combined revealed a higher expression of hepatic progenitor cell markers (e.g. *KRT19*, *EPCAM*, *KRT7*, *TACSTD2*, *CD133*) in the SP than in the main population (MP). Focussing on the individual subtypes, a similar profile was found in each SP when compared to their accordant MP. The SP of HCCs showed a higher expression of *ALB* and *TNFRSF12A* than the SP of the mixed-type. In turn the SP of the keratin 19 positive HCCs could be distinguished from the other SPs by the high expression of *CXCR4*, *SNAI1* and the low expression of *CDH1*.

Conclusions : The proportion SP is higher in hepatic carcinomas thought to originate from progenitor cells. The manifest presence of progenitor cell markers was found to be present in the SP of either hepatocellular carcinoma or mixed hepatocellular/cholangiocarcinoma. Side population analysis provides to be a useful technique to isolate viable cancer stem cells and to better understand the hepatocarcinogenesis.

- A39 -

MORBIDITY AND RISK FACTORS OF CONVERSIONS IN 223 CONSECUTIVE LAPAROSCOPIC LIVER RESECTIONS. R. Montalti, M. Sainz-Barriga, A. Vanlander, F. Berrevoet, X. Rogier, R.I. Troisi. Ghent University Hospital, Ghent, Belgium.

Introduction : As a consequence of continuous technical developments in liver surgery, laparoscopic liver resection (LLR) is increasingly performed worldwide, especially for malignancies.

Aim : We report herein a single institution 7-year experience in LLR, focusing on causes of conversion, technical issues and outcomes.

Methods : Between January 2004 and December 2010, 223 LLR were performed in 200 patients for benign, live donation, primary and metastatic liver disease. Repeat LLR and two-stage hepatectomy were performed in 19 (8.5%) and 3 (1.3%) cases, respectively. Simultaneous resection of colo-rectal primary cancer and synchronous liver metastases was done in 6 (2.7%) patients.

Results : Overall conversion rate was 17/223 (7.6%), mainly due to bleeding episodes (52.9%). Conversions were equally distributed over time and statistically significant factors for conversion were found to be LLR of P-S segments in the converted vs. the non-converted group (14.5% vs. 4.1%, $p = 0.012$ respectively) and major vs. minor hepatectomy (17.5% vs. 5.5%, $p = 0.017$). In malignancy, R0 resection was obtained in 91% of cases. Complication rates were scored as : $n = 15$ grade I ; $n = 9$ grade II ; $n = 7$ grade III and $n = 1$ grade IV. Multivariate analysis revealed resections involving P-S segments as an independent risk factor for conversion ($p = 0.036$, OR = 3.2, CI = 1.1-9.4).

Conclusions : LLR can be safely performed accounting for low overall morbidity and a favourable outcome. LLR in P-S segments most likely lead to conversion to open approach irrespective from the learning curve, needing careful intraoperative management.

- A40 -

RETINOIC ACID DEFICIENCY CONTRIBUTES TO THE INFLAMMATORY STATUS OF ALCOHOLIC CIRRHOTIC PATIENTS. R. Ouziel (1), E. Trépo (1), A. Cremer (2), C. Moreno (1), D. Degré (1), F. Cotton (1), V. Vercruysse (2), E. Quertinmont (2), J. Devière (1), A. Lemmers (1), T. Gustot (1). (1) Erasme Hospital, Brussels, Belgium ; (2) Ulb Faculty Of Medicine, Anderlecht, Belgium.

Introduction : Patients with alcoholic cirrhosis (AC) have an exacerbated immune response which is associated with development of systemic complications. Deficiency of vitamin A (VA) metabolite, all-trans retinoic acid (ATRA), which has anti-inflammatory effects, could contribute to this deleterious proinflammatory reaction.

Methods : VA levels were determined in 65 consecutive patients undergoing transjugular liver biopsy for suspected alcoholic liver disease (all had cirrhosis ; median MELD score 13.4 [6.4-41.2]) ; 10 other AC patients vs. 10 healthy subjects (HS) were recruited for *in vitro* assays. HPLC measured plasma levels of VA and ATRA. Peripheral blood mononuclear cells (PBMCs) were pre-treated with or without ATRA and stimulated with lipopolysaccharide (LPS). TNF- α production was assessed by ELISA. We generated VA-deficient mice and studied LPS-induced peritoneal macrophage activation at baseline and 10 days after oral ATRA supplementation.

Results : AC patients disclosed VA and ATRA deficiency (VA : 143[46-519] ng/ml ; ATRA : 1[0.8-1.2] ng/ml) compared to HS (VA : 1275[1100-1870] ng/ml ; ATRA : 1.2[1-1.4] ng/ml) ($p < 0.001$ and $p < 0.01$, respectively). ATRA levels correlated with VA concentrations ($r = 0.69$, $p = 0.001$) which were negatively correlated with parameters of severity (i.e. MELD score [$r = -0.42$], Child-Pugh score [$r = -0.65$], hepatic venous pressure gradient [$r = -0.48$] ; all $p < 0.001$). *In vitro*, LPS-stimulated PBMCs from AC patients produced more TNF- α than HS (7358[3246-9100] pg/ml vs. 1945[344-5975] pg/ml ; $p < 0.001$). However, ATRA pre-treatment decreased stimulation-induced TNF- α production (LPS+ATRA : 3208[1158-7735] pg/ml vs. LPS, $p = 0.002$). LPS-stimulated macrophages from VA-deficient mice produced more TNF- α and NO₂ than controls while 10 days of ATRA supplementation was able to normalize their production.

Conclusions : AC patients disclose VA/ATRA deficiency that correlates with disease severity. Since *in vitro* addition of ATRA decreases their TNF- α overproduction, VA deficiency in mice reproduces TNF- α overproduction by macrophages and oral ATRA supplementation decreases it, these results suggest that VA deficiency could contribute to TNF- α oversecretion observed in AC patients and that ATRA supplementation in these patients could downregulate their proinflammatory state and might be therapeutically useful.

-A41 -

LAPAROSCOPIC LIVER RESECTIONS VERSUS OPEN APPROACH : AN INTRAOPERATIVE COST-EFFECTIVENESS ANALYSIS. R. Montalti, A. Verhaevert, L. De Gezelle, L. Louagie, X. Rogier, R.I. Troisi. Ghent University Hospital, Ghent, Belgium.

Introduction : The long learning curve and the common opinion that laparoscopic liver resections (LLRs) have higher costs are limiting the widespread utilization of this technique. Aim of the present study was to perform a cost-effectiveness analysis of LLR vs liver resections with standard approach

Aim : Aim of the present study was to perform a cost-effectiveness analysis of LLR vs liver resections with standard approach.

Methods : From August 2011 to November 2011, 25 liver resections were performed, 18 of them (72%) with a laparoscopic approach and 7 (28%) with an open approach. Hepatic resections requiring bile duct or vascular reconstruction or focal lesions not amenable to a parenchyma-sparing technique were not considered for laparoscopic approach. CUSA-Excel[®] was utilized as liver transecting device in open approach whereas Sonosurg[®] was utilized for LLRs. We prospectively collected the number and type of disposable materials used for surgery such as : trocars, vascular staplers and refills, clips, stitches, sutures, irrigation suction, disposable materials for CUSA and Sonosurg, argon beamer, endobags

Results : The mean operation time was 356 ± 123 min vs 186 ± 75 min ($p < 0.001$) whereas the mean postoperative stay was 3.7 ± 2.6 days vs 8.6 ± 2.1 days for laparoscopic and open approach, respectively ($p = 0.001$). Type of resections in LLR vs open patients were respectively : 0 vs 3 right hepatectomy, 3 vs 1 left hepatectomy, 7 vs 2 segmentectomy, 8 vs 1 wedge resection. According to the actual marketing price for Belgium, LLRs resulted in an average intraoperative per/operation surplus costs of 310 € (1406 ± 96 € vs 1096 ± 86 € for LLR and open approach respectively, $p < 0.001$). The main costs for LLR were vascular stapler and refills (391 €), trocars (351 €), and clips (301 €). The main costs for open approach were the disposable CUSA materials (426 €) and sutures/stiches (202 €).

Conclusions : LLRs require significantly higher intraoperative costs and longer OT compared to the open approach. However these higher costs are entirely compensated by the inferior costs of the shorter postoperative stay. A randomized prospective comparative study is necessary to avoid selection bias.

- A42 -

EVALUATION OF TRANSIENT ELASTOGRAPHY DURING ACTIVE ALCOHOL CONSUMPTION AND AFTER WITHDRAWAL. V. Nguenang (1), A. Badaoui (2), E. Danse (1), C. Dragean (1), J. Rahier (1), P. Starkel (1). (1) Clinique Universitaire St Luc, Brussels, Belgium ; (2) Ucl, Mont-Godinne, Belgium.

Introduction : Transient elastography (TE) is a non invasive assessment for liver fibrosis by measuring the liver stiffness. This method was firstly validated for chronic hepatitis C then additional studies have provided new cut off for other chronic liver disease. However, the place and the ideal time to perform TE in alcoholic disease remain unclear.

Aim : The aim of this study was to determine if the results of TE are modified by alcohol withdrawal and when these results are closest to the liver biopsy which is considered to be the gold standard for diagnosis of fibrosis or cirrhosis.

Methods : From 11/10/2010 to 11/10/2011 ; all patients admitted to the unit of alcohol withdrawal were evaluated within twenty four hours by Fibroscan[®] combined to abdominal ultrasound and blood test (AST, ALT, platelet, ---). If the

result of TE was superior or equal to F2 stage (≥ 7.6 KPa) according to new cut off for alcohol disease published in 2008, the patient underwent hepatic venous pressure gradient measure and transjugular liver biopsy. A second TE was performed during the third week of alcohol cessation and compared to the first result and liver biopsy (METAVIR score). Patients with active viral hepatitis or other chronic hepatitis were excluded.

Results : Twenty-three patients out of two hundred patients screened were eligible for the study. Five patients were excluded because of chronic viral hepatitis (2), failure of hepatic catheterism (2) and refusal of liver biopsy in the last patient. Finally we included eighteen patients ($n = 18$), eleven men and seven women. The mean age was 48 years. The average alcohol intake was 204.7 g the first week ; during the second week six patients relapsed with an average consumption of 23 g (10-50 g).

The mean value of liver stiffness (LS) was 13.81 KPa (± 15.13) in the first week and 9.22KPa (± 16.49) at third week with a significant decrease of 4.59 KPa ($p = 0.0003$). Analysis of TE results showed that sixteen (88.8%) of the eighteen patients decreased their fibrosis stage at the third week of weaning. Eleven patients (61%) reduced by one stage and five patients (28%) dropped by two stages. Fibroscan® was comparable to liver biopsy only in two patients (11,11%) the first week. By contrast after detoxification (third week) TE was comparable to liver biopsy in twelve patients (66.6%).

In addition the correlation factor between liver stiffness values and HVP (hepatic venous pressure gradient) was low ($R = 0.4$) during the first week and improved at the third week ($R = 0.69$) after exclusion of outlier (one patient with cirrhosis and liver stiffness > 70 KPa).

No association was found between steatosis or transaminases levels and variation of liver stiffness ; probably due to the low number of patient included in the study.

Conclusions : Fibroscan® performed during active alcohol consumption overstaged the degree of fibrosis. However repetition of exam after a significant period of abstinence permit to reduced this inadequacy. These preliminary data need to be confirmed in a large cohort of patient.

- A43 -

SURVIVAL RESULTS OF HEPATECTOMIES FOR COLORECTAL METASTASIS USING NEW STRATEGIES AIMING R0-RESECTION. A. Dili, J. Lemaire, G. Molle, B. Mansvelt, C. Bertrand. Hôpital De Jolimont, Haine-Saint-Paul, Belgium.

Introduction : The resection of liver metastasis from colo-rectal cancer (CRLM) is actually the only curative treatment. Different artifices have been developed to extend the rate of R0-resection to some patients with initially irresectable or borderline resectable disease.

Aim : The aim of this study is to evaluate the impact on survival of these new strategies and to compare these results with those of the conventional approach, in the era of new systemic chemotherapies

Methods : This is a prospective consecutive series of 68 patients treated between January 2006 and June 2011 by hepatectomy without (group A) and with (group B) new strategies in view to extend the R0-rate resection. Were analyzed, the number of metastasis, the uni- or bilobar localisation, the type of new artifice, the rate of iterative hepatectomies (hepatectomy for a recurrent liver disease), and the global survival (Kaplan-Meier).

Results : 68 patients. Mean age : 64,24 year old (40-89). Mean number of metastasis : 7,4 (1-20), synchronous metastatic disease : 42 patients (61,7%).

Gr. A : 45p (64,2%) : > 3 lésions : 10, bilobar : 14, > 3 lésions and/or bilobar : 16 patients (35,6%).

Gr. B : 23p (33,8%) : > 3 lésions : 14, bilobar : 15, > 3 lésions and/or bilobar : 19 patients (82,6%).

Significantly more patients with a high risk disease were so treated in the group B ($X^2 : p < 0.01$).

In the group B, 39 artifices were used : 14 two-stages hepatectomies (61%), 11 portal vein embolisations (47,8%), 7 thermoablations (30,4%), 7 systemic chemotherapies for unresectable liver disease (30,4%). This group represents 33,8% of the patients.

Iterative hepatectomy for recurrent liver disease were performed in 14 patients, 8 in group A and 6 in group B.

Global survival at 1, 3, 5 years was 94, 60, 56% (group A : 97, 68, 60%. group B : 95, 48 et 48%).

Conclusions : In our experience, the use of new strategies with technical artifices in order to extend the R0-resection rate permitted an increase of 50% the number of resection indications. Despite the fact that these patients had a significantly higher percentage of high-risk hepatic disease (> 3 metastasis and/or bilobar lesions), the 5-years global survival was practically similar to the group where these new strategies were not necessary. In the two groups, near 20% of the patients benefited of an iterative hepatectomy. The 5-year survival of the whole series was 56%.

SYNCOILIN IS A TRUE INTERMEDIATE FILAMENT IN MOUSE HEPATIC STELLATE CELLS. E. Van Rossen (1), Z. Liu (1), D. Blyweert (1), L.A. Van Grunsven (1), H. Reynaert (2). (1) Vrije Universiteit Brussel, Jette, Belgium ; (2) UZ Brussel, Jette, Belgium.

Introduction : Hepatic stellate cells (HSCs) are important in several (patho)physiological conditions. In response to chronic injury, HSCs are activated and change from a quiescent phenotype to myofibroblast-like cells with contractile properties. This makes HSCs an interesting target in the study of portal hypertension. The shift in phenotype is accompanied by a dramatic change in expression of intermediate filaments (IFs). In contrast to most differentiated cell types, HSCs express a broad, but variable spectrum of IFs. In muscle syncoilin was identified as an alpha-dystrobrevin binding protein with sequence homology to IF proteins, but was unable to form filaments in muscle or COS-7 cells. Since then, it has been considered an intermediate filament-like protein.

Aim : We investigated the expression of syncoilin in mouse HSCs and human liver.

Methods : Syncoilin expression in isolated and cultured mouse HSCs was studied by quantitative reverse transcription polymerase chain reaction, Western blotting, immunohistochemistry and fluorescence immunocytochemistry (confocal microscopy). For the latter techniques we generated a syncoilin antibody.

Results : Syncoilin mRNA was present in HSCs and was upregulated during HSC activation. While in quiescent HSCs no syncoilin protein could be detected, this protein was strongly upregulated during *in vitro* activation. We verified the cellular localization of syncoilin using immunocytochemistry and observed filamentous staining in HSCs undergoing *in vitro* transdifferentiation. In diseased human liver samples syncoilin was present in fibrotic septa and blood vessels.

Conclusions : To our knowledge, we are the first to demonstrate that syncoilin is present in mHSCs. In diseased mouse and human liver samples as well as during *in vitro* activation syncoilin was upregulated. In HSC, syncoilin containing filaments can be detected, indicating that syncoilin behaves as a true IF protein in these cells.

ROLE OF CLASS II HDACS DURING HEPATIC STELLATE CELL ACTIVATION AND FIBROSIS. I. Mannaerts (1), N. Eysackers (1), K. Van Beneden (1), O. Onyema (1), A. Mai (2), L. Van Grunsven (1). (1) Vrije Universiteit Brussel, Jette, Belgium ; (2) Sapienza University, Rome, Italy.

Introduction : The conversion of a quiescent vitamin A storing hepatic stellate cell (HSC) to a matrix producing, contractile myofibroblast-like activated HSC is a key event in the onset of liver disease following injury of any etiology. Previous studies have shown that class I histone deacetylases (HDACs) are involved in the phenotypical changes occurring during stellate cell activation in liver and pancreas.

Aim : In the current study we investigate the role of class II HDACs during HSC activation.

Results : We characterized the expression of the class II HDACs in different liver cell types and in *in vitro* and *in vivo* activated HSCs. We addressed their function in HSCs by selective inhibition with MC1568 and by repressing their gene expression using specific siRNAs. Inhibition of HDAC activity leads to a strong reduction of HSC activation markers α -SMA, lysyl oxidase and collagens as well as an inhibition of cell proliferation. Knock-down experiments showed that HDAC4 contributes to HSC activation by regulating lysyl oxidase expression. In addition, inhibition of class II HDAC activity *in vivo* seems to decrease the degree of CCl₄ induced fibrosis in mice.

Conclusions : In conclusion we show that the use of MC1568 has enabled us to identify a role for class II HDACs during HSC activation *in vitro*.

GASTROINTESTINAL REGULATORY MECHANISMS

Invited lecture

- B01 -

THE EOSINOPHIL AND THE GASTROINTESTINAL TRACT-A FRIEND OR A FOE ? A. STRAUMANN. Swiss EoE Research Group, Olten, Switzerland.

Eosinophils originate from pluripotent stem cells in the bone marrow. Under the influence of GATA-1, IL-3, IL-5 and GM-CSF they develop within 8 days to fully differentiated leukocytes and circulate afterwards for some hours in the peripheral blood. Eosinophils and the gastrointestinal tract interact in an intimate and enigmatic relationship insofar as under healthy conditions, the presence of eosinophils is limited almost exclusively to the digestive tract mucosa from the stomach to the rectum. Of note, the healthy esophagus is devoid of eosinophils.

In the mucosa of the gastrointestinal tract eosinophils exert under resting conditions several effector and immunoregulatory functions. So far their precise function in the gastrointestinal tract is not completely understood. Nevertheless, it has becoming clear that, together with different T cell subsets, eosinophils are involved in maintaining the immunologic and bacterial homeostasis across the mucosal barrier. Formation of extracellular traps is one mechanism involved in the anti-bacterial barrier function.

Eosinophils also play a role in several inflammatory conditions, such as intestinal infections, hypersensitivity reactions, primary eosinophilic inflammations and several other chronic intestinal disorders such as celiac disease, Crohn's disease and ulcerative colitis. Eosinophils are late phase inflammatory cells and as such they have prominent repair capacities. In the course of longstanding, unbridled eosinophilic inflammations these repair capacities such as fibrosis and angiogenesis can lead to organ damage with loss of function. Regarding the gastrointestinal tract, this so called remodeling is the main concern in eosinophilic esophagitis, a rapidly emerging disease affecting in westernized areas up to 1 individual among 2500 persons. This chronic process induces a fibrosis of the esophageal wall resulting in loss of elasticity, fragility and narrowing of the esophageal lumen.

Summarized, depending on the responsible triggers and on the micro-environment eosinophils may exert in the gastrointestinal tract either beneficial or detrimental effects.

- B02 -

ADOPTIVE TRANSFER COLITIS MODEL : VALIDATION BY ENDOSCOPY AND μ PET/CT. M. Heylen (1), J. De Man (1), S. Staelens (1), S. Deleye (1), P. Pelckmans (2), T. Moreels (2), B. De Winter (1). (1) University Of Antwerp, Antwerpen, Belgium ; (2) Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Reliable chronic animal models of inflammatory bowel disease (IBD) and techniques allowing longitudinal monitoring of inflammation in the same animal are indispensable for in vivo research.

Aim : To validate inflammation in an adoptive transfer colitis model in which immunodeficient SCID mice are transferred with naive CD4+CD25-CD62L+ T cells using two novel techniques : micro-positron emission tomography (μ PET)/computed tomography (CT) and small animal live endoscopy.

Methods : CD4+CD25-CD62L+ T cells were isolated from normal donor mice using magnetic separation procedures and flow cytometric analysis and transferred into SCID mice, to induce colitis (COLITIS). A control group injected with PBS (CONTROL) and a group injected with splenocytes (SPLEN) were included. Mice were monitored by clinical outcomes (bodyweight, stool consistency, mobility and piloerection). After 4 weeks, colonic inflammation was monitored by μ PET/CT followed 24h later by endoscopy. After the imaging procedures, mice were sacrificed and colonic inflammation was evaluated using the following parameters : a macroscopic and microscopic inflammation score, colonic weight/length ratio and myeloperoxidase (MPO) activity.

Results : COLITIS mice but not CONTROL or SPLEN mice displayed a significant weight reduction at 4 weeks. COLITIS mice showed a significant elevation of the clinical disease score at week 3 and 4 compared to CONTROL and SPLEN mice. PET images of COLITIS mice showed increased distal colonic PET signals at week 4, which was not detected in CONTROL and SPLEN mice. Endoscopic screening of COLITIS mice showed diseased colons characterised by bowel wall thickening, as shown by loss of translucency, alterations of vascular patterns and loss of stool consistency. Consistently, a significant elevation of the endoscopic score was seen in COLITIS mice compared to CONTROL and SPLEN mice. No significant difference was seen for colonic weight/length ratio, while the macroscopic inflammation score tended to increase in COLITIS mice. Microscopically, COLITIS mice showed significant alterations including mucosal hypertrophy, high cell infiltration of the lamina propria and distortion of the crypts, which were absent in CONTROL and SPLEN mice. MPO activity was significantly increased in COLITIS mice at week 4 com-

pared to CONTROL and SPLEN mice. Correlation analysis showed significant correlation coefficients (between 0.85-0.99) between the different techniques assessing inflammation.

Conclusions : The adoptive transfer of CD4⁺CD25⁻CD62L⁺ T cells in SCID mice results in colonic inflammation as evidenced by weight loss, loss of stool consistency, microscopic and endoscopic damage and μ PET/CT images. This transfer model may be useful for studying new treatment strategies for IBD, where small animal molecular imaging (μ PET/CT) and live endoscopy can be used as reliable techniques allowing longitudinal monitoring of inflammation and reducing the need to sacrifice animals.

- B03 -

ANTI-INFLAMMATORY EFFECT OF VAGUS NERVE THROUGH INTESTINAL RESIDENT MACROPHAGES EXPRESSING A7NACHR. P.J. Gomez Pinilla (1), G. Matteoli (1), M. Di Giovangiulio (1), A. Nemethova (1), K. Lambaerts (1), S.H. Van Bree (2), C. Cailotto (2), G. Boeckxstaens (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) Academic Medical Center, Amsterdam, Netherlands.

Introduction : Inflammation of the intestinal muscularis is a crucial mechanism in the pathogenesis of postoperative ileus (POI). We demonstrated that vagus nerve stimulation (VNS) is able to dampen intestinal muscular inflammation and prevent ileus via activation of the alpha 7 nicotinic acetylcholine receptor (a7nAChR). However the neural circuits involved and the cells targeted by the vagus nerve (VN) mediating the anti-inflammatory effect still need to be identified.

Aim : Further investigate the mechanisms of cholinergic anti-inflammatory pathway (CAP) preventing POI.

Methods : Splenic denervation (SPD) was performed to assess the role of the spleen in the cholinergic anti-inflammatory pathway CAP in POI. In addition, chimera mice were generated to identify the cell targeted by the VN via a7nAChR. In brief, a7nAChR wild type mice (WT) were lethally irradiated and adoptively transferred with bone marrow cells isolated from a7nAChR knockout mice (KO) and vice versa. After 8 weeks, mice underwent intestinal manipulation (IM) and the effect of VNS (1ms, 5V, 5 Hz, for 5 min) was studied. Twenty four hours after surgery, mice were gavaged with FITC-Dextran (70KD) and gastrointestinal transit was determined 90 min later. Geometrical center (GC) was used as readout. Intestinal tissues were collected to assess the number of myeloperoxidase positive cells (MPO) and level of cytokine expression. Immune cells from the intestinal muscularis were sorted to quantify a7nAChR expression.

Results : The delay in intestinal transit evoked by IM was prevented by VNS in both sham operated and splenic denervated mice (sham operated ; IM GC : 4.0 ± 0.6 , IM + VNS GC : 8.7 ± 0.3 vs SPD IM + VNS GC : 3.9 ± 0.6 , IM + VNS GC : 8.7 ± 1.0 , $p < 0.05$, IM vs IM + VNS, N = 8). In parallel with the effect on gastrointestinal transit VNS also prevented MPO-positive cell infiltration (269 ± 49 vs 71 ± 15 , $p < 0.05$, IM vs IM + VNS) and reduced gene expression of TNF α , IL1 β and IL6 (71, 67 and 88% respectively) in the muscularis. However, in a7nAChR KO mouse the beneficial effect of VNS on transit (IM GC : 5.3 ± 0.5 , IM + VNS GC : 4.6 ± 0.3 , N = 8) and inflammation was absent. Interestingly, VNS improved intestinal transit and reduced inflammation only in chimera carrying a7nAChR on their immune cells while this effect was absent in WT mice reconstituted with a7nAChR KO bone marrow cells indicative that a7nAChR expression on immune cells is crucial for CAP to prevent POI. Of the immune cells sorted from the intestinal muscularis, only macrophages (CD45⁺ CD11b⁺ F4/80⁺) but not by dendritic cells, neutrophils or T cells expressed a7nAChR.

Conclusions : We showed that VNS reduces manipulation-induced inflammation of the muscularis and POI via a direct action on the intestine, independent of splenic innervation. In addition, we demonstrated that a7nAChR expression on immune cells, in particular resident macrophages, is crucial for this mechanism. We therefore conclude that resident macrophages located within the intestinal muscularis are the ultimate target cells mediating the anti-inflammatory effect of VNS in POI.

- B04 -

MAS-RELATED GENE RECEPTOR MRGD IS A MODULATOR OF MAST CELL RECRUITMENT IN INTESTINAL INFLAMMATION. L.R. Avula (1), R. Buckinx (1), K. Alpaerts (1), D. Adriaensen (1), L. Van Nassauw (2), J.P. Timmermans (1). (1) Laboratory Of Cell Biology And Histology, Department Of Veterinary Sciences, University Of Antwerp, Antwerpen, Belgium ; (2) Laboratory Of Human Anatomy And Embryology, Faculty Of Medicine & Health Sciences, University Of Antwerp, Antwerpen, Belgium.

Introduction : Some members of the Mas-related gene receptor (Mrg) family have been suggested to play a role in nociception, in mediating IgE-independent mast cell activation, and in neuroimmune communication. One such

member, MrgD, so far reported to be expressed only in spinal nociceptive neurons, is encoded by a single copy *MrgD* gene which has clearly defined rodent and human orthologs, can be activated by beta-alanine, and as such constitutes an experimentally attractive therapeutic target for pain. To date, information on the expression of MrgD in the GI tract and its involvement in intestinal inflammation is lacking.

Aim : To investigate the presence of MrgD in the intestine and to unravel its putative function during intestinal inflammation, by comparing the ileum of non-inflamed and *Schistosoma mansoni*-infected wild-type and *MrgD*^{-/-} mice.

Methods : Immunohistochemical analyses were performed on the ileum of non-inflamed and *Schistosoma mansoni*-infected wild-type and *MrgD*^{-/-} mice. Expression of MrgD mRNA was analysed on the afore-mentioned tissues by Real-Time PCR.

Results : In wild-type mice, no MrgD immunoreactivity (IR) was detected in the non-inflamed ileum, whereas MrgD IR was observed in 5% of myenteric neurons in the inflamed ileum. Neurochemical coding revealed that these MrgD-expressing neurons were intrinsic primary afferents. In addition, MrgD IR was detected in almost all mucosal mast cells in the inflamed ileum. In *MrgD*^{-/-} mice, no MrgD IR was found in any tissue, while increased calcitonin gene-related peptide (CGRP) expression in enteric neurons and increased mucosal mast cell infiltration (30% ± 5% increase, $P < 0.01$) were observed in the inflamed ileum. These results were corroborated by Real-Time PCR, demonstrating the absence of MrgD mRNA in the non-inflamed ileum of wild-type mice and in tissues of *MrgD*^{-/-} mice, as well as the *de novo* expression and elevation of MrgD mRNA levels (6 fold, $P < 0.01$) in the inflamed ileum of wild-type mice.

Conclusions : The *de novo* expression of MrgD in sensory neurons and mucosal mast cells during inflammation in wild-type mice, and the increased CGRP expression and mucosal mast cell infiltration during inflammation in mice lacking MrgD, indicate that MrgD is involved in the inflammatory response during intestinal schistosomiasis. Furthermore, since CGRP is known to be involved in mast cell recruitment and enhanced pain responsiveness, our results suggest that MrgD modulates CGRP-mediated mucosal mast cell infiltration, and thereby increased pain perception during intestinal inflammation.

- B05 -

THE VAGUS NERVE AS MODULATOR OF INTESTINAL IMMUNE HOMEOSTASIS. M. Di Giovangiulio, P.J. Gomez-Pinilla, C. Van Heijningen, K. Lambaerts, G. Matteoli, G. Boeckxstaens. Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : As the mucosal surface is exposed to a myriad of antigens, the mucosal immune system should react to harmful antigens (pathogens) but at the same time be tolerant against harmless antigens such as food antigen and microflora. It has been described that the nervous system interacts dynamically with the immune system in a bidirectional manner. Recently, the parasympathetic nervous system via the vagus nerve (VN) has been proposed to play a crucial role in the regulation of the intestinal immune response by activation of alpha-7 nicotinic acetylcholine receptors (α7nAChRs) on immune cells.

Aim : Therefore we investigated the influence of the VN on the induction of oral tolerance and its role in maintaining intestinal immune homeostasis.

Methods : In order to test the ability of the VN to modulate oral tolerance we performed experiments in vagotomized (VXG) and sham operated mice. Briefly, to induce oral tolerance mice were treated with 3 intragastric administration of ovalbumin (OVA) at day 0, 3 and 6 followed by a subcutaneous immunization with OVA in Complete Freund Adjuvant (CFA) at day 14. One week post-immunization, mice were challenged with a subcutaneous injection of OVA in the footpad. Footpad swelling was used as readout for immune tolerance to OVA. To investigate the role of α7AChR in oral tolerance α7nAChR knockout mice (KO) were compared with α7nAChR wild type mice (WT) using the protocol previously described above. Antigen specific T regulatory cells (CD4+CD25+FoxP3+) conversion *in vivo* was performed in VXG and sham operated mice. Mice (Ly5.2) were injected with naïve OVA specific CD4 T cells (Ly5.1) and treated for 5 days with OVA in the drinking water. Conversion of naïve T cells in OVA specific-Tregs was assessed by flow cytometry.

Results : Sham operated mice developed tolerance to OVA when the antigen was orally delivered while VXG mice failed to develop tolerance (OVA sham $0,4 \pm 0,05$ vs OVA VXG $1,2 \pm 0,3$; $p < 0,01$, $n = 9$). In addition, in VXG mice there was a significant lower conversion of OVA specific naïve T cells into FoxP3 T regulatory cells (Sham 31,6% vs VXG 20,7%, $P < 0.01$). In line with these findings, α7nAChR KO mice showed a significant loss of oral tolerance (OVA WT $0,7 \pm 0,05$ vs OVA KO $1,9 \pm 0,3$; $p < 0,01$, $n = 9$).

Conclusions : Our study provides evidence that VN favours the induction of oral tolerance through α7nAChRs. Moreover our data suggest that the vagus nerve regulates the induction and expansion of antigen specific T regulatory cells in the intestine, a key regulatory mechanism to maintain intestinal mucosal homeostasis.

Invited lecture

- B06 -

ROLE OF RAC IN IBD. M. Peppelenbosch. Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : The etiology of Crohn's disease (CD) and Ulcerative Colitis (UC) (collectively denominated as inflammatory bowel diseases (IBD)) involves an inadequate innate immune response and subsequent failure to control gut bacterial homeostasis. The factors driving innate immune inadequacy remain unknown.

Aim : We aim to generate of kinase signatures of the IBD phenotype in an effort to explain the immunology of IBD.

Methods : Here, we generate comprehensive descriptions of kinase activity in inflamed and non-inflamed colonic mucosa from IBD patients in comparison to non-IBD controls. Results are confirmed by conventional technology and the relevance of results obtained is studied using functional immune assays.

Results : p21Rac1 signaling is strongly suppressed in non-inflamed mucosa in both CD and UC compared to non-IBD mucosa. Interestingly, 6-thioguanine (6-TG), the active metabolite of azathioprine and one of the few medications with good efficacy in CD and UC, inhibits p21Rac and increases innate immunity in IBD patients and healthy controls. Stimulation of innate immunity is also achieved by unrelated pharmacological p21Rac1 inhibitors, whereas genetic hyperactivation of p21Rac inhibits innate immunity.

Conclusions : Together, these results demonstrate that inhibition of p21Rac1 and subsequent enhanced innate immune functionality mediates both spontaneous and medication-induced remission in IBD.

- B07 -

ORIGIN OF PHASE 3 OF THE MIGRATING MOTOR COMPLEX DIFFERS BETWEEN LEAN AND OBESE SUBJECTS. E. Deloose (1), P. Janssen (1), M. Lannoo (2), B. Van Der Schueren (2), I. Depoortere (1), J. Tack (1). (1) University Of Leuven, Leuven, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : It has been suggested that phase (ph) 3 contractions of the migrating motor complex in the stomach are associated with hunger feelings.

Aim : In this study we investigated whether ph3s and hunger feelings differ between obese patients and lean volunteers during the interdigestive state.

Methods : In 10 obese patients (43 ± 13 years ; 20% males ; 42 ± 4 kg/m²) and 14 lean volunteers (25 ± 8 years ; 50% males ; 23 ± 4 kg/m²) high resolution antroduodenal manometry was performed after a 12 hour overnight fast. Subjects were positioned in a semi-recumbent position. The manometry catheter was placed with measuring channels located at the lower esophageal sphincter and antroduodenal region with about 7 of the 36 channels located in the duodenum. Recordings lasted for the duration of two complete ph3s, with either gastric or duodenal origin. Ph3 events were visually identified using well-established criteria (Janssen, *APT* 2011 ; 33 : 880-94). During the experiment subjects scored their hunger every 5 min on a visual analogue scale of 10 cm.

Results : Compared to lean volunteers, obese patients had significantly more ph3s with a duodenal origin (63% vs 37% ; $P < 0.0001$), and a lower frequency of contractions during a ph3 in both antrum (2.78 ± 0.37 vs 3.59 ± 0.74 contractions/min ; $P < 0.05$) and duodenum (9.51 ± 2.00 vs 11.18 ± 1.55 contractions/min ; $P < 0.01$). The duration of a ph3 in the duodenum was significantly shorter (5.24 ± 2.14 vs 7.46 ± 1.63 min ; $P < 0.005$) in obese compared to lean subjects, while the duration of antral ph3s did not differ between both groups (4.25 ± 0.50 vs 3.78 ± 0.94 min ; $P > 0.05$). Contraction amplitudes during ph3 in the antrum (104 ± 11 vs 123 ± 7 mmHg ; $P > 0.05$) or duodenum (22 ± 5 vs 20 ± 4 mmHg ; $P > 0.05$) were also not different between obese patients and lean volunteers. Hunger scores associated with ph3, were significantly lower in obese patients compared to lean volunteers ($P < 0.001$).

Conclusions : Origin, frequency and duration of ph3 contractions were different between obese patients and lean volunteers. Consistent with previous reports, hunger was positively correlated to gastric ph3 contractions. Since obese patients have less ph3s with a gastric origin we hypothesize that in obese patients the decrease in hunger is related to the switch of origin of ph3 contractions. This may be a mechanism to compensate for their positive energy balance.

- B08 -

THE EFFECT OF A BITTER RECEPTOR AGONIST ON FOOD INTAKE AND SATIATION IN HEALTHY VOLUNTEERS. S. Verschueren, E. Deloose, S. Janssen, I. Depoortere, J. Tack, P. Janssen. Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : Intragastric administration of bitter receptor agonists decreased food intake in mice (PNAS 108(5) : p2094).

Aim : We set out to investigate the effect of the bitter compound denatonium benzoate (DB) on satiation and food intake during intragastric nutrient drink infusion in humans.

Methods : In a first experiment, an infusion catheter was positioned in the proximal stomach of healthy volunteers (n = 20) after an overnight fast. After a stabilization period DB (0.15 or 1 μ mol/kg) or vehicle was infused intragastrically ; 15-30 minutes later a nutrient drink (1.5 kcal/ml) was intragastrically infused at 60 ml/min until maximum satiation. Satiation and gastrointestinal symptoms such as nausea were scored every minute using visual analogue scales. In a second experiment, healthy volunteers (n = 10) underwent a slow drinking test after intragastric administration of DB (1 μ mol/kg) or vehicle. A peristaltic pump filled one of two beakers at a rate of 15 ml/min with a liquid meal (1.5 kcal/ml). Subjects were requested to maintain intake at the filling rate. Every 5 minutes they scored their satiation and symptoms until maximum satiation was reached. All results are expressed as mean \pm S.E.M. and compared using ANOVA.

Results : Volunteers did not report any symptoms after DB treatment, but satiation scores after administration of the high dose were significantly increased during nutrient drink infusion compared to vehicle treatment (P < 0.01 ; Fig. 1). At maximum satiation the ingested nutrient volumes were 920 \pm 74, 848 \pm 103 and 791 \pm 76 ml after vehicle, 0.15 and 1 μ mol/kg DB respectively (P = 0.06 and 0.01 respectively). DB tended to decrease nutrient ingestion using the slow drinking test (1037 \pm 169 and 897 \pm 119 ml after vehicle and DB respectively). But this effect and the associated satiation scores did not reach significance at the current sample size (respectively P = 0.07 and P = 0.16).

Conclusions : The bitter agonist, DB, dose-dependently increased satiation and reduced the volume of nutrients ingested upon maximal satiation. The contribution of bitter taste receptors (T2Rs) and changes in gastric motor physiology requires further investigation.

- B09 -

ROLE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) AND PACAP IN ADIPOGENESIS. T. Arsenijevic, F. Gregoire, J. Cnudde, M. Waelbroeck, J. Perret, C. Delporte. Ulb Faculty Of Medicine, Anderlecht, Belgium.

Introduction : VIP and PACAP belong to a secretin-glucagon peptide family, and share 68% identity at the amino acid level. The three VPAC/PAC receptors belonging to the G-protein coupled receptors can be distinguished pharmacologically. The physiological action of VIP and PACAP leads preferentially to adenylyl cyclase activation and intracellular cAMP increase

Aim : The aim of our study was to determine the effects of the Vasoactive Intestinal Peptide (VIP)/Pituitary Adenylate Cyclase Activating Peptide (PACAP) peptide family, on the molecular mechanisms of adipogenesis.

Methods : cell culture differentiation *in vitro*, qRT-PCR, Western blotting, adenylyl cyclase activation

Results : Terminal differentiation of 3T3-L1 cell line into adipocyte is induced by a differentiating cocktail made of insulin, dexamethasone and isobutylmethylxanthine. Data obtained in our laboratory have revealed that VIP and PACAP can induce adipogenesis together with insulin and dexamethasone. We confirmed, by RT-PCR, and Western blotting, the presence of VPAC1 and PAC1 receptors on these cells during differentiation. Other results also showed elevated adenylyl cyclase activity in undifferentiated fibroblasts in response to PACAP27 suggesting that PAC1 receptor is functional in confluent undifferentiated cells. As elevation of intracellular cAMP concentration has been associated with induction of adipogenic transcription factors like, C/EBP β and PPAR γ via CREB (cAMP-responsive element-binding protein), we first tried to determine if PACAP induces 3T3-L1 differentiation by altering the expression of these transcription factors. Our qRT-PCR results confirm the up-regulation of the expression of PPAR γ , aP2, c/EBP α and β in the 3T3-L1 adipocytes induced to differentiate with PACAP27, dexamethasone and insulin cocktail.

Conclusions : We show that PACAP can induce adipogenesis with insulin and dexamethasone via cAMP signaling pathway. We hypothesize that VIP is inducing differentiation via another signaling pathway and this hypothesis is currently under investigation.

- B10 -

RESTRICTED FEEDING ACCELERATES GASTRIC EMPTYING INDEPENDENT FROM ITS EFFECT ON GHRELIN SECRETION. J. Laermans, K. Beckers, T. Thijs, S. Janssen, J. Tack, I. Depoortere. Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : The circadian system ensures that all physiological processes are carried out at the optimal time of day or night. While many of these processes are regulated by the master clock in the brain, circadian functions of feeding may depend on a peripheral clock system which is possibly located within the ghrelin-secreting cells of the stomach. Restricted feeding (RF), in which food availability is limited to a short period of time, has been shown to increase plasma ghrelin secretion in anticipation of a regularly scheduled meal.

Aim : Our aim was to study the effect of RF on several functions associated with increased ghrelin signaling : food intake (FI), body weight (BW) and gastric emptying.

Methods : Wild-type (WT) and ghrelin receptor knockout (GHSR-KO) mice were randomly assigned to two groups : an *ad libitum*-fed group with 24-h access to chow and a RF group with access to chow from 12 to 4 PM only. In parallel with the RF group, food was also removed from the *ad libitum*-fed group on day 14 at 4 PM. Blood, stomach and fat were collected from all groups on day 15 at 11:30 AM. Plasma ghrelin levels were determined by radioimmunoassay. mRNA expression of ghrelin, ghrelin O-acyltransferase (GOAT) and PER2 in the stomach was measured by real-time PCR. The effect of RF on gastric emptying was determined by the ¹³C octanoic breath test. *In vitro* contractile responses to ACh and SuP were measured isometrically in strips from the mouse fundus.

Results : In WT mice, RF resulted in a significant increase in plasma octanoyl ghrelin levels compared to *ad libitum*-fed fasted (ALFF) mice (RF : 159 ± 29 pg/ml vs ALFF : 72 ± 10 pg/ml ; n = 8, P < 0.005). Total ghrelin levels were not affected. Ghrelin or GOAT mRNA expression in the stomach was not affected by RF, but the expression of the clock gene PER2 was significantly increased (RF : 1.31 ± 0.14 vs ALFF : 0.23 ± 0.03 ; n = 8, P = 10⁻⁶). BW was significantly diminished in GHSR-KO mice compared to WT mice on RF (P < 0.05), but FI was not affected. RF resulted in a significant decrease (70%) in fat mass in both genotypes. Moreover, RF caused a significant increase in the weight of the fundus (RF : 88 ± 4 mg vs ALFF : 40 ± 1 mg ; P < 0.001), but not of the corpus. Gastric half emptying time was significantly decreased after RF in both WT (RF : 56 ± 3 min vs ALFF : 104 ± 4 min ; n = 12, P = 10⁻⁷) and GHSR-KO mice (RF : 60 ± 4 min vs ALFF : 112 ± 5 min ; n = 10, P = 10⁻⁷). In both genotypes, RF increased the affinity of the *in vitro* contractile response towards ACh and SuP in fundic smooth muscle strips.

Conclusions : RF selectively increases plasma octanoyl ghrelin levels, suggesting that RF targets the biological active form of ghrelin. Ghrelin protects against weight loss during RF. The acceleration in gastric emptying caused by RF is not due to an increase in ghrelin, but might involve local contractility changes in the fundus. Further research is warranted to investigate the role of the increased expression of the clock gene PER2 in the observed changes.

Invited lecture

- B11 -

RATTING OUT IBS ? R.M. Van Den Wijngaard. Academic Medical Center, Amsterdam, Netherlands.

Early childhood factors appear to contribute to development of irritable bowel syndrome (IBS) at adult age in at least part of patients. Several animal models were developed to mimic these predisposing factors. Although neonatal insults such as colorectal distensions and inflammatory stimuli are also being used, most of the current investigations are carried out in the rat neonatal maternal separation (MS) model. MS leads to increased stress-sensitivity at adult age and this is accompanied by visceral hypersensitivity and motility changes. Other changes include alterations in fecal microbiota and immune responses as well as increased mucosal permeability. These same features are currently being investigated in IBS and the MS model may help establish their relevance in this disorder as well as inventory possible connections between these individual characteristics.

Several groups used the MS model to establish the role of mucosal mast cell degranulation in visceral hypersensitivity. These results were translated into a clinical trial in which the mast cell stabilizer ketotifen was able to increase the threshold of discomfort in hypersensitive IBS patients. Follow up investigations in the MS model showed an important role for the histamine-1 (H1)-receptor, suggesting that this receptor can be targeted in IBS. Since H1R-antagonists are frequently being used in allergic rhinitis, clinical trials in IBS should also be feasible in the near future. Interestingly, results obtained in this model also give a possible explanation for the observed lack of effect in two clinical trials with corticotrophin-releasing-factor (CRF)-receptor antagonists. Most preclinical investigations with such antagonists were (successfully) performed in pre-stress treatment protocols. Our recent investigations with alpha-helical CRF (9-41) confirmed these data but also suggested that reversal of post-stress hypersensitivity by the same antagonist is not possible. Future pre-clinical investigations should, therefore, take in to account possible differences in outcome between pre- and post stress treatment protocols. Finally, the recently demonstrated transfer across generations of enhanced stress-induced visceral hypersensitivity in MS rats may be used to further investigate clustering in families which is observed in IBS. This lecture will cover these diverse aspects of the MS model in relation to IBS pathophysiology.

EXPERIMENTAL MODELS TO INDUCE VISCERAL HYPERSENSITIVITY IN RATS. A. Deiteren (1), W. Vermeulen (1), J. De Man (1), T. Moreels (2), P. Pelckmans (2), B. De Winter (1). (1) University Of Antwerp, Antwerpen, Belgium ; (2) Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Visceral hypersensitivity is a hallmark feature of irritable bowel syndrome. The underlying pathophysiological mechanisms have yet to be elucidated, however strong evidence suggests that inflammation and stress are involved.

Aim : The aim of our study was to assess the short- and long-term effects of different combinations of inflammation and stress on visceral sensitivity in rats.

Methods : Colitis was induced by intrarectal instillation of 7.5-15 mg trinitrobenzene sulphonic acid (TNBS) in ethanol. Stress-exposure consisted of one (acute) or multiple (chronic) water avoidance stress sessions. Visceral sensitivity was assessed by electromyographic registration of visceromotor responses (VMR) to colorectal distension (10-80 mmHg, 20 s, 4 min interval) and expressed as the area under the curve. Afterwards, signs of colonic inflammation were assessed by endoscopy, macro- and microscopy and myeloperoxidase activity (MPO). Four series of experiments were conducted to assess 1/ the short-term effects of TNBS-colitis (7.5 mg), acute stress and their combination in Wistar rats, 2/ the short-term effect of chronic stress (10 days) in Wistar rats, 3/ the long-term effects of TNBS (7.5 mg) plus concomitant acute (single) or chronic stress (7 days starting on day 1 post-TNBS) in Wistar rats, assessed after complete resolution of colitis and 4/ the long-term effect of TNBS-colitis (15 mg) after complete resolution of inflammation in Sprague-Dawley rats. Short-term effects were assessed 1-3 days after the latest challenge, long-term effects 13-30 days after the latest challenge in a postinflammatory state.

Results : 1/ In Wistar rats in the acute phase, severe inflammation was present after TNBS with or without stress while inflammatory parameters were normal in control and acute stress groups. Acute colitis, acute stress and their combination all induced visceral hypersensitivity (6571 ± 882 , 6513 ± 650 and $6756 \pm 605 \mu\text{V}$ vs $3061 \pm 348 \mu\text{V}$ in controls ; $p < 0.05$, $n = 7-8$). 2/ Chronic stress-exposure did not induce inflammation or visceral hypersensitivity (1614 ± 203 vs $1215 \pm 358 \mu\text{V}$; ns, $n = 8$). 3/ After TNBS-colitis combined with concomitant acute or chronic stress, post-inflammatory VMR were not significantly different from controls (2807 ± 784 and $2519 \pm 551 \mu\text{V}$ vs $2143 \pm 231 \mu\text{V}$ for controls ; ns, $n = 6-8$). 4/ In Sprague-Dawley rats TNBS-treatment resulted in significant post-inflammatory visceral hypersensitivity compared to controls (4874 ± 841 vs $2181 \pm 307 \mu\text{V}$; $p < 0.05$, $n = 8-10$). The post-inflammatory status (experiments 3-4) was confirmed by endoscopy, macro-, microscopy and MPO.

Conclusions : Different approaches to induce visceral hypersensitivity in rats were assessed. In the acute phase, TNBS-colitis, acute stress-exposure and their combination resulted in visceral hypersensitivity. In our long-term set-up however, only TNBS-treated Sprague-Dawley rats exhibited marked post-inflammatory visceral hypersensitivity.

A LINK BETWEEN STRESS HORMONES, ENTERIC NERVE ACTIVITY AND FASTING IN MICE. K. Lowette, L. Roosen, J. Tack, P. Vanden Berghe. Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : Stress, metabolic and gastrointestinal (GI) disorders have a severe negative impact on quality of life. Moreover, they are interlinked as stress has an important effect on food-intake and GI function. The fact that stress hormones such as corticosterone (CORT) and Urocortin2 (Ucn2) are highly conserved through evolution indicates their importance in survival and adaptation. It is assumed that hunger is a stressor which induces expression of these hormones. However, little is known about cellular mechanisms by which stress-related molecules exert their effect on GI function during fasting and feeding.

Aim : Therefore, we aim to investigate the effects of CORT on synapses and neurons in the myenteric plexus of mouse small intestine and how Ucn2 expression varies during fasting and feeding.

Methods : Mouse myenteric neurons were isolated and grown 4 days in culture medium to which, 20 min prior to the experiment CORT ($10 \mu\text{M}$) or vehicle (DMSO) was added. Subsequently, cells were loaded with the Ca^{2+} indicator Fluo-4 ($5 \mu\text{M}$, 20 min) after which cells were depolarized with high K^{+} solution or stimulated in an electric field in order to monitor their Ca^{2+} responses ($\Delta\text{F}/\text{F}_0$). In addition, mRNA from GI tissue of fasted (20 h) and refeed (3 h) mice was extracted to be used in RT-PCR experiments. CORT plasma levels were determined using an immunoenzymatic assay.

Results : Acute perfusion with CORT did not alter the Ca^{2+} responses of neuronal cell bodies and synapses. However, Ca^{2+} response amplitudes in CORT-incubated neurons were significantly higher compared to vehicle after high K^{+} depolarization ($119 \pm 5\%$ vs. $96 \pm 4\%$, $p < 0.01$, $n = 57$) or electrical stimulation (1 pulse : $10 \pm 0.6\%$ vs. $8 \pm 0.6\%$, $p < 0.01$, $n = 46$; 40 pulses : $66 \pm 3\%$ vs. $52 \pm 3\%$, $p < 0.01$, $n = 56$) without changing the amplitude at varicosities and

vesicle release sites. CORT incubation also prolonged the duration of the Ca²⁺ responses by 50% in neurons (40 pulses : 8.5 ± 0.2 vs. 7.7 ± 0.2 s, p = 0.04, n = 56) and synapses (high K⁺ : 11.5 ± 0.3 vs. 10.2 ± 0.3 s, p < 0.01, n = 387 ; 1 pulse : 4.3 ± 0.3 vs. 4.0 ± 0.2 s, p = 0.02, n = 261 ; 40 pulses : 8.7 ± 0.3 vs. 6.8 ± 0.2 s, p < 0.01, n = 364). We found no differences in CORT plasma levels in fasted and refed mice, but Ucn2 expression, based on the RT-PCR data, was significantly higher in gastric (4.0 ± 1.3 vs. 1.7 ± 0.2, p < 0.02, n = 10) and duodenal (5.6 ± 2.6 vs. 1.2 ± 0.2, p < 0.04, n = 5) tissue of fasted compared to refed mice.

Conclusions : CORT incubation modulates Ca²⁺ responses in myenteric cultures and Ucn2 mRNA expression is increased in the upper GI-tract of fasted mice. These results demonstrate a modulating effect of CORT on enteric neuron- and synapse-activity and suggest a link between stress and fasting. Further research will be needed to investigate the expression of these and other stress hormones *in vitro*, and to unravel their effects on myenteric plexus tissue of fasted and refed animals.

- B14 -

RECTAL SENSITIVITY MEASUREMENT : RECTAL MUCOSAL CAPSAICIN APPLICATIONS AND RECTAL DISTENSIONS. S. Van Wanrooij, W. Vanbrabant, K. Lambaerts, M. Wouters, G. Boeckxstaens. Translational Research Center For Gastrointestinal Disorders (TARGID), KULeuven, Leuven, Belgium.

Introduction : Abnormal sensitivity to distention or visceral hypersensitivity is considered as an important underlying pathophysiological mechanism in 40-65% of patients with irritable bowel syndrome (IBS). Recent evidence suggests that increased expression of the nociceptor TRPV1 may be involved, at least in a subgroup of patients. We hypothesized that this subgroup could be identified by assessment of rectal sensitivity to the TRPV1 agonist capsaicin. Therefore, visceral perception of capsaicin applications to the rectal mucosa was studied in IBS patients and healthy subjects.

Methods : IBS patients and healthy volunteers (HV) were invited to undergo subsequent rectal distensions and capsaicin applications. Visceral sensitivity was evaluated by rectal distensions of 2 minutes at 3, 9, and 21 mmHg above minimal distension pressure. Based on previous studies, subjects unable to tolerate 21 mmHg distention were considered visceral hypersensitive. Secondly, capsaicin was applied (0.01%, 0.1% or solvent in random order) to the rectal mucosa. To this end, a cotton swap with solvent or capsaicin was gently applied to the mucosa during 60 seconds through a proctoscope. Visceral sensations (urge to defecate, pain, burning and warm sensation) were scored on a 100mm visual analogue scale (VAS).

Results : Visceral sensitivity to rectal distention and capsaicin application was assessed in 25 healthy volunteers, (mean age 30 ± 1.9 years, 64% female) and 43 IBS patients (mean age 33 ± 1.7 years, 63% female). Visceral perception to distention was higher in IBS patients compared to HV. Visceral hypersensitivity was present in 47% of IBS patients whereas all healthy subjects tolerated the 21 mmHg distention step. Visceral sensitivity scores of urge and pain increased concentration dependent during rectal capsaicin applications in both IBS and HV, and were significantly higher in IBS patients, in both normo- and hypersensitive IBS patients (Table). After subtraction of sensations scored during solvent applications however, only pain scores during 0.01% capsaicin applications remained significantly higher in IBS patients compared to HV (table), suggesting that increased sensation of urge in IBS was most likely due to increased perception of anal distension by the proctoscope.

Conclusions : We here provide evidence for increased pain perception evoked by rectal capsaicin application in IBS patients compared to HV, suggesting a role for TRPV1 nociceptors in the increased pain perception of IBS patients. The underlying role of TRPV1 in visceral hypersensitivity is currently further evaluated by TRPV1 staining and mRNA expression in rectal biopsies of these patients.

- B15 -

LONG-LASTING IMPAIRED MUCOSA INTEGRITY AFTER ESOPHAGEAL PERFUSION WITH ACID & WEAKLY ACIDIC SOLUTION. N. Pardon (1), M. Vicario (2), H. Vanheel (1), T. Vanuytsel (1), K. Blondeau (1), M. Jimenez (3), J. Tack (1), R. Farré (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium ; (2) Hospital Universitari Vall D'hebron, Barcelona, Spain ; (3) Universitat Autònoma De Barcelona, Barcelona, Spain

Introduction : It has been suggested that impaired esophageal mucosal integrity may be an important contributor to heartburn perception in NERD patients (Farré *et al.*, Gut, 2011). Dilated intercellular spaces (DIS) are proposed to be an early marker of reflux induced-damage. Nevertheless, it has not been established that alterations in mucosal integrity are only due to a direct and repeated effect of aggressive luminal contents. The role of (slower or incomplete) mucosal repair mechanisms has not been addressed.

Aim : The aims of the present study are 1) to assess *in vivo* the effect of an acidic and a weakly acidic solution containing the bile acid deoxycholic acid (DCA) on esophageal mucosal integrity and 2) to evaluate the recovery of the tissue after 24 and 48 h.

Methods : Esophageal perfusion was performed for 30 min in New Zealand rabbits with saline solutions at pH 7.2, pH 5.0, pH 1.0 and pH 5.0 +DCA 500 μ M. Thereafter, subgroups of animals were sacrificed immediately at 0 h, 24 h or 48 h after perfusion. Transepithelial mucosal resistance (TER) was determined in Ussing chambers and mucosa was assessed by transmission electron microscopy.

Results : After esophageal perfusion with solution pH 7.2 the TER was 2156 ± 98 O/cm². Acidic solution (pH 1.0) decreased TER to 457 ± 90 O/cm² ($p < 0.05$, N = 6). After a recovery period of 24h the TER increased to 1660 ± 123 O/cm² ($p < 0.05$, N = 4). After 48h, TER was not different compared to 24 h (1772 ± 122 O/cm², N = 3), but did not recover completely ($p < 0.05$ vs pH 7.2). After esophageal perfusion with solution pH 5.0, TER was 2501 ± 196 O/cm². Solution pH 5.0 +DCA 500 μ M decreased TER to 357 ± 50 O/cm² ($p < 0.05$, N = 6). After a recovery period of 24h, the TER increased to 1143 ± 231 O/cm² ($p < 0.05$, N = 4). After 48h, TER increased to 1847 ± 141 O/cm² ($p = 0.04$ vs 24 h, N = 4) but did not recover completely ($p = 0.04$ vs pH 5.0). At 24 h, TER was lower after +DCA 500 μ M compared to pH 1.0 (1660 ± 123 O/cm² vs 1143 ± 231 O/cm², $p < 0.05$). No macroscopic alterations were observed at the different time points. Impaired mucosal integrity and DIS were present after perfusion with pH 1.0 (0.28 ± 0.06 μ m) and DCA solution (0.15 ± 0.02 μ m) ($p < 0.05$). Ultrastructural abnormalities were present mainly at the basal cell layer at 24 h after DCA solution, characterized by loss of normal cell morphology and abundant cell debris. At 48 h cell morphology was recovered but DIS were still present at the basal cell layer ($p < 0.05$). After 24h of pH 1.0 perfusion, normal ultrastructural cell morphology was observed at the basal cell layer but DIS were still present ($p < 0.05$).

Conclusions : Acidic and weakly acidic solution containing DCA impair mucosal integrity provoking functional and ultrastructural changes that can last at least 48 h. The presence of similar changes in the esophageal mucosa in NERD patients may be due to a direct effect of luminal contents or to a slow/incomplete repair mechanism.

-B16 -

MECHANISM OF ACTION OF BAY 41-2272 IN GASTROINTESTINAL RELAXATION. S. Cosyns, R.A. Lefebvre. Heymans Institute Of Pharmacology, Gent, Belgium.

Introduction : BAY 41-2272 is a heme-dependent NO-independent soluble guanylate cyclase (sGC) stimulator, but its relaxant effect in respiratory and urogenital tissue is only partially dependent on sGC activation. Its mechanism of action has not been studied in the gastrointestinal tract.

Aim : To investigate the effect of BAY 41-2272 in mouse gastric fundus and colon, with special attention for the role of sGC and possible additional mechanisms of action.

Methods : Circular smooth muscle strips from the gastric fundus and distal colon (after removal of the mucosa) of male C57Bl/6J mice (11-15 weeks) were mounted in organ baths under NANC conditions for isometric force recording. cGMP levels were determined by enzyme immunoassay in stored tissues, that had been clamped after exposure to BAY 41-2272.

Results : BAY 41-2272 induced a concentration-dependent relaxation in both tissues (1-10 μ M [fundus] ; 0.3-3 μ M [colon]). BAY 41-2272 (10 μ M [fundus], 3 μ M [colon]) increased basal cGMP levels by 3-fold in the fundus strips and by 4.5-fold in the colonic strips. The sGC inhibitor ODQ (30 μ M) completely abolished this BAY 41-2272-induced increase of cGMP, but only partially reduced the corresponding relaxation. The relaxant responses to BAY 41-2272 (10 μ M [fundus] ; 3 μ M [colon]) were not significantly influenced by apamin (0.3 μ M), charybdotoxin (0.1 μ M), or ouabain (10 μ M) excluding interaction with small, intermediate and large conductance Ca²⁺-activated K⁺-channels and with Na⁺/K⁺-ATPase. Under conditions where all intracellular calcium was depleted from the Ca²⁺-stores, BAY 41-2272 concentration-dependently inhibited contractions evoked by CaCl₂ (0.01-100 mM) in fundus and colonic strips. Pre-treatment with ODQ (30 μ M) did not influence this inhibitory effect of BAY 41-2272 on CaCl₂-induced contractions.

Conclusions : In both mouse gastric fundus and colon, BAY 41-2272 induces relaxation partially through a cGMP-dependent mechanism and by at least one additional cGMP-independent mechanism involving Ca²⁺-entry blockade.

DEEP IMAGING IN THE GUT WALL : EXPLORING TISSUE CLEARING WITH 1- AND 2-PHOTONS EXCITATION MICROSCOPY. J.M. Vanderwinden, P. Hague, F. Bolletquivogne. Ulb Faculty Of Medicine, Anderlecht, Belgium.

Introduction : Histological diagnostic using tissue sections is a most useful two dimensional (2D) technique. However, 2D sampling provides only limited insight into the volumetric relationships between components. Tissues such as the gut wall, in which structural anisotropy (i.e. the property of being directionally dependent) is a hallmark, would benefit from gathering information in three dimensions (3D).

Aim : Here we report on some recent developments in sample preparation for optical sectioning fluorescent imaging in "deep" 3D structures.

Methods : Small intestine and colon wholemounts of wild-type and tamoxifne-inducible KIT-Cre-R26mR/mG mice (a kind gift from D. Saur, TU Munich, Germany) were fixed overnight with paraformaldehyde, and stained with DAPI and/or immunofluorescence markers in various combinations. TDE (Appelton PL *et al.*, J. Microsc., 2009) and Sca/e (Hama H. *et al.*, Nature Neurosci., 2011) tissue clearing protocols for fluorochromes and for fluorescent proteins (FP), respectively, were tested. Fluorescent imaging of z-stacks was carried out in one- and two-photon excitation (1PE and 2PE, respectively) using a line scanning Zeiss LSM510NLO Meta confocal microscope with a 30 mW Argon laser for 1PE and a MaiTai® laser (Spectra-Physics, USA) for 2PE. The resulting stacks were deconvolved using Huygens® (Scientific Volume Imaging, NL) and visualized in 3D using Imaris (Bitplane, CH).

Results : Tissue clearing, by markedly reducing light scattering, allowed imaging of DAPI stained nuclei up to 200 μm (a limitation inherent to the working distance of the objective). KIT+ ICC networks and KIT+ cells in the mucosa could be imaged as a single stack across the whole wall thickness. 2PE allowed deeper imaging while causing less photo-bleaching than 1PE, although at the expense of a moderate drop in Signal to noise ratio. Dylight®488 and Dylight®549 dyes (Dyomics GmbH, Germany), which were bright and photostable in 1PE, could not be excited with the MaiTai® laser and thus appeared unsuitable for 2PE imaging.

Conclusions : Tissue clearing procedures appeared valuable in both 1PE and 2PE during "deep" confocal imaging. Objective working distance and NA, refractive index (mis)match, penetration of immunomarkers and the expression level of fluorescent proteins (FP) are crucial factors that must be optimized for "deep" imaging. 2PE beyond 920 nm (the limit for MaiTai® laser) would likely increase FPs signal. 3D "deep" imaging is a powerful tool but remains a time consuming and demanding technique. Further developments, on fixed samples but also on living tissues in vitro, are ongoing.

VALIDATION OF THE ZEBRAFISH MUTANT *LESSEN* FOR HIRSCHSPRUNG'S DISEASE RESEARCH. L. Uyttebroek (1), I.I. Shepherd (2), G. Hubens (1), J.P. Timmermans (3), L. Van Nassauw (1). (1) Laboratory Of Human Anatomy And Embryology, Faculty Of Medicine & Health Sciences, University Of Antwerp, Antwerpen, Belgium ; (2) Emory University, Atlanta, United States ; (3) Laboratory Of Cell Biology And Histology, Department Of Veterinary Sciences, University Of Antwerp, Antwerpen, Belgium.

Introduction : Hirschsprung's disease (HD) is a congenital disorder characterized by aganglionosis in the distal intestine. The zebrafish mutant, *lessen* (*lsn*), has HD phenotypic characteristics. We use the *lsn* mutant as an experimental model to unravel underlying developmental mechanisms for HD.

Aim : This study aims to compare the neurochemical content of enteric neurons and gastrointestinal (GI)-motility patterns between wild-type zebrafish and *lsn* mutants to further validate this mutant as a suitable model for HD research.

Methods : Immunofluorescence staining was used to detect specific neurochemical markers from 3 to 6 days post-fertilization (dpf) in the proximal (PI), mid (MI) and distal intestine (DI) of both wild-type and mutant embryos. Contractile activity of the GI-tract was filmed and the frequency and direction of contractions in each intestinal region were analyzed.

Results : Both mutant and wild-type embryos showed an increase in enteric neuron numbers over time. In mutants, the number of enteric neurons at each embryonic stage was significantly reduced in DI (absent at 3dpf) and MI, but less in PI. The proportion of nitrergic neurons was significantly reduced in all regions at 3dpf, but nearly unaffected in PI and MI at 4 and 5dpf. In mutants, serotonin, calretinin and calbindin showed a delayed expression and a decrease in both number and proportion at all points of time and in each intestinal region. In both mutant and wild-type embryos, galanin, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) were only detected in nerve fibers. In wild-type, VIP/PACAP-positive and galanin-positive fibers were present at all time points and in each region. Mutants only showed a few immunoreactive fibers at 3dpf in PI, while at 4dpf immunostained fibers appeared in MI and at 5dpf in DI, showing a delayed expression of these neuropeptides. GI-tractility analyses revealed that

wild-type animals have defined motility patterns by 4dpf. When comparing motility patterns, the PI showed little change in when comparing wild-type to mutants. In contrast, we observed a decreased contractility in the MI and DI of mutants. **Conclusions** : The present study reveals abnormalities in the number and proportions of neurons expressing various neurochemical markers in the *lsn* mutant. These results are similar to previously reported data in the intestine proximal to the aganglionic segment and in the aganglionic segment of the *lethal spotted* mutant mice, an experimental HD model. Furthermore, in *lessen* GI-contractility is significantly perturbed in MI and DI. So, this study supports previous studies indicating that the zebrafish mutant *lessen* is a suitable model for HD research.

- B19 -

INFLUENCE OF CORM-A1 ON TNF-ALPHA-INDUCED OXIDATIVE STRESS AND APOPTOSIS IN MODE-K CELLS. D. Babu (1), O. De Backer (1), S. Soenen (2), K. Raemdonck (2), G. Leclercq (2), R. Motterlini (3), R.A. Lefebvre (1). (1) Heymans Institute Of Pharmacology, Gent, Belgium ; (2) Ghent University, Ghent, Belgium ; (3) Inserm U955, Créteil, France.

Introduction : In the mouse postoperative ileus model, our group has previously shown an increase in oxidative stress after intestinal manipulation occurring earlier in the mucosal layer than in the muscular layer ; this might lead to epithelial barrier dysfunction and intestinal inflammation, contributing to ileus (De Backer *et al.*, Gut, 2009). Carbon monoxide (CO)-releasing molecules (CORMs) were shown to markedly reduce oxidative stress and to restore intestinal motility.

Aim : We therefore investigated the effect of CORM-A1 on TNF-alpha-induced oxidative stress and apoptosis in murine Mode-K intestinal epithelial cells.

Methods : Mode-K cells (passage 10-35) were grown for 36 h and then serum starved overnight ; for the preliminary optimization experiments, Mode-K cells were then exposed to TNF-alpha/cycloheximide (CHX) for up to 0-6 h for various experiments. To investigate the effect of CORM-A1, the confluent cells were pretreated with CORM-A1 for 1 h followed by its co-treatment with TNF-alpha/CHX for 3-6 h for different experiments. Reactive oxygen species (ROS) production was directly measured by carboxy-H2DCFDA fluorescence intensity measurement, reduced glutathione level was measured by GSH-Glo bioluminescent assay, caspase-3/7 activity was measured by Caspase-Glo bioluminescent assay, and apoptosis was measured by flow cytometry analysis of propidium iodide stained cells.

Results : TNF-alpha/CHX increased ROS production, reduced the level of the antioxidant glutathione, increased caspase-3/7 activity and induced apoptosis in a concentration-dependent manner (0.1 to 1 ng/ml ; 10 μ g/ml CHX). The effects at 1 ng/ml TNF-alpha were similar to those at 20 ng/ml TNF-alpha, classically used in cell studies. CORM-A1 (100 μ M) was tested versus the effects of 1 ng/ml TNF-alpha. The decrease in reduced glutathione level (to $44 \pm 2\%$ of control) by 1 ng/ml TNF-alpha, was only very partially prevented by pre- and co-treatment with CORM-A1 (to $53 \pm 3\%$) ; the 4.6 (± 0.1) fold increase in ROS production by TNF-alpha was reduced to 2.9 (± 0.1) fold by CORM-A1. The TNF-alpha-induced 6.3 (± 0.1) fold increase in caspase-3/7 activity was only marginally influenced by CORM-A1 (to 6.0 ± 0.1 fold). Mode-K cells in control conditions showed $10 \pm 0.4\%$ apoptosis ; this was increased to $57 \pm 0.3\%$ by TNF-alpha ; CORM-A1 reduced this effect of TNF-alpha to $44 \pm 0.3\%$.

Conclusions : Reduction of oxidative stress by CORM-A1 might contribute to its protective effect on intestinal epithelial cells.

- B20 -

IMPAIRED ENDOPLASMIC RETICULUM STRESS RESPONSE IN INFLAMED BIOBREEDING RATS. T. Masaoka, S. Salim Rasoel, C. Vanormelingen, P. Vanden Berghe, J. Tack. Translational Research Center For Gastrointestinal Disorders (Targid), KULeuven, Leuven, Belgium.

Introduction : Unfolding protein accumulation, a.k.a. endoplasmic reticulum (ER) stress, is emerging as a pathological factor in several diseases, such as diabetes mellitus, motor neuron disease and inflammatory bowel disease. ER stress elicits a signaling cascade known as the unfolded protein response (UPR). The BBDR rat has a mutation in the Gimap5 (GTPase of the immunity-associated protein 5) which localizes to the ER. Increases in ER stress-associated chaperones in T cells from BBDR rats have been reported (Pino 2009). We reported myenteric ganglionitis, inflammation-induced nitergic dysfunction and intestinal dysmotility preceded by increased intestinal permeability and mucosal inflammation in non-diabetic BioBreeding diabetes prone (BBDR) rats (Masaoka DDW 2011, Vanormelingen UEGW 2011).

Aim : To assess the pathophysiological role of the ER stress response system in the intestine of BBDR rats.

Methods : Normoglycemic BBDR rats and BioBreeding diabetes-resistant (BBDR) rats were sacrificed at 220 days. After harvesting jejunum, mucosal layer and longitudinal muscle myenteric plexus (LMMP) preparations were dissect-

ed. Jejunal inflammation, mRNA expression of ER stress chaperone GRP78, and the main regulators of the UPR (IRE1 α , IRE1 β , PERK and ATF6) were analyzed by MPO measurements and realtime PCR, respectively.

Results : Compared to BBDR rats (n = 5), LMMP preparations from normoglycemic BBDR rats (n = 8) had higher MPO levels (0.0 ± 0.0 vs. 5.4 ± 2.2 U/mg, $p < 0.01$), lower GRP78 mRNA expression (1.0 ± 0.1 vs. 0.5 ± 0.1 , $p < 0.01$) and lower PERK mRNA expression (1.0 ± 0.1 vs. 0.4 ± 0.2 , $p < 0.05$). A significant linear correlation was observed between mucosal and LMMP expression of GRP78 mRNA ($p < 0.01$, $r = 0.72$; Figure 1). In LMMP, a significant linear correlation was also observed between MPO levels and GRP78 mRNA expression ($p < 0.05$, $r = -0.65$). Compared to BBDR rats, BBDR rats had lower mucosal GRP78 mRNA expression (1.0 ± 0.1 vs. 0.4 ± 0.1 , $p < 0.001$), lower IRE α mRNA expression (1.0 ± 0.2 vs. 0.5 ± 0.1 , $p = 0.07$), lower IRE β mRNA expression (1.0 ± 0.2 vs. 0.3 ± 0.2 , $p < 0.01$) and lower PERK mRNA expression (1.0 ± 0.2 vs. 0.3 ± 0.1 , $p < 0.01$).

Conclusions : ER stress response was impaired in the inflamed intestine in BBDR-rats, both in mucosa and LMMP. Impaired ER stress response may contribute to the pathogenesis of increased permeability and intestinal dysmotility.

PLENARY SESSION

- D01 -

INHIBITION OF VEGFR2 ATTENUATES STEATOSIS AND INFLAMMATION IN A DIET-INDUCED MOUSE MODEL FOR NASH. S. Coulon (1), F. Heindryckx (1), B. Blomme (1), C. Vansteenkiste (1), L. Libbrecht (1), J.M. Stassen (2), B. Jonckx (2), I. Leclercq (3), N. Lanthier (3), H. Vanvlierberghe (1), I. Colle (1), A. Geerts (1). (1) Universitair Ziekenhuis Gent, Gent, Belgium ; (2) Throbogenics Nv., Heverlee, Belgium ; (3) Université Catholique de Louvain, Brussels, Belgium.

Introduction : The pathophysiology of non-alcoholic steatohepatitis (NASH) should be approached as a multifactorial process. In several stages of NASH there might be a link between disease progression and hepatic microvasculature changes. Given that angiogenesis plays a pivotal role in several chronic liver diseases like cirrhosis and hepatocellular carcinoma, could imply that angiogenesis might also play a role in the disease progression of NASH.

Aim : Characterise the effect of inhibition of vascular endothelial growth factor receptor 2 (VEGFR2) treatment in a diet induced mouse model for NASH.

Methods : Female C57Bl/6 mice, aged 10 weeks, were fed a MCD diet (n = 10/group) or a control diet (n = 10/group). Twice a week anti-VEGFR2 (40 mg/kg body weight) or physiological solution (NaCl) was intraperitoneally injected. Liver and serum samples were taken after 9 weeks of diet and treatment. Sections were stained with H&E and picrosirius red for histology. Hepatic neovascularisation and inflammation was investigated by immunohistochemistry for endoglin and F4/80. Gene expression of stearoyl-CoA desaturase 1 (Scd1), fatty acid binding protein (Fabp) and carnitine palmitoyltransferase 1 (Cpt1) was determined with qRT-PCR.

Results : Serum levels of AST and ALT were significantly higher in MCD fed mice treated with anti-VEGFR2 compared to MCD fed mice treated with NaCl (respectively $p = 0.009$; $p = 0.007$). Liver histology showed a decrease of steatosis ($p < 0.001$) and fewer inflammatory foci ($p = 0.015$) in the liver of anti-VEGFR2 treated mice in comparison to MCD fed mice treated with NaCl. There was no difference in fibrosis. Expression of endoglin in the liver of MCD fed mice treated with anti-VEGFR2 was significantly decreased compared to MCD fed mice treated with NaCl ($p = 0.005$). To investigate the molecular mechanisms, we looked into genes involved in fatty acid metabolism. Scd1 was significantly higher in MCD fed mice treated with anti-VEGFR2 compared to MCD fed mice treated with NaCl ($p < 0.001$). Low Scd1 expression results in excess fat deposition in the liver. Fabp and Cpt1 expression remained the same in both groups.

Conclusions : Our results demonstrate that inhibition of VEGFR2 is protective against the development of steatosis and inflammation in a diet-induced mouse model for NASH. The effect of anti-VEGFR2 might be found in the fatty acid metabolism. These findings might give rise to new therapeutic options for NASH.

- D02 -

DESIGN AND VALIDATION OF SCORES FOR THE PROSPECTIVE ASSESSMENT OF NASH : A LARGE SINGLE-CENTRE STUDY. S. Francque, A. Verrijken, I. Mertens, C. Van Gils, G. Hubens, E. Van Marck, V. Van Marck, P. Pelckmans, P. Michielsen, L. Van Gaal. Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Liver biopsy is still the best standard for diagnosis and staging of NAFLD and NASH. As many patients are at risk, reliable non-invasive tools are warranted. Published scoring systems are mostly designed in selected populations and are poorly validated for the prospective assessment of unselected patients.

Aim : To identify reliable non-invasive predictors of NAFLD and NASH in a large single-centre prospectively included cohort of obese patients and to study their diagnostic accuracy in comparison with established scoring systems.

Methods : Patients presenting to the obesity clinic underwent a metabolic and liver assessment. If NAFLD was suspected a liver biopsy was proposed. The biopsy was scored using the NCRN Scoring System.

Results : Between October 2005 and October 2010, 542 patients (68.8% female) were included after exclusion of other liver diseases. Mean age was 43.5 ± 12.7 y and mean BMI 38.3 ± 6.46 kg/m² (range 24.8-69.1) ; 57.7% underwent a liver biopsy and were randomly separated in a design (n = 200) and a validation (n = 113) cohort. Mean biopsy length was 1.8 ± 0.5 cm and mean number of portal tracts 9.6 ± 3.9 in the overall cohort. Independent predictors for the presence of NASH according to the Brunt definition were elevated ALT (> 40 U/L), elevated AST (> 40 U/L), fasting c-peptide and ultrasound steatosis score (USS) with an AUROC of 0.829 and 0.859 in design and validation cohort respectively. Independent predictors of NASH based on NAS > 5 were ALT, USS and the number of MS criteria according to IDF, with an AUROC of 0.882 and 0.876 in design and validation cohort respectively. NAS could be predicted by ALT, USS and fasting c-peptide ($R^2 = 0.491$). Independent predictors of advanced fibrosis (≥ 3) were waist, AST, elevated AST and HOMA IR with an AUROC of 0.900 and 0.909 in design and validation cohort respectively and a negative predictive value of 98.9 in both for a score cut off of 3.71. NAFLD liver fat score had an AUROC 0.724 for

the diagnosis of NASH and BARD score an AUROC 0.803 for advanced fibrosis. NAFLD fibrosis score, FLI, FIB4 and APRI had significantly lower AUROC. CK18 correlated with both NAS and fibrosis and PNPLA3 polymorphism correlated with NAS and presence of NASH, but both didn't add to the diagnostic accuracy of the scores.

Conclusions : Scores based on routine parameters can predict NASH and advanced fibrosis with high accuracy in the prospective evaluation of obese patients without a priori suspicion of liver involvement. Non-routine tests show significant correlations but do not add to the diagnostic accuracy of the scores. Previously published scores are significantly less accurate.

- D03 -

ANTI-REFLUX SURGERY AFTER CONGENITAL DIAPHRAGMATIC HERNIA REPAIR. T. Verbelen, W. Coosemans, P. Deleyn, P. Nafteux, D. Vanraemdonck, I. Hoffman, J. Deprest, T. Lerut, H. Decaluwe. University of Leuven, Leuven, Belgium.

Introduction : The need for anti-reflux surgery in patients treated for congenital diaphragmatic hernia (CDH) has frequently been reported in literature and its preventive application at the moment of CDH repair has been suggested by some authors, especially in subgroups with a herniated liver or patch requirement.

Aim : The objective of this retrospective single-center observational study was to evaluate the incidence of GERD and anti-reflux surgery.

Methods : We retrospectively reviewed our CDH database. Demographics, prenatal treatment, type of repair, intra-operative findings and incidence of medical and surgical treated GERD were recorded. Since 2002, selected patients are prenatally treated with fetoscopic endoluminal tracheal occlusion (FETO).

Results : Between July 1993 and November 2009, transthoracic CDH repair was performed in 77 infants. Twenty-two of them underwent FETO. Eight died. Seven patients were lost to follow-up. Fifty-six left-sided, 4 right-sided and 2 bilateral CDH patients were included. GERD was diagnosed in 50% of patients, never in right-sided CDH. A herniated liver is significantly more seen in patients with GERD compared to them without (61% vs 23%, $p = 0.0042$). Birth weight was significantly lower in the patients with GERD (2667 ± 560 vs 3035 ± 701 grams, $p = 0.027$). One year after starting GERD treatment, only 42% of GERD patients were still taking medication. 21% of all 62 CDH patients underwent anti-reflux surgery (1 Lind-procedure, 12 Nissen funduplications). Recurrence of a hernia after Nissen fundoplication occurred in 6 patients. Two of these were symptomatic and needed redo-surgery. Patients within the surgical treated group have a significantly earlier gestational age at birth (35.3 ± 3.1 vs 37.4 ± 2.2 days, $p = 0.007$) and significantly more associated pulmonary problems (77% vs 38%, $p = 0.0268$). The incidences of previous FETO-repair and a herniated liver are significantly higher in this group, compared to the other CDH patients (54% vs 13%, $p = 0.0042$ and 75% vs 33%, $p = 0.0257$, respectively). In the subgroups of CDH patients with patch repair, a herniated liver or previous FETO, the incidences of GERD and anti-reflux surgery are 61% and 32%, 62% and 28%, and 71% and 43%, respectively.

Conclusions : In our series 50% of CDH patients develop GERD. Anti-reflux surgery was performed in 21%. The routine performance of an anti-reflux procedure at the time of CDH repair seems not to be justified although incidences of GERD and anti-reflux surgery are raised in certain subgroups. A herniated liver and a lower birth weight are identified as risk factors for the development of GERD. Risk factors for subsequent anti-reflux surgery are an early gestational age at birth and a herniated liver. Also FETO, which creates a new cohort of survivors, is a risk factor for need for anti-reflux surgery. The high incidence of reflux is most likely related to the higher complex surgical physiopathology in these patients.

- D04 -

PERORAL CHOLANGIOSCOPY IN BILIOPANCREATIC INTERVENTIONS : RESULTS OF BELGIAN SINGLE-CENTER COHORT. W. Laleman, W. Vansteenbergen, S. Van Der Merwe, C. Verslype, F. Nevens. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Biliary strictures of uncertain clinical significance and failed conventional therapeutic endoscopic retrograde cholangiopancreatography (ERCP) remain a challenging clinical reality. Direct visualization of the bile and pancreatic duct by cholangioscopy could offer added value in both these situations by visual evaluation of the "indefinite" stenosis combined with targeted biopsies on the one hand and by optically guided-therapy (such elektrohydraulic lithotripsy (EHL) on the other.

Aim : To evaluate the utility of the single operator cholangioscopy (SOC)-system Spyglass in a single-center tertiary biliopancreatic interventional unit.

Methods : All SOC-procedures performed within our unit were reviewed with regard to procedural success (defined as the ability to 1) visualize target lesions and, if indicated, collect biopsy specimens or 2) visualize biliary/pancreatic stones and initiate fragmentation by EHL and removal). Additional evaluation was made with regard to occurrence of adverse events.

Results : Since 3-2010 until 11-2011, 39 SOC-procedures (mean age 57, 18 males, on average 2.5 previous ERCPS before SOC) were performed either for diagnostic (n = 24, 6 primary sclerosing cholangitis (PSC)-18 non-PSC) or therapeutic (n = 15, 13 biliary and 2 pancreatic stones) reason. Overall procedure success was 85% with a specific success rate for therapeutic interventions of 71.4% and SOC-directed tissue sampling of 94.4%. Based on the combination of visual impression and targeted biopsy, all except one of the indeterminate stenoses could be classified as either benign (n = 20) or malignant (n = 3) and therefore influenced management. Overall complication rate was 17.9% with cholangitis (n = 3), mild pancreatitis (n = 3) and EHL-related intraductal bleeding (n = 1). All adverse events were reversible and treated conservatively.

Conclusions : Single operator cholangioscopy by use of the Spyglass-device is of added value in increasing diagnostic yield in patients with indeterminate stenoses and in overcoming failure of conventional therapeutic ERCP.

- D05 -

IMPAIRED INTEGRITY AND ALTERED EXPRESSION OF CELL-TO-CELL ADHESION PROTEINS IN FUNCTIONAL DYSPEPSIA. H. Vanheel (1), C. Martinez (2), T. Vanuytsel (1), N. Pardon (1), M. Vicario (2), J. Soderholm (3), J. Tack (1), R. Farré (1). (1) KULeuven, Leuven, Belgium ; (2) Hospital Universitari Vall D'hebron, Barcelona, Spain ; (3) Faculty Of Health Sciences, Linköping, Sweden.

Introduction : Functional dyspepsia (FD) is an extremely common disorder of gastrointestinal function characterized by chronic epigastric symptoms. Despite intensive research, the pathogenesis is poorly understood. However, it has been shown that low-grade inflammation in the duodenum may contribute to symptom generation (Kindt 2009). Impaired barrier function, which can be caused by altered expression of cell-to-cell adhesion proteins, is suggested to play a key role in the induction of this low-grade inflammation.

Aim : To evaluate duodenal mucosal integrity and changes in expression of cell-to-cell adhesion proteins in FD patients.

Methods : Duodenal biopsies were obtained from 12 non-allergic, non-coeliac FD patients not taking potentially interfering drugs and fulfilling the Rome III criteria (4 men, mean age 29 ± 3 years), and from 12 age- and gender-matched healthy volunteers (4 men, mean age 28 ± 2 years). Transepithelial resistance (TER) was measured *in vitro* during 120 min in four biopsies per subject with the Ussing chamber technique. At the same time, paracellular permeability was assessed using fluorescein isothiocyanate dextran (FITC-dx4, MW 4000Da). Results were compared using two-way repeated measures ANOVA, with Bonferroni correction. Changes in expression of cell-to-cell adhesion proteins were evaluated by qPCR (claudin 1-4, occludin (OCLN), zonula occludens (ZO) 1-3, desmocollin 2 (DSC2) and desmoglein 2 (DSG2)) and western blot (CLDN3-4, OCLN, DSC2 and DSG2).

Results : FD patients displayed lower TER values than healthy controls (ANOVA $p < 0.0001$), with significant differences at time points 0 (22.1 ± 0.9 ?/cm² vs. 25.25 ± 1.0 ?/cm², ANOVA $p < 0.05$) and 30min (19.5 ± 0.9 ?/cm² vs. 22.8 ± 0.9 ?/cm², ANOVA $p < 0.05$). In addition, the passage of FITC-dx4 was higher in FD patients than in healthy volunteers (ANOVA $p < 0.0001$), with significant increases after 90 (50.7 ± 5.7 pmol vs. 29.6 ± 2.7 pmol, ANOVA $p < 0.001$) and 120min (78.5 ± 6.9 pmol vs. 47.6 ± 3.5 pmol, ANOVA $p < 0.001$). Compared to healthy controls, FD patients had a 0.82 fold reduced mRNA level of ZO-1 ($p = 0.03$) and a 0.78 fold decrease in DSC2 gene expression ($p = 0.006$). The expression of DSG2 was significantly reduced at both the gene (0.77 fold ; vs. healthy $p = 0.02$) and protein level (0.82 fold vs. healthy ; $p = 0.009$). The gene expression of OCLN was 0.78 times reduced in FD patients ($p = 0.007$) compared to healthy controls. In addition, patients displayed decreased protein expression of OCLN (0.75 fold vs. healthy ; $p = 0.04$) with reduced phosphorylation of threonine residues (0.40 fold vs. healthy ; $p = 0.007$).

Conclusions : FD patients are characterized by reduced TER and increased permeability to FITC-dx4 in the duodenum, indicative of impaired mucosal barrier integrity. Altered expression of several cell-to-cell adhesion proteins underlies this decreased barrier function in FD patients.

A LONGER INTERVAL BETWEEN CHEMORADIOTHERAPY AND TME FOR RECTAL CANCER IMPROVES RESPONSE AND OUTCOME. A. Wolthuis, F. Penninckx, K. Haustermans, G. Dehertogh, S. Fieuws, E. Vancutsem, A. Dhoore. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : The time interval between neoadjuvant chemoradiotherapy and total mesorectal excision (TME) for locally advanced rectal cancer has arbitrarily been set at 6-8 weeks. However, tumor regression is variable. This study aims to evaluate whether the interval between neo-adjuvant therapy and radical surgery has an impact on pathological response, surgical and oncological outcome.

Methods : A total of 356 consecutive patients with cStage II and III rectal adenocarcinoma were identified. Median age was 63 years and 65% were males. All patients received neoadjuvant chemoradiotherapy (45 Gy) with a continuous infusion of 5-fluorouracil. Data on neoadjuvant-surgery interval, type of surgery, pathology, postoperative complications, length of hospital stay, disease recurrence and survival were reviewed. Patients were divided into 2 groups according to the interval between neoadjuvant therapy and surgery : = 7 weeks (short interval, n = 201), > 7 weeks (long interval, n = 155).

Results : Complete pathological response-rate (ypTON0) was 21%. It was significantly higher after a longer interval (28%) than after a shorter interval (16%, p = 0.006). A longer interval did not affect postoperative morbidity or length of hospital stay. After a median follow-up of 4.9 years, the 5-year cancer-specific survival rate was 83% in the short interval group versus 91% in the long interval group (p = 0.046) and the disease-free survival rate was 73% versus 83%, respectively (p = 0.026).

Conclusions : An interval to surgery of > 7 weeks after neoadjuvant chemoradiotherapy for locally advanced rectal cancer improves pathological response without affecting postoperative morbidity and hospital stay. It has no detrimental effect on oncological outcome.

State of the art lecture

GWAS FOR THE GASTROENTEROLOGIST : CLINICAL IMPLICATIONS. S. Vermeire, KULeuven, Belgium.

THE OCCURRENCE OF SEVERE COLITIS IN THE GRAFT OF INTESTINAL TRANSPLANTATION RECIPIENTS. G. Veereman (1), F. Lacaille (2), F. Ruemmele (2), C. Talbotec (2), C. Chardot (2), O. Goulet (2). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) Hôpital Necker, Paris, France.

Introduction : De novo inflammatory bowel disease has been described post intestinal transplantation.

Aim : Our aim was to determine the incidence of severe chronic colitis in transplanted grafts.

Methods : Medical data from the first 100 pediatric intestinal transplant recipients at Hospital Necker, Paris were reviewed focussing on the occurrence of colitis in the graft.

Results : Over a 17 1/2 yr period (3/1994-9/2011) 100 intestinal transplants were performed in 92 patients. Graft was lost in 49 (53.2%) patients and 46 (50%) were deceased by 11/2011. Fifty seven patients received a combined small intestinal/colon transplant, of these 28 (49,1%) were deceased. Mean age at transplantation was 5,8 yrs (range : 1,5-19,1). Underlying diagnoses were Hirschprung's disease (18), epithelial dysplasia (12), microvillous inclusion disease (11), surgical short gut (6), intestinal pseudobstruction (9) and atresia (1). Nine (15,7%) patients developed colitis. Their mean age at transplantation was 5,4 yrs (range : 2,2-8,5), 3 patients had a liver transplant, familial history of inflammatory bowel disease or allergies was never mentioned. Anti-rejection strategy was similar in all patients (corticoids/tacrolimus). Their indications for transplantation were Hirschprung's disease (4), epithelial dysplasia (2), microvillous inclusion disease (1), surgical short gut (1) and intestinal pseudobstruction (1). Mean interval between transplantation and ostomy closure was 8 mths (range : 1-21). Colitis symptoms appeared after 4,8 yrs (range : 1-12). All presented signs of inflammation (elevated CRP), in 7/9 patients an infectious agent was identified at presentation (Campylobacter : 2, EBV : 2, norovirus : 2, cryptosporidium : 1). Endoscopy showed ulcers of variable severity, histology mixed inflammatory infiltrates often eosinophilic (4/9), ischemia in only 1 case. Stenosis of the graft's ileocaecal valve caused severe persistent problems in 3 patients of which 2 had presented with norovirus. Patients were treated with parenteral nutrition (5), steroids (4), anti-viral agents (3), biologicals (3), pentasa (1) or surgery (2). At follow-up 1 patient was deceased, 6 stable but 2 after graft removal, 2 with residual ileocaecal valve stenosis, one with proximal fistula.

Conclusions : In summary, infections occur frequently but some cases progress to severe chronic colitis and/or ileo-caecal valve stenosis. Since the pathophysiology of his type of colitis is unclear, a rational therapeutic strategy remains to be determined.

- D09 -

GENETIC AND FUNCTIONAL EVIDENCE FOR A ROLE OF CYLD IN CROHN'S DISEASE. I. Cleynen (1), E. Vazeille (2), M. Artieda (3), M. Szczypiorska (3), M.A. Bringer (2), H.W. Verspaget (4), P.L. Lakatos (5), F. Seibold (6), K. Parnell (7), R.K. Weersma (8), J. Mahachie John (9), R. Morgan-Walsh (10), D. Staelens (1), I. Arijis (1), S. Müller (6), A. Tordai (11), D. Hommes (4), T. Ahmad (7), C. Wijmenga (8), S. Pender (10), P. Rutgeerts (12), D. Lottaz (6), S. Vermeire (12), A. Darfeuille-Michaud (2). (1) KULeuven, Leuven, Belgium ; (2) Université D'auvergne, Clermont-Ferrand, France ; (3) Progenika Biopharma, Derio, Spain ; (4) Leiden University Medical Center, Leiden, Netherlands ; (5) Semmelweis University, Budapest, Hungary ; (6) University Hospital Of Bern, Inselspital, Bern, Switzerland ; (7) University Of Exeter & Plymouth, Exeter, United Kingdom ; (8) University Medical Center Groningen And The University Of Groningen, Groningen, Netherlands ; (9) University Of Liege, Liège, Belgium ; (10) University Of Southampton, Southampton, United Kingdom ; (11) Hungarian National Blood Transfusion Service, Budapest, Hungary ; (12) University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Data from both humans and experimental animals underscore the critical role of intestinal bacteria in the establishment and maintenance of inflammatory bowel disease (IBD). Host defense to counteract bacterial colonization and maintaining mucosal integrity involves intestinal proteases and protease inhibitors.

Aim : The aim was to study if, and how, the protease (inhibitor) genes played a role in the pathophysiology of Crohn's disease.

Methods : We performed a genetic association study of all top-ranked protease (and inhibitor) genes, in a previously published systematic review. 185 haplotype tagging SNPs in 23 genes were genotyped in an exploratory dataset of 650 Crohn's disease (CD) patients, and 542 healthy controls (HC). Validation was performed in 1670 CD and 1254 HC. Statistical analysis was performed using SVS v7.5.2 (crude association analysis, additive genetic model), and plink v1.0.7 (meta-analysis of results in the exploration and validation datasets, interaction analysis). A corrected $p < 0.05$ was considered statistically significant. The T84 epithelial cell line was used for functional assessment of CYLD.

Results : 10 markers were found to be significantly associated with CD in the meta-analysis : 4 in *USP40*, 1 in *APEH*, 1 in *USP3*, and 4 in *CYLD*. The top signals were in *CYLD*, a cytoplasmic deubiquitinating enzyme located adjacent to *NOD2* on 16q12 : rs12324931 ($p = 1.64e-18$), rs17314544 ($p = 1.06e-9$), rs7205423 ($p = 1.89e-8$), and rs1861762 ($p = 1.07e-5$). A significant interaction between 'NOD2 overall' and rs12324931 was found. In patients without any *NOD2* risk alleles, a significant CD-risk association with rs12324931 was present ($p = 0.001$, OR = 4.05 [1.68-9.73]). Upon infection of T84 intestinal epithelial cells with the adherent-invasive *Escherichia coli* (AIEC) strain LF82, the prototype strain of AIEC associated with ileal CD, decreased *CYLD* expression was observed, leading to an increased ability of LF82 AIEC to replicate within T84 cells (through *CYLD* siRNA transfection). Together with the AIEC LF82-induced *CYLD* decrease, we observed proteasome-dependent degradation of the NF κ B inhibitor, I κ B- α , in AIEC LF82 infected T84 cells, and an increased translocation of the NF κ B p65 subunit into the nucleus.

Conclusions : Our data provide strong genetic and functional evidence for a role for *CYLD* in CD pathogenesis. We show that AIEC bacteria are able to take advantage of decreased *CYLD* to replicate within host epithelial cells, and that *CYLD* acts as a negative, NF κ B-mediated regulator for *E. coli* colonization.

- D10 -

CALPROTECTIN AS A MARKER OF DISEASE ACTIVITY IN UC PATIENTS UNDER INFLIXIMAB MAINTENANCE TREATMENT. M. De Vos (1), J. Jahnsen (2), J. Vandervoort (3), G. D'haens (4), O. Dewit (5), E. Louis (6), D. Franchimont (7), F. Baert (8), R. Torp (9), P. Potvin (10), P. Van Hooetegem (11), M. Henriksen (12), B. Vander Cruyssen (1), S. Vermeire (13). (1) Ghent University Hospital, Ghent, Belgium ; (2) Oslo University Hospital, Aker, Norway ; (3) OLV Ziekenhuis, Aalst, Belgium ; (4) Imelda Hospital, Bonheiden, Belgium ; (5) Clinique Universitaire St-Luc, Brussels, Belgium ; (6) CHU Sart Tilman, Liège, Belgium ; (7) Erasme Hospital, Brussels, Belgium ; (8) H. Hart Hospital, Roselare, Belgium ; (9) Innlandet Hospital, Hamar, Norway ; (10) St Jozefkliniek, Bornem, Belgium ; (11) AZ Sint-Lucas, Brugge, Belgium ; (12) Ostfold Fredrikstad Hospital, Fredrikstad, Norway ; (13) University Hospital Gasthuisberg, Leuven, Belgium.

Aim : To evaluate the evolution of fecal calprotectin levels in patients with ulcerative colitis (UC) under maintenance treatment with infliximab.

Methods : 113 UC patients, 83 from Belgium and 30 from Norway, in clinical remission under a stable 5 mg/kg Q8W infliximab therapy were followed-up over 1 year. Faecal calprotectin was measured monthly. Clinical and biochemical examination was performed at each infusion. Sigmoidoscopies were performed at inclusion and after 1 year, or earlier if patients dropped out of the study due to flare. “Active disease” was defined by the clinical Mayo score during the study or by an endoscopic score of ≥ 2 at week 52. “Deep remission” was defined by a normal endoscopy at the start of the study and at week 52 associated with clinical Mayo scores at all times < 3 .

Results : After 1 year, 83 (73.5%) of 113 patients remained in clinical remission and continued the study till week 52. Discontinuation was observed in 28 patients : 7 (5.8%) due to flare, 5 (4.4%) due to safety issues (2 hypersensitivity reaction, 1 joint pain, 1 adrenal tumor, 1 polyneuropathy) and 16 due to withdrawal of consent.

“Active disease” was observed in 13/113 (11%) patients and “deep remission” in 30/113 (26.5%) patients.

In patients in deep remission, median calprotectin levels were less than 50 mg/kg at all measured time points. Patients who flared had significantly higher calprotectin levels at the moment of flare (median calprotectin levels of 477 mg/kg). Significant increase was already observed 3 months before flare.

Further ROC analysis (flare vs deep remission at last evaluation) suggested that a calprotectin level > 300 mg/kg showed a reasonable sensitivity (58.3%) and high specificity (93.3%) to model flare and a calprotectin level < 50 mg/kg to model deep remission (sensitivity 83.3% and specificity 83.3%).

Two consecutive calprotectin measurements of > 300 mg/kg predicted a flare with a sensitivity of 61.5% and specificity of 100% (remission vs flare).

Conclusions : In UC patients under infliximab maintenance therapy calprotectin levels highly correlate with disease activity. Deep remission is associated with very low levels. A flare is associated with high levels (median > 300 mg/kg). Two consecutive calprotectin levels of > 300 mg/kg predict a flare.

- D11 -

ADULT LIVER PROGENITOR CELLS DIFFERENTIATE TOWARD MATURE HEPATOCYTES AFTER CDE DIET-INDUCED INJURY. R. Español Suárez (1), R. Carpentier (2), N. Van Hul (1), F. Lemaigre (2), I. Leclercq (1). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) Université Catholique De Louvain, Brussels, Belgium.

Introduction : Homeostasis and regeneration of the adult liver are mediated by self-duplication of mature hepatocytes. However, chronic liver injury impairs this process and a small compartment of otherwise dormant liver progenitor cells (LPC) actively proliferates and generates transit amplifying cells (oval cells). *Ex vivo* and transplantation studies reported the capacity of those transit amplifying cells to differentiate into hepatocytes.

Aim : The aim of the present study is to evaluate the participation of LPC in liver regeneration *in vivo*.

Methods : To follow the fate of LPC, we performed lineage tracing experiments with osteopontin (OPN)-iCreERT2 ; Rosa26RYFP mice. Tamoxifen was injected at weaning to induce Cre recombination and permanent expression of YFP in OPN expressing cells, and choline-deficient ethionine-supplemented (CDE) diet was administered to induce liver injury and expansion of transit amplifying cells. Livers were examined after 3 weeks of CDE, or after 2 weeks of reversal to a standard rodent chow (recovery).

Results : In the normal adult liver, bile duct cells and LPC express CK19, SOX-9 and OPN. After tamoxifen injection, ~35% of bile duct cells and isolated CK19+/SOX9+ LPC, but no hepatocyte or liver non-parenchymal cells, expressed YFP. As previously shown, CDE diet induced liver injury with expansion of CK19+/SOX9+/OPN+ transit amplifying cells from LPC. In tamoxifen-injected CDE-treated mice, 65% of transit amplifying cells expressed YFP as well as 1.25% of hepatocytes, thereby demonstrating their LPC origin. The YFP-positive hepatocytes were found in parenchymal zone 1 close to infiltrating cords of migrating transit amplifying cells. Two weeks after interruption of the CDE diet, the livers recovered from injury : while the number of transit amplifying cells decreased dramatically, the number of YFP+HNF4a+ hepatocytes increased to 2.45%. Those YFP+ hepatocytes mainly located in zone 1/2, along topography reminiscent of that of expanding transit amplifying cells. They were free of the laminin sheet on which transit amplifying cells lie, expressed carbamoylphosphate synthetase like hepatocytes of zone 1/2 but not glutamine synthetase which is restricted to zone 3 hepatocytes, and developed biliary canaliculi (CEACAM1+) with adjacent cells.

Conclusions : Our data provide the first direct proof that transit amplifying cells terminally differentiate into mature, polarized and functional hepatocytes in a model of liver injury induced by the CDE diet.

EFFICACY AND SAFETY ISSUES OF RADIOFREQUENCY ABLATION IN LONG BARRETT'S ESOPHAGUS. J. Carausu (1), A. Mourin (2), H. Piessevaux (2), P. Deprez (2). (1) Université Catholique de Louvain, Brussels, Belgium ; (2) Clinique Universitaire St-Luc, Brussels, Belgium.

Introduction : The use of radiofrequency ablation (RFA) for complete eradication of Barrett's esophagus has shown promise in trials mainly focused on ideal patients with short Barrett's mucosa and limited endoscopic resection before ablation.

Aim : We evaluated the safety and efficacy of RFA in a prospective cohort with long Barrett's (> 3 cm) and mucosal cancer in combination with ER for visible lesions.

Methods : All consecutive patients referred for RFA between 2009 and 2011 were included. Staging of lesions was performed with HRE, NBI and chromoendoscopy with acetic acid, EUS for visible lesions, and CTscan if submucosa invasion was suspected. All visible superficial lesions were resected by EMR or ESD, prior to RFA . RFA technique strictly followed the AMC recommendations of use. Primary RFA was preferentially performed with the circumferential balloon-based device (HALO360, BARRX Medical, USA). After primary RFA, patients underwent follow-up endoscopy every 3 months to assess the degree of eradication of visible Barrett's esophagus. Further ablations were preferentially performed with the focal Halo 90° ablation device. Number of RFA sessions was limited to 1-3, due to the absence of reimbursement of RFA in Belgium. Results are shown as median and range.

Results : 32 pts (28M,4W) with a median age of 67 y (42-84) were studied. 22 of them had ER before RFA (15 cap-EMR and 7 ESD). Median maximal length of BE segment was 7 cm (3-15). Number of RFA sessions was 1 (1-3). Complete remission for dysplasia was 100% and for IM 70.4%, at a follow-up of 11m (3-21) since last treatment (21 m, 4-101 between 1st treatment and last f-up). Short term (< 24 h) complications (31%) included severe pain in 5pts, moderate pain in 3, fever in 3 and confusion in 1 pt. Long term complications included dysphagia in 10pts (31%), needing 2(0-6) dilations in 7 pts, perforation in 1 pt occurring during the 2nd session of dilation, and severe hemorrhage in 1 pt under anticoagulants 8 wks after RFA. Slow healing extensive esophageal ulcer (> 6 months, under 120-240 mg PPI + sucralfate) was observed in 4 pts.

Conclusions : Long Barrett's esophagus can effectively be treated by RFA as primary or adjuvant therapy after extensive ER with similar rates of remission for dysplasia. Complete remission for intestinal metaplasia was however low (70%). Higher rates might however be achieved with repeated RFA sessions (inducing higher costs, without reimbursement). Complication rates (including severe complications) were significantly higher than those reported in reports dealing with shorter Barrett's esophagus.

ASSOCIATION OF PROTECTIVE IL13 POLYMORPHISM WITH IRRITABLE BOWEL SYNDROME. M.M. Wouters (1), D. Lambrechts (2), I. Cleynen (1), P. Whorwell (3), K. Lambaerts (1), L. Agréus (4), A. Dlugosz (4), P. Thelin-Schmidt (4), J. Halfvarson (5), M. Simren (6), B. Ohlsson (7), P. Karling (8), S. Van Wanrooy (1), S. Vermeire (1), G. Lindberg (4), R. Spiller (9), M. D'amato (4), G. Boeckxstaens (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), Kuleuven, Leuven, Belgium ; (2) Vib, Leuven, Belgium ; (3) University Of Manchester, Manchester, United Kingdom ; (4) Karolinska University Hospital, Stockholm, Sweden ; (5) Örebro University Hospital, Örebro, Sweden ; (6) Sahlgrenska Hospital, Göteborg, Sweden ; (7) Lund University, Malmö, Sweden ; (8) Umeå University, Umeå, Sweden ; (9) The University Of Nottingham, Nottingham, United Kingdom.

Introduction : Low grade inflammation and mucosal immune dysfunction may be involved in the pathogenesis of the irritable bowel syndrome (IBS). To what extent genetic factors contribute remains unclear.

Aim : The aim of our study was to evaluate the hypothesis that immune related genes are associated with IBS.

Methods : Two independent cohorts of IBS patients and controls from the UK (provided by GlaxoSmithKline UK : 989 IBS patients, 692 healthy volunteers) and Sweden (515 IBS patients, 900 healthy volunteers) were included in this study. The diagnosis of IBS was based on the Rome II criteria. 245 SNPs covering 190 immune related genes were selected from GWAS studies on potentially related diseases. The UK cohort was genotyped using the GoldenGate platform. SNPs with the strongest association signal were then validated in the Swedish cohort (Sequenom®). Allelic association tests included Bonferroni correction for multiple comparisons. mRNA expression of the genes of interest was evaluated by qPCR on rectal biopsies from healthy volunteers (n = 13) and IBS patients (n = 13). As mRNA expression data were normally distributed, an unpaired student's T test was used for statistical analysis.

Results : 21 SNPs were univariately associated with IBS in the UK cohort, and further evaluated in the Swedish cohort. After Bonferroni correction for 21 SNPs, two SNPs were significantly associated with IBS in the combined cohort : rs2706347 located in *RAD50* (p = 0.05, OR = 0.83 [0.71-0.96]) and rs1881457 located in *IL13* (p = 0.03, OR = 0.79 [0.67-0.92]). Both SNPs are located on chromosome 5 and are in high LD (r² = 0.83). In rectal mucosal biopsies, *IL13*

mRNA expression was not different in healthy volunteers versus D-IBS patients. However, the risk allele significantly increased *IL13* mRNA levels and this was true both in healthy volunteers and in D-IBS patients : delta Ctrisk allele, HV : 18.16 ± 0.64 versus delta Ctprotective allele, HV : 19.22 ± 0.45 ($p = 0.04$) ; delta Ctrisk allele, D-IBS : 18.56 ± 0.40 versus delta Ctprotective allele, D-IBS : 20.04 ± 0.58 ; $p = 0.03$). *RAD50* mRNA levels were not influenced by rs2706347.

Conclusions : We found evidence for genetic association of the *IL13* and *RAD50* genes with IBS. The genetic variant in *IL13*, Rs1881457, furthermore had a significant impact on *IL13* expression. We demonstrate for the first time that the *IL13* protective allele reduces *IL13* expression, thereby modulating IBS susceptibility.

- D14 -

GLEM/LOK REPORT ON CHRONIC PANCREATITIS MANAGEMENT IN BELGIUM. RESULTS OF A NATIONAL SURVEY. M. Delhay (1), W. Van Steenberg (2), M. Arvanitakis (1), J. Deviere (1), P. Deprez (3), R. Fiasse (3), C. De Galocsy (4). (1) Erasme Hospital, Brussels, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) Clinique Universitaire St-Luc, Brussels, Belgium ; (4) CH Joseph Bracops, Brussels, Belgium.

Introduction : A questionnaire on chronic pancreatitis (CP) management was established by 2 experts (M.D. and W.V) and included 10 questions on the following topics : etiology of CP, imaging procedures for diagnosis of CP, complications of CP, therapeutic approach for uncomplicated CP and for complications of CP, follow-up of patients with CP, evaluation and treatment of pancreatic exocrine insufficiency, diagnosis and management of autoimmune pancreatitis (AIP).

Methods : The questionnaire was sent to 630 Belgian Gastroenterologists (GE) (members and leaders of Glems and Loks). Among them, 107 answers were obtained (17%) but only 71 (66%) GE saw patients with CP and managed a mean of 30 patients per GE during the last year. The answers were summarized and statistically analyzed (C.d.G.).

Results : According to the TIGAR-O classification of CP etiologies, most cases (71%) were associated with Toxic factors (alcohol abuse, tobacco smoking).

MRI (36% of GE) and EUS (28% of GE) were considered as the most accurate imaging procedures for the diagnosis of early CP, while MRI (44% of GE) and CT (31% of GE) were used for the diagnosis of severe CP.

Most frequent complications of CP seen by GE were pancreatic pseudocysts (seen by 85% of GE in at least 25% of patients) and common bile duct stricture (seen by 49% of GE in at least 25% of patients). More than 90% of GE had never or rarely seen pancreatic abscess, pancreatic fistula (internal/external), pancreatic carcinoma, bleeding gastric varices from left-sided portal hypertension or arterial pseudo-aneurysm as complications of CP.

More than 98% of GE never or rarely (in less than 25% of patients) proposed surgery as a therapeutic approach for non-complicated CP. More than 70% of GE used often or very frequently pain medications as NSAIDs/paracetamol or mild opioids. Around one third of GE used ESWL combined with endoscopic pancreatic ductal drainage in at least 25% of their patients. Other therapeutic approaches, like celiac plexus block, were used very rarely.

Regarding pseudocysts, the transmural drainage was the therapeutic approach used most frequently by more than 50% of GE.

In biliary stricture complicating CP, biliary stenting with plastic stent(s) was most often used by more than 70% of GE. Nearly all GE proposed to follow patients with CP, by clinical and imaging procedures, mainly those with recurrent pain, those treated by endoscopic therapy and those treated for complications.

Exocrine pancreatic insufficiency was most often assessed by fecal fat quantification or acid steatocrit. The majority of GE adequately treated all patients with demonstrated exocrine pancreatic insufficiency.

The diagnosis of AIP was most often based on serology, pancreatic imaging and response to steroid therapy. The standard schedule for steroid therapy was used in AIP by 75% of consulted GE and nearly half of them would introduce azathioprine in case of relapsing AIP following steroids withdrawal.

Conclusions : A very small proportion of members of Glems and Loks routinely managed patients with CP in Belgium. Many of them applied the recent guidelines about the management of CP

References :

1. Frulloni L. *et al.*, Dig. Liver Dis., 2010.
2. Nguyen-Tang T. *et al.*, Best. Pract. Res. Clin. GE, 2010.

BeSPGHAN

Invited lecture

- E01 -

IBD AND PROBIOTICS. G. Veereman, UZ Brussel, University Paris Descartes, France.

- E02 -

NEW ONSET FOOD ALLERGY IN CHILDREN AFTER LIVER TRANSPLANTATION. S. Vander Plaetsen, K. Robben, S. Van Biervliet, S. Vande Velde, M. Van Winckel, R. De Bruyne. Ghent University Hospital, Gent, Belgium.

Introduction : Food allergy is increasingly reported after paediatric liver transplantation.

Aim : To determine the incidence, clinical characteristics, possible risk factors and prognosis of food allergy in the cohort of paediatric liver transplant patients currently followed in our centre.

Methods : Data were collected from medical records and via a doctor's questionnaire taken from the parents.

Results : The study population consists of 48 children transplanted between 3 weeks and 16 years of age. 27% (13/48) of the patients have been diagnosed with food allergy after transplant, with most frequent food allergens being soy (23%), peanut (31%), cow's milk protein (38%) and egg (38%). 54% (7/13) of patients have multiple food allergies and food allergy is IgE mediated in 77% (10/13). 70% (9/13) presented with gastro-intestinal symptoms (diarrhoea with faltering growth, blood in stools, vomiting), other symptoms were angioedema, urticaria, anaphylaxis, severe atopic dermatitis and treatment resistant cheilitis (present in 3/13 cases). In the food allergic group, 46% (6/13) present atopic dermatitis and 1 patient allergic rhinitis as well. In the non-food allergic group, 6% (2/35) of children suffer from asthma or allergic rhinitis and 20% (7/35) from atopic dermatitis. Hence, in total, 44% (21/48) of the post liver transplant patients have allergic symptoms. Food allergy was diagnosed after a median time interval of 8 months (1-48 months) post-transplant. Median age at transplantation is significantly lower in the food allergic versus non-food allergic group ($p = 0,002$) (10 months (3 weeks-2,9 year) and 3,3 year (1 month-16 year) resp.). The use of tacrolimus as primary maintenance immunosuppression is associated with food allergy ($p = 0,032$). In terms of prognosis, 15% (2/13) of children have outgrown their food allergy and were able to reintroduce the food allergen in the diet.

Conclusions : New onset food allergy is seen in a significant (27%) proportion of our paediatric liver transplant patients. Young age at transplantation and the use of tacrolimus are associated with an increased risk for the development of food allergy. Gastro-intestinal symptoms are present in the majority of patients. However, as post-transplant food allergy is most often IgE mediated, these patients are potentially at risk for severe and life threatening symptoms.

- E03 -

FACTORS DETERMINING THERAPEUTIC STRATEGY AT DIAGNOSIS AND EVOLUTION OF DISEASE SEVERITY IN A COHORT OF PEDIATRIC PATIENTS WITH CROHN'S DISEASE. E. Degreef (1), J. Mahachie (2), I. Hoffman (3), F. Smets (4), S. Vanbiervliet (5), M. Scaillon (6), B. Hauser (1), I. Paquot (7), P. Alliet (8), W. Arts (9), O. Dewit (4), H. Peeters (10), F. Baert (11), G. Dhaens (12), J.F. Rahier (13), I. Etienne (14), O. Bauraind (15), A. Vangossum (16), S. Vermeire (3), F. Fontaine (17), V. Muls (18), E. Louis (19), F. Van De Mierop (20), J. Coche (15), K. Vansteen (2), G. Veeremanwauters (1). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) Montefiore Institute, Liège, Belgium ; (3) University Hospital Gasthuisberg, Leuven, Belgium ; (4) Université Catholique De Louvain, Brussels, Belgium ; (5) Universiteit Gent, Gent, Belgium ; (6) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (7) Chc Clinique De L'esperance, Liège, Belgium ; (8) Virga Jesse Hospital, Hasselt, Belgium ; (9) Zol, Genk, Belgium ; (10) Ghent University Hospital, Ghent, Belgium ; (11) H. Hart Hospital, Roseselare, Belgium ; (12) Imelda Hospital, Bonheiden, Belgium ; (13) Ucl, Mont-Godinne, Belgium ; (14) Chc De La Citadelle, Liège, Belgium ; (15) Clinique St. Pierre, Ottignies, Belgium ; (16) Erasme Hospital, Brussels, Belgium ; (17) St Joseph Hospital, Liège, Belgium ; (18) Ulb Saint-Pierre, Brussels, Belgium ; (19) CHU Sart Tilman, Liège, Belgium ; (20) Sint Augustinus Ziekenhuis, Antwerpen, Belgium.

Aim : To investigate which variables at diagnosis influence treatment strategy and outcome of pediatric Crohn's disease patients .

Methods : Data from pediatric Crohn's disease patients were retrospectively evaluated at inclusion in the BELCRO database (current visit). Outcome parameters were compared to variables at diagnosis and initial treatment. Non-parametric association tests and multinomial logistic regression tests were used.

Results : Data from 152/156 patients with a median follow-up 2.4 yrs (range 0.1-7.9 yrs) showed inactive disease at current visit in patients with L3 at diagnosis ($p = 0.02$; OR 2.8- 95%CI 1.1-7.4). Initial steroid treatment led to less active disease ($p = 0.001$; OR 0.2-95% 0.1- 0.5). Need for surgery was only influenced by disease behavior (S) ($p = 0.001$; OR 6.8 -95% CI 1.8-25.3) not by disease severity, location or treatment. A positive family history was the sole factor influencing initial treatment choice between 5-ASA and immunomodulators ($p = 0.02$; OR 2.1- 95%CI 1.1-4.1 and $p = 0.004$; OR 2.7 - 95%CI 1.3-5.5). L1 patients received less steroids ($p = 0.03$; OR 0.3 -95%CI 0.1-0.9) and therefore had more severe disease at current visit. During follow-up, there was a decrease in the use of steroids and 5-ASA ($p = 0.001$; OR 0.02 -95%CI 0.002-0.3 ; $p = 0.001$; OR 0.03 -0.004-0.3) and an increase in prescription of immunomodulators ($p = 0.03$; OR 1.8-95%CI 0.08-41.1) which parallels a decrease in the disease severity ($p = 0.01$).
Conclusions : In the BELCRO cohort, after a mean of 2.4 yrs follow-up, L1 persisted in severe disease whereas L3 and steroid treated subjects achieved remission. Family history was the sole determinant for initial therapeutic strategy.

Invited lecture

- E04 -

THE GUT MICROBIOTA IN THE PATHOGENESIS OF OBESITY. P.D. Cani. Université Catholique de Louvain, Brussels, Belgium.

Introduction : Obesity and type 2 diabetes are associated with a low grade inflammatory tone. We have provided evidence that the gut microbiota participate to the development of the insulin resistance and the low grade inflammation characterizing obesity. Recently, we described the concept of metabolic endotoxemia (increase in plasma LPS levels) as triggering factor in the development of insulin resistance and low grade inflammation associated with obesity. Following this discovery, we found that the major factor involved in the development of metabolic endotoxemia observed upon obesity is related to the gut barrier function. For instance, we found that both nutritional and genetic obesity are associated with an increased gut permeability leading to the leakage of LPS and possibly other microbiota derived factors.

Aim : The bacterial metabolism of nutrients in the gut is able to drive the release of bioactive compounds (including short-chain fatty acids or lipid metabolites), which interact with host cellular targets to control energy metabolism and immunity. Animal and human data demonstrate the phylogenetic changes occurring in the microbiota upon obesity. They suggest that specific bacteria could be promoted to counteract fat mass development, diabetes and the low levels of inflammation associated with obesity.

Methods : We have modified the gut microbiota composition of obese and type 2 diabetic mice (nutritional or genetic obesity and type 2 diabetes) by using prebiotics (inulin-type fructans). We measured gut permeability, plasma LPS, glucose tolerance, inflammatory markers (in the plasma and in tissues). Gut microbiota has been analyzed by using metagenomic approaches (454 pyrosequencing, phylogenetic microarrays and qPCR).

Results : We found that prebiotic-induced profound changes in the gut microbiota composition, with more than 100 taxa that were affected by the treatment. Among these bacteria, we identified novel putative targets that can shape the metabolism in the context of obesity and type 2 diabetes. Among the mechanisms involved in the bacteria-host interactions, we found that changes in the gut microbiota increases endogenous GLP-2 production (an intestinotrophic peptide), and consequently improves gut barrier functions, by a GLP-2 dependent mechanism. In addition, we found that selective changes in the gut microbiota composition by using prebiotics reduce metabolic endotoxemia and gut permeability via mechanisms involving the endocannabinoid system. Finally, we found that gut microbiota participate to the control of leptin sensitivity in obese animals.

Conclusions : Taken together, the compelling data currently published suggest that specific changes in the gut microbiota occur in overweight or obese patients and are either positively or negatively linked with adiposity, inflammation and glucose or lipid homeostasis.

Invited lecture

- E05 -

ASTHMA AND THE INTESTINAL MICROBIOTIA. A. Vael, Klina Hospital, Antwerp.

EXTRA-INTESTINAL MANIFESTATIONS OF CROHN'S DISEASE IN CHILDREN : INVOLVEMENT OF SKIN AND JOINT. C. Buyck, C. Wouters, I. Hoffman. University Hospital Gasthuisberg, Leuven, Belgium.

Background : Crohn's disease is a chronic transmural inflammatory process that may affect any segment of the gastrointestinal tract (GI) from mouth to anus in a discontinuous way. It is a systemic disease which can be characterized by several extra-intestinal manifestations.

Objective : To determine the prevalence of inflammatory skin and joint involvement in children with Crohn's disease, to find clues for Crohn's disease in case a child presents itself with joint pain, to establish a relationship between these extra-intestinal manifestations of Crohn's disease and the interval between presentation and diagnosis, disease activity and response to medical therapy.

Material and methods : Retrospective observational study of all patients treated at the children's department of the University Hospital in Leuven during the last five years with a diagnosis of Crohn's disease during childhood (between 0 and 16 years) with associated inflammatory skin and joint involvement.

Results : The prevalence of Crohn's disease associated with skin or joint involvement in the University Hospital of Leuven was 18.8% or 16/85 patients (5.9% skin, 17.6% joint). Factors that should refer to Crohn's disease in case of joint complaints are : iron-deficiency anemia, systemic complaints (pallor, fatigue, fever, oral ulcers, malaise and/or kidney stones), a family history of Crohn's disease, an atopic constitution in the personal history, weight loss and/or growth retardation. We found a mean delay of 34 months (median delay of 6.5 months) between presentation with joint complaints and diagnosis of Crohn's disease. The (corrected) Pediatric Crohn's Disease Activity Index-values during flares of Crohn's disease with extra-intestinal manifestations tend to be higher (average 38.2) than the Activity Index-values during flares with isolated gastro-intestinal manifestations (average 31.1). These flares of Crohn's disease with extra-intestinal manifestations did respond equally to therapy than flares without these manifestations.

Discussion : We found comparable values for prevalence of inflammatory skin and joint involvement as values mentioned in literature. We identified some factors that should remind us of possible Crohn's disease when a child presents itself with joint complaints in the future. There is a latency in diagnosis of Crohn's disease when patients present themselves with isolated joint complaints or systemic manifestations of the disease, in comparison with the more obvious gastro-intestinal signs. Flares of Crohn's disease with extra-intestinal manifestations develop in a more aggressive manner but respond equally to therapy than flares without these manifestations.

RESULTS OF ANTI-REFLUX SURGERY AFTER PRIMARY ANASTOMOSIS FOR ESOPHAGEAL ATRESIA. N. Deferm, W. Coosemans, P. Deleyn, P. Naftoux, D. Vanraemdonck, I. Hoffman, T. Lerut, H. Decaluwe. University Of Leuven, Leuven, Belgium.

Introduction : Congenital esophageal atresia (EA) and tracheoesophageal fistula (TEF) are rare and life threatening congenital anomalies. A common problem after EA or TEF repair is gastroesophageal reflux disease (GERD). This can be caused by inadequate acid clearance, shortening of the esophagus and poor esophageal motility. In literature, 30% of the children require anti-reflux surgery.

Aim : The objective of this retrospective single-center observational study was to evaluate our incidence of anti-reflux surgery and the late postoperative outcome in this group of patients.

Methods : We reviewed hospital records of 96 patients who underwent EA or TEF repair at our thoracic centre from January 1993 till February 2010.

Results : Seven patients had an isolated TEF without EA. Twelve had a long gap esophageal atresia. We withheld 77 patients who were treated with a primary anastomosis. Ten (14%) of these patients, five boys and five girls, later underwent a fundoplication for severe GERD. They presented with typical reflux symptoms, airway problems, failure to thrive or recurrent anastomotic strictures related to GERD. Four of these patients were born with a VACTERL syndrome or an isolated cardiac anomaly. In four of them a Livaditis lengthening circular myotomy had to be performed to be able to complete a primary anastomosis. In five a 24hrs pH-study was performed, all showing pathologic reflux. In eight contrast studies showed GERD, and seven had esophagitis on endoscopy. The mean age at anti-reflux repair was 20 months (range 3 - 98 months). A laparoscopic Nissen fundoplication was carried out in 7 children, an open Nissen procedure in 1 patient and 2 Lind procedures were performed. Three, including both Lind procedures, needed a second or third surgical intervention due to persisting GERD symptoms or an intrathoracic migration of the wrap. The mean age at latest follow-up was 122 months (range 12 - 198 months). Four of them were asymptomatic at time of last follow-up. The main complaints of the other six were recurrent pulmonary infections and tracheomalacia, two had some degree of dysphagia. Five stopped all proton pump inhibitors.

Conclusions : GERD is one of the most common problems after primary EA or TEF repair. Severe reflux problems are probably related to short esophagus and impaired esophageal motility. Compared with the literature, however, there is a low need for anti-reflux surgery in our experience. Only 14% of our patients needed a fundoplication after a primary anastomosis. Lind procedures seem to be inadequate and were converted to Nissen funduplications. 40% of them were asymptomatic at time of latest follow-up and 50% were completely off reflux medication.

Invited lecture

- E08 -

COMPOSITION AND FUNCTIONS OF THE HUMAN GUT MICROBIOTA : NOVEL INSIGHTS. F. Guarner. Hospital Universitari Vall D'hebron, Barcelona, Spain.

Introduction : To understand the impact of gut microbes on human health and well-being it is crucial to assess their genetic potential.

Aim : The European MetaHIT project aimed at sequencing the whole genome of microbes from the human gut, identifying functions related with the microbial genes and developing tools for profiling of the gut microbiota.

Methods : The project validated a novel technology based on shot-gun whole genome sequencing of crude DNA extracts from human faecal samples using high throughput sequencing (illumina) with standard Sanger technology. Bio-informatic analysis of large data sets is been developed for identification of genes and functional analysis.

Results : The project consortium recently published the Illumina-based metagenomic sequencing, assembly and characterization of 3.3 million non-redundant microbial genes, derived from 576.7 Gb sequence, in faecal samples of 124 individuals from Denmark and Spain. The gene set is more than 150 times larger than the human gene complement, contains an overwhelming majority of the prevalent microbial genes present in the cohort and likely includes a large proportion of the prevalent human intestinal microbial genes. The gene pool is largely shared among individuals of the cohort. Over 99% of the genes are bacterial, suggesting that the entire cohort harbours between 1000 and 1150 prevalent bacterial species and each individual at least 160 such species, which are also largely shared.

Combining 22 newly sequenced faecal metagenomes of individuals from 4 countries with previously published datasets, MetaHIT investigators have identified three robust clusters (enterotypes hereafter) that are not nation or continent-specific. Same enterotypes were also found in two published, larger cohorts suggesting that intestinal microbiota variation is generally stratified, not continuous. This further indicates the existence of a limited number of well-balanced host-microbial symbiotic states that might respond differently to diet and drug intake. The enterotypes are mostly driven by species composition, but abundant molecular functions are not necessarily provided by abundant species, highlighting the importance of a functional analysis for a community understanding.

Conclusions : MetaHIT tools for analysis of the human gut microbiota are now being applied to the investigation of the role of microbes in two distinct pathologies : obesity and inflammatory bowel diseases.

- E09 -

ENDOSCOPIC ULTRASOUND TO IMPROVE THE MANAGEMENT OF BOWEL DYSFUNCTIONS IN CHILDREN WITH HIRSCHSPRUNG. T. Claeys, M. Miserez, A. Vandenbosch, M. Hiele, P. Schurmans, I. Hoffman. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : In early childhood, up to 85% of the young children with Hirschsprung disease (HD) experience defecation disorders postoperatively such as soiling due to fecal impaction or true incontinuity, irrespective of the surgical technique used.

Aim : The aim of our study is to investigate the use of endoscopic ultrasound as a diagnostic tool to improve the management of these postoperative bowel dysfunctions in children with HD.

Methods : Six patients with HD, aged 2 to 10 years, were examined in our tertiary center because of soiling complaints. One child underwent Duhamel operation, two had a Swenson procedure and three children underwent a laparoscopic assisted Soave pull through. Residual hypo- or aganglionosis at the proximal level of the anastomosis was excluded in all cases. All children had a complete check-up including clinical, radiological (plain abdominal X-ray) and anorectal manometry. In addition, anal endoscopic ultrasound using a sonographic scanner (Hitachi) with a rigid endoprobe (circular and linear, frequency 7.5-10 MHz) was done under sedation with midazolam and pethidine.

Results : All children had a clinical and radiographic confirmation of fecal impaction with soiling. Manometric pressure of the internal anal sphincter (IAS) was > 30 mmHg in all patients. Endoscopic ultrasound showed in 3 of our 6 patients hypo- or atrophic IAS. These patients had an IAS pressure of = 65 mmHg. The other 3 patients had an IAS pressure of = 70 mmHg.

Conclusions : In our 6 children with HD and having postoperative soiling complaints, the results of the endoscopic ultrasound changed our management in 3 cases, avoiding the use of laxatives on the long term. We support the use of endoscopic ultrasound in children with HD and having a manometric IAS pressure of = 65mmHg.

- E10 -

SAFETY AND COST OF INFLIXIMAB FOR THE TREATMENT OF BELGIAN PEDIATRIC PATIENTS WITH CROHN'S DISEASE. E. Degreef (1), I. Hoffman (2), G. Dhaens (3), S. Vanbiervliet (4), F. Smets (5), M. Scaillon (6), O. Dewit (5), H. Peeters (7), I. Paquot (8), P. Alliet (9), W. Arts (10), B. Hauser (1), S. Vermeire (2), A. Vangossum (11), J.F. Rahier (12), I. Etienne (13), E. Louis (14), J. Coche (15), J. Mahachie (16), K. Vansteen (16), G. Veeremanwauters (1). (1) University Hospital Brussel (VUB), Brussels, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) Imelda Hospital, Bonheiden, Belgium ; (4) Universiteit Gent, Gent, Belgium ; (5) Université Catholique De Louvain, Brussels, Belgium ; (6) Queen Fabiola Children's University Hospital, Brussels, Belgium ; (7) Ghent University Hospital, Ghent, Belgium ; (8) Chc Clinique De L'espérance, Liège, Belgium ; (9) Virga Jesse Hospital, Hasselt, Belgium ; (10) Zol, Genk, Belgium ; (11) Erasme Hospital, Brussels, Belgium ; (12) Ucl, Mont-Godinne, Belgium ; (13) Chc De La Citadelle, Liège, Belgium ; (14) CHU Sart Tilman, Liège, Belgium ; (15) Clinique St. Pierre, Ottignies, Belgium ; (16) Montefiore Institute, Liège, Belgium.

Introduction : Biologicals have become an important component in the treatment of Crohn's disease in children. Their increased and long term use raises safety concerns.

Aim : To describe safety and cost of infliximab in a Belgian cohort of pediatric Crohn's disease patients.

Methods : All patients on infliximab as part of the present or past treatment for Crohn's Disease until January 1st 2011 were selected from an existing database. Information on disease phenotype, medication and adverse events were extracted.

Results : Adverse events occurred in 25.9% of patients exposed to infliximab of which 29.6% were severe. In total 31.7% of patients stopped infliximab therapy. The main reasons for discontinuation were adverse events in 45.4% and loss of response in 30.3%. No malignancies or lethal complications occurred over this 241 patient year observation period. Immunomodulators were concomitant medication in 75.9% of patients and were discontinued subsequently in 35.4% of them. The cost of infliximab infusions per treated patient per year in the Belgian health care setting is approximately 9 474 euro, including only medication and hospital related costs.

Conclusions : Even though infliximab is relatively safe in pediatric CD on the short term, close follow-up and an increased awareness of the possible adverse reactions is highly recommended. Adverse reactions appeared in 25.9% of all patients and were the main reason for discontinuation. Treatment cost has to be balanced against efficacy and modifications in disease course. In the Belgian health care system, the medication is available to all patients with moderate to severe CD.

Invited lecture

- E11 -

MAASTRICHT 4-NEW OR OLD TREATMENTS FOR BELGIUM. A. Burette, CHIREC, sites de la Basilique et E. Cavell, Brussels, Belgium.

Invited lecture

- E12 -

H. PYLORI : A FAIRYTALE. S. Cadranet, HUDERF, Brussels, Belgium.

Posters

- E13 -

A RARE CASE OF PROTEIN-LOSING ENTEROPATHY AND CONGENITAL LYMPHEDEMA ? S. Vande Velde (1), S. Van Biervliet (1), M. Van Winckel (1), R. De Bruyne (1), B. Callewaert (1), O. Vanacker (1), J. Degraeuwe (2). (1) Ghent University Hospital, Ghent, Belgium ; (2) Az Maria Middelaes, Ghent, Belgium.

Introduction : Protein-losing enteropathy (PLE), is a rare condition in infants and mostly due to primary intestinal lymphangiectasia (PIL). This case report illustrates PLE as a complication of a more generalized congenital anomaly of the lymphatic system.

Case report : A 3 week old male baby, the first child of healthy unrelated parents, is referred with lymphedema of the lower limbs. At birth, edema of the feet was noted and became more generalized. Laboratory findings include hypoalbuminemia, hypogammaglobulinemia, and normal liver function. Albuminuria is absent. a1-antitrypsin in faeces is elevated. Abdominal ultrasound shows limited ascites with normal duplex sonography. Gastroduodenoscopy indicates white spots. Although these findings suggest intestinal lymphangiectasia, a biopsy of duodenal mucosa did not visualize lymphatic vessels and a transmural duodenal biopsy after 5 weeks of treatment showed a slightly dilated lymphatic system. The protein loss was resistant to a median chain triglyceride (MCT) based diet and to basic -F, an almost completely fat free milk, necessitating total parenteral nutrition (TPN), which stopped intestinal protein loss. Reintroduction of a MCT diet resulted in relapse. Fat-free solid foods were introduced at 4 months. Hypoproteinemia was compensated by regular infusions of albumin (initially twice weekly, now at the age of 9 months, every two weeks) and immunoglobulins (every 4 weeks). Growth and psychomotor development is normal, but lower limb lymphedema persists.

Discussion : The early presentation of PLE and the presence of congenital lower limb lymphedema, suggests a primary congenital lymphedema with intestinal manifestations rather than PIL. PLE is present in only a minority of these patients. Both **Milroy** disease and **Emberger** syndrome should be considered. These autosomal dominant disorders display reduced penetrance and are characterized by edema of the lower limbs, which can be asymmetric . In Milroy disease, lymphedema is congenital and affects primarily the feet in neonates. Mutations have been found in *FOXC2* and *FLT4 (VEGFR3)*. Emberger syndrome associates lymphedema, sometimes congenital with increased risk for acute myeloid leukemia, immunological impairment and sensorineural deafness. The syndrome is caused by mutations in *GATA2*. Mutation analysis is ongoing in our patient.

Conclusion : We present a case of primary congenital lymphedema complicated with severe PLE that only partially responded to LCT free nutrition. Related cases are sparse and the prognosis remains unclear.

- E14 -

DEVELOPING A MODIFIED 'QUALITY OF LIFE' QUESTIONNAIRE FOR CHILDREN WITH SPINA BIFIDA. J. Daelman, J. Laridaen, R. De Bruyne, S. Van Biervliet, S. Vande Velde, M. Van Winckel. Ghent University Hospital, Ghent, Belgium.

Introduction : In order to develop a 'quality of life' (QoL) questionnaire, adapted to the specific needs of the population of children with spina bifida (SB), a pilot study is presented.

Methods : Three versions were made : a parent-scale, an 'adolescent'-scale (11-18 yrs) and a 'child-scale' (5-10 yrs). Questions are answered on a Likert-scale. Questions were based on previous quality of life-scales, especially the standardised and frequently used paediatric QoL 4.0, and missing items relevant to the SB-population.

10 SB patients attending their six monthly consultation to the multidisciplinary SB reference centre, were asked to fill in an 'extended pilot questionnaire'. After completing the questionnaire, child and parent were asked to comment how they experienced it, which questions were relevant to them and which questions were too vague or difficult.

Results : Given the wide diversity of mental abilities in patients with SB, the division in age-dependent scales appeared not useful. The mental age and not the real age was an indicator of the abilities of a child. Several questions were deleted in being unnecessary, too vague or confusing.

One questionnaire was developed for all children, regardless of age. Each question was formulated in a simple way, accompanied by a short clarification. Younger children and children with limited mental abilities were interviewed, showing an adapted picture illustrating each question . Answers were given on a visual 3-points Likert-scale (J K L). Parents, older children and children with enough mental abilities filled the questionnaire themselves, answering on a 5-points Likert-scale.

Conclusions : A quality of life questionnaire was developed, adapted to SB patients. Validation of this questionnaire is in progress.

EOSINOPHILIC ENTERITIS : MORE QUESTIONS THAN ANSWERS. L. Dekeyzer, S. Vande Velde, S. Van Biervliet, R. De Bruyne, M. Van Winckel. Ghent University Hospital, Ghent, Belgium.

Introduction : Eosinophil-associated gastrointestinal disorders (EGID) are characterized by an eosinophilic- infiltration of the gastro-intestinal tract. According to the location, eosinophilic oesophagitis, gastritis, enteritis (EE), and colitis have been defined. Clinical presentation is variable, as illustrated by these case reports.

Case 1 : A 9 year old boy is referred with suspicion of lymphoma. Since two weeks he complains of general malaise, nausea, and anorexia with weight loss (- 20%). At admission, blood tests show leucocytosis, anemia, and hypo-proteinemia. Abdominal ultrasonography demonstrates small intestinal wall thickening, suggestive of inflammatory bowel disease (IBD). There is no eosinophilia, IgE level is normal. Gastroduodenoscopy shows edema of the duodenum without erosions ; ileocolonoscopy is normal. Videocapsule examination shows erosive lesions in the jejunum. Biopsies of duodenum show an eosinophilic infiltration in the lamina propria, other levels are normal. The diagnosis of EE is made. Symptoms resolve on corticosteroids and diet limited to an amino acid formula (Neocate advance). Skin prick tests and RAST tests are negative. Once in remission, food groups are reintroduced and following reintroduction of cow's milk protein the initial symptoms recur. Corticosteroids are tempered and stopped. He continues to do well on a strict cow's milk free diet, 9 months later.

Case 2 : A 13 year old girl presents at the emergency room because of persistent vomiting and a weight loss of 3 kg over 3 weeks. Symptoms have started following a common cold. Clinical examination is normal. Laboratory results show eosinophilia (1300/ μ l) and hyperIgE (1533 kU/l). Abdominal ultrasound is normal. Gastroduodenoscopy shows micro-abscesses in the duodenum. Duodenal biopsies show marked eosinophilic infiltration. Esophagus and stomach are normal. The diagnosis of EE is made. Skin prick tests are positive for fish, peanut, and wheat. An elimination diet is prescribed, but symptoms persist. Methylprednisolon is started, but because of continuing weight loss, she is admitted and enteral feeding with Neocate advance. Weight stabilizes. Laboratory results normalize for eosinophilia but hyperIgE (2000 kU/l) persists. Control gastroscopy shows improvement macroscopically and on biopsies. After three weeks, corticosteroids are tempered. HT3 antagonists and montelukast have no effect. Vomiting persists three months after the initial presentation. Her presentation is atypical since symptoms are resistant to therapy, whereas signs have disappeared.

Conclusions : Despite common laboratory and endoscopic characteristics, presentation and evolution of EE is diverse, as is illustrated by the two case reports. Pathogenesis of this group of conditions is still poorly understood.

EARLY EXPERIENCE WITH SINGLE INCISION LAPAROSCOPIC SURGERY (SILS) FOR THE PLACEMENT OF A GASTROSTOMY. K. De Vogelaere, K. Vanderlinden, N. Vandewinkel, G. Delvaux. UZ Brussel, Jette, Belgium.

Introduction : Access procedures for alimentation have been performed both endoscopically and surgically. In patients in whom endoscopic gastrostomy feeding tubes can not be placed, SILS gastrostomy is an alternative method. This minimally invasive approach is a new technique through a single umbilical incision, without the need for additional laparoscopic ports.

Aim : In this article we present a case of SILS gastrostomy performed with conventional laparoscopic instruments in a 10-year-old female who was not candidate for a PEG-tube because of esophageal varices due to an advanced stage of cystic fibrosis with livercirrhosis and portal hypertension. She also had an umbilical hernia which was repaired during the same procedure through the same incision.

Methods : Access and pneumoperitoneum were obtained through the umbilicus with the SILS port. The selected place for the gastrostomy in the stomach was exteriorized through this incision and the feeding tube was placed. The stomach was returned into the abdomen. The fascial defect, and thus also the hernia, was repaired and the 2-cm umbilical incision was closed with endocutaneous sutures.

Results : Total operative time was 25 minutes. Intraoperative and postoperative course was uneventful. We were able to use the gastrostomy on the first postoperative day with good intestinal function. The patient and her parents were pleased with the cosmetic result.

Conclusions : SILS procedure seems to be a less invasive alternative to open placement of gastrostomy. This approach has the possible advantages of reduced postoperative pain, faster return to normal function, reduced port site complications, improved cosmesis and better patient satisfaction.

IS HIRSCHPRUNG DISEASE DIAGNOSED IN CHILDREN OLDER THAN 1 YEAR OF AGE WITH CONSTIPATION ? B. Hauser, T. Devreker, G. Veerevan, E. De Greef, Y. Vandenplas. UZ Brussel, Jette, Belgium.

Introduction : Constipation is frequent in children. Hirschprung disease is mainly diagnosed in infants.

Aim : We performed a retrospective study to look for Hirschprung disease in children older than 1 year of age with constipation.

Methods : One hundred and one children (49 boys) with an age of 4.3 ± 2.96 years (1.17-15.5 years) presenting with constipation for 1.86 ± 2.32 years (0.04-15.5 years) underwent a rectoscopy with forceps biopsies taken at 3 cm of the anal margin under intrarectal Midazolam between January and December 2010. The biopsies were examined for the presence of muscularis mucosa, submucosa, ganglion cells and hypertrophic fibers.

Results : Muscularis mucosa was present in 58/101 (57.4%), absent in 4/101 (4.0%) and presence unknown in 39/101 (38.6%) of the biopsies. Submucosa was present in 17/101 (16.8%), absent in 74/101 (73.3%) and presence unknown in 10 (9.9%) of the biopsies. Ganglion cells were present in 30/101 (30.3%) and absent in 71/101 (69.7%) of the biopsies. Ganglion cells were present in the muscularis mucosa and submucosa in 13/30 (43.3%), only in the muscularis mucosa because there was no submucosa in 16/30 (53.3%) and it was unknown in 1/30 (3.4%) of the biopsies. Few hypertrophic fibers were present in 8/101 (7.9%), absent in 86/101 (85.2%) and presence unknown in 7/101 (6.9%) of the biopsies. In the group of 71 patients without ganglion cells, there were no hypertrophic fibers in 64/71 (90.1%) and only a few hypertrophic fibers but not enough for a diagnosis of Hirschprung disease in 7/101 (9.9%) of the biopsies.

Conclusions : In a group of children older than 1 year of age with constipation, Hirschprung disease was excluded with certainty in 30% of the patients and was very unlikely in the other 70% of patients using rectal biopsies obtained by rectoscopy.

EARLY EXPERIENCE WITH SINGLE-PORT LAPAROSCOPIC NISSEN FUNDOPLICATION IN CHILDREN. K. De Vogelaere, K. Vanderlinden, A. De Backer, G. Delvaux. UZ Brussel, Jette, Belgium.

Introduction : Single-incision laparoscopic surgery (SILS) is one of the newest developments in minimally invasive laparoscopy in adults. SILS is gaining popularity in the pediatric group for routine operations : the three most common SILS procedures are appendectomy, cholecystectomy and pyloromyotomy. Untill now, reconstructive procedures, requiring sutures, have only sporadically been published.

Aim : We report our first experience with a single incision laparoscopic Nissen fundoplication in children.

Methods : Three patients, aged 9 to 11 years underwent the procedure. A SILS-port was inserted through a 2 cm umbilical incision. Conventional as well as adapted laparoscopic instruments were used. A Veress needle was introduced in the right hypochondrium to retract the left liver lobe.

Results : The operation time ranged from 45 to 70 minutes. There were no intraoperative or postoperative complications. The patients was discharged on the third postoperative day on a fully liquid diet.

We encountered knotting difficulties with the use of the conventional straight laparoscopic instruments. Intracorporal suturing using conventional instruments was challenging because of lack of angulation of the instruments. In the first patient 1 additional 5 mm port was required to complete the procedure.

Conclusions : SILS Nissen is feasible in pediatric patients. Development of laparoscopic instruments specific for children would facilitate this SILS procedure. There is a need for smaller, specialized ports and articulating instruments designed for the pediatric age group.

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

Introduction : Increased gastro-oesophageal reflux (GOR) is common in children with cystic fibrosis (CF). Gastric emptying (GE) can be normal, decreased or increased in children with CF. Delayed GE can be a possible mechanism of GOR in children.

Aim : We performed a prospective study to examine GOR and GE in children with CF.

Methods : Twenty-four CF children (13 boys) with an age of 5.8 ± 4.2 years (0.5-17.1 years) presenting with gastro-intestinal and/or respiratory symptoms suggestive of GOR were studied. They underwent an impedance-pH monitoring for detection of GOR. A ^{13}C -acetate breath test to measure GE of liquids or a ^{13}C -octanoic acid breath test to measure GE of solids using Non Dispersive Infrared Spectrometry was performed

Results : Eleven of the 24 children (45.8%) had an increased acid GOR. Seven of these 11 children (63.6%) had clinical symptoms suggestive of GOR. Eight of the 24 children (33.3%) had a delayed GE. Two of these 8 children (25.0%) had clinical symptoms suggestive of delayed GE. Four patients had increased acid GOR and delayed GE (16.7%), 7 patients had increased acid GOR and normal GE (29.1%), 4 patients had normal acid GOR and delayed GE (16.7%), and 9 patients had normal acid GOR and normal GE (37.5%). Delayed GE was present in 4 of the 11 children with increased acid GOR (36.4%) but also in 4 of the 13 children with normal acid GOR (30.8%).

ACID GOR

Normal Increased Total

GE	Normal	9	7	16
	Delayed	4	4	8
	Total	13	11	

Conclusions : Increased acid GOR and delayed GE are present in respectively 45% and 33% of a population of children with CF presenting with gastro-intestinal and/or respiratory symptoms suggestive of GOR. Delayed GE is present in 36% of the children with increased acid GOR but also in 30% of the children with normal acid GOR.

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

Invited lecture

- G01 -

ACUTE PANCREATITIS : DIAGNOSTIC ENDOSCOPY. P. Deprez, UCL, Saint-Luc, Brussels, Belgium.

- G02 -

THE CLIP-TRACTION-ASSISTED TECHNIQUE - A NEW METHOD FOR SAFE ENDOSCOPIC SUBMUCOSAL DISSECTION. B. Ayang, E. Kim, A. Mourin, H. Piessevaux, P. Deprez. Clinique Universitaire St-Luc, Brussels, Belgium.

Introduction : Endoscopic submucosal dissection (ESD) is the gold standard of care for treating mucosal malignant gastrointestinal epithelial lesions of upper gastrointestinal tract. However, it is seldom performed in Western countries, mainly because it is technically very challenging. Several traction methods have been proposed to facilitate ESD, but they are not widely available, expensive or are difficult to apply.

Aim : Our aim was to evaluate the feasibility of a new method, the clip-traction assisted ESD (CTA-ESD) with the hypothesis it might improve visualization of the submucosal layer during ESD, reduce the complications rate, and reduce the procedure duration.

Methods : A total of 9 patients (40-89 y) with early gastric cancers and Barrett's esophagus with high grade dysplasia were consecutively enrolled. After marking, submucosal injection, circumferential mucosal incision with a DualKnife (Olympus KD-650L/U, Tokyo, Japan), one hemostatic clip (HX-610-090L, Olympus), tied to a thread hanging outside of the scope was applied at the oral side of the specimen in the esophagus and the anal side in the stomach. During submucosal dissection, the thread was slowly pulled to the oral side. Results were compared with a lesion and site matched control group consisting of 9 patients (76 y, 52-88) who had ESD without the CTA technique.

Results : CTA-ESD was performed without any complications in all 9 patients between May and November 2011 : (3 HGD Barrett's esophagus, 2 SCC, 4 gastric adenocarcinoma^o. Mean specimen size was 58 mm (range 30-110), mean dissection time with the CTA technique was 32 min (20-37), and mean general procedure time 102 min (94-167). There were no statistical difference with the matched control group : mean specimen size was 61 mm (35-110) and mean general dissection duration was 121 min (62-118), p = 0.14. **LIMITATIONS** : Short period and a small number of patients. Single endoscopist.

Conclusions : The CTA-ESD technique facilitates dissection of superficial lesions in gastric and esophageal locations with a better view of the submucosal layer and a lesser need for repeated submucosal fluid injection. We were however unable to demonstrate a superiority in speed and safety of dissection.

- G03 -

TAKING BIOPSIES IN ENDOSCOPIC NORMALLY APPEARING OESOPHAGUS TO EXCLUDE EOSINOPHILIC OESOPHAGITIS. B. Hauser, T. Devreker, G. Veereman, E. De Greef, Y. Vandenplas. UZ Brussel, Jette, Belgium.

Introduction : Eosinophilic oesophagitis (EE) generally presents with reflux like symptoms, a specific endoscopic appearance and a histological extensive infiltration of eosinophils in the oesophagus. Some individuals can have a normal endoscopic appearance of the oesophagus making histology the only reliable diagnostic tool.

Aim : We performed a prospective study to look for the presence of histological EE (HEE) in normally appearing oesophagus of children with gastro-oesophageal reflux symptoms.

Methods : One hundred and fourteen children (56 boys) with a mean age of 8 years (0.5-17 years) presenting with gastro-oesophageal reflux symptoms underwent a fibroendoscopy with forceps biopsies taken at the distal (DOB) and proximal (POB) oesophagus between January and December 2010. Endoscopic appearance of the oesophagus was suggestive for EE when longitudinal furrows or vertical lines and/or horizontal rings and/or exudate were present. Refluxoesophagitis (RE) was defined according to the Los Angeles classification. A cut-off of 20 eosinophils per high power field was used to differentiate histological RE (HRE) from HEE (> 20/HPF).

Results : Seven children had an endoscopic appearance of EE : DOB and POB confirmed HEE in 3/7, showed a HRE in 1/7 and a mycosis in 1/7, and were normal in 2/7 patients. Thirty-five children had an endoscopic appearance of RE : DOB and POB were normal in 29/35, showed a HRE in 6/35 and a HEE in 0/35 patients. Six patients had an endoscopic appearance of combined EE and RE : DOB and POB confirmed HEE in 1/6, showed a HRE in 2/6 and were normal in 3/6 patients. Finally, the endoscopic appearance of the oesophagus was normal in 66 patients : DOB and POB were normal in 49/68, showed a HRE in 17/68 and a HEE in 0/68 patients.

Conclusions : The diagnosis of eosinophilic oesophagitis was never made by oesophageal biopsies in an endoscopic normally appearing oesophagus in children with gastro-oesophageal reflux symptoms.

- G04 -

GASTROESOPHAGEAL REFLUX EVALUATION IN ADULT PATIENTS AFFECTED BY CHRONIC COUGH. L. Vandermeulen (1), D. Ummarino (1), B. Roosens (2), B. Hauser (2), Y. Vandenplas (2), D. Urbain (2). (1) UZ Brussel, Jette, Belgium ; (2) UZ Brussel, Jette, Belgium.

Introduction : Gastroesophageal reflux (GER) causes esophageal and extra-esophageal symptoms. Frequent extra-esophageal symptoms are cough, laryngospasm, laryngitis and asthma. Micro- and macro-aspiration are hypothesized pathophysiologic mechanisms.

Aim : To compare the results obtained with two techniques that can measure reflux in the hypo-pharynx.

Methods : The “Restech® technique” evaluate the pH at the level of the hypo-pharynx through a probe with a pH-antimony sensor which is placed behind the soft palate. Multichannel intraluminal impedance/pH-monitoring (MII/pH) records the passage gas and liquid reflux, and the pH of the latter, through a probe with 6 impedance sensors and 2 pH antimony sensors (the lower at 3 cm above the LES and the high at the level of hypo-pharynx). Ten adults patients (age 46.33 ± 9.86 yrs ; range 33.9-66.11) presenting with chronic coughing underwent simultaneous a Restech® and MII/pH recording. A time-interval of 2 minutes was allowed between Restech® and MII/pH to be considered simultaneous episodes. The results were statistically tested with the Chi-square test ; a p value < 0.05 was considered significant.

Results : A total of 515 reflux episodes were measured with the MII/pH (acid : 181 ; weakly acid : 310 ; weakly alkaline : 24). 180 (35%) of these episodes reached the highest impedance channel (hypo-pharynx). However, 74/180 (41%) of these reflux episodes were not related to a change in pH according to the antimony electrode of the MII/pH catheter. With the Restech® technique, we found 87 reflux episodes ; 35 (40%) of these did correlate with swallows (and thus not with reflux) according to the impedance recording, and 39 (45%) were not associated with impedance or pH modification according to MII/pH. Out of all the reflux episodes detected, only 13 (15%) were detected simultaneously with both techniques (2.5% for impedance versus 15% with Restech® ; p : 0.0002). Moreover, we found 49 pH-only reflux events at the pH sensor in the hypo-pharynx with the MII/pH ; of them, only 3 (6.1%) were correlated in time with Restech® detected reflux.

Correlation in time (2 minutes time-interval) between cough and reflux events was positive in 5/10 patients for impedance (symptom index 5/10, symptom association probability 4/10). Correlation in time between cough and reflux was positive in 0/10 patients according to Restech® technique.

Conclusions : Our results show that Restech® detected less reflux episodes than impedance ; 35% of the reflux events according to Restech® were swallows according to impedance. Moreover, time correlation between cough and reflux could not be demonstrated with Restech®.

Invited lecture

- G05 -

ACUTE PANCREATITIS : ENDOSCOPIC THERAPY. O. Le Moine, ULB Erasme, Brussels, Belgium.

CHROMOENDOSCOPY VERSUS VIRTUAL CHROMOENDOSCOPY IN ULCERATIVE COLITIS : A RANDOMIZED CONTROLLED TRIAL. R. Bisschops (1), T. Bessisow (1), F. Baert (2), M. Ferrante (1), V. Ballet (1), H. Willekens (1), I. Demedts (1), K. Geboes (1), G. De Hertogh (1), S. Vermeire (1), G. Van Assche (1), P. Rutgeerts (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) H. Hart Hospital, Roseselare, Belgium.

Introduction : Patients with longstanding ulcerative colitis (UC) have an increased risk for the development of colonic neoplastic lesions. Chromo-endoscopy (CE) has been proven to enhance neoplasia detection while the role of virtual chromo-endoscopy (VC) is still to be defined.

Aim : We compared the performance of CE to VC for the detection of neoplastic lesions in patients with longstanding UC.

Methods : We performed a multi-center prospective randomized trial. A total of 112 patients (age > 18) with longstanding UC (8 years after diagnosis of extensive colitis or 10 years after diagnosis of left-sided colitis) requiring dysplasia surveillance were randomized to CE or VC. Patients with active UC extending > 20 cm, personal history of colon cancer or allergy to methylene blue were excluded. In total, 59 patients were randomized to CE and 53 to VC. Baseline characteristics were similar in both groups. At inclusion, 63% were on mesalazine, 33% on thiopurines, and 37% on biologicals.

Olympus H180Q, Fujinon EC 590 ZW/M or Pentax EC3890Fi colonoscopes were used for CE, during which methylene blue 0.1% was sprayed. VC was performed with standard Olympus H180Q colonoscopes for Narrow-Band Imaging, Fujinon EC 590 ZW/M for Fujinon Intelligent Chromo-Endoscopy, and Pentax EC3890Fi for i-scan. Time to cecum and withdrawal time were recorded. Targeted biopsies of visible mucosal abnormalities were taken. An expert pathologist assessed the histology. Data were analyzed according to the number of patients who had suspected endoscopic lesions (per patient analysis) and also to the number of suspected endoscopic lesions (per lesion analysis).

Results : Bowel preparation was judged good to excellent in all cases. The withdrawal time was significantly longer for CE (median of 27 minutes for CE versus 18 minutes for VC, $p = 0.001$). A total of 271 endoscopically raised lesions were detected in 82 patients (158 lesions in 44 patients with CE and 113 lesions in 38 patients with VC). On histology, 43 were shown to be neoplastic (29 lesions in 11 patients with CE and 14 lesions in 11 patients with VC) including 2 adenocarcinoma, 2 high grade dysplasia, 3 dysplasia associated lesion or mass, and 14 adenoma like mass. The 228 non-neoplastic lesions revealed normal mucosal or inflammatory changes (66%), inflammatory pseudopolyps (7%), and hyperplastic polyps (26%). Comparing endoscopic modalities, we did not observe a significant different detection rate of true neoplastic lesions in endoscopically suspicious raised lesions [per patient analysis : 18.6% for CE vs. 20.8% for VC ($p = 0.779$) ; per lesion analysis : 18.4% for CE vs. 12.4% for VC ($p = 0.185$)].

In addition, age was higher in the group that developed neoplasia (59 versus 49 years, $p = 0.01$) despite a similar disease duration.

Conclusions : CE and VC performed equally for the detection of neoplastic lesions in longstanding UC patients. Given the longer withdrawal time for CE and the easier applicability, VC may possibly replace classical CE.

EFFECT OF NATIONAL GUIDELINES ON QUALITY OF COLONOSCOPY : A SINGLE CENTRE ANALYSIS. E. Macken, T. Van Hoof, N. Messaoudi, P. Pelckmans, T. Moreels. Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Conventional colonoscopy is the gold standard examination to detect mucosal pathology in the colon, including polyps and colorectal cancer. However, the quality of colonoscopy depends on several patient-, endoscopist- and procedure-related quality indices. Beginning 2009, Belgian national quality recommendations for colonoscopy were published accompanied by a national awareness campaign among gastroenterologists. To assess the effect of these recommendations, we retrospectively analyzed the quality of colonoscopy before (2006) and after (2009) publication of the recommendations in our department.

Methods : All reports of endoscopic procedures of the colon performed in 2006 and 2009 were reviewed and the available quality indices were determined and compared. Colonoscopies with no or insufficient bowel cleansing were excluded as were colonoscopies performed in colons with partial surgical resections.

Results : In 2006 a total of 1617 lower gastrointestinal endoscopies were performed of whom 1206 were eligible for analysis, whereas in 2009 there were 1723 lower endoscopies with 1289 eligible colonoscopies. Male / female ratio was 1 : 1 both in 2006 and 2009 with patients' mean ages of 57 ± 1 year and 58 ± 1 year respectively. Caecal intubation rate was 85% in 2006 and rose to 93% in 2009. In 2006 endoscopy trainees achieved a caecal intubation rate of 81% and supervisors 87%. In 2009 these caecal intubation rates rose to 93% and 93% respectively. Polyp detection rate remained stable (31% vs. 33%). Mean withdrawal time was 13 min (range 4-60 min) in 2009, and was not systematically recorded

in 2006. Complication rates also remained stable (4 serious complications in 2006 and 2009) without colonoscopy-related mortality.

Conclusions : The development of national guidelines on quality of colonoscopy and the accompanied awareness campaign resulted in an important increase of caecal intubation rate, an important quality index of colonoscopy. Polyp detection rate and complication rate remained stable and were within the range of the national guidelines. Guidelines and awareness campaigns are necessary to increase and maintain the quality of colonoscopy.

- G08 -

THE REVIVAL OF UNSEDATED COLONOSCOPY THROUGH WATER INFUSION. B. Strubbe, S. Beeckman, M. De Vos, D. De Looze. Ghent University Hospital, Gent, Belgium.

Introduction : Unsedated colonoscopy using water infusion has been performed in a US veterans population, showing feasibility and even enhanced adenoma detection rate (Leung *et al.*, J. Interv. Gastroenterol., 2011, 1 : 8-13). Avoiding sedation or general anesthesia theoretically will avoid sedation related complications, could be time- and cost-saving, could improve patient satisfaction and reduce waiting lists for colonoscopy.

Aim : To study the feasibility of unsedated colonoscopy by means of water infusion in a tertiary hospital setting. The primary endpoint of this trial is reaching the cecum without need for sedation.

Methods : The water infusion technique for colonoscopy consists of using water at body temperature at insertion of the endoscope, instead of air insufflation. It is combined with removal of all residual colonic air by suction and residual feces by water exchange. Once the cecum is reached, air insufflation is used during withdrawal for mucosal inspection. All patients start the endoscopy unsedated but sedation and analgesia are administered if needed or asked for (midazolam 2,5 mg / pethidine 50 mg). When progression with water fails, switch to air insufflation is made. Pain scores are registered on a VAS (0 = no pain, 10 = maximal pain). Patient satisfaction and willing to repeat the procedure are also scored on a VAS (respectively : 0 = very low, 10 = high/ 0 = not willing to repeat, 10 = willing).

Results : 38 patients (18F, 20M) with a mean age of 57 ± 11 yrs are included. Indications for colonoscopy are : cancer screening 24%, polyp surveillance 18%, diagnostic 58%. The primary endpoint, unsedated successful cecal intubation, is reached in 27 patients (71,2%). The global cecal intubation rate is 94,7% (n = 36). Mean insertion time was 14 ± 6 min. Mean length of the colonoscope at the cecum was 82 ± 10 cm. Mean volume of water infused upon arrival to the cecum is 332 ± 172 ml. In 11 patients (28,9%) temporary switch to air insufflation during insertion is needed (6 at the hepatic flexure, 5 in the sigmoid). Men reach the endpoint more readily than women (85% vs. 55,6%, p = 0,046). Age doesn't make a difference (p = 0,62). Maximum mean pain score during insertion is $3,1 \pm 2,7$; pain at time of discharge is $1,5 \pm 2,2$. Patient satisfaction is $9,2 \pm 1,2$, willingness to repeat $9,7 \pm 0,7$. Mean withdrawal time is $8,8 + 4,6$ min and the adenoma detection rate is 21%.

Conclusions : Water-infused colonoscopy is a promising and simple technique to perform complete unsedated colonoscopy. This method may open perspectives for a new era in colonoscopy with high patient satisfaction and lower costs.

- G09 -

A COMPARATIVE STUDY BETWEEN INSUFFLATION WITH CARBON DIOXIDE, AIR OR WARM WATER DURING COLONOSCOPY. E. Macken, T. Moreels. Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Gas insufflation is mandatory for optimal visualisation of the entire colon during colonoscopy. Traditionally standard room air is used to allow an adequate distension of the colon. But accumulation of air can cause significant discomfort and pain for the patient. Carbon dioxide (CO₂) is an inert gas that is absorbed 150 times faster than the nitrogen in the air. Therefore, patients will not suffer from bloating when CO₂ is used for gas insufflation. Warm water infusion can also be used for distension during colonoscopy. Warm water would relieve colonic spasms and facilitate colonoscopy.

Methods : In total 100 patients requiring colonoscopy were enrolled. Patients were randomly assigned to receive insufflation with air, CO₂ or warm water. Procedures were started without sedation, and patients received analgesia if they asked for it. The patient's pain scores during the examination were recorded using a visual analog scale (0-100). Patients were blinded to the type of insufflation.

Results : About 1/3 of patients admitted for colonoscopy was willing to participate. A total of 100 patients were enrolled, 65% men, 35% women. Mean age was 59 (+/- 15) years. 64% of patients completed the colonoscopy without sedation. 29% of patients were sedated with Fentanyl only, 7% with Fentanyl and Dormicum. Mean pain score in the CO₂ group (30 (5-60)) was significantly lower than in the air group (60 (40-70)), there was no statistically significant

difference between the other combinations. Mean score for abdominal cramping was significantly higher in the water group (30 (20-60)) compared with the CO2 (15 (0-30)) and the air group (15 (0-45)). 77% of patients was willing to undergo the same procedure with the same method next time. 84% was satisfied with the procedure as it was performed. Pain score was significantly higher in women (60 (22-80)) than in men (45 (10-60)).

Conclusions : About 1/3 of patients was willing to participate in this study. Reasons for refusal were fear of pain during the examination. However, 64% of patients completed the examination without sedation, and 77% of patients was willing to undergo the same procedure the next time. Advantages of sedationless colonoscopy are lower costs and a quick recovery without need for assistance. We have shown that it is possible to perform colonoscopy without sedation in a well-informed subgroup of patients (preferably men), who accept the greater discomfort and want a quick recovery without assistance. As mean pain score was significantly lower in the CO2 group, we advocate its use for sedationless colonoscopy.

Invited lecture

- G10 -

ACUTE PANCREATITIS : ENDOSCOPIC THERAPY OF ACUTE PSEUDOCYST AND NECROSIS. M. Barthet, Marseille, France.

POSTERS

- G11 -

SCREENING OF PROXIMAL COLON ADENOMAS : CONVENTIONAL COLONOSCOPY VERSUS CHROMO-
ENDOSCOPY. S. Mouzyka. Hospital Lissod, Kiev, Ukraine.

Introduction : The adenoma-carcinoma sequence developed is accepted in principle for colorectal cancer. Small proximal adenomas (above the splenic flexure) are commonly missed during screening colonoscopy.

Aim : The aim of our study was to assess the detection rate of proximal colon adenomas by comparing chromo-colonoscopy with standard white light colonoscopy.

Methods : In one institution, 519 asymptomatic patients (F329, M190 ; mean age 51,5) participated in study of screening video colonoscopy, performed by a single endoscopist. Informed consents were obtained in all cases. Patients were excluded if the bowel preparation was inadequate, if they had an earlier diagnosed colorectal neoplasia or inflammation, or if they were receiving anticoagulant medication. All endoscopic examinations were performed after obtaining permissions from patients. Patients received intravenous propofol prior to intubation of the colonoscope. Complete colonoscopy was performed in 517 (99,6%) cases. Conventional white light colonoscopy was performed in 169 patients. A further 350 patients were examined using pancolonics 0,2% solution indigo carmine dye. All lesions identified during screening colonoscopy in both groups were removed completely by cold forceps or snare polypectomy. The two groups were similar with regard to age, gender and rate of complete colonoscopy. Mann-Whitney U Tests were used to determine differences between conventional colonoscopy and chromoendoscopy.

Results : There was not anything complications after colonoscopy. Totally 151 proximal adenomas (84 patients) were detected during colonoscopy in both groups, with flat lesions 112 (74%) and protuberant lesions 39 (26%). Results of proximal colon lesions detection are shown in Table, the difference between two groups was significant (p value less than 0.05).

	Patients with proximal adenomas only	Patients with proximal adenoma total	Proximal adenoma/ patient
Conventional colonoscopy	4.7%	9%	0.16
Chromoendoscopy	10.3%	19.7%	0.35
P	< 0.005	< 0.005	< 0.005

Conclusions : The results of our comparative study show that for the detection of proximal lesions in asymptomatic patients chromocolonoscopy is superior to standard colonoscopy. Chromoendoscopy with indigo carmine improves the detection rate of proximal colon adenomas.

HETEROGENEITY OF SCORING SYSTEMS IN ODS ASSESSMENT AFTER STARR : A REPORT FROM A SYSTEMATIC REVIEW. A. D'hoore (1), A. Stuto (2), B. Van Geluwe (1), F. Da Pozzo (2), S. Fieuws (1), P.A. Lehur (3). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) Ospedaliera Santa Maria Degli Angeli, Sarone, Italy ; (3) Hôpital Hôtel Dieu, Nantes, France.

Introduction : Stapled transanal rectal resection (STARR) is an increasingly accepted treatment for obstructed defecation syndrome (ODS). A number of publications have reported the efficiency of STARR in correcting symptoms of ODS. However the exact place of STARR procedure in the treatment algorithm of constipated patients is still a matter of debate among the colorectal community.

Aim : This study aimed to evaluate with specific instruments for quality assessment of nonrandomised studies, the results of the literature on the functional outcome of STARR for ODS and determine their consistency.

Methods : A systematic review of the literature up to April 2011 was performed to assess quality and variability of published data on functional outcome after STARR for ODS. Quality of published case series was independently assessed using the Newcastle Ottawa Scoring scale (NOS). Outcome data were pooled and stratified according to the functional scoring system. A meta-analysis was then performed on the standardized effect sizes.

Results : Twenty-eight publications (n = 1700 patients) were identified. Median follow-up was 15 months. In these studies, more than 5 different scoring systems have been used. As a whole, functional scores improved significantly. In 16 studies using Cleveland Clinic Constipation Score and Symptom Severity Score (scores from 0 - normal to 30 - worst points), a decrease from a mean of 14.4 (SD 2.9) at baseline to 6.1 (SD 2.3) postoperative and from 16.3 (SD 6.5) to 7.4 (SD 4.0) was respectively observed. A similar tendency was noticed when other ODS scoring systems were used. However a lack of uniformity in these reports was patent.

Conclusions : The consistent finding of a significant decrease in the various ODS scores confirms a beneficial effect of STARR. However, this meta-analysis is an effort to highlight some of the methodological inconsistencies that may contribute to an overoptimistic view of the functional outcome after STARR for ODS. Heterogeneity in ODS scoring implies the need for standard effect size calculation to compare published results on STARR for ODS, and outlines the urgent need for a more uniform and accurate data reporting.

CHOLANGIOSCOPY IN A PATIENT WITH ROUX-EN-Y LIMB VIA A GASTRIC ACCESS LOOP. G. Mavrogenis, Y. Hoebeke, P. Warzee. Notre Dame, Charleroi, Belgium.

Introduction : A 60-year-old patient with history of minor thalassemia was addressed for recurrent episodes of cholangitis. He had undergone a pancreaticojejunostomy with Roux-en-Y hepaticojejunostomy because of chronic pancreatitis 10 years earlier. Previous episodes of biliary stones had been managed endoscopically, once, through a transient jejunostomy and twice by single balloon enteroscopy. Other possible options of biliary access in patients with altered anatomy include : percutaneous transhepatic cholangiography, laparoscopic assisted ERCP, ERCP through a permanent access loop, creation of gastrostomy by a percutaneous approach into the excluded gastric remnant after exclusion bypass surgery or through a gastro-gastric communication.

Aim : Given the frequent episodes of cholangitis and the continuous need of enteroscopy, we decided to create a gastrojejunal anastomosis by surgical means [1], in order to gain a permanent point of access to the biliary tree. In this way the length of the gastrointestinal tube that has to be traversed to access the common bile duct is short enough for a standard endoscope

Methods : The common bile duct was explored under direct visualization and a few biliary stones were extracted using a balloon.

Conclusions : The creation of a gastric access loop consists in an alternative method of direct biliary access in patients with altered anatomy, when frequent ERCP is needed or when balloon enteroscopy is not available.

IBD RESEARCH GROUP (BIRD)

Invited lecture

- I01 -

THE MICROBIAL FLORA : A REVIEW ON MICROBIOLOGY AND TECHNIQUES. J. Raes, UZ Brussel, Belgium.

- I02 -

PREDOMINANT DYSBIOSIS IN PATIENTS WITH ULCERATIVE COLITIS IS DIFFERENT FROM CROHN'S DISEASE PATIENTS. K. Machiels, M. Joossens, J. Sabino, V. De Preter, I. Arijis, V. Ballet, K. Claes, J. Verhaegen, G. Van Assche, P. Rutgeerts, S. Vermeire. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Bacteria play an important role in the onset and perpetuation of the intestinal inflammation in inflammatory bowel disease (IBD) and intestinal dysbiosis has been described. Most studies characterizing dysbiosis have been done in Crohn's disease (CD). In UC, only small cohorts have been studied and showed conflicting data.

Aim : Our aims were to evaluate in a large cohort if the microbial signature we previously described in CD1 is also present in UC and if we could characterize predominant dysbiosis in UC.

Methods : In total we collected 214 fecal samples from 127 UC patients and 87 age and sex matched controls who had not used antibiotics or probiotics in the last month before sampling. After extraction of the total bacterial DNA, the predominant microbiota per sample was analyzed using denaturing gradient gel electrophoresis (DGGE) analysis. DGGE profiles were processed with BioNumerics software version 4.6. Statistical analyses were performed using Mann-Whitney U tests with SPSS v17.0 software. Sequencing of purified bands was performed with an ABI Prism 3130 Genetic Analyser.

Results : The microbial signature found in CD was not present in UC based on the DGGE analyses. More specifically, no differences between groups were detected for *Ruminococcus gnavus* and *Bifidobacterium adolescentis*. Although *Faecalibacterium prausnitzii* was reduced in UC patients compared to control subjects, the predominant difference was not significant (Uncorrected $p = 0.053$).

When comparing DGGE banding patterns of UC patients with control subjects, 2 band-classes were significantly (p -value < 0.05) different after stringent correction for 49 band-classes. One was identified as *Bifidobacterium longum*, and was more present in UC patients versus controls ($p = 0.01$). The other was identified as *Roseburia hominis* and was reduced in UC patients compared to control subjects ($p = 0.03$).

Conclusions : The predominant bacterial dysbiosis signature found in CD is disease specific as it is not present in UC. The fecal microbiota of UC patients differs however also from healthy individuals. In UC, there is a predominant increase in *Bifidobacterium longum*, known as an important lactic and acetic producer, and a reduction in *Roseburia hominis*, a main butyrate producing bacteria of the Firmicutes phylum. These results suggest that different bacterial species play in the pathogenesis of UC and CD.

- I03 -

TUDCA ALLEVIATES DSS-INDUCED COLITIS BY FORCING IRE1 ACTIVITY AND REDUCING COLONOCYTE APOPTOSIS. D. Laukens, L. Devisscher, P. Hindryckx, H. Peeters, M. De Vos. Ghent University, Ghent, Belgium.

Introduction : Genetic data and mice models point to a direct link between the activation of the unfolded protein response (UPR) and intestinal inflammation. In addition, colonic inflammation in inflammatory bowel disease (IBD) is associated with the induction of key UPR players. A fundamental question that needs to be addressed is how the balance between cytoprotective and apoptosis promoting functions of the UPR can be influenced to alleviate inflammation.

Aim : The dextran-induced model of colitis was used to estimate consecutive UPR events during the onset of acute intestinal inflammation and to evaluate the effect of tauroursodeoxycholic acid (TUDCA), a bile salt with chaperone-like functions that has been shown to alter the UPR.

Methods : C57BL/6 mice received 4% dextran sodium sulfate (DSS) in their drinking water for 7 days to induce colitis. Mice were matched for initial body weight and treated IP with 500 mg/kg/d TUDCA or PBS. Weight loss and mortality were recorded daily. On day 0, 3, 7 and 10, eight mice per group were sacrificed and colon length was assessed. Colonocytes were isolated and lysed for real-time PCR (qPCR) analysis of BIP, the spliced and unspliced form of XBP1, ATF4, PDIA4, CASP3 and CASP12. The effect of TUDCA was evaluated in a TNF-induced apoptosis assay in HT29 cells.

Results : Administration of DSS did not result in substantial activation of the UPR in colonic epithelial cells. A transient activation of IRE1 was seen, measured by the increased ratio of spliced to unspliced XBP1 levels at day 7 and 10. Activation of the PERK and ATF6 pathways was not observed, as ATF4 and PDIA4 expression remained unchanged. The expression of the key chaperone BIP diminished at day 3 and 7 and returned to baseline at day 10. Administration of TUDCA resulted in 100% survival as compared to 60% in vehicle treated mice ($p < 0.05$). Weight loss and colon shortening was significantly less pronounced in TUDCA treated mice ($p < 0.01$ and $p = 0.02$ respectively). Colonocytes from TUDCA treated mice showed a 2.6-fold XBP1 splicing increase at day 3, while BIP levels remained comparable to those in non-DSS treated mice. Interestingly, TUDCA treatment led to significantly reduced CASP3 and CASP12 induction in colonocytes as compared to control mice. These findings were confirmed *in vitro* where TUDCA treated HT29 cells showed increased XBP1 splicing and BIP expression ($p < 0.001$) and were completely protected against TNF-induced cell death (4% vs 12%, $p < 0.001$) and caspase 3 activity ($p < 0.001$).

Conclusions : Acute colitis is accompanied by a transient activation of the IRE1 pathway. TUDCA elicits beneficial effects in epithelial cells subjected to colonic damage by reducing colonocyte apoptosis, forcing IRE1 activation and inducing its downstream target gene BIP, which might further protect cells from stress to the endoplasmic reticulum. Chemical chaperones such as TUDCA merit further clinical investigation for the treatment of IBD.

- 104 -

REPEATED CYCLES OF DSS INDUCING A CHRONICALLY RELAPSING INFLAMMATION :A NOVEL MODEL TO STUDY FIBROSIS. C. Breynaert (1), T. Dresselaers (2), J. Cremer (1), K. Van Steen (3), C. Perrier (1), S. Vermeire (1), P. Rutgeerts (1), J. Ceuppens (4), U. Himmelreich (2), G. Van Assche (1). (1) Translational Research Center For Gastrointestinal Disorders (Targid), Kuleuven, Leuven, Belgium ; (2) Biomedical Nmr Unit - Mosaic, Leuven, Belgium ; (3) University Of Liege, Liège, Belgium ; (4) Experimental Immunology, Kuleuven, Leuven, Belgium.

Introduction : Most experimental animal models of IBD fail to accurately reflect the chronically relapsing inflammation underlying the complications of human Crohn's disease.

Aim : This study investigated whether repeated cycles of DSS adequately reflect the effects of chronic transmural healing. Transmural μ MR imaging with *in vivo* T2 relaxometry was used to differentiate transmural changes.

Methods : DSS colitis was induced in 6 week-old C57BL/6/J mice : acute colitis mice ($n = 10$) received 7 days of DSS prior to sacrifice. One cycle mice ($n = 10$) received 1 cycle of 7 days of DSS followed by 2 weeks of normal drinking water prior to sacrifice, 2-cycle mice ($n = 10$) 2 cycles and 3-cycle mice ($n = 9$) 3 cycles prior to sacrifice. Control mice ($n = 7$) received normal drinking water only. Six mice per group were scanned *in vivo* on a 9.4T MRI Bruker system. T2 weighted images and T2 maps of the distal colon were recorded. Histograms of the colon wall were created from T2 maps using a plugin for ImageJ. After scanning and euthanasia, the distal colon of all mice was harvested for histology and FACS analysis. Collagen deposition was quantified with Martius-Scarlett-Blue staining.

Results : Colon weight, colon weight/length ratio and macroscopic score were significantly higher in the 1-, 2- and 3-cycle group compared to the control group ($p < 0.001$), however, no significant difference was observed within the cycling groups. Although all mice in the 2-, and 3-cycle group had a normal DAI score, the macroscopic score was significantly higher compared to the acute group ($p < 0.001$). CD4+Foxp3+ cells in blood increased with more cycles of DSS ($p < 0.001$). The number of CD4+Foxp3+ cells in MLN was higher in the 2- and 3-cycle model compared to the acute model and controls ($p < 0.001$). A higher number of IFN γ + cells in MLN was observed in the 1-, 2- and 3-cycle model compared to the acute model ($p < 0.001$). The 3-cycle model showed a higher number of IL17+ cells in MLN compared to the acute model and controls ($p = 0.0012$). No significant difference was observed within the different models in IL13. Collagen deposition was significantly higher in the 2- and 3-cycle model compared to the 1-cycle model ($p < 0.001$). T2 mapping of the colon was able to discern between all groups : the increasing number of cycles correlated with a gradual regression of T2 values to those of normal colon.

Conclusions : The immune profile of a cycling DSS model with induction of relapse and remission is clearly different from the acute DSS model inducing an adaptive immune response. The chronic repeated cycles of DSS model opens perspectives to study the effects of healing and fibrosis in a murine model of IBD. *In vivo* T2 relaxometry is a promising non-invasive assessment of inflammation and fibrosis and should be explored in CD patients.

Invited lecture

- I05 -

BACTERIAL HANDLING AND AUTOPHAGY IN HEALTH AND DISEASE. K. Cadwell, ST LOUIS, USA.

- I06 -

CONTRIBUTION OF GENES UNDERLYING MONOGENIC DISEASES WITH IBD-LIKE SYMPTOMS TO PREDISPOSITION TO IBD. L. Amininejad (1), Y. Momozawa (2), B. Charlotiaux (2), E. Trepo (1), M. Mni (2), E. Quertinmont (1), M. Abramowicz (1), J. Deviere (1), E. Louis (3), A. Van Gossum (1), M. Georges (2), D. Franchimont (1). (1) Erasme Hospital, Brussels, Belgium ; (2) University Of Liege, Liège, Belgium ; (3) Chu Sart Tilman, Liège, Belgium.

Introduction : Some rare monogenic disease (chronic granulomatous disease, glycogen storage disease Ib, Chediak-Higashi syndrome, Hermansky-Pudlak syndrome, leucocyte adhesion deficiency and congenital, cyclic and autoimmune neutropenia) are characterized by defective innate immunity and chronic intestinal inflammation, reminiscent of Inflammatory Bowel Disease (IBD).

Aim : The aim of this project is to examine whether common, low frequency or rare variants in the 14 corresponding causative genes identified so far for these diseases may contribute to inherited predisposition to IBD.

Methods : The selected candidate genes were analyzed for the presence of (i) rare variants by means of high-throughput resequencing (HTS) and (ii) low frequency variants by association analysis with CD in meta-data including genotypes from the International IBD Genetics Consortium (IIBDGC) imputed from the 1000 Genomes Project. The HTS were done with the 454 sequencing (ROCHE®) by : 1) amplification from the genomic DNA of 69 open reading frame (ORFs) and intron-exon boundaries of five of the candidate genes (CYBB, CYBA, NCF1, NCF2, NCF4) encoding for the NADPH oxidase complex and muted in chronic granulomatous disease ; 2) constitution of two equimolar DNA pools from 512 Crohn disease (CD) cases and 512 ethnically matched controls ; 3) massive parallel pyrosequencing using the ROCHE® FLX system targeting an average sequence depth of 500 for both the Watson and Crick (W&C) strand ; 4) detection of DNA sequence variants (DSVs) using the Amplicon Variant Analyzer (AVA) software (ROCHE®).

Results : A total of 11 common variants (27,2% of non-synonymous variants) and 14 low-frequency and rare variants (35,7% of non-synonymous variants) were identified. One Indels on CYBA gene with deletion of 11 bp was confirmed by gene scan of 2052 CD patients (allele frequency = 0,22%) and 4468 controls (allele frequency = 0,47%) with a p-value of 0,03 and an odds ratio of 2,15. The association analysis with CD genotypes from the IIBDGC imputed from the 1000 Genomes Project is ongoing for all the 14 candidate genes. The most recent results will be presented.

Conclusions : Replication cohorts are needed to validate this association on CYBA gene. Next step will be done by integrating the results of the association analysis with publicly available epigenomic information and functional information (eQTL) generated in Liège for nine tissue types that are relevant for IBD pathogenesis.

- I07 -

RESTORATION OF FOXP3 REGULATORY T CELL CORRELATES WITH CLINICAL RESPONSE TO ANTI-TNF THERAPY FOR IBD. Z. Li, S. Vermeire, D. Bullens, M. Noman, M. Ferrante, P. Rutgeerts, J. Ceuppens, G. Vanassche. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : It has been reported that Infliximab (IFX), a human-mouse chimeric monoclonal antibody, up-modulates circulating Foxp3 (+) T cells in patients with Inflammatory Bowel Disease (IBD), rheumatoid arthritis, psoriasis and Behçet's disease. Foxp3 expression is not restricted to regulatory T cells (Treg) in humans. CD45RA expression in combination with the strength of Foxp3 expression distinguishes resting and active regulatory T cells (rTreg and aTreg) from Foxp3 (+) effector T cells (Teff). Distribution of the 3 subsets of Foxp3 T cells reflects aging in healthy donors, and mechanisms of some human diseases, such as Sarcoidosis and active Systemic Lupus Erythematosus.

Aim : To investigate the pathogenesis of IBD and the action of anti-TNF therapy in the 3 subsets of Foxp3 T cells.

Methods : In a cross sectional study, blood was taken from healthy controls (n = 39) and patients with IBD (n = 109, ulcerative colitis = 39, Crohn's disease = 70) before or during treatment with IFX (5 mg/kg induction and maintenance IV). The 3 subsets of Foxp3 T cells were assessed by staining with anti-CD4, anti-Foxp3 and anti-CD45RA, and analyzed by flow cytometry.

Results : 28 had active IBD before treatment (BT), 65 were responders (RS) and 16 were non-responders (NRS).

1. Both rTreg and aTreg in blood from patients with IBD before IFX therapy were significantly lower than in healthy controls ($p < 0.0001, 0.0001$, respectively), confirming a deficiency of circulating rTreg and aTreg in IBD.

2. During IFX therapy, rTregs in both RS and NRS were significantly higher than in IBD patients before therapy ($p < 0.0001, = 0.003$), and were in the normal range of HC ($p = 0.32, 0.65$). No significant difference of this population between RS and NRS was observed ($p = 0.29$).

aTregs in RS were significantly higher compared to patients before therapy, to NRS and to HC ($p < 0.0001, = 0.0002, = 0.0007$), while this population in NRS was higher than BT ($p = 0.014$), but remained lower than in HC ($p = 0.0013$).

3. No significant differences in Foxp3 (+) Teff among HC and patients with IBD before or during IFX therapy were detected.

Conclusions : A deficiency of circulating Foxp3 (+) Treg in blood characterizes active IBD and the restoration of circulating activated Tregs correlates with the clinical response to anti-TNF therapy. Foxp3(+)Teff is not involved in the therapy. Further investigation of the pathways by which IFX up-modulates rTreg and aTreg will increase our understanding of the pathogenesis of IBD and the mechanism of action of anti-TNF therapy.

- I08 -

TARGETING METALLOTHIONEIN IN DSS-COLITIS POINTS TO NEW THERAPEUTIC STRATEGIES FOR IBD PATIENTS. L. Devisscher (1), P. Hindryckx (1), K. Olievier (1), H. Peeters (1), M. Lynes (2), C. Cuvelier (1), M. De Vos (1), D. Laukens (1). (1) Ghent University, Ghent, Belgium ; (2) University Of Connecticut, Storrs, United States.

Introduction : Inflammatory bowel diseases (IBD) are chronic intestinal inflammatory disorders. To date, the expression of metallothioneins (MTs), multifunctional acute stress proteins, in IBD patients and their role during intestinal inflammation is indistinct.

Aim : Our aim was to address a functional role to the presence or absence of MTs during gut inflammation.

Methods : Metallothionein knockout (MT^{-/-}), transgenic (MT^{+/+}) and wild type mice (WT) were subjected to 4% Dextran Sulfate Sodium (DSS) for 7 days followed by 7 days of normal drinking water. Body weight and mortality were recorded daily. Anti-MT antibody (or isotype control) treatment was used in a curative setting to study the effect of exogenous blocking of MTs during experimental colitis. Inflammatory response and serum zinc levels were assessed.

Results : Mice lacking MT showed a significant higher survival rate compared to MT^{+/+} mice ($p < 0.05$; 90% survival for MT^{-/-} mice versus 52% for MT^{+/+} mice). At day 10, histological inflammation, neutrophil infiltration and epithelial proliferation were in favor of MT^{-/-} mice ($p < 0.05$). Baseline serum zinc levels were significantly lower in the MT^{+/+} mice ($p < 0.05$). Zinc levels decreased during the course of colitis in all three groups but this was less pronounced in the MT^{-/-} mice. DSS exposed mice treated with the anti-MT antibody tended to lose less body weight and scored better for histological inflammation compared to control treated mice at day 10. Inflammatory cell infiltrate, represented as macrophages, was significantly lower in the anti-MT treated mice compared to control mice ($p < 0.05$). Improved recovery of anti-MT treated mice was modulated by an enhanced hypoxic adaptive response with significant higher levels of the beneficial hypoxia-inducible factor 1 alpha and an increased vascularization compared to the control group ($p < 0.05$).

Conclusions : A low MT profile during colitis was associated with enhanced recovery and prolonged survival of DSS-induced colitis. Targeting extracellular metallothioneins using anti-MT antibody confirmed the beneficial properties of a low MT profile during recovery of colitis and allows extrapolation to future therapeutic opportunities for patients suffering from IBD.

Invited lecture

- I09 -

UC GENETICS AND PATHOGENESIS : HOW DIFFERENT IS UC FROM CD ? S. Danese, Milan, Italy.

Invited lecture

- I10 -

PERSONALIZED MEDICINE : OPTIMIZING ANTI-TNF THERAPIES (THROUGH LEVELS). F. Baert, H. Hartziekenhuis, Roeselaere, Belgium.

COTREATMENT WITH ADALIMUMAB (ADA) MAY BE MORE EFFECTIVE THAN ADA MONOTHERAPY IN CROHN'S DISEASE (CD). C. Reenaers (1), E. Louis (1), J. Belaiche (1), S. Keshav (2), S. Travis (2). (1) Ulg Sart Tilman, Liège, Belgium ; (2) John Radcliffe Hospital, Oxford, United Kingdom.

Introduction : There is clear benefit of combination therapy for infliximab (IFX) with immunosuppressive drugs (IS), whether commenced together, or later (1), but no data are available for ADA.

Aim : Our aim was to assess whether IS combotherapy (CoT) was more effective than monotherapy for Crohn's disease (CD) patients treated with ADA, using the semester approach.

Methods : Retrospective study of patients with CD (n = 181) treated for at least one year with ADA in Oxford, UK or Liege, Belgium. Treatment periods were divided into 6 month semesters and the treatment failure compared between semesters with or without CoT (thiopurines or methotrexate). A multivariate analysis was performed to assess factors associated with treatment failure semesters, including ADA monotherapy vs CoT. Patients receiving 6 months CoT during the first semester were separately analysed. ADA failure was defined as dose modification during therapy, drug modification, perineal complications, or intra-abdominal surgery for active CD.

Results : Overall, 569 semesters were studied in 181 patients (mean age at diagnosis = 21.6y, Oxford n = 98, Liege 83), including 147 semesters in 45 patients having received CoT during the first semester. More patients in Oxford received CoT than Liege (OR : 4.82, p < 0.0001) and fewer females received CoT (OR 0.50, p = 0.01). When considering only patients on CoT during the first semester, treatment failures were less frequent in semesters with IS (20%) compared to semesters without IS (80% ; OR 0.30, p = 0.02) and the protective effect of the CoT was maintained over time (p = 0.01). When considering all the patients, CoT in the first semester was associated with a lower frequency of treatment failures (34% vs 66%, OR 0.69, p = 0.046) in univariate analysis but not in multivariate analysis. Furthermore, CoT later after induction of ADA was not associated with treatment failures. Female gender (OR 1.68, p = 0.01), previous surgery (OR 1.89, p = 0.001) and active perianal disease (OR 1.57, p = 0.02) were also associated with an increased risk of failure on multivariate analysis. Although failures were less common in Oxford (OR 0.52, p = 0.001), more failures in Oxford had surgery (OR 8.85, p = 0.001) or perianal complications (OR 3.33, p = 0.01) and fewer had ADA weekly (OR 0.24, p = 0.0003) on multivariate analysis. The overall number of operations and perianal complications did not differ between CoT and ADA monotherapy. Thiopurines appeared more effective than methotrexate for preventing failure (OR 0.35, p = 0.03). No other factor, including previous IFX or ADA, predicted a semester with failure. The probability of failure did not increase over the semesters (p = 0.86).

Conclusions : When it was given during the first semester, CoT with ADA in CD was associated with fewer semesters with treatment failures (essentially the need for dose escalation or treatment modification, but not the rate of surgery or perianal complications).

Reference :

1. Sokol H. *et al.* Gut, 2010, 59 : 1363-8.

POINT OF CARE QUANTIFICATION OF FAECAL CALPROTECTIN WITH THE QUANTUM BLUE RAPID TEST. L. Coorevits, F. Baert, H. Vanpoucke. H. Hart Hospital, Roseselare, Belgium.

Introduction : Faecal calprotectin (FC) has proven its utility as a non-invasive marker for neutrophilic intestinal inflammation. It can be used in the differential diagnosis between functional and organic bowel disease, thereby avoiding invasive ileocolonoscopy. Within a population of IBD patients, FC has showed to be useful in the follow-up of patients under therapy.

Aim : The objective of this study is to evaluate the recently launched quantitative immunochromatographic point of care test (Quantum Blue Calprotectin (QB POCT), Bühlmann Laboratories AG) regarding its reliability in distinguishing organic from non-organic gastrointestinal disease and its comparability to the current ELISA method (Bühlmann Laboratories AG).

Methods : 142 samples (62 male and 80 female patients) that had been analysed with the ELISA were reanalysed with the QB POCT (normal or high range cartridge). Manufacturer's instructions were strictly followed. During our previous in-house evaluation of the ELISA, a cut-off value of 50 $\mu\text{g/g}$ faeces and a grey zone of 50-200 $\mu\text{g/g}$ faeces were established. These values were revised with the QB POCT, in order to interpret the results of both methods clinically as similar as possible. Different cut-off values were suggested by a ROC analysis and applied to the measurement results. The analogy of interpretation was determined by means of the %agreement and the %mismatches. In addition, a determination coefficient was calculated based upon 122 samples.

Results : A cut-off of 30 $\mu\text{g/g}$ faeces and a grey zone of 30-110 $\mu\text{g/g}$ faeces were found to be the most suitable for the QB POCT, resulting in 89.4% (127/142) agreement and 10.6% (15/142) mismatches. 93.3% (14/15) of those

mismatches concerned minor errors (negative or positive vs. grey zone result) and 6.7% (1/142) were major errors (false negative result (FN)). The FN could not be avoided without resulting in a significant loss of sensitivity. A determination coefficient of 0.89 was found.

Conclusions : Among the 15 mismatches only 1 resulted in a major clinical misinterpretation (FN). The other 14 led to minor interpretation errors (negative or positive vs. grey zone result), which are of limited importance since a FC value is to be interpreted along with the clinical presentation. Therefore we may conclude that the QB POCT can serve as an alternative to the time consuming ELISA in the differential diagnosis between functional and organic bowel disease, using a cut-off value of 30 $\mu\text{g/g}$ faeces and a grey zone of 30-110 $\mu\text{g/g}$ faeces.

- 113 -

MONITORING OF CRP AND CALPROTECTIN TO PREDICT RELAPSE IN CROHN'S DISEASE AFTER INFLIXIMAB WITHDRAWAL. N. De Suray (1), J. Salleron (2), G. Vernier-Massouille (3), J.C. Grimaud (4), Y. Bouhnik (5), D. Laharie (6), J.L. Dupas (7), H. Pillant (8), L. Picon (9), M. Veyrac (10), M. Flamant (11), G. Savoye (12), R. Jian (13), M. De Vos (14), E. Piver (15), J.F. Colombel (3), E. Louis (16). (1) Grand Hôpital De Charleroi, Hôpital Saint-Joseph, Gilly, Belgium ; (2) Centre Hospitalier Régional Universitaire De Lille, Unité De Biostatistique, Lille, France ; (3) Centre Hospitalier Régional Universitaire De Lille, Hôpital Claude Huriez, Lille, France ; (4) Hôpital Nord, Marseille, France ; (5) Hôpital Beaujon, Clichy, France ; (6) Hôpital Haut-Lévêque, Bordeaux, France ; (7) Hôpital Nord, Amiens, France ; (8) Hôpital Henri Mondor, Créteil, France ; (9) Hôpital Trousseau, Tours, France ; (10) Hôpital Saint-Eloi, Montpellier, France ; (11) Hôpital Hôtel Dieu, Nantes, France ; (12) Hôpital Charles Nicolle, Rouen, France ; (13) Hôpital Européen Georges Pompidou, Paris, France ; (14) Ghent University Hospital, Gent, Belgium ; (15) Université François Rabelais, Tours, France ; (16) Centre Hospitalier Universitaire De Liège, Liège, Belgium.

Introduction : In Crohn's disease (CD), predicting clinical relapse in patients in clinical remission by using non-invasive biomarkers could allow early therapeutic intervention. Serum C-reactive protein (CRP) and fecal calprotectin (calpro) have great potential in this regard.

Aim : To assess the value of monitoring CRP and calpro levels to predict a relapse in patients with CD in clinical remission after infliximab (IFX) discontinuation.

Methods : Patients with luminal CD treated for at least one year with scheduled IFX combined with an immunosuppressant (IS) and in stable remission without steroids for at least 6 months were prospectively recruited in the STORI study. IFX was discontinued at baseline and IS treatment was kept at a stable dose over the study period. CRP and calpro were measured every 2 months until 18 months of follow-up or until clinical relapse. CRP and calpro levels were compared between relapsers and non-relapsers at each time point using a linear mixed model. The optimal threshold of each biomarker to predict clinical relapse was determined using ROC curve analysis.

Results : 113 patients were included and analyzed. Among them, 51 presented a relapse after a median follow-up of 10 months. Overall 475 CRP and 454 calpro measurements were performed in relapsers and non-relapsers with a median of 4 measurements/patient for each marker. Median [IQR] CRP at inclusion was 2 mg/l [0.9 ;4.9] and median calpro was 51 $\mu\text{g/g}$ [30 ;224]. The evolution of CRP and calpro levels was significantly different between relapsers and non-relapsers ($p < 0.0001$ and $p < 0.001$). In non-relapsers, a slight but significant increase in CRP and calpro levels was observed throughout the follow-up ($p = 0.0018$ and $p = 0.0016$ respectively) with a median value at maximal follow-up of 3.7 mg/l for CRP and 66.9 $\mu\text{g/g}$ for calpro. In relapsers, after a slight and progressive increase, a sudden and more pronounced increase in CRP and calpro levels was observed during the 4 months preceding clinical relapse ($p < 0.0001$ and $p = 0.0004$ respectively) with a median value before the relapse of 8 mg/l for CRP and 534 $\mu\text{g/g}$ for calpro. Using ROC curve, the best compromise between sensitivity and specificity to predict relapse was 6.1 mg/l for CRP (sensitivity 71%, specificity 66%) and 305 $\mu\text{g/g}$ for calpro (sensitivity 70%, specificity 74%).

Conclusions : After discontinuation of IFX in patients with CD in clinical remission, a sudden increase in CRP and calpro levels predicts the occurrence of a relapse during the next 4 months. Further studies are needed to evaluate the therapeutic implications of these findings.

Invited lecture

- 114 -

COLON CANCER IN IBD : IS THE RISK STILL SIGNIFICANT TODAY ? D. Franchimont, ULB Erasme, Brussels, Belgium.

PROCTECTOMY WITH END COLOSTOMY IS INEFFECTIVE FOR PATIENTS WITH ANORECTAL CROHN'S DISEASE. A. De Buck Van Overtraeten, A. Wolthuis, S. Vermeire, G. Van Asche, P. Rutgeerts, F. Penninckx, A. D'hoore. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Crohn's disease (CD) is an idiopathic chronic inflammatory bowel disease that can affect the entire gastro-intestinal tract. Patients with invasive rectoperineal disease represent a therapeutic challenge. Up to 25% of those patients will eventually need proctectomy as last treatment resort after optimized medical treatment and protracted local surgical therapy.

Methods : Between February 2007 and May 2011, 10 consecutive patients (seven females, median age at diagnosis 21.5 years (range : 11-52)) underwent an intersphincteric proctectomy and end-colostomy for intractable anorectal disease. All patients except one underwent a preoperative colonoscopy to exclude proximal colonic Crohn's involvement. Five patients never had documented proximal disease before and the other five had no active proximal colitis in the years preceding their surgery. Patients did not receive any adjuvant medical treatment after surgery. Endoscopic proximal extension of disease was 35cm (range : 15-50). All patients were prospectively followed and underwent colonoscopy at one year or earlier when indicated.

Results : Median age at surgery was 40 years (range : 22-61). The proximal colon was macroscopically disease free at operation in all patients although five specimens showed microscopic active inflammation at the proximal section margin.

Within a median follow up of 26 months (range : 2-48) nine patients (9/10) developed severe active recurrence of the proximal colon, and starting from the site of the colostomy in all these patients. Despite aggressive medical treatment at recurrence, six patients (60%) needed redo surgery. Five patients received a completion colectomy with end-ileostomy, one patient had a redo segmental colectomy with end transversostomy. No recurrence was observed in the ileostomy group, the patient with a transversostomy demonstrated again colonic recurrence.

Conclusions : In patients with anorectal CD proctectomy with end colostomy is ineffective surgery resulting in early severe recurrence in the proximal colon and disabling peristomal cutaneous lesions. We propose therefore that CD patients with anorectal disease should undergo total proctocolectomy with end ileostomy.

SINGLE ACCESS LAPAROSCOPIC RESTORATIVE PROCTOCOLECTOMY : INITIAL EXPERIENCE AND FEASIBILITY STUDY. D. Leonard, C. Remue, E. Cotte, I. Stefanescu, H. Vincart, A. Kartheuser. Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : With the advancements of minimal-invasive surgery, restorative proctocolectomy with ileal pouch-anal anastomosis (RPC-IPAA) for ulcerative colitis (UC) is increasingly being performed laparoscopically. Through this approach, quality of life preserving principles of the procedure, developed during the laparotomy era, such as inferior mesenteric vessels, autonomic pelvic nerves and Riolan's arcade preservation as well as mesenteric lengthening, could be maintained. Recently, single access laparoscopic surgery (SALS) has emerged as an evolution of the standard multiport laparoscopic approach offering a "single scar laparoscopic" alternative for RCP-IPAA.

Aim : Assess the feasibility of RCP-IPAA through the SALS approach without modifying the basic principles of the procedure.

Methods : Clinical data were prospectively collected during our initial experience of SALS RPC-IPAA.

Results : In our colorectal surgery unit, RPC-IPAA has been performed by multiport laparoscopy for more than 10 years. Since the introduction of the SALS approach in August 2011, 24 colorectal procedures have been performed. Out of those, 4 UC patients (3 female, 1 male), with a median age of 22 years (17-38) and a median BMI of 20 kg/m (29-25) underwent SALS RPC-IPAA by an experienced laparoscopic surgeon. A single-port device (Applied Medical®, Gel Point™) was positioned either peri-umbilically (n = 2) or at the site of the temporary ileostomy (n = 2). Median incision length was 4 cm (3,5-4). All the major principles of RCP-IPAA could be respected. On a subjective scale, intra-operative technical difficulty was perceived as intermediate by the surgeon accustomed to multiport laparoscopy. The colon and rectum were extracted through the SALS site. Pouch-anal anastomosis was hand-sewn and, in 2 patients, a diverting loop ileostomy was created at the SALS port site. There were no intra-operative complications, no conversions or additional ports required. One complication consisting in focal pouch dehiscence required suture reinforcement performed through redo SALS approach with uneventful recovery. There were no hospital readmissions within post-operative day 30.

Conclusions : No technical modifications were necessary to perform RPC-IPAA through SALS approach. For experienced laparoscopic colorectal surgeons, SALS RPC-IPAA is feasible although technically more demanding than straight multiport laparoscopic RCP-IPAA. At this time, SALS may present cosmetic advantages and less trauma to the abdominal wall compared to the multiport laparoscopic RCP-IPAA. However, a randomized controlled trial is required to point out short and long-term advantages in comparison to multiport laparoscopic RCP-IPAA.

PREDICTORS OF COMPLICATED DISEASE IN CROHN'S DISEASE AND ULCERATIVE COLITIS. E. Hoefkens (1), M. Ferrante (2), T. Bessissow (2), I. Cleynen (1), P. Rutgeerts (2), G. Van Assche (2), S. Vermeire (2). (1) University Of Leuven, Leuven, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Several clinical risk factors for a more complicated disease course of Crohn's disease (CD) and ulcerative colitis (UC) have been identified.

Aim : We aimed to validate these factors prospectively in newly diagnosed CD and UC patients.

Methods : In total, 179 patients were followed for a median (IQR) of 32 (17-46) months. CD and UC were diagnosed in 104 and 79 patients, respectively (54% male, median age 26 years). At diagnosis, 36% of CD patients had ileal (L1), 11% colon (L2) and 53% ileocolonic involvement (L3) and 66% had inflammatory (B1), 16% stricturing (B2) and 18% fistulizing behavior (B3), 34% were active smoking and 83% had elevated C-reactive protein level (CRP > 5mg/L). In UC, 27% had proctitis (E1), 21% left-sided (E2) and 52% extensive colitis (E3) at diagnosis, 16% were active smoking and 66% had an elevated CRP. Univariate and multivariate (Cox regression) analyses were performed to study risk factors for disease progression.

Results : During follow-up, 16 CD patients developed a complication (8 strictures, 12 fistula) after a median of 10 (6-24) months. Risk factors were L1 location (LogRank $p = 0.002$) and elevated baseline CRP ($p = 0.043$). Interestingly, none of the 7 patients who started combo therapy within 6 months of diagnosis, developed a complication. In Cox regression multivariate analysis, L1 location was the only predictive factor of complication development [Odds ratio 4.0 (95%CI 1.2-12.9), $p = 0.022$]. In total, 23 CD patients needed resection after a median of 11 (7-26) months. Risk factors were ileal involvement ($p = 0.077$), B3 behavior ($p = 0.053$) and elevated baseline CRP ($p = 0.059$). In multivariate analysis, B3 behavior was the only factor predicting resection [OR 2.9 (1.0-8.3), $p = 0.050$].

Five UC patients needed colectomy after a median of 14 (11-32) months. Risk factors were elevated baseline CRP ($p = 0.084$) and need for combo therapy within 1 year ($p < 0.001$). In multivariate analysis, the need for combo therapy was the only independent predictor [OR 12.0 (1.3-108.3), $p = 0.027$].

Conclusions : In CD, ileal involvement and fistula were predictive of complicated disease behavior. In UC, the need for combo therapy, was predictive for colectomy, probably reflecting a more severe disease course. We will further investigate if genetic variants (*CARD15*, *ATG16L1*, ...) or antimicrobial antibody titers may better predict disease progression.

PROGNOSTIC VALUE OF SEROLOGIC AND HISTOLOGIC MARKERS ON LONG-TERM OUTCOMES IN ULCERATIVE COLITIS. T. Bessissow (1), B. Lemmens (2), M. Ferrante (1), R. Bisschops (1), K. Geboes (1), G. Van Assche (1), S. Vermeire (1), P. Rutgeerts (1), G. De Hertogh (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) KULeuven, Leuven, Belgium.

Introduction : Endoscopic mucosal healing is nowadays considered an important endpoint for the treatment of ulcerative colitis (UC). Due to the suboptimal correlation between endoscopic and histological findings, the role of histologic activity in predicting disease relapse has not been fully assessed.

Aim : We aimed to determine the predictive role of serologic and histological markers on disease relapse in UC patients with endoscopically inactive disease.

Methods : Patients with a confirmed diagnosis of UC (age > 18 years), endoscopically inactive disease (Mayo endoscopic score 0), and a follow up of at least 1 year were retrospectively included. Previous surgical resections, indeterminate colitis or prolonged disease remission (> 10 years) were exclusion criteria. An expert pathologist evaluated all colonic biopsies obtained during endoscopy. Presence of basal plasmacytosis (focal or diffuse) as well as histologic activity according to the Geboes UC score was assessed. At time of endoscopy, blood samples for complete blood count, albumin, liver enzymes, creatinine, and C reactive protein (CRP) were collected. Disease relapse, defined as a clinical Mayo score ≥ 3 , was documented during follow up.

Results : The study cohort consisted of 75 patients (53% male, median age 47 years) of whom 75% had previously extensive colitis. At inclusion, 71% were on mesalazine, 36% on thiopurines, and 53% on biologicals. Elevated CRP (> 5 mg/L) was observed in 15% of patients. Interestingly, although endoscopy was completely normal, histology showed a clear inflammatory activity with a Geboes score ≥ 3.1 (presence of epithelial neutrophils with or without crypt destruction or erosions) in 40% as well as basal plasmacytosis in 21% of patients (13% focal-8% diffuse).

One year after endoscopy, clinical relapse was observed in 20% of patients. Clinical and serological characteristics were not predictive of clinical relapse. However, on univariate analysis, presence of basal plasmacytosis ($p = 0.007$) and a Geboes score ≥ 3.1 ($p = 0.007$) were predictive of disease relapse. Protective factors achieving a cut-off of $p < 0.100$ for inclusion in the multivariate analysis, were baseline CRP ≤ 5 mg/L ($p = 0.093$) and the use of biologicals ($p = 0.083$).

On multivariate analysis, the presence of basal plasmacytosis or a Geboes score ≥ 3.1 was predictive of clinical relapse within 1 year [Odds ratio 4.00 (95% CI : 1.03-15.51), $p = 0.045$] while the use of biologicals was protective [Odds ratio 0.32 (95% CI : 0.05-0.90), $p = 0.035$].

Conclusions : We demonstrate that the presence of epithelial neutrophils in addition to basal plasmacytosis predicts UC clinical relapse in patients with complete mucosal healing. This finding reinforces the need for histological assessment despite normal findings at endoscopy. To strengthen this recommendation, we plan to validate our findings in a prospective cohort.

Invited lecture

- I19 -

NEW SCORES IN IBD : THE DISABILITY INDEX AND LEMANN SCORE. L. Peyrin-Biroulet, Nancy, France.

POSTERS

- I20 -

POUCHITIS HAS A SIMILAR MUCOSAL GENE EXPRESSION PROFILE AS ACTIVE ILEAL CROHN'S DISEASE. I. Arijs, J. Van Der Goten, M. Ferrante, K. Machiels, V. De Preter, L. Van Lommel, G. Van Assche, S. Vermeire, F. Schuit, P. Rutgeerts. University Of Leuven, Leuven, Belgium.

Introduction : Pouchitis is the most important long-term complication of ileal pouch-anal anastomosis (IPAA) for ulcerative colitis (UC). The etiology of pouchitis is not understood and pouchitis is a useful model to study early inflammatory bowel disease.

Aim : We investigated the gene expression profiles of mucosal pouch biopsies in comparison with the profiles of ileal mucosa of Crohn's ileitis (CDi), colonic mucosa of UC and ileal mucosa of healthy controls, focusing specifically on gene expression of cell adhesion molecules (CAMs) and antimicrobial peptides (AMPs).

Methods : Mucosal biopsies were obtained from the pouch of 15 IPAA patients (6 with endoscopic pouchitis and 9 with endoscopically normal pouch), from the (neo-)terminal ileum of 45 CDi patients [18 with mild disease (only aphthous lesions) and 27 with moderate to severe disease (presence of ulcers)] and 12 controls with normal ileum, and from the colon of 21 UC patients [10 with active disease (endoscopic Mayo score 2-3) and 11 with disease in remission (endoscopic Mayo score 0)]. Total RNA isolated from biopsies was used to analyze the gene expression via Affymetrix GeneChip® Human Gene 1.0 ST arrays. Data was analyzed with Bioconductor software. Pair-wise comparisons of the pouch gene expression profiles with the profiles of CDi, UC and control ileums were performed (significant genes : false discovery rate $< 5\%$ and > 2 -fold change).

Results : No CAM and AMP genes were significantly differentially expressed between normal pouch and mild active CDi, and between pouchitis and moderate to severe CDi. In contrast, many significant differences in gene expression of CAMs and AMPs were found between normal pouch/pouchitis and remission/active UC, e.g. gene expression of *TECK*, *NTS*, *REG3A*, *LEAP2*, *DEFA5* and *DEFA6* was > 2 -fold significantly increased, and *SLPI* gene expression was > 2 -fold significantly decreased in both pouch groups vs. UC groups. As compared to control ileums, gene expression of 13 CAMs (*ICAM1*, *PECAM1*, *SELE*, *SELP*, *CCL2*, *CCL28*, *CXCL1*, *CXCL2*, *CXCL5*, *CXCL6*, *IL8*, *CXCR1* and *CXCR2*) and 7 AMPs (*S100A8*, *S100A9*, *S100A12*, *C1R*, *NOS2*, *PI3* and *LCN2*) was > 2 -fold significantly increased, and only 2 AMPs (*NTS* and *LEAP2*) were > 2 -fold significantly decreased in both pouchitis and moderate to severe CDi. Moreover, gene expression of *CCL28*, *NOS2* and *LCN2* was already > 2 -fold significantly increased in normal pouch vs. control ileums.

Conclusions : Our data demonstrate that patients with normal pouch have a similar gene expression profile as mild active CDi, and that the profiles of pouchitis are similar to the profiles of moderate to severe CDi. Many CAMs and AMPs are dysregulated in patients with pouchitis, and there is already upregulation of *CCL28*, *NOS2* and *LCN2* in patients with a normal pouch. This work can help to further refine therapeutic strategies in pouchitis and ileal CD.

GENE EXPRESSION DIFFERENCES BETWEEN EARLY AND POST-OPERATIVE RECURRENT/LATE ILEAL CROHN'S DISEASE. J. Van Der Goten (1), I. Arijs (1), G. De Hertogh (1), M. Ferrante (1), K. Machiels (1), L. Van Lommel (2), G. Van Assche (1), S. Vermeire (1), F. Schuit (2), P. Rutgeerts (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) University Of Leuven, Leuven, Belgium.

Introduction : Clinically, early Crohn's disease (CD) is characterized by an inflammatory phenotype, but in the course of the evolution of the disease most patients develop stricturing and/or perforating complications. Therefore, many patients need resective surgery, often repeatedly. Furthermore, immunosuppressive and biologic treatment seems more effective in the early phase of CD.

Aim : This study compared the ileal mucosal gene expression profiles of newly diagnosed CD, post-operative recurrent CD and late CD to see whether mucosal gene expression differences could explain the changing behavior of the disease over time.

Methods : Ileal mucosal biopsies were obtained from 19 patients with < 2 year diagnosis of CD (= early CD), 31 patients with recurrent CD within 2 year after ileo-colonic resection, 24 patients with > 2 year diagnosis of CD (= late CD). Total RNA extracted from biopsies was used to analyze the gene expression via Affymetrix GeneChip® Human Gene 1.0 ST arrays. Data was analyzed with Bioconductor and Ingenuity Pathway Analysis software.

Results : Unsupervised cluster analyses did not identify different clusters. Comparative analysis (significant gene probe sets : false discovery rate < 5% and > 2-fold change) between early and recurrent CD identified 57 significantly differentially expressed gene probe sets, with 29 probe sets showing an increased signal and 28 probe sets a decreased signal in the former. Twenty-six gene probe sets were significantly different between early and late CD, with 18 probe sets having an increased signal and 8 probe sets a decreased signal in early CD. An overlap of 15 significantly increased and 5 significantly decreased (ASA2, GATA4, GIP, TM4SF4, NPC1L1) gene probe sets was observed between the analyses early vs. recurrent CD and early vs. late CD. The common significantly increased gene probe sets in early vs. recurrent/late CD were mainly involved in homing of naive T lymphocytes (CCL19, CCR7, CXCR5), mobilization of Ca²⁺ (BANK1, CCL19, CCR7, CD180, CD22, FCRL3), quantity of lymphocytes (CCL19, CCR7, CD22, CXCR5, HOXA9, MS4A1) and cell death of immune cells (CCL19, CCR7, CD22, FAIM3, HOXA9, MS4A1). In contrast, only 3 gene probe sets were significantly different between recurrent vs. late CD, with DEFA6 having an increased signal and MUC5B and SLC9A2 a decreased signal in recurrent CD.

Conclusions : Our data demonstrate more differences in mucosal gene expression between early and recurrent ileal CD than between recurrent and late ileal CD. Most of the common differentially expressed genes in early vs. recurrent/late CD were increased in early CD, including genes mainly involved in immune cell trafficking and immune response, indicating that in the early phase of CD the immune reaction is predominant and therefore early aggressive therapy with biologics may be more effective.

MUCOSAL EXPRESSION OF CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 6 (CEACAM6) IN IBD. D. Staelens, I. Arijs, J. Van Der Goten, L. Van Lommel, G. Van Assche, S. Vermeire, F. Schuit, G. De Hertogh, P. Rutgeerts. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Microbial factors play an important role in the initiation and perpetuation of inflammatory bowel diseases (IBD). Barnich *et al.* showed that adherent-invasive *E. coli* (AIEC) colonizes the ileal mucosa of Crohn's disease (CD) patients by CEACAM6 dependent adhesion, suggesting that AIEC can be an entero-invasive bacteria mediating the development of IBD. In addition, TNF- α stimulation of primary enterocytes from CD patients leads to increased expression of CEACAM6.

Barnich N. *et al.* JCI, 2007, 117(6) : 1566-74.

Aim : Given the role of CEACAM6 in CD, we investigated the expression and localization of CEACAM6 in mucosa of IBD patients, and studied the effect of anti-TNF- α therapy and mucosal healing on CEACAM6 gene expression.

Methods : CEACAM6 gene expression was investigated in endoscopic-derived mucosal biopsies from 61 active IBD patients [24 ulcerative colitis (UC), 19 Crohn's colitis (CDc) and 18 Crohn's ileitis (CDi)] before and 4-6 weeks after first infliximab (IFX) infusion and in 12 normal controls (6 colon and 6 ileum). Response to IFX was defined based on endoscopic and histologic findings (Arijs *et al.*, 2009). Total RNA was extracted from biopsies and used to analyze CEACAM6 expression via Affymetrix Human Genome U133 Plus 2.0 Arrays. Microarray data was analyzed with Bioconductor software. Moderated t-statistic was used for comparative data analysis (false discovery rate < 5%). Localization of CEACAM6 was determined by immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded mucosal biopsies of IBD patients and controls.

Arijs I. *et al.* PlosOne, 2009, 4 (11) : e7984.

Results : CEACAM6 gene expression was significantly increased (> 1.5 fold) in both ileum and colon of active CD patients and in colon of active UC patients when compared to controls. IFX therapy significantly decreased the expression of CEACAM6 in colonic IBD responders showing a complete mucosal healing in comparison with the expression

at baseline, while the expression was not altered by IFX therapy in the colon of IBD non-responders nor in the ileum of CDi responders/non-responders. IHC showed a weak expression on the apical surface of epithelial cells in normal ileal mucosa, while a focal epithelial expression deep in the crypts as well as in inflammatory cells was seen in active CDi. A similar staining pattern was seen in UC colonic mucosa. In active CD and UC mucosa there was a clear expression of CEACAM6 on epithelial and inflammatory cells, whereas in mucosa of controls, mild active CD and UC in remission patients the expression of CEACAM6 was mainly restricted to epithelial cells.

Conclusions : Our data shows that there is increased expression of *CEACAM6* not only in active CDi but also in active UC and CDc. Overall, IHC of *CEACAM6* in IBD mucosa indicates that *CEACAM6* expression is not disease specific but is linked with inflammatory activity.

- I23 -

DIFFERENTIALLY EXPRESSED MICRORNAS IN INFLAMED COLON OF PATIENTS WITH ULCERATIVE COLITIS. J. Van Der Goten (1), I. Arijns (1), L. Van Lommel (2), W. Vanhove (1), V. De Preter (1), P. Rutgeerts (1), F. Schuit (2), S. Vermeire (1). (1) University Hospital Gasthuisberg, Leuven, Belgium ; (2) University Of Leuven, Leuven, Belgium.

Introduction : Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by differential expression of genes involved in immune response, barrier integrity and tissue remodeling. MicroRNAs (miRNAs) are small non-coding RNAs which function as negative post-transcriptional regulators of gene expression. Recently, studies have shown their importance in regulating genes involved in immune function.

Aim : In this study, microarray technology was used to investigate the altered miRNA expression in UC colonic mucosa.

Methods : Colonic mucosal biopsies were obtained during endoscopy from 10 active and 8 inactive UC patients, and 10 normal controls. Total RNA, including small RNA, was extracted and used to analyze the miRNA expression via Affymetrix GeneChip® miRNA 2.0 arrays. To assess gene expression, total RNA was isolated from biopsies and analyzed via Affymetrix GeneChip® Human Gene 1.0ST arrays. Data was analyzed with Bioconductor and Ingenuity Pathway Analysis (IPA) software. A false discovery rate < 5% and > 2-fold change was considered as significant.

Results : We identified 53 (26 up- and 27 downregulated) mature miRNAs and 1238 (839 up- and 399 downregulated) genes that were significantly different between active UC and controls. By IPA analysis, we observed an inverse relation between the increased miRNAs and 188 downregulated target mRNAs, and between the decreased miRNAs and 277 upregulated target mRNAs in active UC vs controls. The target mRNAs encode proteins that were predominantly involved in the biological functions : cellular movement, immune cell trafficking, cellular growth and proliferation, cellular development, and hematological system development and function. Several target mRNAs involved in these functions are positional candidate genes located on one of the UC susceptibility loci (*ERRF11*, *FCGR2A*, *IKZF3*, *PRDM1*, *SLC26A3*, *TNFRSF9*, *TNFSF8*). In contrast, no significant miRNA expression differences were found between inactive UC and controls, while 31 miRNAs (14 up- and 17 downregulated) were significantly different between inactive and active UC, with 29/31 miRNAs common to the significant miRNAs between active UC and controls.

Conclusions : This study demonstrates that altered expression of miRNAs plays an important role in the expression of immune-related genes in inflamed UC mucosa. The absence of altered expression in inactive UC supports the potential role of miRNAs in immune activation in UC.

- I24 -

BUTYRICOCOCCUS PULLICAEORUM PREVENTS INFLAMMATION IN THE TNBS-INDUCED COLITIS RAT MODEL. V. Eeckhaut, S. Maes, M. Steppe, R. Ducatelle, F. Van Immerseel, UZ Gent, Belgium.

Introduction : Conventional drug therapy in IBD has quite some disadvantages. Administration of probiotic microorganisms provides an appealing alternative to restore the microbial imbalance of the indigenous flora and to deliver compounds exerting beneficial effects into the intestinal lumen. One such compound having a potential therapeutic value against IBD is butyric acid. Butyric acid exerts a wide variety of effects on intestinal function and is produced by bacterial fermentation. Fecal microbiota from IBD patients have shown a depletion of butyrate-producing *Clostridium* cluster IV and XIVa strains. Further was noticed by Frank et al (2011) who used resected tissues from IBD patients that a shift in the genus *Butyricoccus* could be linked with the disease phenotype.

Aim : Therefore the probiotic potential of *B. pullicaecorum*, a strain belonging to the genus *Butyricoccus* was investigated.

Methods : In a first experiment we showed that *B. pullicaecorum* was superior in decreasing lesions in a TNBS-induced colitis rat model compared to *F. prausnitzii* another, in IBD microbiota, significantly depleted *Clostridium* cluster IV strain. In a second TNBS experiment the curative efficacy of *B. pullicaecorum* to control colitis was shown. Finally, the effect of daily administration of *B. pullicaecorum* was compared to currently used therapeutics (anti-inflammatory drug, corticosteroid, immunosuppressant and anti-TNFa) and showed equivalent to standard therapy in a TNBS rat model.

Conclusions : Based on these results we may conclude that this strain is an attractive candidate for a probiotic used by IBD patients.

RESEARCH GROUP OF CLINICAL NUTRITION AND METABOLISM (SBNC)

- N01 -

SCREENING AND PREDICTING MALNUTRITION IN CANCER PATIENTS UNDERGOING RADIOTHERAPY. N. Barthelemy (1), L. Trokay (2), A.F. Donneau (2), F. Princen (1), P. Coucke (1), A. Albert (2), M. Guillaume (2). (1) University Of Liege, Liège, Belgium ; (2) School Of Public Health, University Of Liege, Liège, Belgium.

Introduction : The assessment and management of nutritional problems are essential to support patients undergoing radiotherapy. Poor nutritional status may occur as the result of pre-existing conditions, treatment side effects, or as the consequence of the cancer itself or the age of the patient. Weight loss, anorexia and cachexia affect many patients with lung, head and neck, or gastro-intestinal cancer. The altered nutritional status afflicts patients and families physically, psychologically and socially. Malnutrition impairs also the outcome of the disease with increased morbidity, mortality, length of hospital stay and healthcare costs.

Aim : This prospective study aimed at developing two simple and easy tools able to screen malnutrition before radiotherapy and to assess before treatment the risk of malnutrition at the end of radiotherapy.

Methods : 151 cancer patients treated in the radiation department of the University Hospital of Liège were recruited and evaluated twice : first before radiotherapy and then at the end of treatment. The evaluation was based on a questionnaire derived from the NRS-2002 and the PG-SGA. It also contained informations specific to the malignancy and its treatment. Malnutrition status was defined using Thoresen's criteria. Multivariate stepwise regression was used to identify risk factors for malnutrition before and after radiotherapy. Baseline data were used to predict the risk of malnutrition after radiotherapy. The validity of the new tools was based on the comparison of anthropometric, biological and nutritional variables between patients classified as being at risk of malnutrition or not.

Results : Among the 144 parameters studied, only two were needed to detect malnutrition before radiotherapy. The score for detection of malnutrition before treatment is given by the following relation : $S = 2.3 - 0.05 \times \text{BMI} - 0.07 \times \text{percentage weight loss in past 3 months}$, with a threshold value of 1.1.

Two others parameters were identified to predict, before radiation, the risk of malnutrition at the end of treatment. The relation giving the score for prediction of malnutrition at the end of radiotherapy writes : $8.58 - 0.13 \times \text{arm circumference} - 0.09 \times \text{Albumin}$ with a threshold value of 1.2. The cut-off values for defining malnutrition were determined using sensitivity and sensibility analysis.

Conclusions : Two simple tools were developed with the capacities (1) to detect malnutrition in cancer patients scheduled for radiotherapy, and (2) to assess the risk for these patients to develop malnutrition during radiation treatment. Further studies are needed to validate these tools in larger samples of different types of cancers.

- N02 -

HIGHER FOOD INTAKE AND APPRECIATION WITH A NEW FOOD DELIVERY SYSTEM IN A BELGIAN HOSPITAL. E. De Wit (1), P. Goeminne (2), C. Burtin (3), Y. Valcke (1). (1) Az Nikolaas, Sint-Niklaas, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) Ku Leuven, Leuven, Belgium.

Introduction : The present structure of meal distribution in hospitals makes it complicated to offer an adequate nutritional support to each patient from admission to discharge. Patients need to estimate their appetite in advance, as ordering food takes place 24 hours beforehand. A new system of meal distribution called Meals on Wheels (MOW), allowing food ordering at mealtime and providing guidance by trained nutritional assistants, might show benefit in offering nutritional support.

Aim : In a prospective cohort trial, we investigated whether MOW improves total food intake per day and whether MOW yielded improved appreciation of food quality and increased access to food and mealtimes.

Methods : Control and intervention groups were taken from all patients hospitalized at the department of respiratory disease. Age, sex, BMI, admission weight, height, reason for admission and discharge weight were noted, as was food intake, supplements, waste per meal and daily total. For food appreciation we used the questionnaire developed by Naithani *et al.* (Naithani S., Thomas J.E., Whelan K., Morgan M., Gulliford M.C. Experiences of food access in hospital. A new questionnaire measure. Clin. Nutr., 2009, 28 : 625-30.)

Results : 83 patients were included in the control group and 106 patients in the MOW group. Mean total daily food intake was 236g higher in patients in the MOW than in controls (95% confidence interval : 163 g to 308 g). There was higher intake of oral nutritional supplements in the MOW group compared to controls ($p = 0.0012$), resulting in significantly less oral nutritional supplements wasted ($p < 0.0001$). There was also significantly less waste in the MOW group ($p < 0.0001$). In the food access and appreciation questionnaire patients appreciated MOW more than the old system in terms of choice, hunger, food quality and physical and organisational barriers.

Conclusions : We showed that the new system of MOW, where food is ordered and delivered at mealtime and guided by trained nutritional assistants, shows promising results in both appreciation by the patient as in total daily food intake.

IS EARLY ORAL ENERGETIC LIQUID DIET DANGEROUS IN ACUTE NON SURGICAL COMPLICATED DIVERTICULITIS ? G. Van Ooteghem, W. Margos, A. El Nawar, J.F. Gallez, A. Patris, J. Kirsch, A. Nakad. Ch Wapi Notre Dame, Tournai, Belgium.

Introduction : Complicated Acute Colonic Diverticulitis (ACD) is usually treated by parenteral way keeping the bowel at rest. Actually there are no clear recommendations about the route of nutrition administration. We study the safety of early feeding by oral energetic fiber free liquids diet in non-surgical complicated ACD patients.

Methods : From February 2008 to October 2011, 25 patients were admitted for complicated ACD (covered perforation and/or abscess) and took part in this prospective study. Surgical and medical assessments were performed at admission. Initial treatment was given with perfusion, intravenous antibiotics and hydric oral diet. Within 72 hours of admission, antibiotherapy was switched to oral administration for 5 up to 15 days depending on the progression of the disease. At the same time the patient received oral liquid fiber free feeding (35 kcal/kg with drinkable bottles of 200 ml of Fresubin 2 kcal drink). Solid but fiber free diet was introduced 24 h before discharge.

Results : 23/25 cases had good recovery and discharge Mean hospitalisation time was 10,4 days. One case progressed to colonic stenosis during his hospitalisation, requiring a sigmoidectomy with a one-time anastomosis and had good recovery. One patient relapsed his abscess in spite of CT scan guided drainage and required sigmoidectomy and transitory ileostomy during the same hospitalisation. The mean daily cost (nutrition and medications) for the non-surgical 23 patients was 30 euros.

Conclusions : Early enteral nutrition in complicated ACD seems not dangerous ; it could reduce the mean hospitalisation time and the cost of the treatment. Further studies comparing enteral with parenteral nutrition are necessary to confirm our hypothesis.

COST/BENEFIT OF PROBIOTICS IN ACUTE INFECTIOUS GASTROENTERITIS : SPEND TO SAVE. Y. VANDENPLAS (1), S.G. DEHERT (2), PROBIOTICAL STUDY GROUP. (1) UZ Brussel, Jette, Belgium ; (2) Ghent University Hospital, Ghent, Belgium.

Introduction : Cost/Benefit of probiotics in the treatment of acute gastroenteritis is debated.

Aim : To evaluate the cost/benefit of the synbiotic food supplement (Probiotal® : combination of 5 probiotic strains (*Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Bifidobacterium infantis*) and fructo-oligosaccharides) in the treatment of acute infectious gastro-enteritis.

Methods : 111 children (median age 40 months) were included in this randomized, prospective placebo-controlled parallel clinical trial in primary health care. All children were treated with oral rehydration solution and with the synbiotic (n : 57) reducing median duration of diarrhea with 1 day compared to placebo (n : 54). Physicians were allowed to prescribe additional medication according to what they considered as “necessary”. Cost of add-on medication and total healthcare cost were calculated.

Results : Significantly more concomitant medication (antibiotics, antipyretics, anti-emetics) was prescribed in the placebo (39 prescriptions in 28 patients) compared to the synbiotic group (12 prescriptions in 7 patients) (p < 0.001). The difference was most striking for anti-emetics : 28 versus 5 prescriptions. The cost of add-on medication in the placebo group was evaluated at 4.04 (median 4.97 (IQ 25 - 75 0 - 4.97)) versus 1.13 €/patient in the synbiotic arm (p < 0.001). If the cost of the synbiotic is considered, median cost raised to 7.15 €/patient (IQ 25-75 : 7.15 - 7.15) (p < 0.001). However, the extra consultations needed to prescribe the concomitant medication resulted in a higher health care cost in the placebo group (14.41 versus 10.74 €/patient, p < 0.001).

Conclusions : Probiotics reduce emesis in acute gastroenteritis. Although probiotics increase substantially medication cost medication (7.15 versus 4.97 €/patient), they reduce the need for add-on treatment, resulting in a reduction of health care cost with more than 30% (10.74 versus 14.41 €/patient, p < 0.001).

Probiotal study group : D. Abrassart ; F. Adriaens ; N. Balduck ; G. Biart ; J.-M. Carbonnelle ; C. Dauge ; P. David ; U. Ehrentreich ; G. Feron ; F. Garbentz ; D. Gob ; R. Graindorge ; B. Hins ; A. Hutsebaut ; S. Jacquart ; I. Jacquemart ; C. Leblanc ; A.-L. Lenoir ; C. Lietaer ; K. Logghe ; A.-S. Loicq ; S. Nouri ; J. Poncelet ; A. Van Damme ; D. Van Damme ; L. Van De Vyver ; A. Verbist ; M. Verboven ; I. Verheyden ; S. Ballard.

Invited lecture

- N05 -

EFFECTS OF AN ARTIFICIAL HYPERCALORIC AND HYPERPROTEIC FEEDING ON NUTRITIONAL STATUS IN POST-SURGERY HEAD AND NECK CANCER PATIENTS. C. Malherbe, J. De Flines, A.-M. Verbrugge, P. Demez, P. Moreau, N. Paquot, ULG, Belgium.

Invited lecture

- N06 -

DEVELOPMENT OF QUALITY OF CARE INTERVENTIONS FOR ADULT PATIENTS ON HOME PARENTERAL NUTRITION (HPN) WITH A BENIGN UNDERLYING DISEASE USING A TWO-ROUND DELPHI APPROACH. M. Dreesen, V. Foulon, K. Vanhaecht, M. Hiele, L. De Pourcq, L. Pironi, A. Van Gossum, G. Wanten, J. P Baxter, F. Joly, C. Cuerda, L. Willems. KULeuven, Brussels, Paris, Bologna, Madrid, Maastricht

- N07 -

FACTORS ASSOCIATED WITH VITAMIN D INSUFFICIENCY IN A POPULATION OF HIV-INFECTED PATIENTS. M. THEODOROU, T. SERSTÉ, M. VAN GOSSUM, S. DE WIT. ULB Saint-Pierre, Brussels, Belgium.

Introduction : The high prevalence of vitamin D (Vit. D) insufficiency in HIV-infected patients has been shown in several studies.

Aim : This study aimed : 1) to identify factors independently associated with Vit. D insufficiency in HIV positive patients and 2) to compare the levels of Vit. D according to the immune status.

Methods : An analysis of a large prospective database including 2044 patients from December 2005 to March 2011 was conducted. General parameters (season, ethnicity, gender, age, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, serum albumin, renal function, liver function tests) and variables related to HIV status (acquisition mode, CDC stage, CD4 count, viral load, duration and modalities of the treatment) were analyzed. If patients had antiretroviral therapy, the dosage of Vit. D had to be performed at least 6 months after the beginning of this treatment. First, factors independently associated with Vit. D insufficiency (Vit. D level inferior to 30 ng/mL) were investigated by logistic regression. Second, the median values of Vit. D were compared according to CD4 levels (cut off : 200 CD4/ μ l), viral load (cut off : 50 copies / ml) and the treatment modalities.

Results : The prevalence of Vit. D insufficiency was 89.2%, median : 13.8 ng/ml (4-102). Winter season, female gender, secondary and multiple lines of antiviral therapy and a longer duration of this treatment were independently associated with Vit. D insufficiency. Median rates of Vit. D were significantly lower in patients with CD4 < 200/ml (11.5 ng/ml versus 14.1 ng/ml, p = 0.0003) and in patients with antiretroviral treatment (13.3 ng/dl versus 15.1 ng/dl, p = 0.0001).

Conclusions : Vit. D insufficiency is frequent in the HIV-positive population. As previously described, the female gender and winter are associated with low Vit. D. Duration of treatment and presence of secondary and multiple lines of antiretroviral therapy are associated with this insufficiency.

Vit. D level is lower when the immune status is poor and in the presence of antiretroviral therapy.

The correction of Vit. D levels on immune status and on the therapeutic response to antiretroviral therapy should be prospectively analyze.

- N08 -

NUTRITIONAL DEFICIENCIES IN PREGNANCY ARE FREQUENT AFTER BARIATRIC SURGERY : A PROSPECTIVE COHORT STUDY. I. Guelinckx (1), G. Vansant (1), S. Bel (2), S. Pauwels (1), C. Verbeke (3), T. Vanderheyden (4), R. Devlieger (2). (1) University Of Leuven, Leuven, Belgium ; (2) University Hospital Gasthuisberg, Leuven, Belgium ; (3) ZOL, Genk, Belgium ; (4) Sint Augustinus Ziekenhuis, Antwerpen, Belgium.

Introduction : Bariatric surgery results in significantly weight loss and improves the patient's quality of life. However, nutritional deficiencies may arise due to the reduced absorptive area and/or decreased gastric acid secretion depending on the type of surgery.

Aim : to analyze the nutritional status of pregnant women with bariatric surgery

Methods : 49 women with restrictive (group R, N = 18) or malabsorption (group M, N = 31) types of bariatric surgery (age 29.9 ± 4.7 ; 39% nulliparae ; 28% smoked at inclusion) were included into a multicenter prospective cohort study. All were prescribed standard prenatal vitamin/mineral supplement and additional patient tailored supplementation if required. A fasting blood collection was performed during 1st and 2nd pregnancy trimester, and 1 non-fasting maternal collection on day of birth. The concentration of vitamin A, 25-OH-D, E, K₁, B₁, B₁₂, ferritine, folate in red blood cells, albumin, hemoglobin and mean corpuscular volume (MCV) was determined.

Results : Prepregnancy BMI in group R was significantly higher than that of group M (31.0 ± 5.7 kg/m² vs. 27.0 ± 4.8 kg/m², p = 0.010). Between both groups the mean concentrations were comparable, except for albumin and vitamin A which were significantly lower in group R. Components that showed significant shifts over pregnancy in the proportion of deficient patients who were deficient for that component are summarized in this table :

	Reference	Deficiencies (%)			
		1 st trimester	2 nd trimester	3 rd trimester	P-value
Vit A	300-650 μ g/l	19	40	58	0.005
Vit K ₁	0.8-5.3 nmol/l	94	67	57	0.002
25-OH-Vit D	7.0-60.0 μ g/l	14	7	6	0.030
B ₁	70-185 nmol/l	2	5	17	0.002
Albumin	35-52 g/l	7	21	74	0.001
Hemoglobin	11-16 g/dl	12	41	59	0.001

The number of vitamin K and D deficiencies decreased due to effective supplementation ; however still 57% of the women had low levels of vitamin K₁ delivery and 36% a 25-OH-vitamin level between 7-20 μ g/l indicating a mild deficiency. There were no vitamin E and folic acid deficiencies. Based on low levels of vitamin B₁₂, ferritine, MCV and hemoglobin the diagnosis of mild iron deficiency and pernicious anemia was made in respectively 22% and 9% of the women during the 1st trimester and 40% and 37% during 3rd trimester. No adverse event occurred expect for 1 miscarriage, 1 termination of pregnancy due to diagnosis of spina bifida and 1 intestinal hernia requiring surgery.

Conclusions : Independently of the type of bariatric surgery, pregnant women with bariatric surgery are at risk for anemia and low concentration levels of vitamin A, 25-OH-D, K₁ and B₁. A routine detection of nutritional deficiencies and a patient tailored prenatal supplementation therefore seems recommended to prevent related complications.

Invited lecture

- N09 -

EFFECT OF IV LIPID EMULSION CONTAINING SOYBEAN OIL, MEDIUM CHAIN FATTY ACIDS AND FISH OIL ON THE SERUM FATTY ACIDS PROFILE IN LONG TERM HPN PATIENTS. M. Arvanitakis, V. Lievin, A. Ballarin, A. Van Gossum, ULB Erasme, Brussels, Belgium.

- N10 -

TREATMENT OF A PATIENT WITH D-LACTIC ACIDOSIS WITH HOME PARENTERAL NUTRITION. K. Boeykens, I. Duysburgh. AZ Nikolaas, Sint-Niklaas, Belgium.

Introduction : D-lactic acidosis or D-lactate encephalopathy is a rare neurologic syndrome that occurs in individuals with short bowel syndrome or after jejunio-ileal bypass surgery. The occurring neurological symptoms can be explained by an overproduction of D-lactate acid produced by colon bacteria. Several therapeutic measures have been proposed in the literature but they have often failed to avoid repeated episodes of D-lactate acidosis. Also in this case report of a patient with proven D-lactic acidosis the recurrence of the metabolic and neurologic symptoms couldn't be resolved with dietary and medical treatment.

Aim : Due to malabsorption, restricted intake, frequent rehospitalisations and acidosis his nutritional status became seriously compromised. The aim was to start home parenteral nutrition to optimize his nutritional status and to prevent new episodes of D-lactic acidosis.

Methods : The Nutrition Support Team (NST) proposed to start with supplemental home parenteral nutrition (HPN). A single lumen tunnelled central venous catheter (Hickman) was inserted for cyclic (14 hours/day) administration. The prescribed total parenteral nutrition regimen included an all-in-one olive oil based admixture with electrolytes (1500 ml, 1800 total kcal, 9,9 g of nitrogen, vitamins and trace elements, extra sodiumchloride (4 g), extra magnesiumsulfate (1 g) and thiamine (100 mg) for 5 days/week.

Results : Three months after the initiation of the home parenteral nutrition the patient had gained 4 kg of body weight. He had sustained resolution of his neurological symptoms, his hunger for sugars was disappeared and his renal function and metabolic status had improved. The NST decided to decrease the HPN regimen from 5 to 3 days/week. Since January 2009 he has continued to do well without any complications and he has gained weight satisfactorily.

Conclusions : Home parenteral nutrition is a possible treatment option for patients with D-lactic acidosis not responding to dietary and medical treatment.

Invited lecture

- N11 -

U-SHAPED RELATIONSHIP BETWEEN CALORIC INTAKE AND OUTCOME OF CRITICALLY ILL PATIENTS. I. Carolina Reis Crosara, C. Melot, J.C. Preiser. ULB Erasme, Brussels, Belgium.

Invited lecture

- N12 -

DETERMINATION OF A SIMPLIFIED METHOD FOR ESTIMATING RESTING ENERGY EXPENDITURE IN CRITICALLY ILL PATIENTS. M. Hviid Simonsen (1,2), H. Steen Andreassen (2), J.C. Preiser (1). (1) ULB Erasme, Brussels ; (2) Aalborg University, Denmark.

POSTERS

- N13 -

COMPARISON OF TWO EXTENSIVELY HYDROLYZED FORMULAS FOR THE TREATMENT OF CHILDREN WITH COW'S MILK PROTEIN ALLERGY. Y. Vandenplas (1), Althera study group. (1) Uz Brussel, Jette, Belgium.

Introduction : Cow's Milk Protein Allergy (CMPA) requires an extensively hydrolyzed formula in formula-fed babies
Methods : 86 infants with symptoms of mild/moderate CMPA, < 6 months, are included in this DBPCR multi-center study, were randomized to receive one of two extensive hydrolysates during 8 months. The non-inferiority boundary was defined in advance. A positive treatment difference points in the harmfully direction, where as a negative difference points in the beneficial direction.

Results : A total of 75 patients were included in the Intent-To-Treat dataset (N = 40 in experimental, N = 35 in control group). The overall CMPH(Hypersensitivity)-score with both groups pooled, showed a significant change from baseline to 4 weeks -8.04 (95%CI = -8.87, -7.21, $p < 0.001$), thereby showing construct validity of the CMPH-score. The change in CMPH-score observed in the experimental group was -8.45 (SD 3.85) and -7.57 (SD 3.33) in the control group. The treatment difference was -0.879, the 95% CI (-2.79, 1.03) showing a statistically significant non-inferiority since the non-inferiority boundary was excluded (+3 CMPH points). Normal growth was observed in both groups. Both formulas were safe and well tolerated. There were no adverse events.

Conclusions : This new extensively hydrolyzed formula is safe and effective for infants with CMPA.

- N14 -

SPECIFIC HUNGER- AND SATIETY-MEDIATED TUNING OF GUINEA PIG ENTERIC NERVE ACTIVITY. L. Roosen, W. Boesmans, M. Dondyne, I. Depoortere, J. Tack, P. Vanden Berghe. Translational Research Center For Gastrointestinal Disorders (Targid), KUleuven, Leuven, Belgium.

Introduction : The regulation of hunger and satiety is a multifactorial, mainly centrally regulated process, but there is convincing evidence that gastro-intestinal motor activity, hormone-fluctuations and luminal factors also contribute to appetite signaling.

Aim : We used a guinea pig model to investigate the effects of fasting and feeding on intestinal peristalsis and enteric nerve activity.

Methods : Animals were fasted for 20 hours, and a subgroup was refed 3 hours prior to the experiment. We tested whether peristaltic activity differed in ileum taken from fasted and refed guinea pigs (N = 19/group), and used Ca²⁺-imaging to investigate whether the feeding state altered neuronal Ca²⁺-signaling (N = 3-6/group).

Results : We found that pressure-induced peristaltic waves occur at a higher frequency in ileal segments of refed guinea pigs (p = 0.028). Ca²⁺-imaging in longitudinal muscle - myenteric plexus preparations (N = 3-4) revealed elevated responses to orexigenic ghrelin in fasted animals (p = 0.013), but the percentage of responders to anorexigenic CCK-8 was higher in refed animals (p < 0.001). High K⁺-evoked Ca²⁺-peaks were consistently higher in refed animals (p < 0.001), suggestive of a hyperexcitable state. We investigated whether a humoral factor was involved by exposing cultured myenteric neurons (N = 3-6) to fasted or refed guinea pig serum and found that the cells acutely exposed to refed serum displayed a higher Ca²⁺-peak (p < 0.001) and more neurons became spontaneously active (p < 0.001). Interestingly, we found that ghrelin-responses were higher in cells incubated with fasted serum (p = 0.023), whereas serotonin-responses proved to be higher in cells incubated with refed serum (p = 0.005). Centrifugal ultrafiltration (MW cut-off 3K) removed a feeding state-independent along with a feeding state-dependent factor from the serum, the latter reverting amplitudes of refed serum- to fasted serum-responses (N = 3). Since glycemia in refed guinea pigs was almost 3 times as high as in fasted guinea pigs, we tested whether these glucose-levels play a role in the fasted-refed differences in neuronal Ca²⁺-signaling (N = 3). We found that ghrelin evoked higher amplitudes in cells incubated with fasted glucose-levels (p < 0.001), whereas CCK-8 (p = 0.005), serotonin (p = 0.006) and high K⁺ (p < 0.001) evoked higher responses in cells incubated with refed glucose-levels.

Conclusions : These observations indicate that the feeding status of an animal remains imprinted *ex-vivo* and humoral feeding state-related factors are implicated. Although the molecular link with hyperactivity is not entirely elucidated yet, we found that a glucose-dependent pathway is involved, probably in combination with other factors. Although responses in the refed state are generally increased, the reduced neuronal responses to ghrelin prove that the effect is signaling pathway-specific and suggests feeding state-related differential tuning of excitability.

RADIOLOGY, PATHOLOGY AND NUCLEAR MEDICINE

Invited lecture

- P01 -

RADIOLOGIC IMAGING IN NET : PITFALL AND CHALLENGES. Annet. UCL St Luc.

Invited lecture

- P02 -

THE DIAGNOSTIC AND THERAPEUTIC ROLE OF NUCLEAR MEDICINE IN NEUROENDOCRINE TUMORS. C. Deroose. Uz Leuven, Leuven, Belgium.

Nuclear medicine plays both a diagnostic and therapeutic role in the management of patients with neuroendocrine tumors (NET). The diagnostic part focuses on the detection of NETs and their metastases based on molecular characteristics of these tumors. These include (i) overexpression of peptide receptors on the cytoplasmic membrane, including the somatostatin receptors (SSR) (Octreoscan®, [68Ga]DOTATOC-PET) and others such as the glucagon-like peptide 1 receptor and the bombesin receptor ; (ii) increased uptake of bioactive amine precursors ([18F]DOPA-PET) ; (iii) increased glucose metabolism in poorly differentiated NET ([18F]FDG-PET). A brief overview of these different techniques and their diagnostic performance will be provided and guidelines regarding SSR imaging will be presented. The therapeutic role of nuclear medicine is based on the overexpression of the SSR and the availability of stabilized radiolabeled SSR-ligands that will accumulate in NET cells upon intravenous administration. Modern protocols use the beta-emitting Yttrium-90 or Lutetium-177-labelled somatostatin analogues. These therapies are associated with little side effects (mainly hematological grade 1 tot 2 and a decrease in kidney function). The latter is prevented by co-infusion of unlabeled amino acids. Objective response rates have been around 50 to 60% disease stabilization, 20 to 30% at least partial response and ~20% progressive disease. Symptomatic control is observed in a large fraction of patients and results in improved quality of life. Recent clinical data, as well as novel targeting strategies and therapeutic protocols will be presented.

Invited lecture

- P03 -

MEDICAL TREATMENT OF NET. A. Hendlisz, ULB Bordet, Brussels, Belgium.

Invited lecture

- P04 -

PATHOLOGIC CLASSIFICATION OF NET. G. Rindi. Rome, Italy.

- P05 -

SIGNET RING CELLS IN ESOPHAGEAL AND GE JUNCTION CARCINOMAS : A STUDY OF BIOLOGICAL BEHAVIOR. P. Naftoux, J. Dhaenens, G. De Hertogh, J. Moons, W. Coosemans, G. Decker, H. Decaluwe, D. Van Raemdonck, T. Lerut, P. De Leyn. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : There has been much controversy surrounding the biologic behavior and prognosis of esophageal signet ring cell containing carcinomas (SRCs).

Aim : To study the biologic behavior of SRCs, we compared the clinicopathologic features and prognosis of SRCs with other adenocarcinomas (AC) of the esophagus and gastroesophageal junction.

Methods : From our prospectively built database we retrieved all adenocarcinoma from 1990 till 2009 who underwent primary surgery with R0 resection. Seven hundred seventy-nine patients were included for further analysis. Pathology reports mentioning signet ring cells (n = 82) were reviewed by our pathologist and, after confirmation, tumors were classified into two groups according to WHO criteria (> 50% SRC or < 50% SRC). The remaining 697 AC patients served as control group.

Results : The fraction of SRC was 10.5% ; 25 patients (3.2%) showed > 50% SRC whereas 57 patients (7.3%) showed < 50% SRC. Fifty-four% of AC, 72% of SRC < 50% and 72% SRC > 50% presented with positive lymph nodes. The mean number of positive lymph nodes was not significantly different between AC and SRC < 50% (5.85 vs. 6.61 ; p = 0.46) but significantly different between AC and SRC > 50% (5.85 vs. 8.89 ; p = 0.049). Likewise, a similar distribution was found for pN3's (> 6 positive LN's) : 30% in AC, not significantly different from pN3's in SRC < 50% (37%, p = 0.39) but highly significant different from SRC > 50% (61%, p = 0.006). Overall cancer specific 5-year survival in AC was 57% (mean survival 120 mo.), where it was 30% in all SRC (mean 47 mo.)(p < 0.001). In < 50% SRC this was 36% (mean 52 mo.) and in > 50% SRC this was 16% (mean 32 mo.) (N.S. p = 0.10). In lymph node positive patients there was no significant difference in cancer specific 5-year survival between AC and SRC < 50% (p = 0.87) but a highly significant difference between AC and SRC > 50% (p < 0.001). SRC showed a higher recurrence rate (56% versus 42% for AC ; p = 0.003) ; this higher rate was not significantly different for metastasis (44% versus 35% ; p = 0.125) but highly significant for local recurrence (29% versus 16% ; p = 0.002). The occurrence of loco-regional recurrence is most prominent in SRC > 50% (44%) and less in SRC < 50% (23%).

Conclusion : Our results suggest that most esophageal carcinomas containing signet-ring cells are aggressive neoplasms associated with a poorer prognosis than other AC after primary esophagectomy, although cancer specific 5-year survival still reaches 30% in SRCs. This worse outcome is related to the subgroup of SRC > 50%, showing a higher mean number of positive lymph nodes and in particular pN3's and having a higher occurrence of loco-regional recurrence. The subgroup of advanced SRC < 50% seems to have a similar behavior and survival rate as advanced AC. Therefore precise preoperative staging as well as a more precise assessment of the proportion of SRC are mandatory to guide adequate therapeutic strategies.

- P06 -

ADVANCES IN THE TREATMENT OF ZOLLINGER-ELLISON SYNDROME. REVIEW OF 45 CASES AND ANALYSIS OF SURVIVAL. N.D. Nguyen Quynh (1), I. Borbath (1), P. Deprez (1), J.F. Gigot (1), P.J. Kestens (1), P. Maldague (2), D. Maiter (1), M. Schapira (3), S. Pauwels (1), R. Fiasse (1). (1) Clinique Universitaire St Luc, Brussels, Belgium ; (2) Clinique Saint-Luc, Namur, Belgium ; (3) Hôpital De Jolimont, Haie-Saint-Paul, Belgium.

Introduction : Zollinger-Ellison syndrome (ZES), a severe ulcer disease, is due to gastrinomas of the pancreas or duodenum, either sporadic (SG) or associated with multiple endocrine neoplasia type I (MEN-I). Previous therapy, i.e. total gastrectomy (TG), evolved to surgical and medical treatment of the tumour, since the use of proton pump inhibitors (PPI).

Aim : We reviewed our therapeutic experience and analyzed prognostic factors of survival.

Methods : Diagnosis of ZES in 45 patients treated since 1965 was based on high basal acid secretion associated with basal hypergastrinemia (> 1000 pg/ml) or positive secretin provocative test. Plasma chromogranin A (CgA) and pancreatic polypeptide (PP) were also determined. After 1983 most patients were treated with PPI. Oncologic treatments consisted of surgery alone, somatostatin (SMS)-analogues, a-interferon, metabolic radiotherapy (PRRT), chemotherapy and combined therapies in case of progressive disease. Non-parametric statistics were used, and univariate and multivariate assessment of prognosis factors using SPSS software.

Results : At diagnosis, the 45 patients (21 women, 24 men) had a median age of 48 years (25-76). There were 27 (60%) cases of SG and 18 (40%) cases of MEN-I. Other tumours were associated with MEN-1 : parathyroid adenomas in 14 cases (78%), pituitary tumours in 7 (39%), insulinoma in 3 (17%) and carcinoid gastric tumours in 3 (17%). Metastases at diagnosis were present in 17 cases (63%) of SG and in 2 cases (11%) of MEN-I (P < 0.05). At last follow-up (Nov 2011), 13/45 (29%) patients were still alive. The median survival of 43 patients was 146 (103-188) months. The survival of the 17 patients with MEN-I was longer than the SG patients : 276 months (138-413) and 85 months (23-147), respectively (P = 0.6). CgA, available at diagnosis for 21 patients, was 3xULN in 14. No difference in survival was observed between normal and pathologic CgA patients. TG was performed in 5 patients (4 SG ; 1 MEN-I) with one postoperative death and a median survival of 336 months (0-560). Resection of gastrinoma was performed in 11 patients with SG who had a median survival of 157 months (0-420), and in 13 patients with MEN-I who had a median survival of 276 months (143-408). Eighteen patients with SG and 12 patients with MEN-1 were treated with SMS-analogues with no difference in survival. Additional a-interferon was administered in 14 patients with progressive disease under SMS-analogues. Chemotherapy with streptozotocine/5-fluorouracile was used in 7 patients. Metabolic radiotherapy was used in 5 patients. The occurrence of other malignancies occurred in 6 patients with MEN-1. Univariate analysis showed that presence of metastases at diagnosis, surgical resection, diagnosis before 1990 and absence of chemotherapy treatment were the prognostic factors to be evaluated in a multivariate analysis ; of these, metastases at diagnosis (HR 5, 1.9-13, p < 0.01) and absence of chemotherapy (HR 2.8, 1.2-6.7, p < 0.01) were the only prognostic factors of survival.

Conclusions : There was a trend towards longer survivals in patients with MEN-I vs. patients with SG. The latter had more frequently metastases at diagnosis than patients with MEN-I. Assessment of prognostic factors demonstrated that metastases at diagnosis and absence of chemotherapy were significantly related to survival.

BIOPSIES INSUFFICIENTLY PREDICT FINAL HISTOLOGY AFTER ENDOSCOPIC RESECTION IN BARRETT'S NEOPLASIA. E. Werbrouck, G. De Hertogh, H. Willekens, X. Sagaert, R. Bisschops. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Endoscopic resection (ER) with or without ablation is the first choice treatment for early Barrett's neoplasia. Adequate staging is important to assure a good oncological outcome. In particular for primary ablative therapy, it is important to rule out any cancer.

Aim : To investigate the accuracy of pre-ER biopsies in patients who undergo ER for high-grade dysplasia (HGD) or early adenocarcinoma (AC) in Barrett's Esophagus (BE) and the cardia.

Methods : Between November 2005 and June 2011, 145 ERs in 120 patients with HGD and/or EAC were performed. All ER data in particular Paris classification of the lesions and histology were prospectively collected. Histological slides were assessed by at least two expert pathologist using the Vienna classification. Worst pre-ER and ER histology were compared to assess diagnostic accuracy of pre-ER biopsies in predicting final ER pathology. For 8 procedures insufficient data were available. Upgrading/downgrading was defined as any more/less severe histological grading on the ER specimen in comparison to pre-ER biopsy.

Results : Pre-ER biopsies revealed no dysplasia (ND) in 4 cases, 5 low grade dysplasia (LGD), 54 HGD and 74 Adenocarcinoma (AC). ER specimens were as follows : ND 12 cases, 15 LGD, 22 HGD, 65 intramucosal carcinoma (IMC), 22 submucosal carcinoma (SMC) and one AC with uncertain invasion depth. The accuracy of pre-ER biopsies in predicting final histology after ER was 61%. ER changed the pre-treatment diagnosis in 53 of the 137 procedures (39%) with downgrading in 25 cases (19%) and upgrading from HGD or even LGD to IMC or SMC in 28 cases (20%). In the majority of upgraded cases, a visible lesion according to the Paris classification (Is, Ila or Iic) could be detected (22/28 or 79%)

Conclusions : The diagnostic accuracy of esophageal biopsies alone in predicting final pathology in Barrett's dysplasia is only 61%, and insufficient to rule out cancer. The majority of upgraded lesions are detectable as slightly elevated lesions according to the Paris classification. When ablative therapy is considered in HGD Barrett a meticulous inspection for and removal of all small visible lesions is mandatory to ascertain a safe oncological outcome.

I-SCAN DETECTS MORE POLYPS IN LYNCH SYNDROME PATIENTS : A PROSPECTIVE CONTROLLED RANDOMIZED TRIAL. R. Bisschops, S. Tejpar, H. Willekens, G. De Hertogh, E. Van Cutsem. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Narrow-band imaging and chromo-endoscopy have been reported to detect more polyps in comparison to (high definition) white light endoscopy (HDWL) in hereditary non-polyposis colonic cancer (HNPPC) patients in a back-to-back study. However, no randomisation for the order of imaging method was applied. I-scan with tone enhancement (TE) is a new form of digital chromo-endoscopy.

Aim : We aimed to assess the additional value of the I-scan TE system in polyp detection in HNPPC.

Methods : 49 HNPPC patients underwent a back-to-back colonoscopy (Pentax EC3890Fi) with two imaging modalities and were randomized in 2 groups. Group 1 underwent HDWL first followed by I-scan, group 2 I-scan first followed by HDWL. For HDWL, standard settings and for I-scan, the I-scan 2 preset (surface enhancement +4, TE c-modus) were used on a Pentax Hi-line processor. Patients with clinical diagnosis or proven gene abnormality for HNPPC were included in the study. Patients with known neoplasia or colectomy with < 50 cm remaining colon were excluded. Bowel preparation after PEG solution was assessed using the Bristol Bowel preparation scale (BBPS). Patients with a BBPS < 6 were excluded. Total inspection time was calculated after subtracting the time needed for polypectomy. Lesion detection rate (total number of lesions for each method/total procedures) and the miss rate (= number of lesions/adenomas detected during second inspection/total number of lesions/adenomas in that group) were assessed.

Results : 25 and 24 patients were included in group 1 and 2 respectively (mean age 45.3 +/- 1.69, 25 male). There was no difference in age or BBPS between the two groups. The lesion detection rate was 0.73 ± 0.19 for I-scan and 0.36 ± 0.12 for HDWL (p = 0.095). In group 1, 14 lesions were detected with HDWL first and 15 with subsequent I-scan. In group 2, 21 lesions were detected with I-scan first and 4 with subsequent HDWL. The miss rate for endoscopic lesions was 52% and 16% respectively and was significantly different in favor of I-scan (p < 0.01 95% CI 0.38 to 0.87). Similarly, 5 adenomas were detected with HDWL vs 7 with I-scan in group 1. In group 2, I-scan first detected 13 of the 15 adenomas, resulting in a miss rate of 58% and 13% respectively (p < 0.05 95% CI 0.24 to 0.96). The higher miss rate in group 1 was not due to a shorter inspection time. On the contrary, in general the second inspection time was significantly shorter than the first one (407 +/- 19 vs 503 +/- 24 sec, p < 0.01). The difference in inspection time during the second pass was not significantly different between group 1 and 2 (427 +/- 24 vs 384 +/- 32 sec resp p = 0.27).

Conclusions : In patients with HNPCC the miss rate for polyps is significantly reduced during colonoscopy performed with I-scan in comparison to HDWL, independently from inspection time. These findings add to the evidence that HNPCC may be a good indication for (virtual) chromo-endoscopy.

Invited lecture

- P09 -

COLORECTAL CANCER. G. Demoulin, ULg, Belgium.

Invited lecture

- P10 -

HEPATOBILOPANCREATIC CANCER. C. Verslype, KULeuven, Belgium.

Invited lecture

- P11 -

OESOPHAGASTRIC CANCER. K. Geboes, UZ Gent, Belgium.

- P12 -

PANCREATIC ENDOCRINE TUMORS : AN OVERVIEW OF TYPICAL AND ATYPICAL PRESENTATIONS AT IMAGING. K. Buccauw, D. Vanbeckevoort, D. Bielen. University Hospital Gasthuisberg, Leuven, Belgium.

Pancreatic endocrine tumors (PETs) are mainly well-differentiated pancreatic or peripancreatic tumors which exhibit an endocrine differentiation. In the past it was assumed that PETs arose from the islets of Langerhans (Islet Cell Tumors), but today we rather suspect that they arise from pluripotential stem cells in ductal epithelium. These neoplasms usually occur sporadically but can be associated with genetic syndromes such as von Hippel-Lindau disease, multiple endocrine neoplasia type 1 (MEN 1), tuberous sclerosis, and neurofibromatosis type 1. PETS are classified based on several parameters : size, clinical symptoms (functioning or nonfunctioning), biological behavior and histological features. In this lecture we attempt to give a brief overview of some typical and atypical presentations of PETs at imaging, in correlation with their histological and clinical presentation.

- P13 -

PERITONEAL CALCIFIED NODULE : A CONDITION WE DO NOT HAVE TO UNDERESTIMATE. E. Danse (1), D. Leonard (2), A. Dragean (2), A. Kartheuser (3), A. Jouret-Mourin (2). (1) Université Catholique De Louvain, Brussels, Belgium ; (2) Ucl Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (3) Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

A pathological-radiological confrontation illustrating preoperative CT and MRI findings frequently considered as non-specific in ordinary situations can be crucial in some cases. A 59 year old man was treated for a neuroendocrine tumor of the rectum. Its preoperative work up included a colonoscopy, an endorectal ultrasound, a pelvic MRI and a chest and abdominal CT-scan. At endorectal ultrasound, the lesion was staged T2. The lesion was not visible with CT and MRI but on both procedures a calcified centimetric nodule was visible close to the location of the rectal tumor. On imagery, this feature is frequent and insignificant but given the past-history, a lymph node metastasis was highly suspected. The patient was operated and this data was confirmed by the pathologist. This case highlights the importance of a radiological and pathological confrontation to improve the quality of diagnosis.

FLUCTUATING CHOLESTASIS IN A 45-YEARS-OLD MAN WITH POLYCYSTIC KIDNEY DISEASE. L. Verset, N. Nagy, C. Moreno, M. Adler, C. Matos, P. Demetter. Erasme Hospital, Brussels, Belgium.

A 45-year-old man was referred for biologic cholestasis. Laboratory data showed increased levels of g-glutamyltransferase (158 UI/l ; N : 8-61 UI/l) and alkaline phosphatase (147 UI/l ; N : 53-128UI/l). The patient's medical history was notable for systemic sclerosis treated by interferon, a familial renal polycystosis, a left jugular vein agenesis and a right jugular vein stenosis. There was no report of alcohol use.

Abdominal MRI revealed a dysmorphic liver with fibrotic areas, and peribiliary and renal cysts. Oesophageal varices of grade I were highlighted at gastroscopy.

A percutaneous liver biopsy was performed. The histological examination displayed a normal lobular architecture with expanded portal tracts in which many ectatic bile ducts could be seen. Reticulin staining showed a nodularity similar to lesions of regenerative nodular hyperplasia. A final diagnosis of congenital hepatic fibrosis (CHF) was, however, proposed.

CHF is a tardive abnormal development of the biliary tract arising from an insufficient remodeling of the ductal plate. There are no isolated genetic alterations but CHF seems to be associated with autosomal recessive polycystic kidney disease. Discriminating CHF from cirrhosis of other origin is a radiologic challenge. Radiologic features can help to distinguish between these 2 entities : the size of the medial segment is normal or enlarged in patients with CHF, but usually small in patients with cirrhosis due to other causes. Furthermore, patients with CHF present other organ abnormalities such as renal cysts.

UNUSUAL LIVER MASSES IN TWO WOMEN. H. Dano (1), I. Theate (1), M. Van Den Eynde (1), M. Lambert (2), J. Rahier (3), L. Annet (2), C. Sempoux (3). (1) Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (2) Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium ; (3) Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Differential diagnosis of liver masses can be challenging for the radiologist and even for the pathologist, especially if only a liver biopsy is available.

Aim : Within one year, we were confronted to such a difficult situation in two patients. Our aim is to report these two cases.

Methods : The first one is a 70-year-old lady with a past history of breast cancer who developed a 4 cm mass in the liver, suspicious for hepatocellular carcinoma or metastasis on MRI. The second patient, a 61-year-old woman with a past medical history of hemolytic anemia, presented a 2 cm mass within the liver, suggestive of a cholangiocarcinoma on MRI. In the first patient, a liver biopsy was performed and in the second one the tumor was removed by surgery.

Results : Histologically, both lesions showed a diffuse parenchymal destruction by a dense lymphoid infiltrate including distinct germinal centres. Numerous plasma cells were observed and variable fibrosis was found. Immunohistochemistry demonstrated the polyclonal nature of the lymphocytes. We also found florid ductal lesions with some granulomas within the portal tracts in the second patient. A diagnosis of reactive lymphoid hyperplasia "pseudolymphoma" type (PL) was proposed. The clinical evolution of both patients is so far reassuring, with stability of the lesions in the first woman, and no recurrence in the second one (follow up of 1 year and 3 months respectively).

Conclusions : Hepatic reactive lymphoid hyperplasia "PL" type is a controversial entity, rare and poorly documented (!), difficult to diagnose clinically and radiologically because mimicking malignant tumors. Histopathology and immunohistochemistry give the nature of the lesion after a consistent needle biopsy or a surgery. An association has been reported with both autoimmune and neoplastic conditions.

Reference :

1. Yoh Zen, Takahiko Fujii and Yasuni Nakanuma. Modern Pathology, 2010, 23 : 244-250.

WHICH GRADING SYSTEM TO BE APPLIED IN PANCREATIC ENDOCRINE TUMORS : SEMI-QUANTITATIVE VS QUANTITATIVE. B. Weynand, I. Borbath, C. Sempoux, J.F. Gigot, A. Jouret-Mourin. Ucl Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Pancreatic endocrine tumors (PETs) are rare, representing 1-2% of all pancreatic tumors. ENETS and WHO have defined criteria for a grading system for PETs to assess their prognosis based on mitotic count or Ki67 label-

ing index (Ki67-LI). Mitotic count should be done on at least 40 fields at 40x magnification (10 HPF = 2 mm²) in areas of highest mitotic density. Ki67-LI should be assessed in 500-2,000 tumor cells in areas of highest nuclear labeling. This recommendation is difficult to implement in routine practice, because of the time-consuming aspect. Therefore many pathologists use a semi-quantitative approach for grading.

Aim : To compare semi-quantitative to quantitative assessment of PETs 'grading system.

Methods : 36 surgically resected PETs from 35 patients were assessed after Ki67 immunohistochemistry. All were counted by 2 independent pathologists (BW = observer 1 and AJM = observer 2) (mean number of counted cells : 200149 ; minimum : 1480, maximum : 2130). The semi-quantitative assessment was done by scanning the tumor at low and high power. The same pathologists did this second evaluation after a 6 months interval.

Results : Semi-quantitative assessment was not concordant for 9 of 36 tumors (25%). Eight cases were discordant between G1 and G2 and one between G2 and G3. In 7 cases, observer 2 was higher than observer 1 and in 2 cases it was the opposite. When assessing Ki67-LI, a discordance was observed in 1 case out of 36 PETs (2.8%), in which observer 1 classified the tumor as G1, whereas for observer 2, it was a G2. Interestingly, this case was discordant in both grading systems. Inter-observer correlation was very good and statistically highly significant for Ki67-LI (Pearson r₂ = 0.95, p < 0.001). Whereas, it was lower in the semi-quantitative assessment (Pearson r₂ = 0.57, p < 0.001).

Conclusions : The grading system proposed by ENETS and WHO for PETs is difficult to apply in routine practice because it is time-consuming. Therefore, many pathologists tend to use a semi-quantitative approach of mitotic count or Ki67 positivity in PETs.

In this study on 36 PETs, it is shown that inter-observer correlation is much better when counting Ki67-LI. We therefore advocate all pathologists to count effectively Ki67 positive cells to assess prognosis of PETs.

Invited lecture

- P17 -

LYMPHATICS IN IBD, FRIEND OR FOE ? J.F. Rahier. Ucl, Mont-Godinne, Belgium.

Abnormalities in lymphatic vasculature have been noted in the original descriptions of CD. Nowadays, the lymphatic system is re-emerging as a critical player in inflammatory and immune processes. Recent studies report lymphangitis, lymphangiogenesis, bacterial infiltration and lymph node infection, immune cell trafficking, and fat-wrapping in CD suggesting altered lymph drainage and implicating the lymphatic system as a player in IBD.

Both forms of IBD show lymphatic remodeling with intestinal vessels proliferating in each layer of the inflamed small and large bowel. This remodeling is also found in non-inflamed sections of IBD suggesting that lymphangiogenesis can be present prior to the appearance of mucosal features of inflammation. Whether lymphangiogenesis in IBD is pathological or protective is an open question.

A close association between inflammation, granulomas, tertiary lymphoid follicles, and the lymphatic vasculature is noted in CD together with lymphangiectasia and lymphocytic perilymphangitis. Beside, unexpected distribution of lymphatic vessels is observed in both forms of colonic IBD. Lymphatic vessels are seen throughout the inflamed colonic mucosa and reach the upper third of the lamina propria, challenging established dogma regarding the absence of lymphatic vessels in colonic mucosa. This may impact luminal antigen sampling and uptake, as well as migration of antigen-presenting cells.

The role of lymphatic vessels in the pathophysiology of IBD remains uncertain. Lymphatic trafficking between the primary sites of inflammation and local draining lymph nodes certainly plays an important role in limiting progression of inflammation. Studies of decoy receptors bring evidence of the lymphatic system as an active player in immune function during IBD. Indeed, D6-deficient mice are more susceptible to DSS colitis and fail to resolve colitis with higher circulating levels of chemokines. Experimental evidence strongly supports the idea that lymph stasis from lymphatic vessels promotes fat accumulation. Disrupted or malfunctioning lymphatic drainage leads to massive edema, increased numbers of fibroblasts, adipocytes, and keratinocytes in the skin and subcutaneous tissue and a mild inflammation. No data are available on the clearance capacities of lymphatic vessels in IBD. Some of the lymphatics might be immature or nonfunctional, as described in tumour-associated lymphangiogenesis. Macroscopic remodeling of mesenteric and serosal lymphatic vessels occurs during CD. Fat-wrapping which is a feature of Crohn's disease patients undergoing ileal resection and which correlates with the extent of transmural inflammation may be another consequence of lymphatic contractile dysfunction or disruption in CD.

Improved knowledge and appreciation of the roles that the lymphatic system plays in immune cell trafficking, infection, fat transport and edema resolution is necessary to better understand the pathogenesis of chronic inflammatory conditions such as CD and may provide the basis for new therapeutic strategies.

ENDOSCOPIC ULTRASOUND-GUIDED FINE-NEEDLE ASPIRATION IN THE DIAGNOSIS OF PANCREATIC LYMPHOMAS. S. Saadi, I. Theate, B. Weynand. Université Catholique de Louvain, Brussels, Belgium.

Introduction : Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is an important tool to diagnose solid pancreatic lesions.

Aim : We evaluated the demographics, histopathologic characteristics, and clinical course of pancreatic lymphomas.

Methods : A review of our database between 2000 and 2010 identified 11 pancreatic lymphomas out of 2360 EUS-FNAs. Data collected included site, tumor size, histological subtype, stage, and outcome. Follow-up ranged from 3 to 78 months (mean 26 months).

Results : Of the 11 lymphoma patients, 6 male and 5 female patients were diagnosed as 8 primary pancreatic lymphomas (PPLs) and 3 secondary pancreatic lymphomas (SPLs). Tumors were predominantly located in the head and body of the pancreas and were revealed by jaundice. Primary pancreatic lymphoma patients were younger with a mean age of 60 years (range, 43-79 years) for PPL and a mean age of 67 years (range 59-77 years) for SPL, with significantly larger tumors in PPL (mean size 54.9 mm ; range, 27-85 mm) than SPL (mean size, 18 mm ; range 16-20 mm) and a female predominance (female to male ratio, 1.61). Histopathologic findings were diffuse large B-cell lymphoma (DLBCL, n = 7), follicular lymphoma (n = 2), marginal zone lymphoma (n = 1), and anaplastic large cell lymphoma (ALCL, n = 1). Patients with SPL seemed much more likely to die of their disease. Interestingly, in the PPL group, patients who died had immunoblastic variants of DLBCL and ALCL ; the 6 remaining patients were alive and disease-free at last follow-up.

Conclusions : This study reports one of the largest series of pancreatic lymphoma diagnosed by EUS-FNA and demonstrates its accuracy in diagnosing. DLBCL was the most frequent encountered histologic subtype. In our series, outcome was better for PPL compared to SPL.

PATHOLOGICAL RESPONSE AFTER PREOPERATIVE TREATMENT FOR RESECTABLE LIVER COLORECTAL METASTASES. G. Pairet, V. Lannoy, A. Jouret-Mourin, Y. Humblet, C. Hubert, J.F. Gigot, C. Sempoux, M. Van Den Eynde. Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

Introduction : Pathological tumor response (PTR) of colorectal liver metastases (CLM) after preoperative chemotherapy and curative surgery has recently been recognized as a promising prognosis factor for patient survival. Adjunction of bevacizumab to chemotherapy seems to increase the PTR. Nevertheless, no data are available for cetuximab's use.

Aim : The aim of this study was to analyse whether adjunction of cetuximab to preoperative chemotherapy could increase the PTR evaluated by the Rubbia-Brandt pathological classification of tumor regression grade (TRG) (1).

Methods : Patients curatively operated of their CLMs after 1 line of preoperative chemotherapy regimen between 01/01/2008 and 15/04/2011 at Cliniques Universitaires St-Luc, were retrospectively included in this study. Patients with multiple lines of treatment (= 2) before surgery were excluded unless they underwent resection after > 6 months of complete remission before liver recurrence. Control group was defined as operated patients without any preoperative treatment between 01/01/2009 and 15/04/2011. PTR of all resected CLMs was evaluated according to the Rubbia-Brandt pathological TRG classification. Overall PTR (OPTR) included major (TRG 1 and 2) and minor response (TRG3). Patients with multiple CLMs showing different grades of TRG were categorized according to the morphological aspect of the metastasis having the worst response (highest TRG) (1).

Results : Sixty-seven patients (52.2% men, 30% rectum, median age 61.4 y-old, 21% metachronous metastases, 181 total CLMs with a mean of 2.7 CLMs/pts) were eligible and separated into 4 groups : chemotherapy alone (CHIM ; 13 pts, 19.4% ; 33 CLMs), chemotherapy + bevacizumab (BEVA ; 23 pts, 34.3% ; 81 CLMs), chemotherapy + cetuximab (CETUX ; 10 pts, 14.9% ; 29 CLMs) and control group (CONTR ; 21 pts, 31.3% ; 38 CLMs). Among the 181 analysed CLMs, OPTR was seen in 51.5, 49.4, 62.1 and 2.6% respectively in the CHIM, BEVA, CETUX (NS) and CONTR group and > 50% of CLM fibrosis was described in 36.4, 31.3, 58.6 and 5.3% respectively in the CHIM, BEVA, CETUX (p = 0.033) and CONTR group. Among the 67 analysed patients, OPTR was seen in 38.5, 43.5, 50 and 4.8% respectively in the CHIM, BEVA, CETUX (NS) and CONTR group.

Conclusions : The addition of cetuximab in a preoperative chemotherapy regimen seems to increase the OPTR. Further investigations and correlation with patient clinical outcome are mandatory before any conclusion.

Reference :

1. Rubbia-Brandt L., Giostra E., Brezault C., *et al.* : Importance of histological tumor response assessment in predicting the outcome in patients with colorectal liver metastases treated with neo-adjuvant chemotherapy followed by liver surgery. *Ann. Oncol.*, 2007, 18 : 299-304.

BARRETT'S METAPLASIA, DYSPLASIA AND ESOPHAGEAL ADENOCARCINOMA : AN INADEQUATE ANTITUMOUR IMMUNITY ? J. Somja (1), S. Demoulin (2), L. Herman (2), M. Herfs (2), P. Roncarati (2), E. Dortu (2), E. Louis (1), P. Hubert (2), P. Delvenne (1). (1) Centre Hospitalier Universitaire De Liège, Liège, Belgium ; (2) University Of Liege, Liège, Belgium.

Introduction : Barrett's esophagus (BE), which is the replacement of the normal esophageal squamous epithelium by a metaplastic columnar epithelium, is a premalignant lesion leading to a 30-125 fold increase in the risk to develop an esophageal adenocarcinoma (EAC). We hypothesize that the initiation and progression of cancer in this metaplasia is associated with alterations of the density and the functions of immunocompetent cells due to the change of local environment within the metaplastic epithelium.

Aim : The aim of our study was to compare the density and functions of immunocompetent cells in the normal esophagus (NE), BE, low-grade Barrett's esophagus (LGB), high-grade Barrett's esophagus (HGB) and EAC.

Methods : Endoscopic biopsy and operative surgical specimens were obtained from patients with NE (n = 13), BE (n = 20), LGB (n = 15), HGB (n = 8) and EAC (n = 12). Immunohistochemical stainings using CD1a (dendritic cells), FoxP3 (T regulatory cells), BDCA2 (plasmacytoid dendritic cells), NKp46 (Natural Killer cells), CD68 (macrophages), CD4 (T Helper cells), CD8 (T cytotoxic cells), CD3 (T cells), CD20 (B cells) and CD138 (plasma cells) antibodies were performed on paraffin-embedded sections.

Dendritic cells (DC) generated from CD34+ precursors cells (n = 5) were co-cultivated with several tumor cell lines (Het-1A (NE epithelial cells), CPA (BE cells), CPB (HGB cells), CPC (HGB), CPD (HGB), JhEsoAd1 (EAC), and OE33 (EAC)) using 0.4 mm pore size inserts. After incubation with LPS, DC were analysed for maturation (CD80, CD83, CD86, HLA-DR, HLA-ABC) and migratory (CCR7) markers expression.

Results : We observed some modifications of DC density within the sequence normal-metaplasia-dysplasia-cancer (NMDC). BE was associated with a significant reduction in the density of DC compared to NE ($p < 0.001$). In contrast, the development of EAC was associated with a relative increased density of DC, compared to LGB ($p < 0.001$). T regulatory cells number increased during the NMDC sequence and was significantly different from NE, BE et EAC ($p < 0.001$). Natural killer cells, plasmacytoid dendritic cells, macrophages, T cells, B cells and plasma cells also showed an increase during the NMDC sequence.

HGB and EAC cell lines inhibited DC expression of costimulatory molecules (CD80, CD83, CD86) and HLA-DR with a significance ($p < 0.05$) between EAC cell lines and NE or controls.

Conclusions : Our study suggest that the immunosurveillance within the epithelium of BE may be intrinsically perturbed due to the altered maturation and the concomitant decreased density of DC and other immune cells. Moreover, immature DC have been described to be involved in the induction of T regulatory cells mediating tolerance. A better knowledge of the regulatory mechanisms of the immune response induced or inhibited by DC could be interesting for the development of new immunotherapeutic approaches.

HEPATIC SINUSOIDAL OBSTRUCTION SYNDROME REDUCES EFFECT OF OXALIPLATIN IN COLO-RECTAL LIVER METASTASES. C. Vreuls (1), M. Van Den Broek (1), A. Winstanley (2), G. Koek (1), E. Wisse (1), C. Dejong (1), S. Olde Damink (1), F. Bosman (3), A. Driessen (1). (1) Maastricht University Medical Centre, Maastricht, Netherlands ; (2) University College London, London, United Kingdom ; (3) Erasmus Medical Center, Rotterdam, Netherlands.

Introduction : Oxaliplatin is an important chemotherapeutic agent, used to reduce hepatic colorectal metastasis, resulting in tumour reduction and permitting surgical resection. This treatment has significant side effects as oxaliplatin can induce sinusoidal obstruction syndrome (SOS) in the nontumour-bearing liver, resulting in increased morbidity. SOS is induced by toxic damage to the sinusoidal endothelial cell leading to detachment and obstruction of the sinusoid.

Aim : The aim is to investigate the relationship between SOS and tumour reduction. We hypothesized that SOS might impede hepatic perfusion, thereby interfering with the tumour environment and attenuate the response to the chemotherapy.

Methods : From the prospective database of the Maastricht University Medical Centre we collected 50 patients (mean age 61.2 yrs (range 40-79), M/F = 1) with hepatic colorectal carcinoma metastases. All patients received neo-adjuvant oxaliplatin followed by partial hepatectomy. Metastases and nontumour-bearing liver were studied histopathologically.

Results : Thirty-two of 50 (64%) patients showed SOS lesions, classified as mild (26%) and moderate-severe (38%). The response to treatment, as expressed in the tumour regression grade (TRG), was grade 1 (10%) ; grade 2 (14%) ; grade 3 (28%) ; grade 4 (32%) and grade 5 (16%). Statistical analysis showed that a higher grade of SOS lesions was associated with a higher grade of TRG ($p = 0.016$).

Conclusions : Developing SOS goes along with lower tumour response to neo-adjuvant oxaliplatin treatment. Hepatic hypoperfusion due to sinusoidal obstruction syndrome might induce hepatic hypoxia, diminishing response to chemotherapy.

- P22 -

AUTO-IMMUNE GASTRITIS CHARACTERISTICS IN A LARGE SERIES OF PATIENTS WITH AUTO-IMMUNE THYROIDITIS. H. Valdes (1), M. Tomegarcia (2), L. Lutteri (1), C. Reenaers (1), M. Polus (1), V. Geenen (1), E. Louis (1), A. Beckers (1). (1) CHU Sart Tilman, Liège, Belgium ; (2) University of Liege, Liège, Belgium.

Introduction : Autoimmune thyroiditis (AIT) may be associated with other organ-specific autoimmune disorders, including autoimmune gastritis. However, the prevalence of this association is probably underestimated in retrospective studies. Moreover, the possible link between *H pylori*, gastric and thyroid auto-immunity had seldom been studied.

Aim : This is a prospective study to determine the prevalence, the clinical and the pathological features of gastric autoimmunity in patients recently diagnosed with AIT. A second objective is to assess clinico-pathological and/or biological significant predictive factors of gastric auto-immunity to improve diagnosis in these patients.

Methods : A total of 410 consecutive patients with autoimmune thyroiditis -AIT- (Hashimoto's Thyroiditis : n = 360 and Graves Disease : n = 50) were studied in our clinic from 2008 to 2010. These patients had no other clinical features of autoimmunity (Vitiligo, Addison, Candidiasis, etc). All patients underwent TSH, FT4, FT3, ATPO, ATG, TBII, parietal cell autoantibodies (PCA), intrinsic factor antibodies (IFA), antigliadin and antitransglutamin Ab, gastrin, vitamin B12 determinations and thyroid ultrasounds. Patients with PCA and/or IFA were invited to have a gastric endoscopy. Biopsies for histology and immunohistochemical studies including *H pylori* detection were done. To determine predictive factors of autoimmunity, they were compared to a control group of 45 patients matched for sex and age (these patients had Hashimoto's Thyroiditis or Graves disease but no serologic gastric autoimmunity).

Results : Patients with both AIT and gastric autoimmunity, had a mean age of 51 ± 17 years (47F/9M) at inclusion. Parietal cell autoantibodies were present in 14% (56/410). Intrinsic factor autoantibodies were found in 30% (9/30). One caeliac patient was identified in this series and he was excluded from this study. Hypergastrinemia (> 120 pg/ml) was present in 36% (20/56) of patients with a mean of 190 ± 572 pg/ml. One patient had anemia, whereas only six patients had macrocytosis and 6 patients had microcytosis. Vitamin B12 was less than 200 pg/ml in 18% (10/56) of patients with PCA. A total of 61% (34/56) with PCA and no gastric symptoms underwent a gastroscopy. Gastroscopy revealed mucosal abnormalities in% (25/33). Gastric and duodenal biopsies showed in 76% (25/33) histological signs of lymphocytic infiltration. Another 33% (11/33) had signs of metaplasia and ECC hyperplasia, and only 27% (9/33) of patients had signs of mucosal atrophy. Interestingly, *H pylori* infection was found in 21% (7/33) of patients : follow-up in some of these patients showed PCA negativization after *H pylori* eradication. No gastric carcinoid was found in this prospective series.

Conclusions : The Thyro-Gastric syndrome should be differentiated of classical forms of auto-immune polyendocrine syndrome (type I, II or III), that are uncommon. *H pylori* can mimics gastric auto-immunity in AIT, being reversible after eradication. Prevalence of gastric autoimmunity in the general population is less than 1%. The high prevalence of autoimmune gastritis and vitamin B12 deficiency in this series provide a strong rationale for an early serologic screening and gastroscopic diagnosis in patients with AIT.

Invited lecture

- P23 -

CORRECT HISTOPATHOLOGICAL STAGING OF COLORECTAL CANCER : A CHALLENGING ISSUE. P. Demetter. Erasme Hospital, Brussels, Belgium.

Cancer staging systems codify the extent of tumour to provide clinicians and patients with the means to quantify prognosis for individual patients and to compare groups of patients in clinical trials and who receive standard care around the world.

Since the classical proposal from 1932 by Dukes, significant improvements have been made in the histopathological staging system of colorectal cancer. The Dukes classification was based on the extent of diseases, as evaluated by the degree of tumour infiltration through the bowel wall, and the presence or absence of lymph node involvement. Although this staging underwent several modifications, problems included the lack of consideration for the extent of lymph node involvement, the tumour grade, and other pathologic features of tumours. In 1987, Jeremy Jass added two biologically oriented tumour characteristics : the nature of the expanding front of the tumour (pushing or infiltrating) and the presence or absence of lymphocytic infiltration at the advancing edge.

The current TNM staging system is essentially based on anatomic descriptors referring to intestinal wall and peritoneal infiltration, the number of involved regional lymph nodes, and occurrence of distant metastasis. With regard to tumour deposits or satellites in pericorectal tissues, too many changes have, however, taken place. Moreover, the absence of studies that could validate reproducible criteria of the nature of satellites resulted in placing the interpretation of such lesions back into the hands of individual pathologists, leading to unacceptable subjective variability. Instead of facilitating international standards, we have created a situation that makes great databases inaccurate ; if we want to ensure that this is avoided in the future, the principles of staging should be reviewed and debated.

POSTERS

- P24 -

CISPLATIN-MODIFIED DE GRAMONT IN SECOND LINE THERAPY FOR PANCREATIC ADENOCARCINOMA. V. Ky (1), M. Hav (1), E. Monsaert (2), E. Vanderstraeten (2), D.B. Katrien (2), N. Van Damme (1), S. Laurent (1), K. Geboes (1). (1) Universitair Ziekenhuis Gent, Gent, Belgium ; (2) Maria Middelaers Ziekenhuis, Gent, Belgium.

Introduction : Today there is growing evidence supporting the benefit of 2nd line chemotherapy after gemcitabine failure in pancreatic cancer. However, which type of chemotherapy is preferred has not been defined yet. Different therapeutic regimens have been tested in small trials and have shown a limited significant clinical benefit. In Belgium, combination chemotherapy of cisplatin and 5-fluorouracil (5FU) + leucovorin according to the modified de Gramont schedule is the treatment of choice. Combination therapy with cisplatin and 5FU has yielded positive results in 3 trials.

Aim : We analyzed retrospectively the data in two Belgian centres in a non-selected population.

Methods : Between January 2004 and October 2011, 48 patients with histologically proven recurrent, or unresectable pancreatic adenocarcinoma who had received cisplatin and 5FU - leucovorin (modified de Gramont) as 2nd line therapy were identified. We retrospectively analyzed the following parameters : progression free survival and overall survival for each line (after the start of 1st and 2nd line). We also assessed the efficacy of the 2nd line regimen by the growth modulation index or progression free survival ratio.

Results : The median progression free survival after the start of 1st line was 5,4 months (95% CI 4,1 - 6,6). The median progression free survival after the start of 2nd line was 3,6 months (95% CI 1,8-5,5). More interestingly, the median overall survival after the start of 1st line was 12 months (95% CI 9,4-14,6). The 2- and 1-year survival rate after the start of 1st line therapy were 8% (4/48) and 50% (24/48), respectively. Twenty three percent of patients had a growth modulation index > 1,33, referring to a benefit in progression free survival of 2nd line therapy that was greater than that of treatment in 1st line.

Conclusions : We show an overall survival close to 12 months with cisplatin-5FU in 2nd line therapy in a retrospective analysis, an overall survival that is higher than what has been described before. This good result was not due to selection of a patient population that responds well to chemotherapy, since 23% of patients showed a longer progression free survival in 2nd line than in 1st line. These results are in agreement with what is found in the literature : both combination therapy with oxaliplatin and cisplatin show promising results in pancreatic cancer. Oxaliplatin may be preferred because of its lower toxicity, but a recent meta-analysis shows more efficacy for cisplatin. Sequential therapy with good overall survival and good quality of life may be preferred to strong upfront therapy in an incurable disease such as pancreatic cancer.

- P25 -

AMBULATORY ADMINISTRATION OF DCF IN GE CANCER, COMMUNITY HOSPITAL EXPERIENCE. M. Criel, K. Bulte, F. Geurs, I. Hilderson, J. Ponette. Sint Maria Ziekenhuis, Halle, Belgium.

Introduction : Phase II and III trials of docetaxel, cisplatin and fluorouracil (DCF) have shown superior efficacy versus cisplatin and fluorouracil alone but high rates of hematologic toxicity in advanced gastric and gastroesophageal cancer ^(1,2).

Aim : To reduce toxicity while maintaining the efficacy of DCF, we investigated split doses of docetaxel (T), cisplatin (P), leucovorin (L) and fluorouracil (F). Furthermore, this schedule should allow for ambulatory administration of this regimen. With the advent of potent antiemetics like granisetron and aprepitant, ambulatory administration of cisplatin has become feasible for dosages of cisplatin administration up to 40mg/m² per week. Neulasta was administered at the end of the FU administration during the second week.

Methods : We reviewed the files of all 24 patients (from 1/11/2005-1/11/2010)) who were treated with cisplatin-docetaxel -5FU (DCF regimen) for locally advanced or metastatic gastroesophageal cancer. The patients were treated with T 40 mg/m², P 40 mg/m², L 200 mg/m² and F 2000 mg/m² week 1 and 2 of a three week schedule, on an outpatient basis with ambulatory pumps.

Results : The actual median survival of our patients presenting with st III-IV gastric and gastroesophageal cancer is actually similar to the most recently published data : 6 months median.

This dose of cisplatin was well tolerated, no increase in creatinin noted, and all courses were given on an ambulatory basis.

Conclusions : This DCF regimen, a modification of the initial 5 day regimen by Van Cutsem (ref 1) has similar activity, can be given entirely on an outpatient basis and has no significant febrile neutropenia thanks to the growth factor support.

References :

1. Van Cutsem E. *et al.* Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer : a report of the V325 Study Group. *J. Clin. Oncol.*, 2006 Nov 1, 24 (31) : 4991-7.
2. Lorenzen S. *et al.* Split-dose Docetaxel, Cisplatin and Leucovorin/fluorouracil as First-line Therapy in Advanced Gastric Cancer and Adenocarcinoma of the Gastroesophageal Junction. *Annals of Oncology*, 2007, 18 (10) : 1673-1679.

- P26 -

CRS+HIPC TO TREAT PERITONEAL CARCINOMATOSIS FROM UPPER GI CANCER ; 1-Y ANALYSIS OF THE HIPCUPP-TRIAL. B. Topal, F. Huysentruyt, A. Van Den Broeck, G. Sergeant, R. Aerts, C. Verslype, E. Van Cutsem, W. Laleman, S. Tejpar, W. Van Steenberghe, V. Vandecaveye, T. Roskams, H. Prenen. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Many patients with upper gastrointestinal cancer, such as biliary, gastric, or pancreatic carcinoma develop peritoneal metastases. Peritoneal carcinomatosis (PC) from these cancers is highly resistant to systemic chemotherapy and results in extremely poor survival.

Aim : The aim of the current ongoing monocentric phase II study is to evaluate the effectiveness of cytoreductive surgery (CRS) plus hyperthermic intra-operative peritoneal chemotherapy (HIPC) with cisplatin in patients with PC from biliary, gastric, or pancreatic carcinoma. (NCT01116791)

Methods : From August 2010 till November 2011, twenty-three patients (F/M ratio 9/14 ; median age 56 y.) underwent CRS+HIPC for PC from biliary (n = 3), gastric (n = 13), or pancreatic (n = 7) adenocarcinoma. Systemic chemotherapy was administered in 18 patients before and in 13 after CRS+HIPC. Median PC index (PCI) was 6 (range 3-18). An open coliseum technique was used, with perfusion of cisplatin at a concentration of 100 mg/m² for 60 minutes at intracavitary temperatures around 40°C.

Results : Complete cytoreduction (CCR-0) was obtained in all patients. Median intra-operative blood loss was 300 ml, and operating time 360 minutes. No postoperative mortality occurred. Re-operation was needed in 3 patients. The median duration of planned ICU stay was 2 (1-9) days. The severity of postoperative complications ranged between TOSGS grade 1 and 4b. Surgical site complications (SSC) were observed in 6 patients and non-SSC in 15, including 5 patients with cisplatin nephrotoxicity (serum creatinin level > 2 mg%). No patients needed haemodialysis, while renal function spontaneously normalized in 2/5 patients with postoperative renal insufficiency. Postoperative length of hospital stay was 14 days (range 9-70 d.). Cancer recurrence was observed in 11 patients of whom 8 were diagnosed in patients with a PCI higher than 10, though current follow-up time is too limited. Six out of 7 patients, who had a follow-up of at least 1 year, are still alive. Three patients died at 6, 7, and 9 months after CRS+HIPC, respectively. Updated data will be presented at the BWG.

Conclusions : The interim 1-year evaluation of CRS+HIPC with cisplatin to treat PC from upper gastrointestinal cancer shows acceptable morbidity, no mortality, and promising survival rates.

LONG-ACTING OCTREOTIDE AS SECONDARY PREVENTION OF CHEMOTHERAPY-INDUCED DIARRHEA. A. Hendlisz (1), M. Van Den Eynde (2), G. Machiels (1), I. Dero (3), K. Geboes (3), M. De Man (4), K. Hendrickx (4), T. Delaunoy (5), E. Monsaert (6), E. Vanderstraeten (6), J.L. Van Laethem (7), W. Lybaert (8), S. Holbrechts (9), K. Wouters (10), M. Peeters (10). (1) Institut Jules Bordet, Brussels, Belgium ; (2) Université Catholique De Louvain, Brussels, Belgium ; (3) Ghent University Hospital, Ghent, Belgium ; (4) OLV Ziekenhuis, Aalst, Belgium ; (5) Hôpital De Jolimont, Haine-Saint-Paul, Belgium ; (6) Maria Middelaers Ziekenhuis, Gent, Belgium ; (7) Erasme Hospital, Brussels, Belgium ; (8) Az Nikolaas, Sint-Niklaas, Belgium ; (9) Chu Ambroise Paré Mons, Mons, Belgium ; (10) Antwerp University Hospital, Antwerpen, Belgium.

Introduction : Chemotherapy-induced diarrhea (CID) is one of the most disturbing side effects of chemotherapy with respect to the patient's quality of life, often dose-limiting if not calling into question the entire therapeutic strategy. CID prevention could enhance treatment safety and outcome by enabling the administration of optimal therapeutic doses. Diarrhea prevention is a major challenge for future oncological therapies, as CID is a frequent side effect of modern biological agents. In this multicentric, Belgian, prospective non-randomized trial, we tested the secondary prevention of CID with long-acting octreotide (LAO) in patients receiving cytotoxic chemotherapy associated with a high risk of digestive toxicity.

Methods : In the study's observational phase, all patients treated with a high-risk chemotherapy regimen were prospectively screened after having provided written consent. Patients experiencing a CID = 2 were proposed to enter the interventional phase, after having signed a second informed consent. They received a monthly Sandostatin LAR30 IM injection and the next chemotherapy course was administered with a 25% dose decrease. If no grade = 2 CID occurred, subsequent chemotherapy doses were increased to the initial 100% values. The main endpoint of the study was the diarrhea control rate for patients participating in the interventional phase of the study and receiving the optimal dose of chemotherapy for a minimum of 2 cycles. The statistical plan used a 2-step Simon design, with a first step after successful secondary CID prevention in 19 out of 25 patients. LAO would be considered as efficient in secondary prevention of CID if no diarrhea > grade 1 had been observed in at least 62 out of 79 patients treated with full-dose chemotherapy.

Results : From March 2007 to March 2009, a total of 57 patients were included in the trial. The study was terminated before reaching its target size population because of poor accrual. 29 patients did not develop any CID and participated only in the observational phase. Of the 28 patients included in the interventional part after the first onset of a = grade 2 CID, 5 patients (17.8%, 95% CI = 6.1% - 36.9%) experienced no improvement after a 25% decrease in chemotherapy dose. 9 patients (32.1%, 95% CI = 15.9% - 52.4%) did not continue for the following reasons : rapid tumor progression (7), local reaction after LAO injection (1) and refusal (1). The screen failure rate is thus 14/28 (50%, 95% CI = 30.6% - 69.4%). Of the 14 patients whose CID was resolved after chemotherapy dose reduction, only 2 experienced a = grade 2 CID recurrence after receiving full dose chemotherapy. The remaining 12 patients were treated at the optimal chemotherapy dose without significant digestive side effects (85.7%, 95% CI = 57.2% - 98.2%). The overall success rate of the tested strategy is 12/28 (42.8%, 95% CI = 24.5% - 62.8%).

Conclusions : While this trial did not recruit enough patients to answer the study question, available data suggest that LOA is very effective in allowing the return to optimal doses of chemotherapy in patients whose moderate to severe CID was improved after an initial chemotherapy dose reduction. Moreover, the high rate of screen failures emphasizes the need for more aggressive management of acute CID in addition to secondary prevention.

SMALL BOWEL METASTASES FROM MELANOMA : DOES VIDEOCAPSULE PROVIDE ADDITIONAL INFORMATION AFTER PET-CT ? M. Aerts (1), F. Mana (1), B. Neyns (1), D. De Looze (2), C. Reenaers (3), D. Urbain (1). (1) Uz Brussel, Jette, Belgium ; (2) Ghent University Hospital, Ghent, Belgium ; (3) Chu Sart Tilman, Liège, Belgium.

Introduction : Small bowel capsule endoscopy (SBCE) is a useful tool for detecting small bowel tumors, primary or metastatic. Finding small bowel metastases of melanoma can be important because surgical removal of unique small bowel metastasis of melanoma could improve survival. FDG PET-CT is the most employed technique for the follow-up of melanoma patients. Both methods could be complementary.

Aim : To evaluate if SBCE can provide additional information after FDG PET-CT has been performed. The potential clinical impact of SBCE findings near FDG PET-CT scanning was also evaluated.

Methods : The files of patients referred in the context of known melanoma were collected from January 2006 to February 2011 in 4 Belgian academic medical centers. In all patients, a FDG PET-CT was first performed. When Pet scan suspected abdominal involvement, and / or in the presence of ATCD of small bowel resection for melanoma metas-

tasis or iron deficiency anemia (IDA) or obscure gastrointestinal bleeding (OGIB), a SBCE was performed. Information was collected about age, sex, results of the FDG PET-CT, indication for performing SBCE, and influence on the further staging and/or therapeutic modalities.

Results : Nine patients were referred to the centers. In 5 patients, there was a story of former small bowel resection for metastasis. In 6 patients SBCE was performed for IDA/OGIB. In two of these patients, there was a combination of former bowel resection for metastasis and IDA/OGIB. PET CT was positive in 4 and dubious in one case. Capsule was positive in 5 cases (4 jejunal lesions and 1 duodenal lesion). Capsule examination failed to identify metastasis in one case. Capsule identified diffuse superficial intestinal metastases in one case while Pet CT result was dubious. SBCE was positive in 5/6 of the patients with IDA or OGIB and influenced therapeutic decision in 2/9 patients. Endoscopic aspects were heterogeneous.

Conclusions : We recommend to perform SBCE in case of dubious Pet CT (intraluminal localization or not, uncertain localization) and in patients with advanced disease and IDA and/or OGIB. The impact on therapy is of course limited in this category of patients with advanced disease, but it can influence the clinician in taking decision of small bowel resection.

- P29 -

PRIMARY SMALL BOWEL ADENOCARCINOMA : RETROSPECTIVE ANALYSIS OF 29 CASES IN THE ANTWERP REGION. T. Moreels (1), T. Botelberge (2), J. Callens (3), B. De Schepper (2), J. Holvoet (4), J. Maus (3), B. Naessens (5), I. Ruytjens (4), D. Sprengers (2), P.H. Steger (6), L. Terriere (4), L. Verbist (7), H. Büscher (8). (1) Antwerp University Hospital, Antwerpen, Belgium ; (2) Sint Augustinus Ziekenhuis, Antwerpen, Belgium ; (3) Klina, Brasschaat, Belgium ; (4) ZNA Middelheim, Antwerpen, Belgium ; (5) AZ Nikolaas, Sint-Niklaas, Belgium ; (6) Sint-Vincentiusziekenhuis, Antwerpen, Belgium ; (7) ZNA Jan Palfijn, Merksem, Belgium ; (8) AZ St Jozef, Malle, Belgium.

Introduction : Primary small bowel adenocarcinoma is a rare gastrointestinal malignancy. Due to a lack of clinical trials, evidence-based guidelines on diagnosis and treatment are missing. Therefore, it is unclear how this type of malignancy is dealt with in daily clinical practice.

Aim : To evaluate daily practice in the diagnosis, treatment and outcome of primary small bowel adenocarcinoma, in order to optimise diagnosis and treatment.

Methods : On behalf of the Antwerp Gastroenterologist Club (AGC) we retrospectively analysed 29 cases of primary small bowel adenocarcinoma which occurred in the Antwerp region between January 2006 and December 2010. Data on risk factors, diagnosis, treatment and outcome were collected and analysed.

Results : We were able to obtain data on 29 patients diagnosed with primary small bowel adenocarcinoma during the previous 5 years. The male/female ratio was 55/45% with a mean age of 69 ± 2 years (range 45-86) at diagnosis. Three (10%) patients previously smoked tobacco and 7 (24%) were active smokers. Predetermining risk factors like Crohn's disease, celiac disease and colorectal cancer were present in 2 (7%), 1 (4%) and 2 (7%) patients respectively. Abdominal pain (65%), anemia (38%), weight loss (35%) and gastrointestinal obstruction (35%) were the most prevalent presenting symptoms. Diagnosis was suspected based on medical imaging (X-ray, computed tomography or magnetic resonance) in 48%, endoscopy in 37% and during laparotomy in 15%. The tumor was located in the duodenum (papillary carcinoma excluded) in 41%, the jejunum in 17% and in the ileum in 41% and the majority presented as locally advanced (stage III : 38%) or metastatic (stage IV : 31%) disease, mostly peritoneal metastases. Treatment consisted of surgical resection of the tumor (both R0 and R1 resections) in 18 (62%) patients and palliative surgical derivation in 8 (28%) followed by chemotherapy in 11 (38%) patients. Folfox was chosen as first line chemotherapy, followed by Folfiri and then Avastin, Cisplatinum or Gemcitabine. Overall, 10 (35%) patients show a mean disease-free survival of 33 ± 6 months (range 6-60) up until today, whereas 6 (21%) patients are in palliation for 22 ± 9 months (range 2-65) all treated with palliative chemotherapy. Tumor-related mortality was seen in 11 (38%) after a mean time of 11 ± 5 months (range 0-56), the majority (73%) of these patients were diagnosed as stage III-IV.

Conclusions : Even with the availability of new and accurate radiological and endoscopic tools, primary small bowel adenocarcinoma is still diagnosed at late stage disease in the majority of patients, and thus reducing survival rates. Surgical R0 tumor resection leads to disease-free survival, whereas surgery (R1 tumor resection or palliative derivation) followed by chemotherapy may increase survival duration. Although there are currently no guidelines available on chemotherapy for primary small bowel adenocarcinoma, several types of chemotherapy (Folfox, Folfiri, Avastin, Cisplatinum or Gemcitabine) are used in daily practice.

CYTOREDUCTIVE SURGERY AND HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY FOR COLORECTAL CARCINOMATOSIS. E. Cotte (1), S. Van Den Broeck (2), C. Remue (1), D. Leonard (1), F. Lois (1), P. Forget (1), M. De Kock (1), P.F. Laterre (1), X. Wittebole (1), Y. Humblet (1), M. Van Den Eynde (1), E. Danse (1), C. Dragean (1), C. Sempoux (1), A. Jouret-Mourin (1), B. Crispin (1), G. Leclercq (1), L. Delcarte (1), J. Jamart (3), A. Kartheuser (1). (1) Clinique Universitaire St Luc, Brussels, Belgium ; (2) Antwerp University Hospital, Antwerpen, Belgium ; (3) Ucl, Mont-Godinne, Belgium.

Introduction : Peritoneal carcinomatosis (PC) from colorectal cancer has long been considered as the terminal stage of the disease. Over the last 20 years, cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has progressively become a therapeutic option for PC thanks to its favourable oncologic results.

Aim : The aim of this study is to review the preliminary results of the association of the closed abdomen technique and Oxaliplatin for HIPEC.

Methods : Between October 2007 and December 2010, 24 patients (median age 56 years (range : 36-77)) underwent 25 cytoreductive surgeries and closed abdomen HIPEC with Oxaliplatin for the treatment of PC arising from colorectal cancer. Eleven patients (46%) presented synchronous resectable hepatic metastasis.

Results : All patients were considered to have undergone a CCR-0 resection (no residual tumour after cytoreductive surgery). Postoperative mortality and morbidity rates were respectively 4% and 52%. The median follow-up was 219 months and the 3-year overall survival rate was 82%. Five patients (21%) presented an isolated PC recurrence and 13 patients (54%) distant metastasis after HIPEC. Fourteen patients (58%) received postoperative adjuvant systemic chemotherapy.

Conclusions : The use of closed abdomen HIPEC with Oxaliplatin to treat PC of colorectal origin offers favourable oncologic results. Despite satisfying survival rate, the recurrence rate is high, principally due to systemic dissemination and not to PC relapse. This seems to confirm that HIPEC is effective and warrants further study to evaluate the potential benefit of adjuvant systemic chemotherapy.

NATURAL HISTORY AND PROGNOSTIC FACTORS OF PATIENTS WITH HIGH RISK pT4 STAGE II COLON CANCER. B. Vos (1), R. Marechal (1), P. Demetter (1), L. Verset (1), M. Fernandez (2), A. Hendlisz (2), J.L. Van Laethem (1). (1) Erasme Hospital, Brussels, Belgium ; (2) Institut Jules Bordet, Brussels, Belgium.

Introduction : Stages II colon cancer disease can be cured by surgical intervention but may harbour various prognosis related to clinico-pathological and molecular characteristics.

Aim : The aim of this study was to analyse the pattern of recurrence and the outcome of patients with resected stage II colon cancer focusing on pT4 lesion, an understudied population in large trials.

Methods : We retrospectively analyzed, between 1990 and 2010, 286 consecutive resected stage II colon cancers, including 253 pT3N0 and 33 pT4N0. Additionally, 48 pT4N+ colon cancers were analyzed to evaluate the specific influence of T status. We compared both groups including Kaplan-Meier survival curves analysis.

Results : In the pT4N0 group, as compared to the pT3N0 group, age, sex ratio, tumor location and preoperative CEA level were similar in both groups while tumor size and macroscopic tumor perforation were significantly higher in pT4N0 group ($p = 0.002$ and < 0.001 , respectively). About histology, lymphatic and perineural invasion were more important in pT4N0 than in pT3N0 group ($p = 0.034$ and 0.002 , respectively). Adjuvant treatment was more frequent in pT4N0 group ($p < 0.001$). Patients with pT4N0 stage disclosed a close recurrence rate to those with pT3N0 : 21 vs 15%, $p = 0.231$ and a similar pattern of recurrence site (local/distance) : 50/50 vs 43/57%, $p = 0.674$ while pT4N+ patients have a clear significant increase of recurrence rate : 21 vs 52%, $p = 0.009$, with a predominance of distant recurrence in 80%, as compared to pT4N0. About outcome, three-year disease-free survival (DFS) and OS were significantly lower in high risk pT4N0 than in pT3N0 : 85 vs 60%, $p = 0.031$ and : 89% vs 65%, $p = 0.001$, respectively.

Conclusions : pT4N0 colon cancer lesions are rare as compared to pT3N0 or stage III colon cancer ; our follow up study showed that recurrence rate and site are similar to those of pT3N0 with no recurrence site predominance ; when compared to pT4N+ population, we observed that lymph nodes invasion led to more frequent relapse, predominantly distant ; Further research should focus on biomolecular markers of microinvasiveness of this specific pT4 population, aiming at better defining the risk profile of relapse.

LAPAROSCOPIC RESECTION OF GASTRIC GIST IS SAFE AND EFFECTIVE, IRRESPECTIVE OF SIZE. K. De Vogelaere, A. Hoorens, I. Van Loo, G. Delvaux. UZ Brussel, Jette, Belgium.

Introduction : Feasibility and long-term safety of laparoscopic removal of gastric gastrointestinal stromal tumors (GISTs) of the stomach is well established for lesions smaller than 2 cm.

Aim : Our specific aim was to explore if laparoscopic treatment is equally applicable for gastric GISTs larger than 2 cm.

Methods : Between 1997 and 2010, 31 consecutive patients presenting with a gastric GIST were scheduled for laparoscopic resection, irrespective of tumor size. Prerequisites for laparoscopic approach were : the absence of metastases, and a well-defined tumour on CT scanning without involvement of adjacent organs, the esophagogastric junction, or the pylorus of the stomach. Data were retrieved retrospectively from a prospectively collected database, including information on patient demographics, surgical procedure, complications, hospital stay, and recurrence.

Results : All 31 laparoscopic resections were carried out successfully. Tumor size was less than 2 cm in 5, and more than 2 cm in 26 patients. Median blood loss was identical in both groups (20 mL), but duration of operation (60 vs. 103 min) and duration of hospital stay (6 vs. 8 days) were lower when tumor size was less than 2 cm. Only 1 patient (with tumor size < 2 cm) experienced a postoperative hemorrhage. After a median follow-up period of 64 months, there were no recurrences or metastases.

Conclusions : The low morbidity rates and the long-term disease free interval of 100% observed in our cohort indicate that laparoscopic resection is safe and effective in treating gastric GISTs, even for tumors larger than 2 cm.

SEVEN SOCIETIES POSTGRADUATE COURSE

- S01 -

PROGNOSTIC FACTORS IN LIVER BIOPSY FOR HCC. J. Zucman Rossi, Paris, France.

- S02 -

STAGING AND LOCAL TREATMENT OF HCC. B. Op de Beeck. Antwerp University Hospital, Antwerpen, Belgium.

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer and the third leading cause of cancer-related death. A careful multidisciplinary assessment of tumour characteristics, liver function, and physical status is required for proper therapeutic management.

Several classification systems are available for HCC. The Barcelona Clinic Liver Cancer (BCLC) classification has emerged in recent years as the standard classification that is used for clinical management of patients with HCC. This classification links stage stratification with a recommended treatment strategy and defines standard of care for each tumour stage.

In this presentation, treatment strategy according to tumour stage will be discussed, underlining advantages and limitations of current local-regional treatments with respect to surgical and systemic approaches.

Image-guided tumour ablation (RFA) is recommended in patients with early-stage HCC, according to the BCLC staging system (preserved liver function, Child-Plugh A and B with solitary HCC or up to three nodules less than 3 cm in size), when patients are excluded from surgical options.

For patients with multinodular HCC, relatively preserved liver function, no cancer-related symptoms, and no vascular invasion or extrahepatic spread (intermediate-stage HCC, according to BCLC system) transcatheter arterial chemo-embolization (TACE) is the current standard of care.

A growing body of literature suggests that interventional treatments, including radioembolization, might be an effective treatment approach for selected categories of patients with advanced HCC.

Response rate and time to progression represent surrogate endpoints for survival in cancer research, and the limitations of the WHO criteria and the RECIST when applied to local-regional therapies in HCC or to molecular-targeted therapies are well known. Therefore the modified RECIST (mRECIST) assessment, based on the concept of viable tumour or tumoural tissue showing uptake during the arterial phase of contrast-enhanced radiologic imaging, should be used for the measurement of response rate in HCC.

MRI is the best and the most sensitive non-invasive radiological technique to diagnose and stage HCC as well as to evaluate the response after local-regional therapies.

- S03 -

INDICATIONS FOR TRANSPLANTATION FOR HCC AND MANAGEMENT OF HCC WHILE ON WAITING LIST/ DOWNSTAGING ON. J.F. DUFOUR. University Hospital Of Bern, Inselspital, Bern, Switzerland.

- S04 -

MEDICAL TREATMENT OF ADVANCED HCC. C. Verslype (KULeuven).

- S05 -

PATHOGENESIS OF PORTAL HYPERTENSION. H. Reynaert. University Hospital Brussel (VUB), Brussels, Belgium.

Cirrhosis is a widespread disease with a high morbidity and mortality, which is characterized by hepatocellular insufficiency and portal hypertension (PHT). Portal hypertension is a frequent and potentially lethal complication of cirrhosis, which is characterized by increased pressure in the portal vein, and results in serious complications such as bleeding from esophago-gastric varices, ascites, renal insufficiency, encephalopathy etc.

As in any vascular system, Ohm's law ($DP \sim Q \times R$), is applicable, and thus is portal venous pressure proportional to blood flow in the portal vein and resistance along the vessel. In the setting of PHT, both an increase in intra-hepatic resistance and in portal blood flow contribute to the onset and aggravation of PHT. Until recently, it was generally accepted that the increase in portal blood flow was caused by a hyperdynamic circulation, high splanchnic blood flow, and hyporesponsiveness of the splanchnic arteries for circulating vasoconstrictors. More recently, it was shown that angiogenesis in the splanchnic vascular bed adds to the high splanchnic blood flow.

Apart from increased portal blood flow, cirrhotic patients have a high intra-hepatic resistance, which is caused by fixed structural factors (fibrosis, regeneration nodules, capillarization of sinusoidal endothelial cells, deposition of extracellular matrix around sinusoids) and variable, dynamic factors (changes in vascular tone), which account for 20-30% of the total resistance. Moreover, it has been demonstrated recently that intra-hepatic angiogenesis contributes to the rise in intra-hepatic resistance.

Conclusions : in recent years, the pathogenesis of PHT has been elucidated and new mechanisms have been unraveled. It has become clear that PHT is a multifactorial process. Many new treatment options, based on our new pathophysiological knowledge and acting on new targets, are under investigation. We can anticipate, that the arrival of these new drugs, will enable us to treat our patients with more success.

- S06 -

MANAGEMENT OF ACUTE VARICEAL BLEEDING AND GAVE. O. Le Moine (ULB Erasme, Brussels).

- S07 -

PRIMARY AND SECONDARY PROPHYLAXIS OF VARICEAL BLEEDING. P. Stärkel, Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

A substantial proportion of patients with cirrhosis will develop varices and up to a third of patients with high-risk varices will eventually bleed. Current strategies aim at preventing a first variceal bleed (primary prophylaxis) as well as recurrent bleeding after an initial bleed (secondary prophylaxis). Primary prophylaxis in patients with medium-to large oesophageal varices has been reported to reduce the risk of bleeding by 50%. Noncardioselective beta-blockers (propranolol, nadolol) are the preferred first-line drug therapies in most patients. Recently, carvedilol has gained substantial interest in this setting, with promising initial data. There is no role for drug therapy in patients without varices and likely also not in patients with small varices. Variceal band ligation may be an alternative in patients who do not tolerate or do not respond to noncardioselective beta-blockers. Gastric varices extending from the oesophagus (GOV1) are generally treated like oesophageal varices whereas primary prophylaxis in patients with high risk fundic (GOV2) and isolated gastric varices (IGV1) is more controversial. Although most guidelines recommend the use of non-cardioselective beta-blockers, compelling evidence for their efficacy is lacking. Recently, prophylactic injection of cyanoacrylate (tissue glue) showed some promise in preventing a first bleed in carefully selected patients with a high risk of bleeding. To date, no clear recommendation can be given concerning the best prophylactic strategy to prevent a first variceal hemorrhage in patients with high risk gastric varices.

Once a first bleeding has occurred, prevention of re-bleeding (secondary prophylaxis) must be considered. A combination of variceal band ligation with addition of pharmacotherapy with noncardioselective beta-blockers is considered standard of care for oesophageal varices and GOV1. The aim is to achieve eradication of varices through iterative endotherapy. Recent data suggests that early portosystemic shunting (Tipps) might be more effective than combination therapy in patients with active bleeding on endoscopy and/or with hepatic decompensation at the time of bleeding. Although there is some data that tissue glue obliteration might prevent re-bleeding from gastric varices, Tipps also seems to be the best option for secondary prophylaxis in patients with GOV2 and IGV1.

- S08 -

MANAGEMENT OF CHILDREN WITH GI HAEMORRHAGE DUE TO PORTAL HYPERTENSION. Xavier Stéphane and Etienne Sokal, Cliniques St Luc, Université Catholique de Louvain, Service de Gastroentérologie et Hépatologie Pédiatrique, Louvain, Belgium.

Portal vein pressures exceeding 5 mm Hg or portal vein to hepatic vein gradient of greater than 10 mm Hg are defined as portal hypertension. Portal hypertension is caused by modification of flow through the portal system and can be

categorized into intra-hepatic and extra-hepatic aetiologies. Extra-hepatic causes can be further classified by pre-hepatic and post-hepatic localisations. Intra-hepatic aetiologies include pre-sinusoidal, sinusoidal, or post-sinusoidal obstruction. Portal hypertension in children is predominantly caused by an intra-hepatic aetiology, cirrhosis (more frequently in the evolution of children with biliary atresia). Extra-hepatic causes include obstruction of the portal vein, typically after catheterisation of the umbilical vein (venous access during the neonatal period). Other aetiologies include infection (omphalitis), intra-abdominal abscess, severe dehydration, and blunt trauma to the abdomen. Budd-Chiari syndrome, veno-occlusive disease, and cardiac disease can also cause portal hypertension.

Portal hypertension can be first identified by clinical history and physical examination. Some limited blood tests can be helpful in the differential diagnosis of portal hypertension and to evaluate consequences of variceal, if suspected, bleeding : haemoglobin and platelet counts, clotting and liver function tests. Ultrasound can demonstrate heterogeneity of the liver in chronic liver disease, and Doppler examination provides information about portal vein patency and directionality of flow. Upper gastrointestinal tract endoscopy is the best mode to screen for oesophageal and gastric varices. The variceal grade identified and the presence of gastric varices is shown to be predictive of the risk of gastrointestinal bleed. Angiography is used selectively in patients when the bleeding is so massive that endoscopy evaluation and therapy are difficult.

Management of portal hypertension should be adapted according to the aetiology of the disease and liver function. If bleeding, the hemodynamic state of the patient will guide the management. In children, age adjusted tachycardia is the most sensitive indicator of severe blood loss. If blood volume reconstitution is needed, this has to be done cautiously to avoid overcorrection. Vaso-active drugs can be used before endoscopy in variceal bleeding children (more classically somatostatin). Once the child is stabilized, sclerotherapy and variceal band ligation can be used to stop active bleeding. Within 12 hours, the endoscopy has to be performed. Endoscopy treatment can also prevent bleeding from occurring. When technically feasible, band ligation is preferred as it is associated with a lower rate of complications. The smallest size multiband ligator can be adapted for an 8.5 mm endoscope. Angiography can provide a therapeutic approach by placement of coils for embolization of the bleeding vessel. In children, the use of β -blockade in the management of portal hypertension requires new investigation and the benefit of this therapy has to be proven. When used, the dose of the β -blocker is adjusted until the heart rate is reduced to 75% of its baseline value, which is in practice difficult. Transjugular intrahepatic portosystemic shunts and surgical shunts are reserved for those who are not candidates for liver transplantation or have refractory bleeding despite medical or endoscopic treatment. The porto-portal meso-Rex shunt is preferred when feasible.

- S09 -

UPDATE IN THE TREATMENT OF ACHALASIA. G. Boeckxstaens. University Hospital Gasthuisberg, Leuven, Belgium.

Introduction : Achalasia is a rare motility disorder of the oesophagus characterized by dysphagia, chest pain, regurgitation of undigested food and weight loss. These symptoms result from the absence of peristalsis and impaired relaxation of the lower oesophageal sphincter leading to esophageal stasis. Treatment of this disorder aims to reduce the resistance at the oesophago-gastric junction to enhance emptying.

Aim : To review and discuss the available treatment options of achalasia.

Results : Pharmacological treatments, such as botuline toxin injection or smooth muscle relaxants have a modest and short lasting clinical effect, and should be reserved to treat patients at high risk to undergo surgery or pneumatic dilation, or to bridge the time to definite therapy while on a waiting list. Pneumatic dilation (PD) and surgical myotomy (laparoscopic Heller myotomy ; LHM) on the other hand have both proven to be effective. PD is preferentially performed using graded dilation with a Rigiflex balloon of increasing diameter. This procedure carries the risk of oesophageal perforation (2-4%), but this complication, when diagnosed early, can be treated conservatively. LHM is gaining interest especially in view of the minimal invasive approach and the excellent results published. A recent prospective randomized trial however indicated that LHM is not superior to PD. Risk factors for treatment failure of both treatments are preexisting daily chest pain, poor emptying following treatment and a width of the oesophagus of less than 4 cm before treatment. Recently, peroral endoscopic myotomy (POEM) has been introduced as new treatment. This involves endoscopic myotomy through a submucosal tunnel with excellent short term results.

Conclusions : Both PD and LHM are efficient treatments for achalasia. POEM may be attractive alternative although studies with longer follow-up are required. More research is however required to develop approaches aiming to restore oesophageal function rather than destroying the lower oesophageal sphincter.

- S10 -

ASCITES AND HYPONATREMIA. S. Francque, Universitair Ziekenhuis Antwerpen, Belgium.

- S11 -

RENAL DYSFUNCTION IN CIRRHOSIS. M. Wissing, UZ Brussel, Belgium.

- S12 -

SPONTANEOUS BACTERIAL PERITONITIS AND OTHER INFECTIONS IN PATIENTS WITH CIRRHOSIS. T. Gustot. Erasme Hospital, Brussels, Belgium.

Patients with cirrhosis have a higher incidence of bacterial infections than the general population. The most common infection is spontaneous bacterial peritonitis (SBP), followed by urinary tract infection (UTI), pneumonia, bacteremia following a therapeutic procedure, cellulitis and spontaneous bacteremia. There is evidence that in cirrhosis, sepsis is associated with a markedly imbalanced cytokine response, which converts responses that are normally protective for fighting infections into excessive, damaging inflammation. In patients with cirrhosis and severe sepsis, high production of proinflammatory cytokines seems to play a role in the worsening of liver function and the development of organ failures, such as shock, renal failure, acute respiratory distress syndrome, coagulopathy and hepatic encephalopathy. Bacterial infections become the first cause of death. Sepsis is associated with a twofold increase of mortality rate in cirrhosis with hospital mortality about 73% for septic shock. Prompt and adequate empirical antibiotic treatment and early resuscitation are essential in determining patient's outcome. In cirrhotic patients with SBP, early use of antibiotics and intravenous albumin administration decreases the risk for developing renal failure and improves survival. Unfortunately, at this time, there is no randomized study that has been specifically performed in patients with cirrhosis and severe sepsis to evaluate treatments that have been shown to improve outcome in patients without cirrhosis.

- S13 -

HEPATOPULMONARY SYNDROME AND PORTOPULMONARY HYPERTENSION. H. Van Vlierberghe, UZ Gent, Belgium.

- S14 -

HEMOSTASIS IN CHRONIC LIVER DISEASE : SHIFTING THE BALANCE. J. Schouten. Erasmus Medical Center, Rotterdam, Netherlands

Most coagulant factors, with exception of factor VIII and von Willebrand factor, are produced in the liver. As a result, liver function impairment is characterized by a defective synthesis of coagulation factors leading to prolonged conventional coagulation tests (APTT, prothrombin time and INR) due to factor deficiencies. These coagulation tests have been included in many prognostic scores of chronic and acute liver disease (Child-Pugh score, MELD score, King's College criteria of acute liver failure). Until recently chronic liver disease was considered a prototype of acquired coagulopathy. In comparison to patients with clotting disorders, prolongation of the laboratory tests of coagulation in patients with end stage liver disease have been falsely used as though they were predictive of the patient's hemorrhagic risk and attempts to correct these test abnormalities by means of fresh frozen plasma and procoagulant agents transfusion prior to invasive procedures have been performed in order to prevent bleeding. However, similar to the procoagulant factors, their anticoagulant counterparts are also reduced to the same extent in patients with liver failure. Consequently, thrombin generation in these patients is comparable to healthy subjects and the prolongation of the conventional coagulation tests does not adequately reflect the bleeding risk in these patients since these tests only take into account the procoagulant hemostasis pathway.

Several observations can be viewed in this context. First, conventional coagulation tests poorly correlate with clinical bleeding after liver biopsy or other hemorrhagic procedures. Second, powerful procoagulant agents such as recombinant activated factor VII failed to control bleeding from upper intestinal tract even though the postinfusion prothrombin time was considerably shortened.

Currently available experimental and clinical data even suggest that chronic liver disease should rather be regarded a condition with an increased thrombin generation instead of a coagulopathy. Despite the prolongation of the conventional coagulation tests, plasma from patients with liver cirrhosis is more resistant to the action of anticoagulant factors than plasma from healthy subjects. In line with this experimental observation, a high incidence of deep venous thrombosis and portal vein thrombosis has been demonstrated in patients with end stage liver failure.

Finally, thrombophilia has been attributed a pathophysiological role in the development and progression of hepatic fibrosis. Epidemiological studies demonstrated that carriage of the Factor V Leiden mutation and protein C deficiency are associated with accelerated progression to cirrhosis in hepatitis C patients.

These interesting pathophysiological insights will soon introduce a new era in the treatment of liver disease.

- S15 -

INDICATIONS FOR LIVER TRANSPLANTATION IN END STAGE LIVER DISEASE. J. Delwaide, ULg, Liège, Belgium.

BELGIAN PANCREATIC CLUB (BPC)

Invited lecture

- T01 -

DIFFUSION-WEIGHTED MR IMAGING OF THE PANCREAS. C. MATOS. Erasme Hospital, Brussels, Belgium.

Definition : Diffusion-weighted imaging (DWI) is a functional magnetic resonance (MR) imaging technique that displays quantitative information about the displacement of tissue water due to random, thermally driven motion over distances of about 1-30mm. Water movement in tissues is dependent on blood flow and on interactions with cellular membranes, intracellular organelles and macromolecules, and is of larger magnitude in vessels than in the extravascular compartments. Thus, DWI provides information about extracellular space tortuosity, tissue cellularity and the integrity of cell membranes.

Rationale : MR imaging has a high contrast resolution to detect the majority of pancreatic diseases through changes in T1 and T2 relaxation. However these changes might be insufficient to detect or characterize lesions that are of small size or occur in a background of chronic or acute inflammatory changes. DWI provides another mechanism for developing image contrast that may increase the sensitivity and the specificity of MRI of the pancreas. In this lecture the added value of DWI will be discussed and illustrated.

Technique : DW images are obtained by applying diffusion-sensitized gradients within a T2-weighted sequence. The motion of water molecules is detected as attenuation of the measured signal intensity at DWI. The amount of signal loss is proportional to the degree of water motion. The sensitivity of the DWI sequence to water diffusion is characterised by its b-value (in s/mm²) and can be adjusted by changing the gradient amplitude, the duration of the applied gradient and the time interval between the application of the gradients. Within the intravascular space water molecules have a large degree of motion and will show decreased signal (black blood effect) with small b-values (b = 10-100 s/mm²). At higher b-values (b = 1000s/mm²) slow-moving water molecules or small diffusion distances will show signal attenuation. For visual qualitative analysis DWI should be performed using b values, which result in sufficient background suppression to allow signal intensity differences in the target tissue to be observed. By acquiring DWI with different b values (at least 2) quantitative analysis is available and an apparent diffusion coefficient (ADC) map can be generated. ADC is dependent on the number of b values. With a single DW sequence flow-sensitive and flow-insensitive ADC maps can be generated by using respectively all the acquired b-values or simply those greater than 150-200 s/mm².

Imaging display and interpretation : In addition to conventional TSE T2-weighted, 3D GRE fat suppressed T1-weighted and multiple b-values DW sequences and ADC maps, we match anatomical information on fused images (combining TSE T2-w and DWI) for interpretation.

A highly cellular tissue (tumour, coagulative necrosis or an abscess) will show high-to-intermediate signal intensity on T2-w imaging, high signal intensity on high b-value DWI and low signal intensity on ADC map.

Fluids and low cellularity tissues will show high signal intensity on T2-w imaging, low signal intensity on high b-value DWI and high signal intensity on ADC map.

Fibrous tissue w/o tumour cells and w/ low water content will show low signal intensity on T2-w imaging, low signal intensity on high b-value DWI and low signal intensity on ADC map.

Fluid w / high protein content will show high signal intensity on T2-w imaging, high signal intensity on high b-value DWI and high signal intensity on ADC map.

Clinical results : Investigating the full range of pancreatic diseases (benign and malignant) shows that DWI improves the sensitivity and negative predictive value of MRI. It is reported that pancreatic cancer showed lower ADC values compared with normal pancreas because of increased cellularity and fibrosis of the tumor, which cause restricted water diffusion, and that mean ADC values of malignant lesions are significantly lower than those of benign lesions. Promising results have also been reported concerning the usefulness of DWI for characterizing mucinous lesions and when applying flow-sensitive and flow insensitive measurements for differentiating pancreatic adenocarcinoma and mass forming pancreatitis including autoimmune pancreatitis. DWI is also a useful non invasive tool for the follow-up of inflammatory diseases and for detecting a lesion that may be suitable for biopsy in a patient w/ chronic pancreatitis.

References :

1. Kartalis N. *et al.* DW magnetic resonance imaging of pancreatic tumours. *Eur. Radiol.*, 2009.
2. Kamisawa T. *et al.* Differentiation of autoimmune pancreatitis from pancreatic cancer by diffusion-weighted MRI. *Am. J. Gastroenterology*, 2010.
3. Klauss M. *et al.* Intravoxel incoherent motion MRI for the differentiation between mass forming chronic pancreatitis and pancreatic carcinoma. *Invest. Radiol.*, 2011.
4. Fatima Z. *et al.* Magnetic resonance diffusion-weighted imaging in the characterization of pancreatic mucinous cystic lesions. *Clinical Radiology*, 2011.

HOW ACCURATE IS ENDOSCOPIC ULTRASOUND IN THE DIFFERENTIATION OF CYSTIC LESIONS OF THE PANCREAS ? E. Cesmeli (1), B. Pauwels (1), D. Laukens (1), M. De Vos (2). (1) Ghent University Hospital, Ghent, Belgium ; (2) Ghent University Hospital, Ghent, Belgium.

Introduction : Cystic lesions of the pancreas are increasingly diagnosed due to the widespread use of high-quality cross-sectional imaging. Even when found incidentally, they may represent a pre-malignant condition or even harbour clear malignancy. Endoscopic ultrasound (EUS) is considered to be an important tool in the diagnosis and management of these lesions.

Aim : To assess the accuracy of EUS in the differentiation of the cystic lesions of the pancreas in our center because long term Belgian data are scarce.

Methods : For this retrospective study we reviewed the data of all consecutive patients undergoing an EUS examination for a cystic lesion of the pancreas in the period of January 2005-June 2010. Data collected included clinical history, cytology of fine needle aspirations (FNA) and cyst fluid analysis. These results were compared to definitive (pathologic) diagnosis if available. CEA > 192 ng/mL in the cyst fluid was considered a non-benign lesion, amylase > 5000 U/L with low CEA a pseudocyst.

Results : A total of 176 patients (91 M, 85 F) underwent EUS and in 117 patients an FNA was performed. In 45 patients we found data on cyst fluid analysis. Mean age of the patients was 61 yrs and mean cyst diameter was 2.6 cm (range : 0.1-10). In 48 patients we had a definitive diagnosis after surgery, endoscopic drainage or clinical evolution in some cases of malignancy.

Benign pathology was found in 22 patients, including serous cystadenoma in 3 patients, pseudocysts in 17 patients and no cystic lesion in 2 patients.

Non-benign pathology was found in 26 patients, with intraductal papillary mucinous neoplasia or IPMN in 7 patients, mucinous cystadenoma in 1 patient, adenocarcinoma in 14 patients, neuroendocrine tumour in 3 patients and solid pseudopapillary neoplasia or SPPN in 1 patient. In 38 of these patients an FNA was performed and in 13 of the 38 (34%) FNA-results were non-contributive.

To differentiate benign from non-benign when compared to the definitive diagnosis, the performance results for EUS were :

sensitivity : 64.3%, specificity : 85% and accuracy : 72.9%. For EUS-FNA the sensitivity, specificity and accuracy were respectively : 94.4%, 85.7% and 92%. For cyst fluid analysis (in 17 patients) these results were respectively : 66.7%, 100% and 82.4%.

For the diagnosis of a pseudocyst the accuracy results were : EUS : 86.7%, EUS-FNA : 84% and cyst fluid analysis : 84.6%. For the diagnosis of adenocarcinoma the accuracy results were : EUS : 85.4% (sensitivity : 57.1%) and EUS-FNA : 81.8% (sensitivity : 64.3%). Mean CEA was significantly higher in cysts with adenocarcinoma versus other cysts ($p = 0.024$).

Conclusions : EUS alone is not accurate enough to differentiate benign from non-benign cystic lesions of the pancreas, with the exception of the pseudocysts. For the management of the cystic lesions, FNA and cyst fluid analysis are essential. Potential shortcomings are non-contributive FNA's and low aspirate volumes in the smaller cysts.

DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING AND CHARACTERISATION OF PANCREATIC FLUID COLLECTIONS. M. Arvanitakis (1), H. Duarte (1), S. El Bacha (1), A. Lemmers (1), E. Toussaint (2), P. Eisendrath (1), J. Deviere (1), M. Delhaye (1), M.A. Bali (1), C. Matos (1). (1) Erasme Hospital, Brussels, Belgium ; (2) Institut Jules Bordet, Brussels, Belgium.

Introduction : Diffusion-weighted magnetic resonance imaging (DW-MRI) can help detect tissues with dense cellularity by demonstrating low apparent diffusion coefficient (ADC) values.

Aim : The aim of this study was to assess the utility of DW-MRI in characterizing pancreatic fluid collections (PFC) and determining the presence of infection.

Methods : All patients with post-pancreatitis PFCs requiring endoscopic transmural drainage were prospectively included. Exclusion criteria were previous drainage or surgery. Before the endoscopic procedure, patients underwent DW-MRI with ADC measurements in the PFC. PFC infection was suspected in case of restriction in diffusion with low ADC. During drainage under endoscopic ultrasound (EUS) guidance, ultrasound characteristics of the PFC were noted, along with the aspect of the aspirated contents. The fluid was sent for biochemical evaluation (CEA, lipase and protein levels), as well as bacteriological culture. A PFC was considered infected if cultures were positive (gold standard). Continuous values were expressed in median and range and comparisons were performed using non-parametric tests.

Results : Between January 2010 and November 2011, 26 patients (17 males, 65%) were included. Median age was 50 years. The underlying disease was acute (AP) ($n = 15$, 58%) or chronic pancreatitis ($n = 11$, 42%). Median time

between onset of symptoms and drainage was 56 days (14-300) for AP patients. Antibiotics had been previously administered in 9 patients (35%). Indications for drainage included pain (n = 13, 50%) and clinical suspicion of infection (n = 9, 35%). The median delay between DW-MRI and EUS drainage was 0 days (0-14). Based on DW-MRI, median PFC size was 86mm (25-960) and the content was considered to contain high protein fluid (blood or pus) in 13 cases (50%). Restriction in diffusion in the PFC suggested infection in 6 patients (23%). Based on EUS, most of PFCs were hypoechogenic (n = 22, 84.6%) and contained debris in 19 cases (73%). Aspirated fluid was described as pus in 7 cases (27%). Median CEA, protein and lipase levels were, respectively, 9 ng/ml (0.3-350), 2.7 gr/dl (0.4-4.1) and 31915 U/L (25-717270). Bacteriological cultures were positive in 9 patients (35%). Sensitivity, specificity, accuracy, positive and negative predictive values of DW-MRI for predicting PFC infection were respectively 55.5% (5/9), 94% (16/17), 80% (21/26), 80% (16/20) and 83% (5/6). These values increased if we considered only patients without previous antibiotics (n = 17) (respectively 75%, 92%, 88%, 92% and 75%). Median ADC was significant lower in infected PFCs (2.8 vs 1.1, p = 0.045).

Conclusions : DW-MRI is a promising diagnostic tool concerning PFC assessment before drainage. Preliminary results disclose high specificity and negative predictive values, which can help in excluding PFC infection and determining time of drainage.

- T04 -

IS AMOXICILLIN-CLAVULANATE ANTIBIOPROPHYLAXIS BEFORE PANCREATIC CYSTIC LESION EUS-FNA EFFECTIVE ? P. Leclercq (1), F. Xuereb (2), I. Mohammedi (3), E. Boselli (4), E. Zaoui (3), F. Fumex (3), C. Lefort (3), R. Legeron (2), D. Breilh (2), D. Ribeiro (3), B. Napoleon (3). (1) Centre Hospitalier Chrétien, Liège, Belgium ; (2) Hôpital Haut-Lévêque, Bordeaux, France ; (3) Hôpital Privé Jean Mermoz, Lyon 08, France ; (4) Hôpital Edouard Herriot, Lyon, France.

Introduction : French antibioprophyllaxis guidelines concerning echoendoscopic ultrasound guided pancreatic cystic lesion fine needle aspiration (EUS-FNA) are based on the advice of experts. The aim of such prophylaxis is to prevent a cystic iatrogenic bacterial infection. Despite scarce data, intravenous amoxicillin/clavulanate is recommended 30 minutes before pancreatic cystic lesion EUS-FNA. However, to our knowledge, antibiotic diffusion has never been studied in this indication.

Aim : The aim of this study was to determine amoxicillin/clavulanate diffusion into the pancreatic cystic liquid when performing an EUS-FNA.

Methods : 19 consecutive patients (9 women - 10 men, 58-85 kg), mean age : 64 +/- 19 years, admitted for pancreatic cystic lesion EUS-FNA (5 serous cystadenoma, 1 mucinous cystadenoma, 8 IPMN, 2 pseudocysts, 5 others) were included in this prospective study between March and December 2010. According to the guidelines, they received a 30 minutes amoxicillin 1g/clavulanate 200mg intravenous infusion 30 minutes before the procedure. At the time of the cystic EUS-FNA, a blood sample was collected. High Performance Liquid Chromatography-Mass Spectrometry quantitative dosage of amoxicillin/clavulanate were performed on both cystic and blood samples.

Results : The delay between antibiotic infusion start and cystic/blood samples ranges from 48 To 98 Minutes (mean : 72.7 +/- 13.1). Mean amoxicillin and clavulanate blood levels were 27.2 +/- 12.5 mg/L (12.3 to 53.4 mg/L) and 8.9 +/- 3.9 mg/L (3.3 to 18.5 mg/L), respectively. Amoxicillin cystic levels range from 0 to 3.23 mg/L (mean : 0.59), null in 10/19 patients. By comparison with blood levels, tissue penetration was 2.7% for amoxicillin and 2,6% for clavulanate.

Conclusions : Antibiotic cystic diffusion after 1g amoxicillin/200mg clavulanate infusion is very low, leading to inefficiency to decrease bacterial proliferation. These results suggests that an increase in antibiotic dose or an antibiotic class switch should be evaluated in this setting.

- T05 -

EVALUATION OF THE NUTRITIONAL STATUS OF PATIENTS WITH ALCOHOLIC CHRONIC PANCREATITIS. J. Rigaux, M. Arvanitaki, M. Delhay, J. Devière. Erasme Hospital, Brussels, Belgium.

Introduction : Malnutrition is generally recognized to be frequent in patients with chronic pancreatitis (CP). However, few good studies have been published and very few data are available on the nutritional status of these patients. By another hand, alcohol consumption is a well-known risk factor for malnutrition, suggesting that most patients with alcoholic CP should be malnourished.

Aim : The aim of this study was to evaluate prospectively the nutritional status of patients with alcoholic CP in our tertiary centre.

Methods : Between March and June 2011, 25 patients with alcoholic CP have been included during a hospital stay. Their clinical data have been recorded by a short questionnaire and by search in the medical records. Blood analyses included markers of malnutrition and measurement of vitamins and trace-elements.

Results : Most patients were male (80%), young (median age 49 years), and have been diagnosed with CP for several years (median interval 6 years), with severe parenchymal and/or ductal damages for most of them. Only 36% of the patients admitted an active consumption of alcohol at the time of the study. At least one complication associated with a risk of malnutrition as diabetes, steatorrhea or chronic abdominal pain had been observed in 76% of the patients. Twenty-eight percent of the patients were receiving pancreatic enzymes. Body mass index (BMI) was normal in most patients (median 22.1 kg/m², with 4/25 patients with a BMI < 20 kg/m²) but it was lower than the median BMI observed for other hospitalized patients (25 kg/m²). Biological analyses demonstrated a frequent deficiency in vitamin D, zinc, selenium and iron, while prealbumin (transthyretin) was just at the lower limit and other biological markers remained normal (table 1). Statistical analysis did not permit to demonstrate a significant association between clinical and biological markers.

Table 1

	Median (min-max)	Patients with abnormal values (%)	Normal values
Prealbumin (mg /dL)	21,0 (7,0-36,0)	10/25 (40%)	20-40
Vitamin A (mg/L)	0,4 (0,1-0,7)	3/25 (12%)	0.2-1.0
Vitamin E (mg/L)	9,4 (3,2-16,1)	4/25 (16%)	5.0-20.0
Magnesium (mg/dL)	1,8 (1,1-2,6)	3/25 (12%)	1.6-2.6
Phosphore (mg/dL)	2,9 (2,2-4,2)	3/25 (12%)	2.5-4.5
Copper (μg/dL)	122,0 (61,0-228,0)	7/25 (28%)	70-140
Iron (μg/dL)	50,0 (16,0-137,0)	12/25 (48%)	50-150
Zinc (μg/dL)	70,0 (44,0-135,0)	12/25 (48%)	70-120
Selenium (μg/L)	59 (25-86)	13/25 (52%)	60-120
Cobalamin (pg/mL)	529 (212-1563)	10/25 (40%)	180-700
Folate (ng/mL)	6,5 (1,9-18,5)	4/25 (16%)	1.5-12
Vitamin D (ng/mL)	12,0 (6,0-149,0)	22/25 (88%)	30-80

Conclusions : This study shows that the majority of patients with alcoholic CP develop nutritional deficiencies that are multifactorial. The low prealbumin level reflects that a poor nutritional protein intake contribute to deficiencies in 40% of the patients. As a liposoluble compound, the vitamin D deficiency is typically related to fat malabsorption. Trace-elements (zinc and selenium) deficiencies are frequently observed in alcoholic patients. A larger sample size is necessary to assess these results and to intent to identify statistical association between clinical and biological markers of malnutrition.

- T06 -

MESOPANCREAS : THE «HOLY PLANE» OF PANCREATIC RESECTION FOR CANCER CONTAINS 20% OF ALL LYMPH NODES. C. Bertrand, A. Dili, M.O. Peny, R. Gerard, G. Molle, B. Mansvelt. Hôpital De Jolimont, Haine-Saint-Paul, Belgium.

Introduction : Poor prognosis of pancreatic cancer is attributed to local spread of tumor cells into the retropancreatic tissue along neural plexus as well as to early lymph node metastasis.

Aim : The aim of this study was to show the importance of a perfect clearance of the mesopancreatic tissue, not only to improve R0 resection rate, but also in order to obtain a correct lymphatic staging. Surgical principles with an “artery first” approach are reviewed.

Methods : Immediately after cephalic pancreatectomy, resection of the firm, well-vascularized structure extending from the posterior surface of the pancreatic head to behind the mesenteric vein and artery was performed in 14 consecutive fresh specimen.

9 of these 14 pancreatectomies were performed by a left posterior approach of the mesenteric artery.

Results : 562 lymph nodes were found and analyzed from the complete lymphatic clearance performed (40 nodes/patient). From these nodes, 490 belong to a standard duodenopancreatectomy lymphatic clearance, most of them present in the peripancreatic tissue(42,7%). In the celiac and hepatic lymph node clearance 110 nodes were identified (22%). In the mesopancreas 97 nodes were identified (19,7%).

Conclusions : The recently described mesopancreas contains an important number of lymph nodes, and seems at least as important as the celiac trunc. Its incomplete resection could understage the patients with a consequently high risk of misdiagnosed R1 resection.

Invited lecture

- T07 -

LAPAROSCOPIC PANCREATIC RESECTIONS. C. Hubert. Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium.

The first laparoscopic pancreatic resection was performed by GAGNER in 1992. Currently, more than 800 cases have been reported. However laparoscopic pancreatic resection is not an established treatment for all pancreatic tumors. Indeed, the laparoscopic approach has some limitations due to the retroperitoneal position of the gland and due to the loss of palpation, compared to open surgery. Otherwise, patients who are suffering from aggressive pancreatic tumors like primary adenocarcinomas need a radical oncological surgery to have a chance of cure.

According to the world literature reviewed between 1992 and 2010, including more than 26 retrospective studies, 90% of laparoscopic pancreatic resections have been performed for benign disease located in 89% of cases in the body or in the tail of the pancreas. Eighty percents of laparoscopic pancreatic resections are therefore distal pancreatectomies. For those distal pancreatectomies, the conversion rate into laparotomy is 9% (7-18%). The 2 months peri operative mortality rate is 0.8% (0-8.3%), the post-operative complications rate is 30% (9-53%) and the surgical reoperation rate is 4% (0-12%). Surprisingly, in majority of series of distal laparoscopic resections for malignant tumors, late oncological follow-up are rare and data regarding oncological recurrence are lacking.

Regarding laparoscopic pancreatico-duodenectomy, 150 cases have been reported and 90% were performed for malignant tumors. The conversion rate was 22%, the 2 months peri operative mortality rate is 4.1% (0-4.5%) and the post-operative morbidity rate is from 29% to 60%. However, and while it concerns malignant tumors in 90% of cases, data regarding the oncological aspect (surgical margins, lymph nodes clearance, late survival,...) are lacking.

The oncological benefit of laparoscopic pancreatic resections for high grade malignancies remains therefore unproved.

- T08 -

INTRADUCTAL TUBULOPAPILLARY NEOPLASM OF THE PANCREAS : A RARE AND SEPARATE ENTITY. L. Verset, B. Doukoure, M. Arvanitakis, C. Matos, M.A. Bali, J. Closset, P. Loi, M. Delhaye, N. Nagy, P. Demetter. Erasme Hospital, Brussels, Belgium.

Pancreatic intraductal neoplasms are relatively uncommon and the classification of primary pancreatic intraductal neoplasms proposed by the World Health Organization includes 3 distinct categories : pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN) and intraductal tubulopapillary neoplasm (ITPN). This last one, previously called intraductal tubular neoplasm, has been described recently and is characterised by a predominant tubulopapillary growth without evidence of secreted mucin. These tumours account for only 3% of pancreatic intraductal neoplasms and less than 1% of all pancreatic exocrine neoplasms. Here, we report two cases of ITPN.

The first case concerns a 68 year-old woman followed since 5 years for a main pancreatic duct stenosis without evidence of chronic pancreatitis or malignancy. Tumoural and autoimmune markers were negative. In January 2011, she developed an acute pancreatitis. Magnetic resonance cholangiopancreatography showed a solid mass filling the main pancreatic duct and positive in diffusion-weighted images. An EUS guided fine needle aspiration (EUS guided FNA) and intraductal biopsies displayed cohesive epithelial cells organised in papillae. A total duodenopancreatectomy with splenectomy was carried out and the final diagnosis was ITPN associated with an invasive carcinoma.

The second patient is a 51 year-old woman with bilateral breast cancer. During her oncologic follow-up, a mass of 84 mm was discovered in the pancreatic head and body at abdominal MRI. The lesion was hypermetabolic at PET-scan. An EUS guided FNA highlighted neoplastic epithelial cells. A total duodenopancreatectomy with splenectomy was performed and histopathological examination revealed the diagnosis of ITPN with a minimal invasive component limited to the pancreas.

Limited data in the literature suggest that ITPNs are relatively indolent, with a prognosis that is significantly better than that of ductal adenocarcinoma. Therefore, invasive carcinomas associated with ITPNs should be considered a separate entity, and reported as such.

- T09 -

PANCREATIC MASS OF UNKNOWN ORIGIN. H. Vafa, M. Arvanitakis, P. Demetter, P. Eisendrath, E. Toussaint, J. Devière, M. Delhaye. ULB Erasme, Brussels, Belgium.

- T10 -

OBSTRUCTIVE JAUNDICE FROM UNKNOWN ORIGIN IN A 39 YEAR OLD MAN. A. Thoof, M.A. Bali, E. Coppens, M. Arvanitakis, J. Devière, P. Demetter, M. Delhay. ULB Erasme, Brussels, Belgium.

- T11 -

BILE DUCT OBSTRUCTION BY AN AMPULLARY MASS LESION OF THE PAPILLA OF VATER : RARE CAUSE OF BENIGN BILIARY OBSTRUCTION. K. Nelissen, G. De Hertogh, W. Van Steenberg. KULeuven.

- T12 -

SEVERE PANCREATITIS OF APPARENTLY UNKNOWN ORIGIN IN A YOUNG FEMALE PATIENT. W. Van Moerkercke, W. Van Steenberg. KULeuven.

XXIVth Belgian Week of Gastroenterology
February 9-11, 2012

ABSTRACTS

A01 – A45	Belgian Association for the Study of the Liver (BASL)/Belgian Liver Intestine Committee (BLIC)
B01 – B20	Research Group “Gastrointestinal Regulatory Mechanisms (OG-FWO)”
D01 – D14	Plenary Session Belgian Week of Gastroenterology
E01 – E19	Belgian Group of Pediatric Gastroenterology, Hepatology and Nutrition (BeSPGHAN)
G01 – G13	Belgian Society for Gastrointestinal Endoscopy (BSGIE) and Small Bowel Group
I01 – I24	IBD Research Group (BIRD)
N01 – N14	Research Group of Clinical Nutrition and Metabolism (SBNC and VVKVM)
P01 – P32	Radiology, Pathology and Nuclear Medicine
S01 – S15	Seven Societies Postgraduate Course
T01 – T12	Research Group “Belgian Pancreatic Club” (BPC)

CONTRIBUTORS

A

ABRALDES J.	A08
ABRAMOWICZ M.	I06
ADLER M.	P14
ADRIAENSEN D.	B04
AERTS M.	P28
AERTS R.	A02, A21, A24, A28, A31, A35, A36, A38, P26
AGRÉUS L.	D13
AHMAD T.	D09
ALBERT A.	N01
ALBRECHT J.	A18
ALLIET P.	E03, E10
ALPAERTS K.	B04
AMININEJAD L.	I06
ANNET L.	P15
APPENRODT B.	A08
ARIJS I.	D09, I02, I20, I21, I22, I23
ARSENIJEVIC T.	B09
ARTIEDA M.	D09
ARTS W.	E03, E10
ARVANITAKIS M.	D14, T03, T05, T08
AUDET M.	A32
AUSLOOS F.	A37
AVULA L.R.	B04
AYANG B.	G02

B

BABU D.	B19
BADAQUI A.	A42
BAERT F.	D10, E03, G06, I12
BALI M.A.	T03, T08
BALLET V.	G06, I02
BAMMENS B.	A20, A35
BARTHELEMY N.	N01

BAUMERT T.	A32
BAURAIND O.	E03
BECHMANN L.P.	A09
BECKEBAUM S.	A14
BECKERS A.	P22
BECKERS K.	B10
BEDOSSA P.	A37
BEECKMAN S.	G08
BEL S.	N08
BELAICHE J.	I11
BERREVOET F.	A06, A33, A39
BERT F.	A37
BERTRAND C.	A43, T06
BESSISSOW T.	G06, I17, I18
BEST J.	A09
BIELÉN D.	P12
BIMENYA G.	A03
BISSCHOPS R.	G06, I18, P07, P08
BLOMME B.	A23, A26, D01
BLONDEAU K.	B15
BLYWEERT D.	A44
BOECKXSTAENS G.	B03, B05, B14, D13, S09
BOESMANS W.	N14
BOEYKENS K.	N10
BOLLETQUIVOGNE F.	B17
BORBATH I.	P06, P16
BOSCH J.	A08
BOSELLI E.	T04
BOSMAN F.	P21
BOTELBERGE T.	P29
BOUHNİK Y.	I13
BRASS C.	A18
BREILH D.	T04
BREYNAERT C.	I04
BRINGER M.A.	D09
BRONOWICKI J.P.	A18
BUCCAuw K.	P12
BUCKINX R.	B04
BULLENS D.	I07
BULTE K.	P25
BUREAU C.	A08
BURTIN C.	N02
BUYCK C.	E06
BÜSCHER H.	P29

C

CACA K.	A08
CAEKELBERGH K.	A19
CAILOTTO C.	B03
CALLENS J.	P29
CALLEWAERT B.	E13
CALLEWAERT N.	A26
CANBAY A.	A09
CANI P.D.	E04
CARAUSU J.	D12
CARPENTIER R.	D11
CASSIMAN D.	A20, A21, A24, A35, A36

CASTELEYN C.	A17
CESMELI E.	T02
CEUPPENS J.	I04, I07
CHARDOT C.	D08
CHARLOTEAUX B.	I06
CHATTERJEE S.	A03
CLAES K.	I02
CLAEYS T.	E09
CLEYNEN I.	D09, D13, I17
CLOSSET J.	T08
CNUUDE J.	B09
COCHE J.	E03, E10
CODARIN E.	A33
COLLE I.	A06, A09, A15, A19, A23, A26, A27, A33, D01
COLOMBEL J.F.	I13
COLTESCU C.	A32
COOREVITS L.	I12
COOSEMANS W.	D03, E07, P05
COSYNS S.	B16
COTTE E.	I16, P30
COTTON F.	A40
COUCKE P.	N01
COUDYZER W.	A20
COULON S.	A15, A23, D01
CREMER A.	A40
CREMER J.	I04
CRIEL M.	P25
CRISPIN B.	P30
CUVELIER C.	I08

D

D'AMATO M.	D13
D'HAENS G.	D10
D'HONDT M.	A17
D'HOORE A.	G12, I15
DA POZZO F.	G12
DABO C.	A32
DAELMAN J.	E14
DANO H.	P15
DANSE E.	A42, P13, P30
DARFEUILLE-MICHAUD A.	D09
DARIUS T.	A35
DAVIS M.	A18
DE BACKER A.	E18
DE BACKER O.	B19
DE BRUYNE R.	E02, E13, E14, E15
DE BUCK VAN OVERTRAETEN A.	I15
DE CARLIS L.	A14
DE GALOCSY C.	D14
DE GEZELLE L.	A41
DE GREEF E.	E17, E19, G03
DE HERTOOGH G.	G06, I18, I21, I22, P05, P07, P08
DE KOCK M.	P30
DE LEYN P.	P05
DE LOOZE D.	G08, P28
DE MAN J.	B02, B12
DE MAN M.	P27

DE PRETER V.	I02, I20, I23
DE SCHEPPER B.	P29
DE SCHUTTER I.	E19
DE SIMONE P.	A14
DE SURAY N.	I13
DE VOGELAERE K.	E16, E18, P32
DE VOS M.	D10, G08, I03, I08, I13, T02
DE WACHTER E.	E19
DE WINTER B.	A17, B02, B12
DE WIT E.	N02
DE WIT S.	N07
DE WOLF E.	A33
DEBOULLE M.	A27
DECALUWE H.	D03, E07, P05
DECKER G.	P05
DEFERM N.	E07
DEGALOCY C.	A29
DEGRAEUWE J.	E13
DEGRE D.	A04
DEGREEF E.	E03, E10
DEGRÉ D.	A01, A40
DEHERT S.G.	N04, N13
DEHERTOGH G.	D06
DEITEREN A.	B12
DEJONG C.	P21
DEKEYZER L.	E15
DELAUNOIT T.	P27
DELCARTE L.	P30
DELCROIX M.	A36
DELEYE S.	B02
DELEYN P.	D03, E07
DELHAYE M.	D14, T03, T05, T08
DELOOSE E.	B07, B08
DELPORTE C.	B09
DELTENRE P.	A01
DELVAUX G.	E16, E18, P32
DELVENNE P.	P20
DELWAIDE J.	A29
DEMEDTS I.	G06
DEMETTER P.	A04, P14, P23, P31, T08
DEMOULIN S.	P20
DENG W.	A18
DEPAUW M.	A06
DEPOORTERE I.	B07, B08, B10, N14
DEPREST J.	D03
DEPREZ P.	D12, D14, G02, P06
DERO I.	P27
DEROOSE C.	P02
DESMET V.J.	A02
DESSCHANS B.	A24
DEVIERE J.	A04, D14, I06, T03
DEVISSCHER L.	I03, I08
DEVIÈRE J.	A01, A40, T05
DEVLIEGER R.	N08
DEVREKER T.	E17, E19, G03
DEWIT O.	D10, E03, E10
DHAENENS J.	P05

DHAENS G.	E03, E10
DHOORE A.	D06
DI GIOVANGIULIO M.	B03, B05
DI PASCOLI M.	A08
DILI A.	A43, T06
DLUGOSZ A.	D13
DOFFOEL M.	A32
DOLLÉ L.	A07, A09
DONDEYNE M.	N14
DONNEAU A.F.	N01
DORTU E.	P20
DOUKOURE B.	T08
DRAGEAN A.	P13
DRAGEAN C.	A42, P30
DRESSELAERS T.	I04
DRIESSEN A.	P21
DRITSAS S.	A32
DUARTE H.	T03
DUCATELLE R.	I24
DUFOUR J.F.	S03
DUPAS J.L.	I13
DURAND F.	A10
DURNEZ A.	A02
DUYSBURGH I.	N10

E

EECKHAUT V.	I24
EISENDRATH P.	T03
EL BACHA S.	T03
EL NAWAR A.	N03
ELLERO B.	A32
EMPSÉN C.	A38
ESMAT GAMIL M.	A24
ESPAÑOL SUÑER R.	D11
ETIENNE I.	E03, E10
EYSACKERS N.	A45

F

FARRELL G.	A11
FARRÉ R.	B15, D05
FERNANDEZ M.	P31
FERRANTE M.	G06, I07, I17, I18, I20, I21
FIASSE R.	D14, P06
FIEUWS S.	A28, D06, G12
FISCHER L.	A14
FLAMANT M.	I13
FLAMM S.	A18
FONTAINE F.	E03
FORGET P.	P30
FRANCHIMONT D.	A01, D10, I06
FRANCOIS J.	A21
FRANCOZ C.	A10
FRANCQUE S.	A03, A12, A17, A23, A34, D02
FUMEX F.	T04
FUNG J.	A14

G

GALLEZ J.F.	N03
GARCIA-PAGAN J.C.	A08
GEBOES K.	G06, I18, P24, P27
GEENEN V.	P22
GEERTS A.	A06, A15, A23, A26, A27, D01
GEORGES M.	I06
GERARD R.	T06
GERVY C.	A01
GEUBEL A.	A37
GEURS F.	P25
GIGOT J.F.	P06, P16, P19
GOEMINNE P.	N02
GOMEZ PINILLA P.J.	B03, B05
GORDON S.	A18
GOULET O.	D08
GOVAERE O.	A02, A31, A38
GREGOIRE F.	B09
GREMEAUX L.	A38
GRIMAUD J.C.	I13
GUARNER F.	E08
GUELINCKX I.	N08
GUILLAUME M.	N01
GUIMARÃES E.L.	A07
GULBIS B.	A01
GUSTOT T.	A01, A04, A10, A16, A40, S12

H

HABERSETZER F.	A32
HAENTJENS I.	A25
HAGUE P.	B17
HALFVARSON J.	D13
HAUSER B.	E03, E10, E17, E19, G03, G04
HAUSTERMANS K.	D06
HAV M.	P24
HEINDRYCKX F.	A09, A15, A23, D01
HENDLISZ A.	P27, P31
HENDRICKX K.	P27
HENRIKSEN M.	D10
HERFS M.	P20
HERMAN L.	P20
HEYLEN M.	B02
HIELE M.	E09
HILDERSON I.	P25
HILLAIRES S.	A37
HIMMELREICH U.	I04
HINDRYCKX P.	I03, I08
HO E.	A34
HOEBEKE Y.	G13
HOEFKENS E.	I17
HOFFMAN I.	D03, E03, E06, E07, E09, E10
HOLBRECHTS S.	P27
HOLVOET J.	P29
HOMMES D.	D09
HOORENS A.	P32
HORSMANS Y.	A18

HUBENS G.	A12, B18, D02
HUBERT C.	P19, T07
HUBERT P.	P20
HUMBLET Y.	P19, P30
HUYSENTRUYT F.	P26

I

IEVEN M.	A34
----------	-----

J

JACOBSON I.	A18
JAHNSEN J.	D10
JAMART J.	P30
JANSSEN P.	B07, B08
JANSSEN S.	B08, B10
JIAN R.	I13
JIMENEZ M.	B15
JONAS S.	A14
JONCKX B.	D01
JOOSSENS M.	I02
JOURET-MOURIN A.	P13, P16, P19, P30

K

KARLING P.	D13
KARTHEUSER A.	I16, P13, P30
KATRIEN D.B.	P24
KESHAV S.	I11
KESTENS P.J.	P06
KIM E.	G02
KIRSCH J.	N03
KNEISELER G.	A09
KOEK G.	P21
KOMUTA M.	A02, A31, A38
KUYPERS D.	A35
KWANTEN W.	A17
KY V.	P24

L

LACAILLE F.	D08
LAERMANS J.	B10
LAHARIE D.	I13
LAKATOS P.L.	D09
LALEMAN W.	A08, A17, A20, A21, A24, A35, A36, D02, D04, P26
LAMBAERTS K.	B03, B05, B14, D13
LAMBERT M.	P15
LAMBRECHTS D.	D13
LAMOTTE M.	A19
LANNOO M.	B07
LANNOY V.	P19
LANTHIER N.	A11, D01
LARIDAEN J.	E14
LATERRE P.F.	P30
LAUKENS D.	I03, I08, T02
LAURENT S.	P24

LAWITZ E.	A18
LEBREC D.	A10
LEBRUN V.	A05
LECLERCQ G.	B19, P30
LECLERCQ I.	A05, A09, A11, D01, D11
LECLERCQ P.	T04
LEFEBVRE R.A.	B16, B19
LEFORT C.	T04
LEGERON R.	T04
LEGRY V.	A11
LEHUR P.A.	G12
LEMAIGRE F.	D11
LEMAIRE J.	A43
LEMMENS B.	I18
LEMMERS A.	A01, A40, T03
LEONARD D.	I16, P13, P30
LERUT T.	D03, E07, P05
LI Z.	I07
LIBBRECHT L.	A15, D01
LIEM J.	A30
LINDBERG G.	D13
LIU Z.	A44
LOI P.	T08
LOIS F.	P30
LOTTAZ D.	D09
LOUAGIE L.	A41
LOUIS E.	D10, E03, E10, I06, I11, I13, P20, P22
LOWETTE K.	B13
LUCA A.	A08
LUTTERI L.	P22
LUTUN P.	A32
LYBAERT W.	P27
LYNES M.	I08

M

MACHIELS G.	P27
MACHIELS K.	I02, I20, I21
MACKEN E.	G07, G09
MAES S.	I24
MAHACHIE J.	D09, E03, E10
MAI A.	A45
MAITER D.	P06
MALDAGUE P.	P06
MALFROOT A.	E19
MANA F.	P28
MANNAERTS I.	A07, A45
MANSVELT B.	A43, T06
MARECHAL R.	P31
MARGOS W.	N03
MARTINEZ C.	D05
MASAOKA T.	B20
MATOS C.	P14, T01, T03, T08
MATTEOLI G.	B03, B05
MAUS J.	P29
MAVROGENIS G.	A32, G13
MERTENS I.	A12, D02
MESSAOUDI N.	G07

METSELAAR H.J.	A14
MICHELSEN P.	A03, A12, A17, A19, A29, A34, D02
MIELI-VERGANI G.	A22
MISEREZ M.	E09
MNI M.	I06
MOHAMMEDI I.	T04
MOLENDI-COSTE O.	A05
MOLLE G.	A43, T06
MOMOZAWA Y.	A01, I06
MONBALIU D.	A21, A24, A35, A36
MONSAERT E.	P24, P27
MONTALTI R.	A39, A41
MOONS J.	P05
MOREAU R.	A10
MOREELS T.	B02, B12, G07, G09, P29
MORENO C.	A01, A04, A16, A19, A37, A40, P14
MORGAN-WALSH R.	D09
MOTTERLINI R.	B19
MOURIN A.	D12, G02
MOUZYKA S.	G11
MULS V.	E03
MUSISI E.	A03
MUYOMBYA G.W.	A03
MÜLLER S.	D09

N

NAESSENS B.	P29
NAFTEUX P.	D03, E07, P05
NAGY N.	A04, P14, T08
NAJIMI M.	A07
NAKAD A.	N03
NAPOLEON B.	T04
NEMETHOVA A.	B03
NEVENS F.	A02, A08, A14, A19, A20, A21, A24, A28, A31, A35, A36, D04
NEYNS B.	P28
NGUENANG V.	A42
NGUYEN QUYNH N.D.	P06
NJIMI H.	A10, A16
NOMAN M.	I07

O

OHLSSON B.	D13
OLDE DAMINK S.	P21
OLIEVIER K.	I08
ONYEMA O.	A45
OP DE BEECK B.	S02
OUZIEL R.	A01, A40

P

PADALKO E.	A26, A27
PAIRET G.	P19
PAQUOT I.	E03, E10
PARDON N.	B15, D05
PARNELL K.	D09
PATRIS A.	N03

PAUWELS B.	T02
PAUWELS S.	N08, P06
PEDICONE L.	A18
PEETERS H.	E03, E10, I03, I08
PEETERS L.	A09
PEETERS M.	P27
PELCKMANS P.	A17, B02, B12, D02, G07
PENDER S.	D09
PENNINCKX F.	D06, I15
PENY M.O.	T06
PEPPELENBOSCH M.	B06
PERRET J.	B09
PERRIER C.	I04
PICON L.	I13
PIESSEVAUX H.	D12, G02
PILLANT H.	I13
PIRENNE J.	A02, A20, A21, A24, A28, A31, A35, A36
PIVER E.	I13
PLESSIER A.	A37
POEKES L.	A11
POLUS M.	P22
PONETTE J.	P25
POORDAD F.	A18
POTVIN P.	D10
PRADAT P.	A01
PRENEN H.	P26
PRINCEN F.	N01
PROBIOTICALSTUDYGROUP .	N04

Q

QUERTINMONT E.	A01, A40, I06
----------------	---------------

R

RAEMDONCK K.	B19
RAEVENS S.	A06
RAHIER J.	A42, P15
RAHIER J.F.	E03, E10, P17
RAMON A.	A17
RAUTOU P.E.	A10
REENAERS C.	I11, P22, P28
REMUE C.	I16, P30
REYNAERT H.	A33, A44, S05
REYNTJENS K.	A06
RIBEIRO D.	T04
RIGAUX J.	T05
ROBAEYS G.	A19
ROBBEN K.	E02
ROBERTS L.R.	A02
ROGIERS X.	A06, A25, A26, A33, A39, A41
ROHR-UDILOVA N.	A15
RONCARATI P.	P20
ROOSEN L.	B13, N14
ROSENS B.	G04
ROSKAMS T.	A02, A28, A31, A38, P26
RUEMMELE F.	D08
RUPPERT M.	A12

RUTGEERTS P. D09, G06, I02, I04, I07, I15, I17, I18, I20, I21, I22, I23
RUYTJENS I. P29

S

SAADI S. P18
SABINO J. I02
SAGAERT X. P07
SAINZ-BARRIGA M. A33, A39
SALIBA F. A14
SALIM RASOEL S. B20
SALLERON J. I13
SAULEAU E. A32
SAVOYE G. I13
SCAILLON M. E03, E10
SCHAPIRA M. P06
SCHOUTEN J. S14
SCHUIT F. I20, I21, I22, I23
SCHURMANS P. E09
SEIBOLD F. D09
SEMPOUX C. P15, P16, P19, P30
SERGEANT G. P26
SERSTÉ T. A10, N07
SHEPHERD I.I. B18
SIMREN M. D13
SMETS F. E03, E10
SODERHOLM J. D05
SOENEN S. B19
SOKAL E. A07
SOMJA J. P20
SOWA J.P. A09
SPEE B. A02
SPILLER R. D13
SPRENGERS D. P29
STAELENS D. D09, I22
STAELENS S. B02
STARKEL P. A05, A42, S07
STASSEN J.M. A15, D01
STEFANESCU I. I16
STEGER P.H. P29
STEPPE M. I24
STRAUMANN A. B01
STRUBBE B. G08
STUTO A. G12
SUDAN D. A14
SZCZYPIORSKA M. D09

T

TACCONE F. A16
TACK J. B07, B08, B10, B13, B15, B20, D05, N14
TALBOTEC C. D08
TEJPAR S. P08, P26
TELL G. A33
TEMMERMAN F. A20, A35
TERRIERE L. P29
THEATE I. P15, P18
THELIN-SCHMIDT P. D13

THEODOROU M.	N07
THIJS T.	B10
THOEN L.F.	A07
THORGEIRSSON S.S.	A02
TIEK J.	A28
TIMMERMANS J.P.	B04, B18
TOMEGARCIA M.	P22
TOPAL B.	A02, A28, A31, A38, P26
TOPAL H.	A28
TORDAI A.	D09
TORP R.	D10
TOUSSAINT E.	T03
TRAVIS S.	I11
TREITEL M.	A18
TREPO E.	A04, I06
TROISI R.	A06, A25, A26, A33, A39, A41
TROKAY L.	N01
TRÉPO E.	A01, A40

U

UIJTTERHAEGEN G.	A25
UMMARINO D.	G04
URBAIN D.	G04, P28
URSI D.	A34
UYTTEBROEK L.	B18

V

VALCKE Y.	N02
VALDES H.	P22
VALLA D.	A10, A37
VAN ASSCHE G.	G06, I02, I04, I15, I17, I18, I20, I21, I22
VAN BENEDEN K.	A45
VAN BIERVLIET S.	E02, E13, E14, E15
VAN BREE S.H.	B03
VAN CUTSEM E.	P08, P26
VAN DAMME N.	P24
VAN DE MIEROP F.	E03
VAN DEN BROECK A.	A38, P26
VAN DEN BROECK S.	P30
VAN DEN BROEK M.	P21
VAN DEN EYNDE K.	A38
VAN DEN EYNDE M.	P15, P19, P27, P30
VAN DEN WIJNGAARD R.M.	B11
VAN DER GOTEN J.	I20, I21, I22, I23
VAN DER MERWE S.	A36, D04
VAN DER SCHUEREN B.	B07
VAN DIJCK C.	A17
VAN GAAL L.	A12, D02
VAN GELUWE B.	G12
VAN GILS C.	D02
VAN GOSSUM A.	I06
VAN GOSSUM M.	N07
VAN GRUNSEN L.	A45
VAN GRUNSVEN L.	A07, A09, A38, A44
VAN HEIJNINGEN C.	B05
VAN HOOF T.	G07

VAN HOOF V.	A34
VAN HOOTEGEM P.	D10
VAN HUL N.	A09, D11
VAN IMMERSEEL F.	I24
VAN LAETHEM J.L.	P27, P31
VAN LOMMEL L.	I20, I21, I22, I23
VAN LOO I.	P32
VAN MALENSTEIN H.	A24
VAN MARCK E.	A03, A12, A17, D02
VAN MARCK V.	D02
VAN NASSAUW L.	B04, B18
VAN OOTEGHEM G.	N03
VAN PELT J.	A20
VAN RAEMDONCK D.	P05
VAN ROSSEN E.	A44
VAN STEEN K.	I04
VAN STEENBERGEN W.	A20, A21, A24, A31, A35, A36, D14, P26
VAN STEENKISTE C.	A17
VAN VLIERBERGHE H.	A06
VAN WANROOIJ S.	B14
VAN WANROOY S.	D13
VAN WINCKEL M.	E02, E13, E14, E15
VANACKER O.	E13
VANASSCHE G.	I07
VANBECKEVOORT D.	P12
VANBIERVLIET S.	E03, E10
VANBRABANT W.	B14
VANCUTSEM E.	D06
VANDE VELDE S.	E02, E13, E14, E15
VANDECAVEYE V.	A31, P26
VANDEN BERGHE P.	B13, B20, N14
VANDENBOSCH A.	E09
VANDENBUSSCHE S.	A21
VANDENPLAS Y.	E17, E19, G03, G04, N04, N13
VANDER BORGHT S.	A02
VANDER CRUYSSSEN B.	D10
VANDER PLAETSEN S.	E02
VANDERHEYDEN T.	N08
VANDERLINDEN K.	E16, E18
VANDERMERWE S.	A21
VANDERMEULEN L.	G04
VANDERSTRAETEN E.	P24, P27
VANDERVOORT J.	D10
VANDERWINDEN J.M.	B17
VANDEWINKEL N.	E16
VANGAAL L.	A23
VANGOSSUM A.	E03, E10
VANHEEL H.	B15, D05
VANHOECKE F.	A27
VANHOVE W.	I23
VANKELECOM H.	A38
VANLANDER A.	A39
VANMAELE G.	A25
VANMAERKEN T.	A27
VANORMELINGEN C.	B20
VANPOUCKE H.	I12
VANRAEMDONCK D.	D03, E07
VANSANT G.	N08

VANSLEMBROUCK R.	A20
VANSTEEN K.	E03, E10
VANSTEENBERGEN W.	D04
VANSTEENKISTE C.	A15, D01
VANUYTSEL T.	B15, D05
VANVLIERBERGHE H.	A15, A23, A25, A26, A27, D01
VAZEILLE E.	D09
VEEREMAN G.	D08, E17, E19, G03
VEEREMANWAUTERS G.	E03, E10
VERBEEK J.	A36
VERBEKE C.	N08
VERBEKE L.	A17
VERBELEN T.	D03
VERBIST L.	P29
VERCRUYSSSE V.	A01, A40
VERHAEGEN J.	I02
VERHAEGEN M.	A24
VERHAEVERT A.	A41
VERHELST X.	A26
VERMEIRE S.	D09, D10, D13, E03, E10, G06, I02, I04, I07, I15, I17, I18, I20, I21, I22, I23
VERMEULEN W.	A17, B12
VERNIER-MASSOUILLE G.	I13
VERRIJKEN A.	A12, A23
VERSCHUEREN S.	B08
VERSET L.	A04, P14, P31, T08
VERSLYPE C.	A02, A20, A21, A24, A28, A31, A35, A36, D04, P26
VERSPAGET H.W.	D09
VEYRAC M.	I13
VICARIO M.	B15, D05
VIERLING J.	A18
VINCART H.	I16
VINCENT J.L.	A16
VINEL J.P.	A08
VOS B.	P31
VREULS C.	P21

W

WAELEBROECK M.	B09
WAMUTU S.	A03
WARZEE P.	A32, G13
WEERSMA R.K.	D09
WERBROUCK E.	P07
WEYLER J.	A03
WEYNAND B.	P16, P18
WHORWELL P.	D13
WIJMENGA C.	D09
WILLEKENS H.	G06, P07, P08
WILLEMSEN F.	A30
WING-KIN S.	A09
WINSTANLEY A.	P21
WISSE E.	P21
WITTEBOLE X.	P30
WOEHL M.L.	A32
WOLF P.	A32
WOLTHUIS A.	D06, I15
WOUTERS C.	E06
WOUTERS J.	A38

WOUTERS K.	P27
WOUTERS M.	B14, D13
WYFFELS V.	A19

X

XUERE B F.	T04
------------	-----

Y

YOSHIDA E.	A18
------------	-----

Z

ZAOUI E.	T04
----------	-----