ABSTRACTS

XXIth Belgian Week of Gastroenterology 2009

ABSTRACTS

BASL

- A01 -

ADVANCED GLYCATION END PRODUCTS INDUCE PRODUCTION OF REACTIVE OXYGEN SPECIES VIA ACTIVATION OF NADPH OXIDASE IN HEPATIC STELLATE CELLS. E. Guimaräes, C. Empsen, L. Van Grunsven, A. Geerts. VUB Brussels.

Background/aims : Advanced glycation end products (AGEs) are known to play an important role in the metabolic syndrome, and were recently suggested to contribute to liver fibrosis development. However, little is known about the effect of AGEs on hepatic stellate cells (HSCs), the major contributors to liver fibrosis development. Therefore, we studied the effect of AGEs on reactive oxygen species (ROS) generation, a main feature on HSCs activation.
Methods : three different types of AGEs were generated by BSA incubation with different substrates. The presence of AGE receptors was examined by RTq-PCR, immunofluorescense and western blotting. ROS production was measured using DCFH-DA.
Results : HSCs express five AGE receptors, Galectin-3, CD36, SR-AI, SR-BI and RAGE. All three AGE types induced ROS generation. DPI and NSC, respectively a NADPH oxidase and a Rac1 inhibitor, abrogated ROS production. Rottlerin, a molecule often used as a PKC´ inhibitor, also abrogated ROS production. Knockdown of $p47^{phox}$, Rac1 and PKCdelta decreased ROS production induced by AGEs, establishing a role for these proteins on ROS induction in HSCs.
Conclusions : The demonstration of several AGE receptors and AGE induced ROS generation in HSCs puts forward a potential route through which AGEs could induce liver fibrosis in the metabolic syndrome.

- A02 -

HEPATITIS B-DELTA VIRUS CO-INFECTION IN BELGIUM : PRELIMINARY DATA OF THE BASL HDV REGISTRY. E. Ho (1), P. Michielsen (1), J.P. Mulkay (2), M. Nkuize (2), J. Henrion (3), C. De Galocsy (4), H. Orlent (5), P. Deltenre (3), L. Lasser (6), J. Delwaide (7), I. Colle (8), M. Ngassa (6), J. Holvoet (9), S. Francque (1). (1) UZ Antwerp ; (2) ULB Saint-Pierre ; (3) Hôpital de Jolimont ; (4) Hôpital Bracops ; (5) St Jan Hospital, Bruges ; (6) Hôpital Brugmann ; (7) CHU Sart Tilman ; (8) UZ Gent ; (9) ZNA Middelheim, Antwerp.

Introduction/aim : Hepatitis Delta virus (HDV) is a subviral satellite, detected in 1977, requiring hepatitis B virus (HBV) to propagate, usually leading to severe, chronic liver disease. Although in the 1990s reported in decline, recent data suggest increasing prevalence in Europe. As data on epidemiology and management practice of HDV infection in Belgium are lacking, a retrospective and prospective, multi-centric questionnaire-based registry is being performed under the aegis of the Belgian Association for the Study of the Liver (BASL) during 1 year starting March 1, 2008.
Methods : Active hepatitis B replication is defined as HBeAg + or HBV DNA > 2000 IU/mL HBeAg being negative. Fibrosis was scored according to the Metavir scoring system (F0-F1 : mild, F2-F3 : moderate, F4 : cirrhosis).
ALT levels were characterized as being normal, < 2 X normal, and > 2 x normal. Currently, 12 centres are participating. Preliminary data from 36 patients from 8 centres are presented.
Results : *Gender*, male : 29/36 (80.6%) ; *age* (mean, range) : 38 (19-58) ; *Ethnic origin* : Caucasian 19/36 (52.8%), Black African : 11/36 (30.6%), Oriental : 4/36 (11.7%), Turkish 2/36 (5.9%) ; *Mode of transmission HBV* : intravenous drug use : 7/36 (20.6%), sexual : 2/36 (5.9%), other/unknown : 25/36 (73.5%) ; *Mode of transmission HDV* : intravenous drug use : 7/36 (20.6%), sexual : 1/36 (2.9%), other/unknown : 26/36 (76.5%) ; *Active HBV replication* : HBeAg + : 6/36 (16.7%) ; HBeAg neg and HBV DNA > 2000 IU/mL : 1/36 (2.8%) ; *Coinfection* : HCV 9/36 (25.0%) (all HCV RNA undetectable) ; HIV : 0/34 ; *ALT* : N : 8/36 (22.2%), N-2N : 13/36 (36.1%), > 2N : 15/36 (41.7%) ; *Fibrosis* : F0-F1 : 9/29 (31.0%), F2-F3 : 15/29 (51.7%), F4 : 5/29 (17.2%) ; *Treatment* : No treatment : 10/36 (Interferon : 5/24 (20.8%), Pegylated interferon : 13/24 (54.1%), Lamivudine : 2/24 (8.3%), Adefovir : 1/24 (4.2%), Pegylated interferon + adefovir : 1/24 (4.2%), Pegylated interferon + ribavirine : 2/24 (8.3%) ; *Outcome* : liver transplant : 2/36 (6.3%), death 2/36 (6.3%)
Conclusions : 1. HDV-HBV co-infection in Belgium presents mostly as moderate to advanced liver disease ; 2. About 20% of the patients show active HBV replication ; 3. A majority of patients were infected outside Belgium ; 4. A majority of patients have received or are receiving treatment for HBV and/or HDV. Currently, the effect of pegylated interferon in selected patients on HDV replication is being evaluated including quantitative HDV RNA testing.

- A03 -

VITAMINS C AND E COMBINATION THERAPY SIGNIFICANTLY IMPROVES MOST OF THE BIOCHEMICAL AND HISTOLOGICAL CHANGES OF LIVER DAMAGE IN CCL4 INDUCED CIRRHOSIS IN RATS. S. Wamutu (1), S. Francque (2), S. Chatterjee (3), E. Musisi (1), B. Ambayo (1), G. Muyomba (1), J. Weyler (2), E. Van Marck (2), P. Michielsen (2), G. Bimenya (1). (1) Makarere University, Kampala, Uganda ; (2) UZ Antwerp ; (3) UA Antwerp.

Background : Vitamins C and E have antioxidant properties. A combination of these may effectively offset the effects of CCl4 induced liver damage in rats. There are no current reports on this model.
Aim : To examine the role of vitamins C and E combination therapy on chemically induced liver cirrhosis in rats.
Methods : Three groups (each n = 10) male albino rats were studied. Group I (controls) and Groups II and III were treated every third-day, respectively, with 0.5 mL/kg saline (IP) and 0.5 mL/kg CCl4 (IP) + 0.3 mg pentobarbital in drinking water, for ten weeks. Thereafter, CCl4 and pentobarbital treatments were stopped. Group III was treated with vitamins C and E (50 mg/kg/day) (IM) combination for further 2 weeks, while group II remained untreated. Liver homogenates and blood samples were, respectively, assayed for malondialdehyde (MDA) and glutathione (GSH) and liver-function tests. Liver histology was scored using a modification from Brunt and Metavir systems : necrosis 0-2, ballooning 0-3, steatosis 0-3 and fibrosis 0-4. Biochemical results were analysed using Student's t-test and scores for histology by Pearson Chi-Square.
Results : The Liver/Body weight ratio was significantly ($p < 0.001$) higher in group II compared to controls (0.049 ± 0.002 vs. 0.029 ± 0.001). ALT and ALP were more elevated in group II compared to controls, 132.5 ± 8.9U/L vs. 80.7 ± 4.7 U/L($p < 0.001$) and 237.7 ± 14.5 U/L vs. 99.0 ± 3.7 U/L($p < 0.001$) respectively. GGT was more elevated (39.1 ± 4.8 U/L vs. 30.1 ± 3.9 U/L) without significance. Combination therapy resulted in a significantly lower L/Bwt ratio (0.035 ± 0.001, $p < 0.001$), ALT (97.6 ± 5.6 U/L, $p < 0.005$) and ALP (130.8 ± 11.2 U/L, $p < 0.001$) compared to group II. Hepatic MDA (nmol/mg tissue) was significantly higher (3.52 ± 0.13 vs. 1.43 ± 0.09, $p < 0.001$) and GSH (nmol/mg tissue) lower (38.12 ± 3.42 vs. 66.92 ± 4.55, $p < 0.001$) in group II compared to controls. The combination therapy was associated with a significantly lower tissue MDA (1.96 ± 0.28 nmol/mg tissue, $p < 0.001$) and a higher GSH (56.40 ± 3.19 nmol/mg tissue, $p = 0.001$) compared to group II. Group III also showed significantly lower histology scores for ballooning ($p = 0.028$), steatosis ($p = 0.043$) and fibrosis ($p = 0.009$).
Conclusion : Combination therapy with vitamins C and E is associated with significantly lower biochemical and histological signs of liver damage and with a lower level of oxidative stress in the CCl4 model.

- A04 -

IMPACT OF PEGYLATED BITHERAPY ON MORBIDITY AND MORTALITY IN PATIENTS WITH CHRONIC HEPATITIS C AND NORMAL TRANSAMINASES. P. Deltenre (1), S. Deuffic (2), C. Moreno (3), I. Lonjon-Domanec (4), G. Babany (4), A. Louvet (5), V. Canva (5), S. Dharancy (5), H. Castel (5), F. Roudot-Thoraval (6), J. Henrion (1), P. Mathurin (5). (1) Hopital de Jolimont ; (2) LEM-CNRS, Lille, France ; (3) ULB Erasme ; (4) ROCHE, Neuilly-sur-Seine, France ; (5) Hopital Huriez, CHRU Lille, France ; (6) Hopital Henri-Mondor, Créteil, France.

Background : Patients with chronic hepatitis C (CHC) and normal transaminases (ALT N, between 10 and 46% of CHC) have less severe lesions and a slower progression to cirrhosis than patients with elevated transaminases (ALT E). The impact of virological response to treatment on morbidity and mortality in this population is controversial.
Aims of study : To predict the impact of pegylated bitherapy on progression to cirrhosis and its complications, and on mortality, in patients with ALT N.
Methods : A previously published Markov model was adapted to simulate separately patients with ALT N (30%) and ALT E (70%). The model estimates fibrosis progression rates according to age, sex and whether ALT are normal or elevated, considering that patients with ALT E have a 2.6-time higher progression than patients with ALT N. It takes into account improvement of HCV screening and treatment, and competitive mortality. It is calibrated on reported HCC mortality (CepiDc). Treatment data were obtained in real-life from two multi-centric observatories (HEPATYS and PERSEE) assuming that patients with ALT N are treated 80% lower between 2002 and 2004 and 70% lower from 2005. Two other scenarios of treatment are also assessed.
Results : Repartition of treated ALT N according to genotype and fibrosis predicted by the model is similar as the one observed in HEPATYS and PERSEE. Antiviral treatment on the whole HCV population (ALT E and ALTN) might reduce 2006-2025 morbidity and mortality by 36400 cirrhosis (35%, 35600-36500), 23100 complications (26%, 22400-23200) and 18000 deaths (23%, 16800-18600) of whom 3000 cirrhosis (20%, 2800-3700), 1200 complications (14%, 1000-1700) and 1000 deaths (13%, 900-1300) would be observed in ALT N population. This specific impact of antiviral therapy in ALT N would be observed despite a probability to be treated 3 to 5 times lower than in ALAT E. In a scenario assuming from now that therapeutic guidelines recommend not treating ALT N patients, the treatment impact over the next twenty years would be reduced by 80% in this population. By contrast in a scenario assuming that patients with ALT N will be treated in the same proportions than patients with ALT E, morbidity and mortality over the next twenty years could be further reduced by 1400 cirrhosis (12%, 1300-2500), 600 complications (9%, 600-1000) and 500 deaths (8%, 500-1000).
Conclusion : Treatment of CHC patients with ALT N would have a long-term impact on HCV morbidity and mortality. Therapeutic guidelines need to be proposed after considering their potential impact on HCV morbidity and mortality.

- A05 -

RELATION BETWEEN LIVER PROGENITOR CELL EXPANSION AND EXTRACELLULAR MATRIX DEPOSITION IN A CDE INDUCED MURINE MODEL OF CHRONIC LIVER INJURY. N. Van Hul, J. Abarca-Quinones, C. Sempoux, Y. Horsmans, I. Leclercq.

Background and aims : In chronic liver injury, liver progenitor cells (LPCs) proliferate in the periportal area and migrate inside the lobule where they undergo further differentiation. This process is associated with extracellular matrix (ECM) remodeling. Here we analyzed, in a time-line fashion, LPCs expansion and matrix accumulation in the choline deficient, ethionine supplemented (CDE) murine model of LPC proliferation.
Methods : A CDE diet was administrated to C57Bl6 mice for 0, 3,7,10,14,18 or 21 days. Livers were retrieved and gene expression was evaluated by RT-PCR while (immuno)histochemistry was used to reveal the LPCs and ECM.
Results : After three days of CDE diet, excess collagen deposition is already observed in the periportal area. Expansion of LPCs, as assessed by an increase of cytokeratin 19 (CK19) positive cells, did not occur until day 7. At that time, ECM already accumulated to levels ten times higher than in controls. Thereafter, LPCs and ECM increased in parallel. Further, ECM not only appears prior to the increase of LPCs, but is also found in front of LPCs along the porto-veinous gradient of lobular invasion. Double immunostaining revealed that, at all time, LPCs are embedded in ECM. Moreover, LPCs infiltrating the liver parenchyma are chaperoned by ±-SMA positive cells. Gene expression analyses confirmed those observations. Albumin expression took a fall at day 3, confirming the selective damage to hepatocytes due to the CDE diet, and showed slow recover through the following days. The expression of CK19, ±-fetoprotein, E-cadherin or CD49f mRNA, largely over-expressed by LPCs, significantly increased starting from day 7. By contrast, there was a rapid burst in the expression of components of the ECM, collagen I, laminin and CTGF mRNA at day 3, as well as in ±-SMA expression.
Conclusions : Our data demonstrate that, in the CDE model, ECM deposition and activation of matrix producing cells occurred as an initial phase, prior to LPCs expansion, and in front of LPCs along the porto-veinous gradient of lobular invasion. Those observations may reveal a fundamental role for the established hepatic microenvironment or niche during the process of activation, proliferation and differentiation of liver progenitor cells.

- A06 -

CHRONIC ADMINISTRATION OF VALPROIC ACID INHIBITS COMPLETE ACTIVATION OF HEPATIC STELLATE CELLS IN VITRO AND IN VIVO. I. Mannaerts, N. Nuytten, A. Geerts, L. Van Grunsven. VUB.

Background : Transdifferentiation of hepatic stellate cells (HSC) to myofibroblastic cells (MF) is a central event in liver fibrogenesis. Understanding molecular mechanisms that underlie this cellular event provide pivotal insights into development of new therapeutic modalities for cirrhosis. Stellate cell activation by liver injury leads to a phenotypic transdifferentiation characterized by loss of vitamin A and extensive production of extracellular matrix. This process can be mimicked in vitro by culturing freshly isolated hepatic stellate cells. The use of the histone deacetylase inhibitor (HDAC-I) trichostatin A in these cultures has shown that histone deacetylases (HDACs) might play a role in the pathogenesis of liver fibrosis. Here we investigated the influence of the class I specific HDAC-I, Valproic acid (VPA), on the transdifferentiation from quiescent to activated HSCs in vitro and in vivo.
Methods : Cultured HSCs were exposed to VPA. Cell proliferation was assessed by EdU incorporation assay. Histone acetylation was studied by immunocytochemistry. RNA levels were evaluated by RT-qPCR. Carbon tetrachloride intoxication was used to obtain in vivo HSC activation.
Results : The overall extend of septa formation in livers stained by Sirius Red was smaller in the VPA drinking animals compared to control animals. RNA analysis of the livers showed that VPA co-treatment inhibits the CCl4-induced upregulation of the classical profibrogenic markers ±-SMA, Col1a1, TIMP-1 and MMP13. In vitro treatment of HSCs with VPA enhances histone H4 acetylation. It also strongly inhibits cell proliferation and the expression of HSC activation markers ±-SMA, Lox, Spp1 and Myh11. To specifically study the in vivo effect of VPA on HSC transdifferentiation, stellate cells were isolated from mice treated with CCl4 or CCl4 + 0.5% VPA for 2 weeks. Stellate cells isolated from VPA treated mice display lower RNA levels of activation markers ±-SMA, Lox and Spp1 compared to cells isolated from CCl4 treated mice.
Conclusions : Together, these results suggest that VPA hampers in vitro and in vivo HSC activation. The mechanism of VPA action is still under investigation, since an siRNA mediated knock-down of the class I HDACs could not completely mimic the effect of VPA on in vitro HSC activation.

- A07 -

PROLIFERATION OF HEPATIC STELLATE CELLS IN RATS WITH ACUTE HEPATITIS IS DEPENDENT ON VAGAL INNERVATION VIA MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPE 2. I. Bockx, N. Sinelli, I. Vander Elst, T. Roskams, D. Cassiman. KULeuven Gasthuisberg.

Background : In galactosamine induced hepatitis, hepatocytes lose their replicative capacity and hepatic progenitor cells (HPC) and hepatic stellate cells (HSC) support liver tissue regeneration. We have shown that HPC express M3 and VIP2 receptors, mRNA for several acetylcholine (ACh) receptors and that hepatic branch vagotomy (HV) inhibits HPC proliferation. The aim of our study was to examine whether HSC proliferation could also be neurally modulated.
Methods : RT-PCR with receptor-specific primers was performed on total RNA from isolated HSC, with rat brain RNA as a positive control. In the first experiment, male Wistar rats were subjected to HV or sham operation (SHAM-HV), while another group underwent electrical stimulation of the vagus nerve (STIM vs SHAM-STIM). In the second experiment, rats received IP injections of atropine (ATR), mecamylamine (MEC) or saline (Control). In all rats ($n > 6$ for all groups), acute hepatitis was induced by a single intravenous injection of galactosamine and lipopolysaccharides. Rats were sacrificed 12 and 48 hours later, the time points with maximal necrosis and maximal numbers of HSC, respectively. Frozen liver tissue sections were stained for HSC using polyclonal antibodies against desmin (for periportal HSC, D-HSC) and GFAP (for pericentral HSC, G-HSC). HSC were quantified using image analysis software.
Results : We found that HSC express mRNA for muscarinic receptor subtype 2, for nicotinic receptor subtype ± 1, ± 5 and ²1, and for VIP1 receptor. HV had significantly less D-HSC than SHAM-HV at 12 and 48h ($p = 0{,}008$ and $p = 0{,}01$ respectively), and significantly less G-HSC at 48h ($p = 0{,}03$). STIM on the other hand had more D- and G-HSC at 12 h ($p = 0{,}01$ and $p = 1{,}68.E^{-5}$) than SHAM-STIM. Administration of ATR (blocking of muscarinic receptors) also resulted in less D-HSC at 12h ($p = 0{,}009$) and 48h ($p = 0{,}03$), and less G-HSC at 48h ($p = 0{,}002$) than Control. Blocking of nicotinic receptors by MEC caused less D- and G-HSC at 12h ($p = 3{,}9.E^{-9}$ and $p = 1{,}07.E^{-11}$) but no differences were found at 48 h. These results show that vagal neurotransmission can modulate the proliferation of HSC in acute hepatitis. The stimulatory effect of the vagus nerve on HSC might occur via the direct action of neurotransmitters on the receptors expressed on these cells. Moreover, since the effect of ATR seems to be more pronounced than the effect of MEC, the vagus nerve might exert its effect on HSC mainly through muscarinic receptors.
Conclusion : Intact vagal innervation appears necessary to provoke a proliferative stimulus for HSC, an effect that is most likely exerted through binding of ACh on muscarinic receptor subtype 2, expressed by HSC.

- A08 -

ANTI-VIRAL THERAPY IN HAEMODIALYZED HCV PATIENTS : EFFICACY, TOLERANCE AND TREATMENT STRATEGY. P. Deltenre (1), D. Thabut (2), A. Tran (3), C. Moreno (4), H. Castel (5), M. El Nady (5), V. Canva (5), A. Louvet (5), F. Provot (6), F. Glowacki (6), S. Dharancy (5), H. Ben Ali (5), F. Stanke (7), J. Henrion (1), C. Noel (6), P. Mathurin (5). (1) Hôpital de Jolimont ; (2) AP-HP, Hôpital Pitié-Salpetrière, Paris, France ; (3) CHU Nice, France ; (4) ULB Erasme, Bruxelles ; (5) Hôpital Huriez, CHRU Lille, France ; (6) Hôpital Calmette, Lille, France ; (7) CHU Grenoble, France.

In haemodialyzed HCV patients (HDP), viral eradication may improve survival after renal transplantation. In this prospective study of HDP treated with PegIFN and RBV (1000 mg/week), our **aims** were to : A/ analyse virological response (VR) ; B/ evaluate tolerance and propose a strategy for EPO use ; C/ compare RBV concentrations between HDP and HCV controls with normal renal function.
Methods and study design : In the first part of the study, EPO was increased when HB was < 10 g/dl (strategy 1). In the second part, EPO was doubled from the start of the treatment and then adapted to HB level (strategy 2). Treatment duration was 6-12 months (M) according to genotype (G).
Results : 31 HDP (21 G1/4, 10 G2/3) waiting for renal transplantation were included. Median viral load was 350000 IU/ml (95%CI : 146100-2200000). Fibrosis was dF2 in 27 HDP. Median PegIFN ± 2a/ ± 2b doses were 150 (95%CI : 135-180) and 50 μg/week (95%CI : 35-50). Median RBV dose was 112 mg/day (95%CI : 86-142). A/ 31% of HDP had rapid VR (G1/4 : 17%, G2/3 : 62%, $p = 0.02$), 79% early VR (G1/4 : 74%, G2/3 : 89%, $p = 0.4$), and 48% sustained VR (G1/4 : 40%, G2/3 : 60%, $p = 0.3$). B/ When compared to strategy 1, HDP treated with strategy 2 had higher median EPO doses during M1 (20000 vs. 6000U/week, $p = 0.0001$), higher median HB levels at week 2 (12.7 vs. 11.4mg/dl, $p = 0.04$), M1 (12.8 vs. 11.2, $p = 0.01$), M2 (12.2 vs. 10.0, $p = 0.01$) and M3 (10.8 vs. 9.8, $p = 0.03$). There was a trend for less patients transfused with strategy 2 (18 vs. 40%, $p = 0.2$). C/ Median RBV concentrations were not different in HDP and healthy controls from M2-12 (1.2 vs. 2.4 mg/l at M2, 2.1 vs. 1.9 at M3, 2.1 vs. 2.1 at M6, 2.4 vs. 1.8 at M9, and 1.8 vs. 2.6 at M12, all $p > 0.05$). However, time to reach steady-state RBV concentration was longer in HDP than in healthy controls as shown by median RBV concentrations at weeks 2 (0.5 vs. 1.3mg/l, $p = 0.01$) and 4 (0.8 vs. 1.7, $p = 0.03$).
Conclusions : 50% of HDP treated with PegIFN and adapted doses of RBV reached sustained VR. Doubling EPO from the start of the treatment provides better tolerance than adaptation on demand. 5 pills of RBV/week lead to adequate concentrations. However, the delay to reach steady-state concentrations has to be considered to determine the ideal RBV dose in HDP.

- A09 -

SATAVAPTAN, A V2-RECEPTOR ANTAGONIST AND AQUARETIC DRUG, IMPROVES PORTAL HYPERTENSION IN NON-ASCITIC THIOACETAMIDE-INDUCED CIRRHOTIC RATS. L. Van Landeghem, W. Laleman, I. Vander Elst, J. Van Pelt, D. Cassiman, F. Nevens. KULeuven Gasthuisberg.

Background/aims : Cirrhotic portal hypertension is maintained and aggravated by splanchnic hyperemia and the hyperdynamic circulation. Both phenomena lead to a secondary increase of vasopressor hormones, such as vasopressin (AVP), and a subsequent increase in plasma volume. We aimed to investigate the direct effect of Satavaptan (SR121463B), a selective vasopressin-receptor-2-antagonist and aquareticum, on portal hypertension in a rat model of toxic cirrhosis with portal hypertension but without ascites.
Methods : Different concentrations of satavaptan (0.01 to 0.1mg/kg), in comparison to placebo, were injected intraperitoneally 6 hours prior to hemodynamic measurements and in vivo evaluation in a rat model of thioacetamide-induced cirrhosis (n = 8 per condition). Besides invasive hemodynamic characterization, aquaretic effects and liver and renal function tests were evaluated. Plasma volume was assessed by dilution of administered Evans blue (n = 4). The effect of satavaptan on intrahepatic vascular resistance (IHVR) was evaluated in an in-situ liver perfusion model (n = 8). Sensitivity of hepatic stellate cells (HSC) to V2-receptor-antagonism was studied by a stress-relaxed-collagen-lattice model.
Results : In vivo, portal pressure decreased significantly with 0.1 mg/kg satavaptan, (TAA + satavaptan vs TAA : 5.7 ± 0.9 mmHg vs 8.7 ± 1.7 mmHg, P = .004). Satavaptan did not affect splanchnic flow (4.7 ± 0.9 mL/min/100 g vs 5.2 ± 0.7 mL/min/100 g, P = .383) nor mean arterial pressure (P = .429). Total urine volume (19.8 ± 11.9 mL/6 h vs 2.5 ± 1.1 mL/6 h, P = .04) was increased while urinary osmolality (744 ± 323 mmol/kgH2O vs 1403 ± 137 mmol/kgH2O, P = .06) decreased inversely. Serum levels of sodium and creatinine remained stable. No hepatic or renal toxicity was observed in the range of doses used. During cirrhotic liver perfusion, increasing doses of satavaptan did not aggravate the increased IHVR nor did satavaptan reduce the AVP-induced dose-related increase in perfusion pressure. Plasma volume was significantly decreased in rats treated with 0.1mg/kg satavaptan (4.79 ± 0.15 mL/100 g vs 5.45 ± 0.24 mL/100 g, P = 0.008). In vitro, preincubation with increasing doses of satavaptan did not significantly alter AVP-promoted HSC contraction (P = 0.925).
Conclusion : Satavaptan improves portal hypertension by means of an extrahepatic effect, including a decrease in plasma-volume.

- A10 -

ANTIVIRAL TREATMENT IMPROVES SURVIVAL OF PATIENTS WITH CHOLESTATIC FIBROSING HEPATITIS AFTER LIVER TRANSPLANTATION FOR HEPATITIS C. G. Robaeys, D. Cassiman, D. Monbaliu, R. Aerts, J. Pirenne, F. Nevens. KULeuven Gasthuisberg.

Background : Among the ± 25% of orthotopic liver transplantation (OLT) recipients who develop progressive liver allograft injury within the first 5 years related to theirhepatitis C virus (HCV) recurrence, the subset of patients who develop cholestatic fibrotic hepatitis (CFH) has an extremely high mortality. Little data are available about outcome of antiviral treatment in CPH patients.
Aim : Compare the outcome of antiviral treatment during a fixed period of 18 months in patients developing CFH vs. patients developing rapidly progressive hepatitis (RPH) (defined as more than F2 fibrosis in the absence of cholestasis).
Methods : From 8.2000 to 11.2007, all patients developing CFH and RPH due to HCV infection after OLT were treated with peginterferon alpha-2-a 135 μg once a week and ribavirin 400 × 800 mg a day during 18 months and evaluated in a monocentric, prospective, cohort study for SVR, side effects, survival.
Results : Respectively 13 (M/F = 8/5, age = 59.2 ± 3.5 y) and 14 patients (M/F = 8/6 ; age : 54,8 ± 19,1 y) developed CFH and RPH from resp. 8.9 ± 5 and 22 ± 13 months after OLT. In CFH patients genotype 1 was present in 6 patients, 3 in 1, 4 in 2 ; while in RPH patients genotype 1 in 6, 2 in 1, 3 in 1, 4 in 1, 5 in 2. Genotype was unknown in 4 of CFH and 3 of RPH group. During antiviral treatment ribavirin had to be stopped temporarily because of anaemia in resp. 15% (2/13) and 21% (3/14) (P = 0.53).
There was no statistical significant difference in SVR between both groups (P = 0.46). In CFH patients SVR was 33% (4/12) and HCV RNA is absent in one patient after 12 months still on treatment. Antiviral treatment was associated with improvement of cholestasis in all but in 2/13 there was a need of retreatment due to recurrence of cholestatic hepatitis. In the RPH group SVR was reached in 43% (6/14) of the patients.
No statistical difference in survival (P = 0.34) was obeserved (mean follow-up of CFH :35months vs. RPH : 47 months). In CFH patients 38% (5/13) died (3 : nonresponders ; 2 : recurrence of HCC) while 21% (3/14) in RPH (1 : recurrence of HCC).
Conclusion : 18 month lasting antiviral treatment offers similar survival for CFH and RPH patients and is higher than what has been previously reported for CFH. The results of this pilot study must be confirmed in larger studies.

- A11 -

KUPFFER CELL DEPLETION IMPROVES HEPATIC INSULIN SENSITIVITY. N. Lanthier, M. Petit, H. Pélerin, J. Abarca, V. Lebrun, O. Molendi, D. Campard, Y. Horsmans, I. Leclercq. UCLouvain.

Objectives : We hypothesize that inflammatory cells in the liver participate to metabolic disturbances and hepatic insulin resistance (IR). In a model of high fat diet (HFD)-induced steatosis and IR, we therefore analysed the activation of macrophages and studied the effect of Kupffer cells (KC) depletion on hepatic lipid storage and insulin sensitivity.
Methods : Male mice were fed a standard or a HFD (60% calories from lipids) for 3 days preceded by injection of liposome-encapsulated PBS (controls) or clodronate to deplete KC. Liver macrophage populations (F4/80, CD11b, CD11c and CD68) were evaluated by flow cytometry and immunohistochemistry (IHC). The insulin signalling pathway upon insulin stimulus was analysed by western blot. RT-qPCR was used to examine mRNA expression of macrophage activation, lipid metabolism and inflammatory markers.
Results : Upon HFD, hepatic lipids increased 4-fold and IHC of liver sections revealed enlarged F4/80 + KC in close proximity to fat-laden hepatocytes. FACS analysis demonstrated an increased proportion of activated F4/80 + CD68 + KC. In response to insulin, phosphorylation of insulin receptor and intermediates of the insulin signalling cascade was significantly lower in HFD than in control livers. Liposome-encapsulated clodronate depleted (by 70%) F4/80 + CD68 + KC and restored phosphorylation levels of insulin signalling in HFD-fed animals close to those seen in controls, without affecting hepatic lipid content. PCR analyses showed that increased gene expression of F4/80, CD68, TLR4 seen in HFD-fed animals was blunted by clodronate.
Conclusions : High fat feeding induces steatosis, hepatic IR and an early KC activation. KC depletion by clodronate restores hepatic insulin sensitivity, suggesting that targeting their activation may represent an attractive tool to relieve HFD-induced hepatic IR.

- A12 -

THE INTERLEUKIN-17 PATHWAY IS INVOLVED IN HUMAN ALCOHOLIC LIVER DISEASE. A. Lemmers (1), C. Moreno (1), T. Gustot (1), R. Maréchal (1), D. Degré (1), P. Demetter (1), P. De Nadai (2), A. Geerts (3), E. Quertinmont (1), V. Vercuysse (1), O. Le Moine (1), J. Devière (1). (1) ULB Erasme ; (2) ULB IRIBHM ; (3) VUB.

Background & aims : Immune dysregulations in alcoholic liver diseases are still unclear, especially regarding alcoholic hepatitis inflammatory burst. Interleukin-17 (IL-17) is known to enhance neutrophil recruitment. We studied the IL-17 pathway in alcoholic cirrhosis (AC) and alcoholic hepatitis (AH).
Methods : Patients with alcoholic liver disease (n = 76) were compared to patients with chronic HCV infection (n = 16), autoimmune liver disease (n = 18) and healthy controls (n = 10). IL-17 plasma levels and peripheral blood mononuclear cells (PBMC) secretion were assessed by ELISA and T cell phenotype by flow cytometry. IL-17 staining and co-staining with CD3 and myeloperoxidase were performed on liver biopsies. IL-17 receptor expression was studied on liver biopsies and in human hepatic stellate cells as well as their response to recombinant IL-17 by chemotaxis assays.
Results : IL-17 plasma levels were increased in alcoholic liver disease patients (55.9[0-265.9]pg/ml) ; $p < 0.001$ vs healthy subjects). PBMCs of patients with alcoholic cirrhosis produced higher amounts of IL-17 than healthy subjects (1470.5[703.4-2695] vs 727.9[406.7-1406.7]pg/ml, respectively ; $p < 0.001$) and their CD4 + T lymphocytes disclosed an IL-17 secreting phenotype (AC 2.1[0.7-3.8] vs healthy subjects 1.0[0-3.9]%CD3 + CD4 + gated cells, $p < 0.05$). In the liver, IL-17 + cells contributed to inflammatory infiltrates in autoimmune, HCV related, and alcoholic chronic liver diseases. Alcoholic hepatitis foci disclosed many IL-17 + cells, including T lymphocytes and neutrophils. In alcoholic liver disease, liver IL-17 + cells infiltrates correlated to MELD score (Á = 0.5), and in alcoholic hepatitis to modified Discriminant Function (Á = 0.5). AH was associated with lower IL-17 plasma levels compared to alcoholic liver disease patients without AH (AH 41.4[0-200.4]pg/ml vs no AH 106.6[0-265.9]pg/ml ; $p < 0.05$) and no difference with controls in terms of IL-17 + cells among circulating CD4 + lymphocytes or IL-17 secretion upon PBMCs stimulation (AH 855.6[469.7-2000]pg/ml vs healthy subjects 727.9[406.7-1406.7]pg/ml, $p = 0.4$). IL-17 receptor was expressed in alcoholic liver disease by hepatic stellate cells and these cells recruited neutrophils following IL-17 stimulation in a dose-dependent manner through IL-8 and GRO-± secretion in vitro.
Conclusions : Human alcoholic liver disease is characterized by the activation of the IL-17 pathway. In alcoholic hepatitis liver infiltration with IL-17 secreting cells infiltrates is a key feature that might contribute to liver neutrophil recruitment.

- A13 -

KERATIN 19 EXPRESSION IN HEPATOCELLULAR CARCINOMA (HCC) IS CORRELATED WITH POST-OPERATIVE TUMOUR RECURRENCE AND METASTASIS MARKERS. B. Spee (1), A. Durnez (1), S. Vanderborght (1), O. Govaerde (1), V. Desmet (1), S. Thorgeirsson (2), T. Roskams (1). (1) KULeuven ; (2) LEC/NIH, USA.

Background : The presence of keratin(K)19, a progenitor cell/biliary marker, in HCC has been reported to predict early recurrence after resection and a worse overall survival after surgical therapy.
Materials & methods : To investigate the clinicopathological correlations and relevance of *KRT19* expression (mRNA) in HCCs, and to discern potential clues for a more aggressive behaviour, a micro-array was performed on a data-set of 139 well-characterised HCC from patients (61 from China, 49 from Belgium, and 29 from U.S.). The relationship between *KRT19* expression and various clinicopathological parameters (e.g. age, gender, aetiology) was analysed using the Mann-Whitney test. The prognostic relevance of variables was assessed using the log rank test and the univariate and multivariate Cox proportional hazards model (HZ). Genes, associated with expression of *KRT19* were identified using Pearson's correlation, with a cut-off of > 0.5 or < -0.5 correlation. Quantitative PCR (qPCR) on a selection of markers confirmed differential expression in K19 positive HCCs.
Results : *KRT19* expression is significantly associated with a highly elevated serum alpha-feto protein (AFP) (> 300 ng/ml) (p = 0.027), poor tumour differentiation (Edmondson Steiner differentiation grade III-IV versus I-II, p = 0.0018), and a larger tumour size (e 5cm) (p = 0.041). *KRT19* expression as a continuous variable significantly correlates with poor survival (p < 0.0001, HR :1.43) and with tumour recurrence (p = 0.0001, HR :1.56). In addition, *KRT19* significantly correlated with gene-expression of non-tumoral progenitor cells/biliary markers (*K7, PROM1, NCAM1, GSTP1, LIF*), a lower expression of hepatocyte markers (e.g. *HNF4A, albumin, F2, F8, F11, ABCC2*), and an increase in known metastasis markers (*S100A6, CTBP2, CT120, TACSTD2, MTSS1, RAB27B*).
Conclusions : *KRT19* expression in HCC is an independent predictor of tumour recurrence after resection. Furthermore, *KRT19* expression does not appear as an isolated progenitor cell trait, but correlates significantly with the expression of well-known markers of hepatic progenitor cells and with a highly elevated serum AFP level. In addition, HCCs with *KRT19* expression are decreased in hepatic differentiation and are more prone to metastasis.

- A14 -

DISTINCT ROLES OF NONMUSCLE MYOSIN II ISOFORMS IN THE REGULATION OF MOUSE HEPATIC STELLATE CELL CONTRACTION AND MIGRATION. Z. Liu (1), H. Reynaert (1), E. Van Rossen (1), L.A. Van Grunsven (1),B. Schroyen (1), J.P. Timmermans (2), A. Geerts (1). (1) VUB, (2) U Antwerp.

Background : Contraction and migration of hepatic stellate cells (HSC) during hepatic injury is essential for wound-healing, liver fibrosis and portal hypertension.
Aims : To investigate the role of nonmuscle myosin II (NMMHC-II) isoforms in mouse HSC.
Methods : First all, we identified which NMMHC-II(s) is expressed in HSC by QRT-PCR and Western blot. Then their subcellular localization was visualized by immunofluorescence and/or confocal microscopy after immunofluorescence staining. The interaction with actin isoforms was explored by dual-immunostaining and further conformed by co-immunopericiptation. Under the condition of isoform-specific siRNA gene silencing or treatment with chemical inhibitors, morphological and functional assays were performed. Changes were visualized with phase contrast microscopy. ET-1-induced contractile force generation was examined by collagen gel contraction and silicone wrinkle formation assay. Intracellular Ca2+ increase induced by ET-1stimulation was measured using Fluo-4. Cell migration was evaluated using the 'wound healing' assay.
Results : NMMHC-IIA and -IIB are expressed in cultured HSC at both mRNA and protein level. NMMHC-IIA mRNA and protein levels were roughly constant. It was located in the subcortical area of quiescent HSC and colocalized with ±-SMA in stress fibers in activated HSC. Knockdown of NMMHC-IIA by siRNA decreased cell size, stress fibers and vinculin-containing focal adhesion. Knockdown of NMMHC-IIA completely impaired wrinkle formation on silicone substrate, and also blocked intracellular [Ca2 +] release response to ET-1, but accelerated wound-induced cell migration. NMMHC-IIB was upregulated at both mRNA and protein levels. It was present in cytoplasmic processes of quiescent HSC, lamellipodia and cytoplasm in activated HSC. Colocalization of NMMHC-IIB and ^{2}-actin was observed in the leading edge of lamellipodia. We also found expansion of ±-tubulin in lamellipodia (but not the leading edge) following spreading of the leading edge. Knockdown of NMMHC-IIB by siRNA decreased the formation of lamellipodia, impaired expansion of ±-tubulin in lamellipodia, and slowed down cell migration. We did not observe a significant effect of NMMHC-IIB on stress-fiber formation, cell contraction and intracellular Ca2 + release.
Conclusions : myosin IIA and IIB are differentially expressed and localized in mHSC. They play distinct and complementary roles : myosin IIA controls cell body, focal adhesions and cell contraction by regulating stress-fiber formation which contain myosin IIA and ±-SMA. Myosin IIB mediates lamellipodia spreading and cell migration by distribution of ^{2}-actin in the leading edge and by inducing ±-tubulin-expansion in lamellipodia.

- A15 -

ENTERAL NUTRITION WITH OR WITHOUT N-ACETYLCYSTEINE IN THE TREATMENT OF SEVERE ACUTE ALCOHOLIC HEPATITIS : A BELGIAN RANDOMIZED, MULTICENTER, CONTROLLED TRIAL.
C. Moreno (1), P. Langlet (2), A. Hittelet (1), S. Evrard (1), L. Lasser (2), I. Colle (3), A. Lemmers (1), J. Devière (1), O. Le Moine (1). (1) ULB Erasme ; (2) ULB Brugmann ; (3) UZ Gent.

Background and aims : Severe acute alcoholic hepatitis (AAH), defined by a mDFe32, is associated with a high mortality rate. Oxidative stress is involved in the pathogenesis of AAH. One major effect of N-acetylcysteine (NAC) is the gluthation stores restoration, limitation of oxidative stress and decrease of cytokine production as TNF. Evidence was also provided that enteral nutritional support might increase survival in patients with severe AAH. Therefore, the aim of the present study was to evaluate the efficacy of NAC in combination with adequate nutritional support in patients with severe AAH.
Methods : Patients with biopsy proven AAH and a mDFe32 were randomized to receive NAC (300 mg/kg) intravenously (NAC group) or a placebo perfusion (control group), and enteral nutrition (27 kcal/kg/day) in addition to usual meals for 14 days. Primary enpoint was 6 months survival ; secondary endpoints were biological parameters evolution and rate of infections.
Results : 44 patients were randomized in the study (25 in NAC arm, 19 in control arm). Both groups did not differ for baseline characteristics : mean age : 50 ± 1.7 vs 47 ± 1.7 years (p = 0.19) ; males : 68 vs 78.9% (p = 0.42) ; mean mDF : 57.5 ± 3.5 vs 58 ± 5.4 (p = 0.70) ; mean MELD score : 24.1 ± 1.1 vs 24.3 ± 1 (p = 0.70) ; mean bilirubin : 16.8 ± 2.3 vs 22 ± 3 mg/dl (p = 0.14) ; mean PT : 40.6 ± 1.8 vs 44.5 ± 2.4% (p = 0.25) ; mean creatinin : 1.1 ± 0.2 vs 0.9 ± 0.1 mg/dl (p = 0.81) ; mean albumin 29.2 ± 1 vs 28.5 ± 0.9 g/l (p = 0.63) ; mean AST : 111.8 ± 9.7 vs 147.8 ± 25.6 IU/l (p = 0.34) ; mean leukocytes : 11.8 ± 1.4 vs 11.7 ± 1.1x10^3/mm^3 (p = 0.68) in NAC vs control group, respectively. Survival rates at 1 and 6 months in NAC and control groups were 70.8 vs 83.3% (p = 0.35) and 62.5 vs 66.7% (p = 0.78), respectively. Mean bilirubin changes at day 3, 8 and 14 did not differ between both groups. Documented infection rate at one month was 40% vs 28% (p = 0.41) in NAC vs control group, respectively.
Conclusions : In this study, the addition of NAC to enteral nutrition in patients with severe AAH did not improve 1 and 6 months survival, 1 month infection rate, and bilirubin changes from day 3 to day 14. Survival rates observed in this study are comparable to those reported in studies with current recommended therapies, suggesting a major role of systematic use of enteral nutritional support in severe AAH.

- A16 -

THE PRESENCE OF AN INFLAMMATORY GENE EXPRESSION PATTERN IN PERITUMORAL LIVER TISSUE PROTECTS AGAINST DEVELOPMENT OF MACROVASCULAR INVASION AND IS DECREASED AFTER ANTIANGIOGENIC TREATMENT WITH SUNITINIB IN CIRRHOTIC RATS.
H. Van Vlierberghe, I. Colle, M. Praet, L. Libbrecht. UZ Gent.

Recently, Hoshida *et al*. (New Engl. J. Med., 359: 1995) described a 186-gene signature that predicts survival and late (> 2 years) recurrence after partial liver resection for early-stage hepatocellular carcinoma (HCC). The authors suggest that this signature is related to metachronic, multicentric development of HCC, explaining its association with late recurrence and survival. They also described that a considerable proportion of genes correlating with poor prognosis were associated with inflammatory pathways, while good prognosis genes were more related to liver function.
To further evaluate the significance of this gene signature, we assessed its pattern and average expression in two independent microarray datasets : 1) peritumoral liver samples surrounding 20 HBV-associated more advanced HCCs, of which 9 with macrovascular invasion and poor survival and 11 without macrovascular invasion or intrahepatic metastasis and better survival (GSE5093 ; Cancer Cell 10 :99) and 2) samples of CCl4-induced cirrhosis in rats treated with vehicle (n = 4) or with the antiangiogenic compound sunitinib (n = 4) (GSE6929 ; Hepatology, 46 : 1919).
Hierarchical clustering of the 20 peritumoral liver samples using the 186 genes revealed two very distinct clusters : one cluster with 9 samples of which 8 surrounded HCC without macrovascular invasion, while 8 of 11 cases in the other cluster were liver samples surrounding HCC with macrovascular invasion (p = 0.0059). We also calculated an inflammatory score by subtracting the average expression of good prognosis genes from that of the poor prognosis (i.e. inflammatory) genes. This inflammatory score was lower in peritumoral liver around HCC with macrovascular invasion than in liver adjacent to HCC without macrovascular invasion or intrahepatic metastasis (p = 0.0367). Furthermore, the inflammatory score was negatively correlated with tumor size (r = -0.484, p = 0.0296).
Hierarchical clustering of the eight CCl4-induced cirrhotic rat liver samples also showed two clusters based on sunitinib treatment, although cluster size was too small for statistical analysis. Moreover, the inflammatory score was lower in sunitinib-treated liver than in vehicle-treated liver (p = 0.0285), which is in line with the finding that sunitinib decreased inflammatory infiltrates.
In conclusion, our analyses strongly suggest that the inflammatory liver environment which stimulates metachronic and multicentric HCC development also protects against development of macrovascular invasion. Since this inflammatory pattern is subsided by sunitinib in a rat model of liver cirrhosis, it is possible that antiangiogenic treatment in the neoadjuvant setting (e.g. bridging therapy for patients on transplant waiting list) might lead to increased development of macrovascular invasion and intrahepatic metastasis.

Invited lecture
The BASL/BLIC Spring Meeting Lecture

-A17-

REJECTION AND IMMUNE TOLERANCE. A.D. Billiau. University of Leuven, Leuven, Belgium.

Fifty years after the first allogeneic kidney transplant under immunsuppressive cover of azathioprine, allotransplantation has become a major success. Progress in surgical techniques and the introduction of modern immunosuppressive agents have led to a dramatic improvement in survival rates of all solid organ grafts and their recipients. Currently, for most solid organ grafts, short-term graft loss due to acute rejection can be adequately controlled. However, despite the use of novel immunosuppressants, chronic graft loss remains a significant problem and is principally due to drug-related toxicity and chronic immune-mediated rejection. In this respect, liver grafts are known to be exceptional because of their unique tolerogenic properties, although the 'liver-tolerance' effect is as yet incompletely understood. The problem of chronic graft loss has shifted the focus of transplant research toward long-term graft function and survival, and toward quality of life of the recipient. Indeed, in addition to graft-specific toxicity, prolonged immunosuppressive treatment leads to significant patient-related side-effects, including infections, renal and cardiovascular disease, infections and malignancy. In theory, induction of transplantation tolerance would both obviate the need for immunosuppression and preclude the drug-related side-effects. 'Transplantation tolerance' is defined as the specific and definitive acceptance of a transplanted graft in the absence of ongoing immunosuppression. 'Prope tolerance' refers to graft acceptance and stable graft function under significantly reduced immunosuppression. Major advances have been made in the understanding of the immunological mechanisms of rejection and tolerance. In particular, induction of mixed allogeneic hematopoietic chimerism using hematopoietic stem cell transplantation (HSCT) has been shown in rodents to provide robust donor-specific tolerance, even with non-myeloablative conditioning regimens. Following successful translation of this approach in a large animal model and in a series of patients receiving a combined (single-donor) HSCT and living kidney transplant for multiple myeloma, the first clinical pilot trials of intentional induction of donor-specific tolerance for renal allografting have been undertaken, with encouraging results. Non-HSCT approaches to induce transplantation tolerance, relying on T cell depletion, (co)signaling blockade of donor antigen infusion are under investigation. With this respect, prope tolerance has been achieved in a recently published series of patients receiving allogeneic intestinal transplantation under a tolerizing regimen that includes donor-specific blood transfusion.

- A18 -

CONFIRMATION OF CANDIDATE SERUM MARKERS OF HEPATITIS C-RELATED FIBROSIS FOLLOWING SERUM PROTEOMICS. K.J. Cheung, K. Tilleman, D. Deforce, I. Colle, H. Van Vlierberghe. UZ Gent.

Background : Serological fibrosis markers have gained much attention over the past ten years and have generated several commercial diagnostic tests. These approaches are much more preferable than the liver biopsy, given the non-invasive properties. In our previous proteome study, several candidate serum markers for hepatitis C-related fibrosis were suggested : Mac-2-binding protein, ±2-macroglobulin, hemopexin and ±-1B-glycoprotein were increased in late fibrosis stages, while ±1-antitrypsin, fetuin-A, leucine-rich ±2-glycoprotein and haptoglobin were decreased. Mac-2-binding protein expression has already been confirmed.
Aim : The aim of this study was to confirm these candidate markers based on an enzyme-linked immunosorbent assay (ELISA) approach for ±2-macroglobulin (±2M), hemopexin (Hx), haptoglobin (Hp) and fetuin-A (FetA).
Materials & methods : Hepatitis C patient distribution (n = 82) according to METAVIR : F0 (n = 6 ; age = 31 [17-37] yrs) ; F1 (n = 25 ; age = 37 [20-69] yrs) ; F2 (n = 24 ; age = 47.5 [21-62] yrs) ; F3 (n = 9 ; age = 52 [22-73] yrs) ; F4 (n = 18 ; age = 64.5 [43-79] yrs) (median [min _ max]). ELISAs for ± 2M (n = 40), Hx (n = 42), Hp (n = 24) and FetA (n = 71) were performed and protein concentrations were measured by an UV-VIS spectrophotometer at 450 nm. These levels were compared between all METAVIR scores and between early (F0F1) and late (F2F3F4) fibrosis using the Kruskall-Wallis test and Mann-Whitney U-test at $p < 0.05$, respectively.
Results : A distinction betweenfibrosis stageswas observed in the Hx-levels ($p < 0.05$), characterized by the decrease in F1 (0.4 [0.4-0.6] g/l) and F4 (0.5 [0.2-0.6] g/l) opposite to F2 (0.6 [0.4-0.8] g/l). Thisis rather the opposite of what we saw inour previous proteome analysis. In addition, an upwards trend in ± 2M-levels was observed. A2M-levels were increased in F3 (4.2 [3.0-5.9] g/l) and F4 (3.7 [3.0-5.2] g/l) opposite to F0 (3.2 [2.6-3.5] g/l) ($p < 0.05$), which is consistent with our previous data. Hp- and FetA-levels were not significantly altered, although Hp-levels showed a certain tendency. Comparison of early versus late fibrosis resulted in an expected increase of the ±2M-levels in late stage (3.2 [2.4-4.5] g/l) opposite to early stage fibrosis (3.8 [2.4-5.9] g/l) ($p < 0.05$).
Conclusions : In conclusion, an increase in serological ±2M-levels was confirmed by ELISA, whileHx-levels following ELISA wereinconsistent with our previous proteome data and might suggest the discrepancy between these approaches. No distinction was found for Hp- and FetA-levels. Despite throughput, robustness and ease, ELISA and antibodies, in general, are unableto distinguish specific isoforms of a certain protein (e.g. FetA, Hx). Further specification will enable the outlining of better approaches for confirmation and measurement as well as giving functional insightsof these proteins.

- A19 -

LIVER TRANSPLANTATION FOR SUBACUTE HEPATOCELLULAR FAILURE AFTER SCOPINARO BILIO-PANCREATIC DIVERSION. S. Rogge, H. Van Vlierberghe, A. Geerts, R. Troisi, F. Berrevoet, X. Rogiers, B. De Hemptinne, I. Colle. UZ Gent.

Background : The prevalence of obesity has increased dramatically over the last decades. Comorbidities related to obesity, such as non-alcoholic fatty liver disease (NAFLD) are also increasing. Therapeutic options for obesity include restrictive bowel surgery and surgery that promotes malabsorption, such as the Scopinaro (biliopancreatic diversion) technique. Bariatric surgery is effective in promoting weight loss with control of comorbidities. Nevertheless complications associated with these procedures, such liver failure, have been observed.
Patients and methods : We report here 4 patients – 25 to 59 years old – who developed hepatic failure 13 to 84 months after bariatric surgery (Scopinaro procedure).
Results : Due to irreversible hepatic failure, liver transplantation was indicated for our 4 patients. Liver transplantation was performed in 2 of the patients. In the first patient also a reversal of the bariatric surgery was performed. There were no immediate post-operative complications and this patient is now 6 years and 9 months after transplantation. The hepatic state is stable, but there is a significant gain of weight. The second patient is now 2 months after transplantation, the post-operative period was uneventful. The 2 other patients are on the waiting list for liver transplantation.
Conclusion : Surgical procedures for the treatment of obesity have resulted in satisfactory ameliorations of the associated metabolic abnormalities. The Scopinaro procedure has been advocated by some surgeons as a safe and effective treatment for severe obesity. Nonetheless severe liver disease and hepatocellular failure have been observed as a rare but sometimes fatal complication of biliopancreatic diversion. The possible cause of this complication of surgery for obesity is poorly understood. This complication may be secondary to rapid and excessive weight loss after surgery (starvation hepatitis). An alternative concept is that it arises as a result of progression of pre-existing steatohepatitis. The real incidence of severe liver disease following Scopinaro is probably underestimated.

- A20 -

MYCOPHENOLATE MOFETIL MONOTHERAPY IN STABLE LIVER TRANSPLANT PATIENTS WITH CALCINEURIN INHIBITOR RELATED TOXICITY. K. Tilleman, A.M. Geerts, X. Rogiers, R. Troisi, B. De Hemtpinne, I. Colle, H. Van Vlierberghe. UZ Gent.

Aim : We report our experiences with calcineurin inhibitor (ciclosporin, tacrolimus (CNI) withdrawal and mycophenolate mofetil (MMF) monotherapie in 6 adult orthotopic liver transplantation recipients (OLT) with CNI-related toxicity.
Patients and methods : 6 patients (mean age 55,2 years), who received a OLT between 1991 and 2006 (median of 93,3 months after OLT), were converted to MMF monotherapy due to CNI toxicity : chronic renal dysfunction (CRD) in 3 patients (2 with arterial hypertension), hypertension in 4 patients (1 with diabetes and 2 with CRD) and neurotoxicity in 1 patient. Immunosuppressive therapy 1 year for conversion was a combination of MMF with tacrolimus in 4 and ciclosporin in 1 and sirolimus monotherapy in 1.
Doses were adapted to the AUC of MMF, calculated with a 3-point limited sampling strategy (along to the protocol of the Univerity Hospital of Limoges with a target of 60 h.mg/L in stead of a fixed dose. Serum creatinine level, blood pressure and HbA1c were measured at starting and after 3 and 6 months monotherapy.
Results : The mean time between starting MMF therapy and conversion to MMF monotherapy was 42 months and the mean follow-up on monotherapy was 8 months. The mean daily dosis before conversion was 1600 mg, to reach the AUC was 2800 mg and to remain the AUC was 2300 mg.
No acute rejections were histologically proven. Creatinine levels fell progressively in 2 of 3 patients, with significant differences between baseline levels and levels at 6 months ($p < 0,05$), but not compared with levels at 3 months ($p > 0,05$) of monotherapy.
Of the 4 patients with hypertension, 2 (one with CRD) remained normotensive without changing the antihypertensive drugs, 1 became normotensive by adding other antihypertensive drugs and the diabetic stayed hypertensive. Blood glucose control was better.
MMF was generally well tolerated, only 1 patient needed a dosis reduction because of diarrhea. No other adverse events were recorded.
Conclusion : MMF monotherapy improved, but did not normalize, renal function in 66% of patients and was not associated with a significant risk of rejection. Arterial hypertension did not improve under MMF monotherapy. Side effects

were mild with dose regimens up to 1500 mg twice daily.

- A21 -

INFLUENCE OF ADIPOCYTOKINES ON SINUSOIDAL LIVER CELLS. B. Schroyen, S. Knops, C. Empsen, L. Van Grunsven, A. Geerts. VUB.

Background & aim : Obesity is a well-known risk factor for the development of non-alcoholic fatty liver disease (NAFLD). Therefore, we studied the relationship between adipocytokines and the sinusoidal liver cell population. We investigated the expression of leptin and adiponectin receptors, and studied the effects of both adipocytokines on the cells.
Methods : Primary mouse cells were obtained by enzymatic digestion (pronase, collagenase, DNase) through *in situ* perfusion and subsequent density gradient centrifugation, combined with fluorescent activated cell sorting (FACS). The expression of the different receptors was examined by RTQ-PCR and Western blotting. Mouse sinusoidal liver cell cultures were exposed for different periods of time to leptin and adiponectin.
Results : The presence of short leptin receptor isoforms can be clearly demonstrated on stellate cells, sinusoidal endothelial cell and Kupffer cells,but signalling through these receptors was negligible. Adiponectin receptor 1 is upregulated during stellate cell activation, while adiponectin receptor 2 was undetectable. Short-term incubation of hepatic stellate cells with globular adiponectin did not result in significant phosphorylation of AMPK±.
Conclusion : In our opinion leptin and adiponectin only have minor effects on commonly activated adipokine pathways.

- A22 -

EVALUATION OF THE JAK2 V617F MUTATION IN A SERIES OF PATIENTS WITH IDIOPATHIC SPLANCHNIC VEIN THROMBOSIS. V. Michels, O. Plomteux, B. Bastens, H. Charlier, D. Brisbois, F. Fontaine. CHC Liège.

Introduction : Splanchnic vein thrombosis (SVT) is a multifactorial disease resulting from one or several prothrombotic disorder, whether or not a local precipating factor is found. The JAK2 V617F mutation was described in 2005 and implied in the development of myeloproliferative disorders who are themselves a cause of SVT. The aim of the present work is to determine the prevalence and the role of the JAK2 V617F gene mutationand other prothrombotic abnomalities in cases of SVT described as idiopathic, ie without local precipating factor.
Method : Between January 1999 and November 2007, 20 patients have been hospitalized for an idiopathic SVT. Blood samples were obtained to analyze genetic and acquired prothrombotic abnormalities. JAK2 V617F genemutation was screenedamong 12 of them (6 died and 2 lost of follow-up) in 2008.
Results : Among the 10 men and 10 women of the original series, the average age at diagnosis of TVS was 51.9 years (30-85). The thrombosis undertook portal vein only in 12 cases, superior mesenteric and splenic veinsin 2 patients and thethree splanchnic veins in 6 patients. Protein C deficiency wasfound inone patient. No patient had a deficit in protein S nor in antithrombin III. Anticoagulant antibodies and paroxysmal nocturnal hemoglobinuriawere not found in any patient. The APC resistance was present infive patients. All were heterozygous for factor V Leiden gene mutation (ARG 506 > GLN). No patient had the G20210A factor II gene mutation. Two patients were homozygous forMTHFR C677T gene mutation. Only 1 patient had the JAK2 V617F gene mutation. Bone marrow biopsy showed trilinear hyperplasia and megakaryocytic dystrophy.
Conclusion : Despite exhaustive screening, eleven cases of SVT of this seriesremained unexplained,even after screening for JAK2 V617F gene mutation. Nevertheless, JAK2 gene mutationscreening is mandatory among the etiological factors of SVT, even ifits place in themanagement of SVTremains to be defined in prospective studies.

- A23 -

ISOLATION AND FACS-BASED PURIFICATION OF LIVER SINUSOIDAL CELLS. C. Empsen, E.L. Guimaraes, B. Schroyen, S. Knops, L. Van Grunsven, A. Geerts. VUB.

Background : The Metabolic Syndrome is a major risk marker for type 2 diabetes and for cardiovascular disease. The hypothesis of the HEPADIP project for which this work is carried out, is that this reflects an alteration in metabolic relationships or the signaling between adipose tissue and liver. The objectives of the project are to address the role of adipose tissue and the liver, and the interaction between them, in the development of the Metabolic Syndrome in order to identify, validate and develop novel targets for diagnosis, characterization, prevention and treatment of the syndrome.
Aim : Our first task in the HEPADIP-project is the optimization of the isolation and purification procedures of hepatic stellate cells (HSC), liver sinusoidal endothelial cells (LSEC) and Kupffer cells (KC), to provide the consortium with the needed pure cell populations for omics analyses.
Methods : The traditional method of enzymatic digestion (pronase, collagenase, DNase) through perfusion and subsequent density gradient centrifugation was combined with fluorescence-activated cell sorting (FACS), using a custom BD FACSAria with UV. For the purification of HSC, the auto fluorescence of vitamin A, resulting from excitation by UV light, was used. For the purification of LSEC, anti-LSEC (Miltenyi) was used. We tested CD11b and F4/80 for KC. After sorting KC were further purified more by selective adherence.
Results : Using only auto fluorescence, we could obtain very pure, viable HSC populations. With the aid of an 18% Nycodenz density gradient centrifugation step, followed by incubation with anti-LSEC-FITC, the same high purity could be obtained for LSEC. The KC however proved to be difficult to isolate from mouse using perfusion. We found that the KC were washed out of the liver during the first minutes of perfusion. We therefore decided to digest the liver *in vitro*. We compared CD11b and F4/80 as markers and found morphologically similar sorted populations. It is much more difficult to obtain pure KC isolations in sufficient quantities.
Conclusion : We conclude that the purification of HSC and LSEC using FACS is an improvement over density gradient based procedures in terms of purity. The procedure for KC needs further optimisation. These procedures will also be adapted to other mice strains.

- A24 -

UPPER GASTROINTESTINAL BLEEDING IN CIRRHOSIS : WHAT HAS CHANGED DURING THE LAST 20 YEARS ? J. Henrion, P. Deltenre, S. De Maeght, J.M. Ghilain, J.M. Maisin, M. Moulart, T. Delaunoit, D. Verset, C.P. Yeung, M. Schapira. Jolimont.

Aims : To compare in cirrhotic patients, the epidemiological and clinical characteristics as well as the management and prognosis of upper gastrointestinal bleeding (UGIB) between 2 periods 20 years apart.
Methods : Eighty episodes of UGIB in outpatients with cirrhosis collected in 1984-1989 (cohort A) were compared with 80 episodes collected in 2004-2008.
Results : Sex ratio was identical (M/F : 53/27) while mean age was higher in cohort B (58 y versus 54.4 y). It was the first episode of bleeding for 48 cases in cohort A and 47 in cohort B. The ratio *alcoholic cirrhosis/ other origin* was similar (65/15 cohort A versus 62/18 cohort B). Severity of cirrhosis assessed by child pugh score was significantly higher in cohort B (mean score 7.5 in cohort A versus 8.7 in cohort B). Consumption of any gastrotoxic drug (ASA and/or NSAID) was similar (cohort A : 18, cohort B : 20), but the intake of NSAID was significantly more frequent in cohort B (14 versus 3). The median delay between admission and initial endoscopy was significantly shorter in cohort B (3 h versus 10 h). Aetiology of UGIB was more often variceal haemorrhage in cohort A (52 versus 44), but the difference was not significant. Therapeutic intervention during initial endoscopy was significantly more frequent in cohort B (38 versus 10). By contrast, balloon tamponnade was far more frequent in cohort A (25 versus 4). Patients from cohort A were more often transfused (66 v 45) and the number of blood units given on the first day was higher (median 4 versus 2 in cohort B). Bleeding relapse and requirement for surgery were significantly more frequent in cohort A (33 versus 8 and 7 versus 0, respectively). Death during hospitalisation was more frequent in cohort A (18 versus 9, $p = 0.07$).
Conclusions : Alcoholic cirrhosis remains the main aetiology but the severity of cirrhosis is higher today. Initial endoscopy is performed earlier with therapeutic intervention more frequent today. Frequency and volume of blood transfusion have decreased. Bleeding relapse, surgical rescue and death are less frequent despite the higher degree of cirrhosis severity.

- A25 -

RESULTS OF LIVER TRANSPLANTATION FROM CONTROLLED DONATION AFTER CARDIAC DEATH (DCD) DONORS : A SINGLE CENTRE EXPERIENCE. O. Detry, B. Seydel, C. Veys, A. Deroover, M.F. Hans, M.H. Delbouille, J. Monard, J. Delwaide, A. Lamproye, S. Lauwick, P. Damas, F. Damas, J.P. Squifflet, M. Meurisse. CHU Liège.

Introduction : Donation after cardiac death (DCD) donors have been proposed to partially overcome the organ donor shortage. Liver transplantation (LT) from DCD donors remains controversial, with reported increased risk of graft failure and ischemic type biliary tract lesions. We retrospectively reviewed our experience with DCD LT and compared the DCD results with the 'classical' donation after brain death (DBD) LT in the same period.
Patients and methods : From 2003 to June 2008, amongst 176 consecutive LT, 19 (10.7%) DCD LT were performed. These 19 cases were compared to the 113 primary DBD whole LT, excluding combined or partial graft procedures. Liver graft allocation was patient driven in the DBD group, and centre driven for the DCD patients. Primary endpoints were graft failure and patient death. Graft survival was defined as time from liver transplantation to graft loss and patient death at follow-up. Patient survival was considered from time to first transplantation to patient death. Data are presented as mean ± SEM. P value < 0.05 was considered as significant.
Results : Procurement DCD warm ischemia was 20 ± 1.5 min. The DCD donors were significantly older (53.3 ± 3 vs 41.5 ± 1.3 min), had more cardiac arrest phases, had higher BMI (26.2 ± 0.7 vs 23.9 ± 0.3), and longer ICU stay (6.1 ± 0.7 vs 3.8 ± 0.2 days). Due to brain death, DBD donors had higher 24 hr urine output, higher sodium and increased need for pressors. Liver tests were similar. MELD score was significantly lower in the DCD group (13.3 ± 0.8 vs 18.8 ± 1.3). Suture time was longer in the DCD group (39 ± 1.6 min vs 34 ± 0.9 min), and cold ischemia was shorter in the DCD group (304 ± 25 min vs 421 ± 15 min). Posttransplant peak AST was 3,078 ± 1,162 U/L in the DCD group, compared to 1,730 ± 325 U/L in the DBD group ($p < 0.01$). Peak biliribins were not significantly different. There was no difference in patient and graft survival at 1 and 5 years. Graft and patient survivals were 100% and 100% at one year in the DCD group, and 83% and 78% in the DBD group (ns). There was no PNF in the DCD group. There were 2 deaths in the DCD group, related to malignancy. Two DCD patients developed biliary stenoses requiring endoscopic and/or surgical management. No DCD patient underwent retransplantation.
Discussion : In this series, DCD LT appears to provide results equal to DBD LT. The procurement DCD warm ischemia time was not different from the reported series in the literature. Short cold ischemia and recipient selection may be the keys to good results in DCD LT.

- A26 -

ROLE OF THE CIRRHOSIS RISK SCORE FOR THE PREDICTION OF FIBROSIS PROGRESSION IN HEPATITIS C PATIENTS WITH MINIMAL LIVER DISEASE. E. Trepo (1), P. Pradat (2), B. Young (3), R. Lagier (3), C. Moreno (1), J. Sninsky (3), A. Lemmers (1), T. Gustot (1), D. Degre (1), V. Vercruysse (1), E. Quertinmont (1), C. Trepo (2), M. Adler (1). (1) ULB, Brussels, Belgium ; (2) INSERM U871, Lyon, France ; (3) Celera, Alameda, CA, USA.

Background and aims : Fibrosis progression in patients with chronic hepatitis C viral (CHC) infection is highly variable and those factors associated with progression remain poorly understood. A Cirrhosis Risk Score (CRS) based on seven genetic variants and gender has been recently developed (Celera, Alameda, CA) for identifying patients at risk for cirrhosis. The objective of this study was to assess the role of the CRS for predicting fibrosis progression in CHC patients with mild liver fibrosis and e5 years of follow-up.
Methods : CHC patients from Hôpital Erasme, Brussels, Belgium, were retrospectively analyzed. Only patients with a fibrosis METAVIR score of F0-F1 at first biospsy were included. Patients were classified as progressors if they showed an increase of at least 2 fibrosis stages at the second histological evaluation. If the decrease was below 2 points after at least 5 years of follow-up or if patients remained stable, they were classified as non-progressors. After DNA extraction, the CRS was assessed using a multiplex PCR and Oligonucleotide Ligation Assay based on the Luminex® 200TM system. Patients with confounding progression factors such as heavy alcohol consumption were excluded.
Results : Twenty-five patients were studied with a mean age of 51 years. Twelve patients were classified as progressors (48%) and 13 (52%) as non-progressors. The CRS was significantly associated with fibrosis progression (0.72 in progressors vs 0.49 in non-progressors, $p = 0.050$).
Conclusions : Although conducted on a limited number of patients, this study confirms that an increased CRS is associated with fibrosis progression. Thus, the CRS could help to identify those CHC patients with mild liver disease at the highest risk for fibrosis progression to cirrhosis. This association and the potential relevance of CRS for the management of treatment decisions in patients at risk of progression should be further evaluated.

- A27 -

DIFFERENT N-GLYCOSYLATION PATTERNS IN MICE MODELS OF PORTAL HYPERTENSION AND CIRRHOSIS. B. Blomme (1), C. Van Steenkiste (1), J. Vanhuysse (1), I. Colle (1), N. Callewaert (2), H. Van Vlierberghe (1). (1) UZ Gent ; (2) VIB, UGent.

Introduction and objective : The golden standard to asses liver fibrosis is through a liver biopsy, but this technique is invasive and it is associated with several disadvantages. A non-invasive test based on alterationof *N*-glycosylation of serum proteins was developed by researchers of the Flemish interuniversity institute of biotechnology (VIB). This test, the GlycoCirrhoTest, is the logarithmic proportion of the peak heights of abisecting *N*-acetylglucosamine modified-glycan(increased in cirrhosis) and a triantennary glycan (decreased in cirrhosis) in the electroferogram. Our study further validates this test by investigating *N*-glycan profiles of serum samples obtained from 3 mice models of chronic liver diseases : common bile duct ligation, partial portal vein ligation and CCl4 injections SC. All models were induced in C57Bl/6 mice.
Methods : Glycosylation patterns of serum proteins were obtained by the DNA sequencer assisted fluorophore assisted capillary electrophoresis (DSA-FACE) technology. Additional information about the relationship between glycosylation and inflammation was provided by two ELISAs on IgG and serum amyloid A.
Results : The CCl4 model showed an increased abundance of multi-antennary glycans. The CBDL model was characterized by a clear augmentation of (core-) fucosylated glycans which was not present in the CCl4 model. Next to thesedifferences, also similarities were observed between the two models : the most abundant peak in the electroferogram(and its derived structures) were significantly lowered in abundance. In the PPVL model, no major changes in glycosylation patterns compared with control samplescould be observed. The alteration of glycosylation can predominantly befound on IgGs in human patients with cirrhosis. This could not be demonstrated in our mice models. Possible explanations could be the much lower IgG concentration in mice (0,5-2 ml) or a different physiopathogenesis in which the liver plays a more prominent role than in the human situation.
Conclusions : Portal hypertension is not a factor that contributes to the alteration of glycosylation in liver diseases. Etiology might be a factor to take into consideration when interpreting a clinical test based on glycosylation of serum proteins.

- A28 -

SERUM PROTEOMICS IN FIBROTIC HEPATITIS C PATIENTS. K.J. Cheung (1), K. Tilleman (2), D. Deforce (2), I. Colle (1), H. Van Vlierberghe (1). (1) UZ Gent ; (2) UGent.

Background : Liver fibrosis/cirrhosis is a serious health issue caused by long-term liver damage (e.g. hepatitis C). Liver biopsy is the current diagnostic procedure for fibrosis, but it is invasive method and it has an estimated 20% chance of error. Non-invasive serological markers are gaining more interests ; in this regard, proteomics proves to be a useful in the discovery of such markers.
The aim of this study was to discover useful fibrosis markers based on the serum proteomes of fibrotic Hepatitis C virus (HCV) infected patients.
Materials & methods : 30/77 HCV-patients with different METAVIR F-scores (F0-F1-F2-F3-F4) were selected for serum proteome analysis (mean age = 44 [27-69] yrs, mean duration of infection = 22 [6-52] yrs and the mean body mass index = 25 [19-40]). 69/77 were used for confirmation by dot blot and enzyme-linked immunosorbent assay (ELISA). Albumin and immunoglobulin were depleted from the patient's sera prior to protein separation by two-dimensional gel electrophoresis according to charge (pH 4-7) and molecular weight on a 10% polyacrylamide gel, resulting in individual proteome profiles.
Profile comparison was conducted between F-stages (F0-F1-F2-F3-F4) and between early (F0F1) and late (F2F3F4) stage by PD-Quest v.7.3. (Bio-Rad, Hercule, CA, USA) and univariate analyses (student's T-test/Mann-Whitney U-test) and Jonckheere-Terpstra trend-test. Resulting protein spots with p d 0.05 were identified by mass spectrometry. Proteins of statistical and clinical interest in line with fibrosis progression were selected.
Results : 8 from the 93 identified differentially expressed proteins were selected. Mac-2-binding protein, ± 2-macroglobulin and hemopexin were increased in F4 opposite F0/F1. ±1-antitrypsin, leucine-rich ±2-glycoprotein and fetuin-A were decreased in F4 opposite F0/F1. Mac-2-binding protein, ±2-macroglobulin and ±-1B-glycoprotein were increased in F2F3F4, while haptoglobin was decreased.
Dot blot assay (n = 66) and ELISA (n = 69) confirmed the Mac-2-binding protein proteomic data expression : F0 ($p < 0.001$, = 0.037), F1 ($p < 0.001$, = 0.001) and F2 ($p < 0.001$, = 0.02), respectively. The mean concentration (± standard deviation) in F4 was 17.8 (± 7.7) ¼ g/ml.
Conclusions : Existing and new candidate markers were identified in line with fibrosis progression in HCV-patients. The identified proteins might reflect early liver changes/dysfunction and therefore predict fibrosis severity. Mac-2-binding protein has been confirmed by two independent assays in a larger patient population.

- A29 -

INSULIN INDUCES OXIDATIVE STRESS, BUT NO METABOLIC EFFECTS ON MOUSE HEPATIC STELLATE CELLS. S. Knops, B. Schroyen, L. Van Grunsven, E.M. Guimaraes, H. Reynaert, A. Geerts. VUB.

Background & aims : The pathogenesis of non-alcoholic fatty liver disease (NAFLD) is not yet fully understood. It is known that the disease is characterized by insulin resistance and hyperinsulinemia and that during the progression of NAFLD, transdifferentation of quiescent into activated hepatic stellate cells (HSCs) plays an important role. We investigated insulin signaling in mouse HSCs and the effects of insulin on HSC transdifferentation.
Methods : Transdifferentiation of HSCs was obtained by culturing primary mouse HSCs on polystyrene dishes in presence of 10% fetal bovine serum. The presence of key molecules of the insulin receptor signaling pathways was determined by quantitative, absolute RT-PCR and Western blot. Proliferation was analyzed by BrdU incorporation. Glucose uptake was measured using 2-[^{3}H]deoxyglucose and fatty acid uptake by the fluorescent fatty acid analogue BODIPY-FA. ROS production was determined using DCFH-DA.
Results : Mouse hepatic stellate cells express both insulin receptor isoforms, mainly insulin receptor substrate-2 (IRS-2), FATP-1 (the insulin responsive FATP), but almost no GLUT4 (the insulin responsive glucose transport). Insulin stimulates the PI3K pathway, whereas the MAPK pathway was only weakly activated and no activation of the Gbl/TC10 pathway could be detected. Insulin is able to increase ROS production in activated HSCs, but has no effect on glucose uptake, fatty acid uptake or proliferation. Long term insulin exposure caused downregulation of its receptor and did not affect the HSC activation markers ±-SMA and GFAP.
Conclusions : These results suggest that hyperinsulinemia observed during NAFLD does not lead to increased proliferation of HSCs nor an increased glucose or fatty acid metabolism in these cells. The insulin-induced ROS production in HSCs could have an impact on the progression of NAFLD, but further studies are needed.

OG-FWO

- B01 -

THE CHARATERIZATON OF NEURONAL CONDUCTION AND THE FUNCTIONAL ROLE OF TACHYKININS IN COLONIC PERISTALTIC ACTIVITY OF MICE. A. Deiteren, B. De Winter, P. Pelckmans, J. De Man. UA Antwerp.

Objectives : Previous observations show that in inflammatory bowel disease, colonic peristaltic activity is disturbed and tachykinin receptors are upregulated. The role and interplay of NK1, NK2 and NK3 tachykinin receptors in colonic peristalsis is however not well understood. The aim of this study was to characterize neuronal conduction and investigate the functional role of tachykinin receptors in the *in vitro* colonic peristaltic activity of mice.
Material and methods : Colonic peristaltic activity was assessed by quantifying the amplitude and interval of distension-induced pressure waves in proximal and distal colon segments using a modified Trendelenburg set-up. In preliminary experiments, the effect of hexamethonium, atropine and tetrodotoxin (TTX) was studied on colonic peristaltic activity. A second series of experiments focused on the contribution of tachykinins to peristalsis. We investigated the cumulative effect of blockade of tachykinin NK3, NK2 and NK1 receptors with SR142801, nepadutant and RP67580 respectively.
Results : Gradual distension of the colon resulted in spontaneous and repetitive rhythmic pressure waves which were virtually abolished by TTX (1¼M). The amplitude was significantly reduced by hexamethonium (100 μM) in the proximal (6.18 ± 1.10 to 0.79 ± 0.41 cmH2O, n = 7) and distal (6.43 ± 0.34 to 0.56 ± 0.28 cmH2O, n = 6) colon. Atropine (1¼M) also successfully reduced the amplitude (proximal 5.83 ± 0.43 to 2.15 ± 0.48 cmH2O, n = 6 ; distal 5.46 ± 0.51 to 2.98 ± 0.89 cmH2O, n = 5). The interval was abridged by hexamethonium in the distal colon (49.83 ± 4.85 to 16.11 ± 9.11 s ; n = 6) with a trend toward shortening proximally (p = 0.07). Atropin had no effect on the interval.
SR142801 (0.1- 0.3¼M) had no effect on the amplitude or interval. Nepadutant (1¼M) showed a trend towards diminishing the amplitude in the proximal colon (p = 0.08), but no effect was seen distally. Also there was no effect noticeable on the interval. RP67580 (2¼M) significantly reduced the amplitude (6.51 ± 0.94 to 1.60 ± 0.83 cmH2O, n = 6) and widened the interval (63.25 ± 4.80 to 106.06 ± 22.42 s, n = 6) proximally but the effect in the distal colon was not as pronounced.
Conclusion : *In vitro* colonic peristalsis in mice is almost entirely mediated by neuronal nicotinic neurotransmission. Colonic peristaltic activity has a strong tachykininergic component that is mediated mainly by NK1 receptors and to a lesser extent by NK2 receptors. We could not demonstrate a role for NK3 receptors.

- B02 -

SOMATOSTATIN CONTROLS MAST CELL-INDUCED RESPONSES OF MURINE SPINAL NEURONS. J. Van Op den Bosch, L. Van Nassauw, E. Van Marck, J.P. Timmermans. UA Antwerp.

The onset and maintenance of intestinal inflammatory responses is in many cases associated with an extensive bidirectional communication between enteric neurons and mast cells embedded within the intestinal wall. The recently demonstrated expression of multiple somatostatin receptor (SSTR) subtypes in mucosal mast cells and in the extrinsic and intrinsic nerve fibres in the murine small intestine, suggests that somatostatin (SOM) is able to modulate this self-reinforcing communication network by simultaneously suppressing the inflammatory activities of both neurons and mast cells. Therefore, we assessed the modulatory effects of SOM on the short-term and long-term effects induced by the main mast cell mediators histamine (HIS) and 5-HT on spinal neurons of the mouse. As demonstrated by enzyme-linked immunosorbent assays and calcium live cell imaging in spinal cultures in vitro, HIS and 5-HT induced neuronal CGRP release and calcium-mediated activation in both neurons and non-neuronal cells. Both of these mast cell-induced effects were strongly reduced by preincubating the spinal cultures with SOM. Moreover, quantitative real-time PCR and quantitative analysis revealed a profound inhibitory effect of SOM on the increased neuronal expression of substance P, CGRP and SOM induced by long-term exposure to HIS and 5-HT. Immunocytochemical and molecular-biological experiments suggest the involvement of somatostatin receptor 1 (SSTR1) and SSTR2A in these profound SOM-dependent effects. Moreover, intestinal inflammation was accompanied not only by increased numbers of spinal neuronal somata immunoreactive for substance P and CGRP, but also by enhanced expression levels of SOM, SSTR1, SSTR2A and SSTR4 in spinal ganglia. These data reveal that intestinal inflammation not only induces the onset of pro-inflammatory cascades, but simultaneously triggers endogenous systems destined to prevent excessive tissue damage. Moreover, these data provide for the first time functional evidence that SOM is able to directly modulate intestinal inflammatory responses by interference with the coordinating mast cell-neuron communication. This study was supported by an IWT fellowship (SB53449) to JVOdb, IUAP project P5/20 and FWO grants G.0377.04 and G.0179.08.

- B03 -

MOTILITY PECULIARITIES OF FOETAL OESOPHAGUS AND INTESTINE USED FOR CERVICAL OESOPHAGUS REPLACEMENT. V. Coulic (1), P. Delrée (2), E. Dekoster (3). (1) ULB/CHU Brugmann ; (2) IRSPG Gosselies, (3) CHU Brugmann.

Taking into account the up to now remaining difficulties and frequent complications of cervical oesophagus replacement, an attempt was made to use for this aim foetal intestine and oesophagus.
Methods : Experiments were carried on 20 Rats (Wistar and Fischer). The following operations were performed. As a first step, a foetal intestine or oesophagus segment (donor aged 15-19 days of intra uterine development) was implanted between muscles of the anterior part of the neck. The second step consisted in resection of a 2-5 mm segment of the host cervical oesophagus and anastomosis of the oesophageal cut edges with the grown implant end to end or end to side. In pre and post operation period, ultrasounds investigation of the neck was realized, as well as biopsies of the implant and repaired oesophagus. General condition of the animals (body weight, capacity of taking food and water) was evaluated. After death or sacrifice, internal organs and the site of oesophageal operation were investigated. Histology wasperformedwith 12% formalin fixation, paraffin embedment and hematoxylin eosin staining. Observation delays were up to 2 months. All the manipulations on the animals were realized with respect of the Bioethics rules and allowed by the local Ethic Committee.
Results : The proposed 2-stage operation was technically possible. After implantation, foetal intestine and oesophagus gave growth to adult like organs with tendency to form retention cysts (especially intestinal). The optimal moment for the second stage to be performed was 6-8 weeks after the first one, when the implant was mature enough but not distended or hyperplasic, and with the correct dimensions. Anastomosis between organs had to be large enough to allow easy food and water transit. The implant position was also important to prevent its blood supply alteration and necrosis. Some problems arose with the peristalsis of the graft (triggering, orientation, efficiency of the motility activity) and its adaptation to the deglutition movements of the host oesophagus. The possible respective roles of extra- and intramural innervations of the graft were examined.
Conclusion : Use of foetal intestine or oesophagus for replacement of cervical oesophagus circular defects is theoretically and technically possible but some physiological problems of food transit can arise and must be solved.

- B04 -

ESOPHAGEAL MUCOSAL DAMAGE INDUCED BY WEAKLY ACIDIC SOLUTIONS CONTAINING UNCONJUGATED BILE ACIDS, SIMILAR TO REFLUX IN GERD PATIENTS ON PPI, CAN BE PREVENTED WITH ANTI-OXIDANTS. R. Farré, L. Cardozo, R. De Vos, K. Blondeau, P. Vanden Berghe, J. Tack, D. Sifrim. KULeuven.

Altered esophageal mucosa integrity (low electrical resistance and dilated intercellular spaces) may play a role in heartburn perception both in patients with non-erosive reflux disease and GERD patients refractory to PPI. Patients ‘on’ PPI have gastric content with a higher pH (weakly acidic), bacterial overgrow and increased proportion of unconjugated bile acids. Weakly acidic solutions with the unconjugated bile acid deoxycholic (DC) disrupt esophageal mucosa integrity and induces dilated intercellular spaces (DIS) (Farré 2008). It is known that exposure of esophageal epithelial cells to unconjugated bile acids increases intracellular reactive oxygen species (ROS) and this effect is blocked by anti-oxidants. We aimed to further assess possible mechanisms by which weakly acidic reflux can provoke impairment of esophageal mucosa integrity in patients on PPI. We hypothesized that anti-oxidants may also prevent disruption of esophageal mucosa integrity induced by unconjugated bile acids in weakly acidic solutions.
Methods : New Zealand rabbits were euthanized and segments from the esophageal mucosa were mounted in Ussing chambers. The luminal side was exposed for 30 min to solutions containing 0.5, 2 and 5 mM of DC and chenodeoxycholic acid (CDC) at pH 5.0. Transepithelial resistance (Rt) was measured for 90min. The anti-oxidants N-acetylcysteine (NAC) and vitamin C were added 30 min before exposure of the tissue to bile acids. After transepithelial resistance measurements, mucosa was assessed using transmission electron microscopy for diameter of intercellular spaces.
Results : The unconjugated bile acids DC and CDC in weakly acidic conditions dose-dependently decreased Rt. At 5 mM the drop in Rt was 84.3 ± 2.8% and 75.3 ± 2.1% respectively ($N = 4$, $P < 0.05$). Application of NAC and vitamin C in the mucosal side (5, 10 and 20 mM), dose dependently attenuated the drop in Rt induced by DC ($N = 4$). Application of both anti-oxidants in the serosal side at the highest concentration did not produce any effect ($N = 4$). Pretreatment with NAC 20mM prevented the dilation of intercellular spaces induced by DC (0.004 ± 0.001 ¼m vs 0.048 ± 0.004 ¼m, $N = 3$, $P < 0.05$). Similar prevention was observed when antioxidants were applied before exposure to the unconjugated bile acid CDC.
Conclusion : Esophageal mucosal damage induced by weakly acidic solutions with unconjugated bile acids, similar to reflux in GERD patients on PPI, can be prevented with anti-oxidants. Our experimental results suggest that anti-oxidants might be considered in the development of pharmachological strategies for patients with symptoms on PPI associated to persistent microscopic impairment of esophageal mucosa integrity.

Invited lecture

- B05 -

GASTROINTESTINAL ANTI-INFLAMMATORY EFFECTS OF HYDROGEN SULFIDE AND HYDROGEN SULFIDE-RELEASING MOLECULES. J. Wallace. McMaster University, Hamilton, Ontario, Canada.

Hydrogen sulfide has long been recognized as an industrial pollutant, but in recent years has been identified as an endogenous regulator of physiological and pathophysiological processes. Hydrogen sulfide in produced throughout the gastrointestinal tract. Suppresson of hydrogen sulfide synthesis leads to diminished mucosal defence against agents such as nonsteroidal anti-inflammatory drugs, and results in significant mucosal inflammation. Hydrogen sulfide synthesis in the GI tract is markedly up-regulated in response to mucosal injury, and appears to contribute to the healing process. Indeed, administration of hydrogen sulfide donors has been shown to protect the mucosa from injury and to promote the healing of pre-existing damage. In experimental colitis, hydrogen sulfide donors can acclerate the resolution of tissue injury and inflammation, while inhibitors of endogenous hydrogen sulfide synthesis have the opposite effects. Chemical modification of anti-inflammatory drugs so as to incorporate a hydrogen sulfide-releasing moiety has been shown to greatly enhance the activities of these drugs. Thus, a hydrogen sulfide-releasing derivative of mesalamine exhibits markedly greater effects than mesalamine in experimental colitis.

- B06 -

ROSIGLITAZONE, A PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ AGONIST, REDUCES THE DEVELOPMENT OF POSTOPERATIVE ILEUS INDUCED BY SURGICAL MANIPULATION OF MURINE COLON. O. De Backer, E. Elinck, B. Blanckaert, R.Lefebvre . U Gent.

Objective : Peroxisome proliferator-activated receptors (PPARs) are a family of ligand-activated nuclear transcription factors, members of the nuclear hormone receptor superfamily. The PPAR-γ subtype is predominantly expressed in skeletal muscle, adipose tissue, macrophages, and the gastrointestinal tract. Besides its well-known role in glucose metabolism and lipid storage, the PPAR-γ subtype has also been demonstrated to play a pivotal role in the regulation of inflammation and oxidative stress. This study was designed to evaluate the effect of the synthetic PPAR-γ agonist rosiglitazone in a murine model of POI.
Methods : Postoperative gut dysmotility (ileus) was induced by surgical anaesthesia and manipulation of murine colon. Rosiglitazone was administered intraperitoneally (0.3, 1, 3, 10 mg/kg, i.p.) 1 h before surgery. Intestinal contractility and transit were evaluated 24 h postoperatively using spatiotemporal motility mapping and fluorescence imaging. Molecular changes in small and large intestinal tissue samples were evaluated 6 h after surgery.
Results : Intestinal contractility and transit were markedly suppressed after colonic manipulation. This was associated with the release of pro-inflammatory mediators and the recruitment of leukocytes into the colonic and small intestinal wall. Pre-treatment of mice with the PPAR-γ agonist rosiglitazone led to a dose-dependent improvement in intestinal contractility and transit ; reaching significant improvement from 1 mg/kg, i.p. onwards. This beneficial effect was reflected by a decrease in interleukin (IL)-1β, IL-6, MCP-1 and ICAM-1 levels, diminished myeloperoxidase activity and reduced oxidative stress (MDA/HNE) levels. Remarkably, levels of PPAR-γ binding activity were significantly decreased in colonic tissue samples following surgical manipulation, but markedly enhanced by rosiglitazone.
Conclusions : Surgical manipulation of the colon leads to a marked decrease in colonic PPAR-γ binding activity. Pre-treatment with the PPAR-γ agonist rosiglitazone significantly increases PPAR-γ binding activity and is effective in ameliorating POI through the reduction of surgically-induced inflammation and oxidative stress. These results indicate that PPAR-γ agonists are potentially useful agents in the prevention of POI.

- B07 -

TNBS-INDUCED COLITIS INFLUENCES COLONIC T CELL CYTOKINE PROFILES AND CAUSES GASTROINTESTINAL MOTILITY DISORDERS IN MICE. N. Ruyssers (1), J. De Man (1), N. Ruyssers (1), A. Van Gils (1), P. Pelckmans (1), T. Moreels (2), B. De Winter (1). (1) UZ Antwerp ; (2) Tufts New England Medical Centre, Boston, USA.

Aim : In this study, we investigated the effect of acute experimental colitis on *in vivo* gastrointestinal motility and *in vitro* colonic peristalsis in mice. Furthermore, we measured the cytokine profiles of colonic T cells after induction of colitis.
Methods : Colitis was induced by intrarectal administration of 10 mg trinitrobenzene sulphate (TNBS) in 30% ethanol. Three days after TNBS enema, colonic inflammation was verified and quantified using macroscopic and microscopic inflammation scores as well as myeloperoxidase measurements. We measured gastric emptying (expressed in% GE) and intestinal transit (expressed as geometric centre, GC) of 25 glass beads (30, 120 and 360 min after intragastric gavage) and of the semiliquid Evans blue (15 min after intragastric gavage). Peristaltic activity of distal colonic segments was studied by quantifying the amplitude and frequency of distention-induced pressure waves. Focussing on the balance between Th1, Th2, Th17 and Treg cells in the colon, we measured the relative mRNA expression of respectively IFN-γ and IL-12, IL-4 and IL-5, IL-17, IL-10 and TGF-β by real-time RT-PCR.
Results : Colitis did not delay GE of Evans blue or the glass beads at any time point. However, colitis significantly decreased GC only 360 min after beads gavage from 7.3 ± 0.2 in control mice to 5.5 ± 0.6 in TNBS-injected mice (n = 7-10). The GC of the Evans blue bolus was not altered by TNBS. Peristaltic pressure waves were observed in each segment of the distal colon from control mice. In mice with colitis, only 3 out of 7 segments of the distal colon showed peristaltic activity. The amplitude of the colonic peristaltic waves was decreased from 4.8 ± 0.7 cmH_2O (n = 6) in control mice to 2.8 ± 0.4 cmH_2O in mice with colitis (n = 3) ; whereas the interval between the waves significantly increased from 46.4 ± 3.4 s (n = 6) in control mice to 197.8 ± 32.6 s (n = 3) in mice with colitis. Induction of colitis significantly increased the relative expression of IFN-γ mRNA from 1.0 ± 0.0 in controls to 11.2 ± 3.6 in colitis mice while the relative expression of TGF-β and IL-5 mRNA was significantly downregulated after induction of colitis (n = 4-6).
Conclusions : TNBS-induced colitis in mice did not affect *in vivo* gastric emptying and small intestinal transit whereas colonic transit was significantly delayed. This coincides with an impaired *in vitro* colonic peristaltic activity in mice with colitis. The induction of colitis caused an upregulation of Th1 and a downregulation of Th2 and Treg cytokines.

- B08 -

ENTERIC 5-HT1A-LIKE RECEPTOR DENSITY IN COLIC HORSES SUFFERING FROM SMALL INTESTINAL STRANGULATION IS ASSOCIATED WITH DURATION AND SEROTONIN (5-HT) LEVELS IN PLASMA AND PERITONEAL FLUID. C. Delesalle (1), N. Van Acker (2), K. De Ceulaer (3), P. Deprez (4), G. Van De Walle (4), C. Nolten (5), A. Van Hemelrijck (5), L. Verdonck (5), J. Dewulf (4), R. Lefebvre (4). (1) U Gent & Utrecht University, (2) HistoGeneX nv, Edegem, (3) UA Antwerp, (4) U Gent, (5) Johnson & Johnson, Beerse.

Background : 5-HT_{1A}-like receptors located on smooth muscle have been shown to mediate the 5-HT-induced contractile response of longitudinal and circular smooth muscle in equine jejunum. *In vitro*, these 5-HT_{1A}-like receptors show tachyphylaxis upon repeated activation with 5-HT. *In vivo*, increased plasma and peritoneal fluid 5-HT levels have been reported in colic horses suffering from small intestinal strangulation, a colic type that significantly predisposes horses to develop post-operative ileus (± 42% of cases). Our aim was to investigate the 5-HT_{1A}-like receptor density in the jejunum of healthy and colic horses. Moreover, we wanted to investigate a possible relation between (i) the duration of small intestinal strangulation, (ii) the plasma and peritoneal fluid 5-HT levels and (iii) the small intestinal 5-HT_{1A}-like receptor density in colic horses.
Methods : The localization and density of the 5-HT_{1A}-like receptors was assessed immunohistochemically with rabbit polyclonal rat 5-HT_{1A} receptor antibodies in the jejunum of healthy horses (n = 10). In addition, plasma 5-HT levels, beta-thromboglobulin (β-TG) and platelet factor 4 (PF4) levels, plasma coagulation profile, packed cell volume (PCV), Base Excess (BE) and lactic acid levels were determined in plasma, along with lactic acid and 5-HT levels in peritoneal fluid. Such analyses were also performed in small intestinal strangulating colic cases (n = 18). Immunohistochemistry was performed on the outer viable parts of resected small intestinal segments (n = 18) and on viable small intestinal segments localized > 1 m orally to the strangulation (n = 3).
Results : Immunohistochemistry confirmed the presence of muscular 5-HT_{1A}-like receptors in the muscularis mucosae, and both longitudinal and circular smooth muscle layers of the equine jejunum. The 5-HT_{1A} receptor density showed a tendency to decrease with increasing duration of small intestinal strangulation and increasing peritoneal and plasma 5-HT levels. Decreased 5-HT_{1A} receptor density was also seen in the three small intestinal segments localized orally to the strangulation.
Conclusion : The density of the equine enteral 5-HT_{1A}-like receptor decreases *in vivo* in small intestinal strangulating colic horses apparently in correlation with increasing duration of strangulation and increasing peritoneal fluid and plasma 5-HT levels. These findings corroborate a possible role for 5-HT in the pathophysiology of ileus in horses and moreover, show that the pathophysiological processes that occur in colic horses can lead to downregulation of enteric contractile receptors.

- B09 -

EXPRESSION OF THE PROSTAGLANDIN E2 RECEPTOR EP4 IN CROHN'S DISEASE. C. Reenaers, C. Libioulle, J. Belaiche, P. Delvenne, M. Georges, E. Louis. CHU Liège.

Introduction : Crohn's disease is an inflammatory bowel disease characterized by an inflammation of the bowel wound due to a combination of environmental, genetic and immunological factors. A lot of progresses took place recently concerning the discovery of susceptibility genes in CD particularly a gene desert mapped on 5p3.1, closed to many genes involved in inflammation, particularly PTGER4 coding for the receptor EP4 for prostaglandin E2 (PGE2). EP4 acts as an immunomodulator, inhibits the production of LPS-induced cytokines and can inhibit Th1 and Th2 responses. Moreover, EP4 agonists also protect epithelial cells against apoptosis, reduce the production of proinflammatory cytokines and improve severe colitis in rat dextran sulphate colitis.
Aims : The aims of our work were to study the EP4 expression in the intestine and in peripheral blood mononuclear cells (PBMC) in IBD and to compare it with inflammatory and non inflammatory controls.
Material and methods : Total RNA was extracted from gut samples (5 controls). Fixed colonic samples from 13 CD patients (8 active CD, 5 non active CD), 6 non inflammatory controls, 17 inflammatory controls (7 acute diverticulitis, 9 active ulcerative colitis) were studied by immunostaining. We studied the cells expressing EP4 in the serum by flow cytometry on PBMC isolated from 4 CD patients, 3 inflammatory controls and 4 healthy controls.
Results : Transcripts for EP4 were confirmed in the human colon by RT-PCR. We observed by immunostaining a high expression of EP4 by epithelial cells of the colon and of the ileum. This expression was the same in crypts and villosity. No difference was observed between CD, inflammatory controls and non inflammatory controls. No expression of EP4 by immune cells from the lamina propria was described in controls but also in active and non active CD. The study of PBMC in flow cytometry demonstrated that EP4 positive cells corresponded to 15% of PBMC. The cells expressing EP4 corresponded to monocytes positives for CD4. Some of them also expressed CD69 and CD 14 but there were negative for CD1a, CD8 and CD25. The preliminary results showed no differences between CD, inflammatory controls and non inflammatory controls.
Conclusion : In the bowel, we showed an expression of EP4 only by epithelial cells but no differences was demonstrated between CD and controls. Surprisingly, we found no expression of EP4 by lamina propria mononuclear cells suggesting a role of EP4 in the permeability of the bowel wound. PBMC corresponded to monocytes CD4 + , CD69 + and CD14 + also expressed EP4 suggesting a possible role in systemic inflammation. Further studies are needed to quantify the expression and explore the role of EP4 in epithelial cells and PBMC in CD.

- B10 -

THE IMPACT OF INFLIXIMAB THERAPY ON COLONIC MUCOSAL EXPRESSION OF BARRIER GENES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE. I. Arijs (1), R. Quintens (2), L. Van Lommel (2), K. Van Steen (3), G. De Hertogh (1), G. Van Assche (1), S. Vermeire (1), K. Geboes (1), F. Schuit (2), P. Rutgeerts (1). (1) KULeuven Gasthuisberg ; (2) KULeuven ; (3) ULg.

Introduction and aim : Intestinal epithelial barrier function is impaired in inflammatory bowel disease (IBD), but it remains unclear whether this dysfunction is a primary event of IBD or a consequence of mucosal inflammation. This study investigated the impact of anti-inflammatory therapy with infliximab on the colonic mucosal expression of genes related to intestinal epithelial barrier function in IBD patients using oligonucleotide arrays.
Methods : The expression of 121 genes related to intestinal epithelial barrier function, including IBD candidate susceptibility genes, mucins, trefoils, E-cadherin, genes involved in the apical junction complex function, were investigated in colonic mucosal biopsies obtained at endoscopy from 43 IBD patients with active colitis (24 ulcerative colitis (UC) and 19 Crohn's disease (CD)) before and 4-6 weeks after first infliximab infusion. Response to infliximab was defined as endoscopic and histologic healing : 20 responders (12 CD and 8 UC) and 23 non-responders (7 CD and 16 UC). Six control patients undergoing colonoscopy for screening were included. Total RNA was isolated, labelled and hybridized to Affymetrix Human Genome U133 Plus 2.0 Arrays. Microarray data was analyzed using Bioconductor software. Moderated t-statistic was used for comparative data analysis. A false discovery rate < 5% combined with a > 2 fold-change were considered statistically significant.
Results : There was no significant difference between UC and CD at baseline. In UC and CD, the mRNA expression levels of MUC1, MUC4, MUC5B, MCAM, TFF1, CLDN1, CLDN2, JAM2 and TLR4 were all upregulated at baseline compared to controls, while the mRNA expression levels of MUC20, CLDN23, CLDN8, MPP5, MPP7, CDH1, SLC22A4, SLC22A5, ABCB1 and PDZD3 were all downregulated at baseline compared to controls. In responders to anti-TNFalpha treatment, the expression levels of TFF1, TFF2, CLDN2, CLDN8, SORBS1, ABCB1 and DSG3 in UC and CLDN1 in UC and CD normalized after treatment. In CD (not in UC) responders, MUC1 and MUC4 mRNA expression remained increased and CXADR and SLC22A5 mRNA expression remained decreased after complete healing in comparison with controls.
Conclusion : Our data demonstrate that the expression of many barrier genes is dysregulated in the colon of active IBD. After healing of the mucosa with infliximab therapy, the expression of most of these genes was restored in UC, while in CD the expression of a number of barrier genes remained dysregulated. We do not find arguments for a primary defect in barrier in UC whereas in Crohn's disease some barrier defects persist in healed colonic mucosa.

Invited lecture

- B11 -

INTESTINAL FACTORS INVOLVED IN THE METABOLIC EFFECTS OF DIETARY FRUCTANS.
N. Delzenne. UCL Drug Research Institute.

Dietary fructans are non digestible carbohydrates, which are highly and selectively fermented by certain strains of bacteria in the gut microbiota, thus having prebiotic properties. Experimental data in animals, and intervention studies in humans suggest their potential to lessen metabolic disorders associated to obesity. In rats and mice, the addition of fructans in the diet improves glycemic response and steatosis and decrease fat mass development, those events being clearly related to the increased secretion of gut peptides. The fermentation of short chain fructans leads to an increase in the differentiation of stem cells into endocrine L cells in the proximal colon of rats, and therefore promotes the production of glucagon like peptide 1 (GLP-1) in this organ. The relevance of this peptide in the improvement of metabolic disorders is shown through experiments performed in mice lacking functional GLP-1 receptor : those mice are resistant to the beneficial effect of fructans on obesity and glucose metabolism. Moreover, recent studies have indicated that the addition of fructans in the diet, lowers endotoxemia and the serum concentration of proinflammatory cytokines (IL1a, IL6, MCP1…) in high fat-fed mice. The improvement of diabetes and insulin secretion by the prebiotic was significantly correlated with the lower endotoxemia (LPS level) and a higher Bifidobacterium spp gut content. Recent data suggest that another gut peptide – GLP-2- is involved in the lower gut permeability towards LPS observed in fructans-fed animals, a mechanism that could be involved in the improvement of metabolic endotoxemia. Thus, experimental data demonstrate that events occurring in the colon (fermentation ; modulation of gut microbiota) through prebiotics ingestion exert a key influence on the development of metabolic diseases associated to obesity .
The relevance of those effects in humans is supported by several studies, showing that dietary supplementation with inulin-type fructans increases satiety and decreases hepatic triglyceride secretion in adult healthy volunteers, decreases body mass index in adolescents, and improves lipidemia and non alcoholic steatohepatitis. The effect of fructans-type prebiotics on the inflammatory status and GLP production would be interesting to test in overweight and obese people, knowing that gut microbiota is disturbed upon obesity.

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- B12 -

ROLE OF TASTE RECEPTORS IN THE REGULATION OF GHRELIN SECRETION.
S. Janssen, B. De Smet, P.J. Verhulst, T. Peeters, J. Tack, I. Depoortere. KULeuven.

Introduction : Ghrelin is a hunger hormone released by the stomach. Plasma ghrelin levels increase before a meal and decrease after ingestion of nutrients. The mechanisms that regulate ghrelin secretion are unknown. Recently, a family of taste (sweet, bitter and umami) receptors have been identified in the gastrointestinal tract that couple through the G protein, ±-gustducin, to specific second messenger cascades. It has been suggested that taste receptors play an important role in molecular sensing by gastrointestinal cells.
Aim : To investigate whether ±-gustducin plays a role in the effect of nutrients (chow, glucose and bitter) on ghrelin secretion.
Methods : The co-localization of ghrelin and ±-gustducin was investigated by immunohistochemistry. Sections from the mouse stomach were taken at the limiting ridge and double stained with a rabbit anti-gustducin and goat anti-ghrelin antibody. The effect of nutrients on plasma ghrelin levels was measured in male wild-type (±-gust $^{+/+}$) and ±-gustducin knockout (±-gust$^{-/-}$) mice. Mice (n = 5) were fasted for 18 h, re-fed with mouse chow for 2 h after which blood samples were taken. Another group of mice were gavaged with 150 μl glucose (5 g/kg) (n = 5) or 150 μl of a bitter taste receptor agonist mixture (denatonium benzoate(10 mM), phenylthiocarbamide(10 mM), 6-propyl-2-thiouracil (5 mM), quinine (5 mM), D-[-]salicin (5 mM)) (n = 5) after an overnight fast. Blood samples were taken at 0, 10, 20, 30 and 40 min after gavage. Plasma total ghrelin levels were determined by radioimmunoassay.
Results : Several ghrelin-positive cells were visualized in close proximity to ±-gustducin-positive cells. No co-localization was observed. The brush cells containing ±-gustducin were spanning the epithelium and reaching the central lumen whereas ghrelin cells were of the 'closed type' and did not reach the apical surface of the mucosa. Fasting caused a significant increase in plasma ghrelin levels in both genotypes, but the increase was more pronounced in the wild-type mice ($P < 0{,}001$). Re-feeding for 2 h with mouse chow decreased plasma ghrelin levels to a lesser extentin ±-gust$^{-/-}$ mice (38 ± 4%) than in ±-gust $^{+/+}$ mice (72 ± 3%). Nevertheless, food intake after 2 h of re-feeding did not differ between both genotypes. To specifically investigate the role of sweet or bitter taste receptors in the regulation of ghrelin secretion,mice were gavaged with a glucose or a bitter taste solution, respectively. Glucose suppressed plasma ghrelin levels in ±-gust $^{+/+}$ mice by 33 ± 9% (40 min). In ±-gust$^{-/-}$ mice plasma ghrelin levels remained unaffected during glucose administration. Oral gavage of a bitter taste solution significantly increased plasma ghrelin levels by 87 ± 21% (40 min) in ±-gust $^{+/+}$ mice but not in ±-gust$^{-/-}$ mice (40 min : 24 ± 13%).
Conclusions : Our findings suggest that sweet and bitter taste receptors coupled to ±-gustducin play a role, either directly or indirectly, in the regulation of ghrelin secretion by nutrients.

- B13 -

INTRAGASTRIC PRESSURE DURING THE DRINKING TEST. P. Janssen, R. Vos, J. Tack. KULeuven.

Introduction : Gastric accommodation allows food intake without a rise in intragastric pressure (IGP). We have previously shown that impaired gastric accommodation is associated with enhanced meal-induced satiation and may lead to weight loss. The aim of our study was to investigate the relationship between IGP and satiation during food intake.
Methods : 16 healthy volunteers (8 males, 30.9 ± 1.4 years, BMI of 21.6 ± 0.4) were studied after an overnight fast. IGP was measured with a solid-state manometry catheter positioned in the proximal stomach through the mouth. After a stabilization period, the subjects drank a liquid nutrient meal (Nutridrink®, Nutricia ; 1.5 kcal/ml) from beakers that were filled by a peristaltic pump at a rate of 60 ml/minute. Satiation was scored on a 0-5 scale (1 = threshold, 5 = maximum) at 1-minute intervals, until a score of 5 was reached. Values are given as mean ± SEM ; correlation analysis was performed using analysis of variance.
Results : Maximum satiety was reached after 16.6 ± 1.7 minutes or 996 ± 102 ml. Immediately after the start of nutrient ingestion, IGP decreased to a maximum drop of 8.2 ± 1.0 mmHg, 4.1 ± 0.4 minutes after the start. Subsequently, a gradual increase in IGP with 0.4 ± 0.1 mmHg per minute occurred. When maximum satiety was reached, the IGP was still 3.5 ± 1.0 mmHg decreased vs. the IGP during the stabilization period. Satiation scores and the increase in IGP from nadir pressure were significantly correlated ($P < 0.005$; $R^2 = 0.50$). No significant correlations were found between satiation scores and the maximum IGP decrease or the time to reach maximum IGP decrease.
Conclusion : During nutrient ingestion IGP decreases initially, and gradually recovers thereafter. We showed that this IGP recovery is closely associated with rising satiation scores. Since gastric accommodation plays a major role in the control of IGP, we postulate that IGP during food intake can be used as a marker for gastric accommodation.

- B14 -

RIMONABANT-INDUCED EARLY SATIETY IS ASSOCIATED WITH DECREASED GASTRIC COMPLIANCE AND INHIBITION OF GASTRIC ACCOMMODATION. K. Ameloot, R. Vos, E. Scarpellini, P. Vanden Berghe, I. Depoortere, J. Tack. KULeuven.

Background : Satiety is partly controlled by the gastric accommodation reflex and by the activation of descending endogenous anti-nociceptive pathways, putatively through mediators like endogenous opioids and endocannabinoids. The endocannabinoid receptor antagonist rimonabant has been shown to decrease food intake, mainly attributed to an effect on the central nervous system.
The **aim** of the present study was to investigate how suppression of endocannabinoid signalling by rimonabant would influence the gastric response to meal ingestion and the sensitivity to gastric distension in normal volunteers.
Methods : 9 healthy subjects (mean age 32 yrs, 4 men) participated in a placebo-controlled, double blind, randomized, crossover gastric barostat study. After 3 days of pretreatment with rimonabant 20 mg/day or placebo, stepwise distensions were performed (2 mmHg steps at 2 minute intervals, until discomfort or pain). To quantify gastric sensitivity, upper abdominal sensation was scored on graded scales (0 = no sensation, 6 = maximal) at the end of every distension step. To quantify gastric accommodation, the mean gastric volume over consecutive 5 minute intervals was measured 30 minutes before and 60 minutes after a standardized meal (Nutridrink, 200 ml, 300 kcal). Subsequently, another series of stepwise distensions was performed. To quantify satiety, volunteers were requested one day later to a slow liquid nutrient drink test (15 ml/min). Visual analogue scales (VAS) were used to quantify dyspeptic symptoms. Numbers are shown as mean ± SEM.
Results : During the drinktest, rimonabant induced satiation earlier compared to placebo (AUC 139 ± 30 vs. 74 ± 26, $p = 0.02$). During the first series of barostat distensions, gastric compliance was significantly decreased by rimonabant compared to placebo (38 ± 5 vs. 60 ± 7 ml/mmHg, $p < 0.05$). The pressures needed to induce first perception (score = 1) or discomfort (score e5) and corresponding intra-balloon volumes did not differ between placebo or rimonabant. Meal-induced gastric relaxation (difference between pre- and postprandial volumes) was significantly lower after rimonabant (72 ± 39 vs.176 ± 38 ml, $p < 0.05$). Postprandial phasic motility, expressed as a motility index, was significantly higher after rimonabant compared to placebo (31.7 ± 3.0 vs. 28.1 ± 3.8 ml*mmHg, ANOVA $p < 0.05$). Postprandial compliance did not differ significantly between placebo and rimonabant (78.1 ± 13.6 vs. 63.2 ± 5.2 ml/mmHg, NS). VAS scores did not reveal alterations in epigastric symptoms after rimonabant .
Conclusion : Rimonabant causes early satiation which is at least in part attributable to decreased gastric compliance and inhibition of gastric accommodation.

- B15 -

ROLE OF UPPER GASTROINTESTINAL MOTILITY STIMULATION IN THE OCCURRENCE OF HUNGER PEAKS. Scarpellini, R. Vos, H. Nicolai, D. Ang, P. Vanden Berghe, I. Depoortere, J. Tack. KULeuven.

Background : Recently, we reported that hunger ratings in the fasting state in man were closely correlated with gastric motor activity, and that hunger peaks coincided with gastric phase 3 of the migrating motor complex (MMC) (Ang *et al.*, DDW 2008). It is unclear whether intense stimulation of gastric motility is sufficient to induce a sensation of hunger, or whether this requires the highly organised pattern of gastric phase 3.
Aim : To further elucidate the relationship between motor activity and hunger ratings by comparing the influence of the cholinesterase inhibitor neostigmine and the motilin agonist erythromycin on upper gastrointestinal motor activity and hunger ratings in man.
Materials and methods : Twenty-five fasted healthy subjects (11 males ; 32.6 ± 2.0 years) underwent antroduodenojejunal manometry. Twenty minutes after a full MMC cycle, neostigmine (NEO) 0.5mg (n = 13) or erythromycin (EM) 40mg (n = 12) were administered i.v. Phases of the MMC were visually identified. Computer-aided baseline reconstruction was used to quantify phasic contractions as a motility index (MI), reflecting the area between signal and baseline normalized over time. Hunger scores (on 100 mm visual analogue scales (VAS)) were measured throughout the study. Comparisons were made between 20 minutes before and 60 minutes after start of drug administration.
Results : Prior to drug administration, a significant correlation was found between antral MI and hunger scores ($r = 0.6021$, $p < 0.001$), and gastric phase 3 was associated with a hunger peak compared to phase 2 scores (35.9 ± 5.4 vs. 62.5 ± 7.5, $p < 0.005$). Administration of NEO was associated with a significant increase in antral MI (0.32 ± 0.09 to 3.25 ± 0.62, $p < 0.0001$), which resulted in a typical phase 3 pattern in only 1 subject. Hunger scores were not significantly affected by NEO (46.8 ± 6.7 vs. 47.2 ± 7.2, NS) and no significant correlation was found between antral MI and hunger scores after NEO ($r = 0.03$, NS). Administration of EM was followed by a gastric phase 3 in all subjects after 17±2 min, with a significant increase in antral MI (1.22 ± 0.18 to 3.49 ± 0.35, $p < 0.0004$), and this was associated with peak hunger scores (29.2 ± 7.0 vs. 61.7 ± 8.0, $p = 0.02$). A significant correlation was found between antral MI and hunger scores after EM ($r = 0.24$, $p < 0.05$).
Conclusions : Phasic gastric contractions, as induced by a cholinesterase inhibitor, do not induce a hunger signal. Peak hunger seems to require the induction of a typical gastric phase 3 pattern.

- B16 -

RETURN OF HUNGER AFTER A MEAL : RELATION TO GASTRIC EMPTYING AND GASTROINTESTINAL MOTOR PATTERNS. H. Nicolai, E. Scarpellini, R. Vos, I. Depoortere, P. Vanden Berghe, J. Tack. KULeuven.

Introduction : Gastric emptying rate is considered a major determinant of the speed of return of hunger after a meal. Recently, we reported the occurrence of hunger peaks in close association with gastric phase 3 activity during fasting. The **aim** of the present study was to investigate the role of hunger peaks in the return of hunger after a meal in healthy volunteers.
Methods : 17 healthy subjects (7 males ; age 30.1 ± 1.5 years) underwent antroduodenojejunal manometry on 2 separate occasions at least 1 week apart. After the occurrence of a spontaneous phase 3, a standard gastric emptying breat test-meal (bread and egg, 250 kcal) was administered and 2 hours later EM 40mg i.v. or OCT 100ug s.c. were administered. Phasic contractions were quantified as a motility index (MI), reflecting the area between signal and baseline normalized over time. Gastric emptying rate was expressed as solid half emptying time (t1/2). Hunger was scored on 100 mm visual analogue scale (VAS) at 5-min intervals throughout the study, which lasted up to 6 hours after meal ingestion. Hunger peaks were identified as rises in hunger scores above the mean value + 2SD.
Results : Preprandially, and during the first 120 minutes postprandially, hunger ratings and antral MI did not differ between the 2 conditions. Antral MI decreased from 1.49 ± 0.16 preprandially to 1.03 ± 0.04 the first 2 hours postprandially ($p < 0.001$). During the 2 hours after drug administration, antral MI was significantly increased by EM (2.09 ± 0.1,$p < 0.01$) and significantly inhibited by OCT (0.66 ± 0.05, $p < 0.001$). EM induced and OCT suppressed gastric phase 3. Hunger scores dropped from 32.1 ± 2.4 preprandially to 18.9 ± 0.7 during the first 120 minutes postprandially ($p < 0.001$). During the 2 hours after drug administration, hunger scores were significantly higher after EM (36.3 ± 1.3) compared to OCT (27.5 ± 1.3, $p < 0.05$). The rise in hunger after EM was attributable to the occurrence of hunger peaks. Prior to the meal, 7 hunger peaks were recorded. Administration of EM 2 hours postprandially induced the occurrence of 17 hunger peaks after 30 ± 6 min, of which 14 (77%) coincided with antral phase 3 motor activity. Administration of OCT was associated with 8 hunger peaks after 58 ± 10 min, none of which coincided with phase 3 motor activity, and 3 with small bowel phase 3 activity. Gastric emptying rate did not differ significantly between both treatments (t1/2 90.4 ± 13.9 vs. min, NS).
Conclusions : In the late postprandial phase, hunger peaks, associated with gastric phase 3, rather than gastric emptying rate, determine the return of hunger. Suppression of gastric phase 3 by OCT is associated with lower hunger scores.

- B17 -

THE COLD AND MENTHOL RECEPTOR TRPM8 IS EXPRESSED IN EPITHELIAL CELLS OF THE HUMAN AND MURINE GASTRIC ANTRUM. W. Boesmans, V. Van Den Abbeel, G. Owsianik, T. Voets, J. Tack, P. Vanden Berghe. KULeuven.

Introduction : Menthol, a secondary alcohol produced by the peppermint herb, *Mentha piperita*, is widely used in food industry as a cooling and soothing tastant or odorant. Menthol is also present in herbal drugs designed to treat abdominal discomfort and pain (eg. Iberogast®). Peppermint oil, with menthol as major constituent, reduces gastric spasms during upper endoscopy and slows small intestinal transit. By activation of the transient receptor potential melastatin 8 channel (TRPM8), a Ca^{2+}-permeable, cold-activated member of the TRP superfamily of cation channels, menthol induces Ca^{2+} influx in a subset of sensory neurons from dorsal root and trigeminal ganglia. Knowledge about the presence of TRPM8 and its possible function in the gastrointestinal tract is however rare.
Objectives : To study the distribution of the menthol receptor TRPM8 in the human and murine gastrointestinal tract.
Methods : Immunohistochemistry using a rabbit-hosted antibody directed against a highly conserved region in the TRPM8 C terminus (C + ^{1078}MRHRFRQLDTKLNDL^{1092}), was performed on cryosections of human mucosal biopsies and on cryosections of whole murine gut. Double labeling was performed using antibodies for serotonin and ghrelin. RT-PCR using primers for mouse TRPM8 was carried out on mRNA isolated from mouse stomach, ileum and colon.
Results : In biopsies from human antral mucosa, TRPM8 immunoreactivity was found in cells scattered throughout the epithelium. TRPM8 positive cells were not found in duodenal or fundic mucosa. In frozen sections from C75BL6 mice, TRPM8 immunoreactivity was also restricted to cells in the gastric oxyntic mucosa. TRPM8 immunoreactivity was absent in frozen sections derived from mouse small intestine and colon. Furthermore, TRPM8 immunoreactive mucosal cells did not stain with antibodies for serotonin and ghrelin, indicating that these cells differ from enterochromaffin cells and the ghrelin containing X/A cells. Using RT-PCR, the specific presence of TRPM8 in the gastric mucosa was confirmed as an amplicon was only obtained with mRNA isolated from stomach and not with mRNA derived from ileum or colon.
Conclusions : We found evidence for expression of the menthol sensitive receptor TRPM8 in epithelial cells residing in the human and murine gastric antrum with immunohistochemistry and confirmed this on the mRNA level. The presence and location of TRPM8 in the antral mucosa could explain the effects of menthol containing compounds on gastrointestinal motility. Further research is needed to identify the 'menthol sensing' mucosal cell type and to unravel the function of TRPM8 in gastrointestinal physiology.
Support : FWO, Belgium.

- B18 -

NEUROCHEMICAL IDENTIFICATION OF SPECIFIC SUBPOPULATIONS OF ENTERIC NEURONS IN THE LARVAL AND ADULT ZEBRAFISH (DANIO RERIO) INTESTINE. L. Uyttebroek (1), M. Dirckx (1), F. Harrisson (1), G. Hubens (1), I.T. Shepherd (2), J.P. Timmermans (1), L. Van Nasssauw (1). (1) UA Antwerp ; (2) Emory University, Atlanta, USA.

Introduction : In the last decade, the zebrafish has emerged as a leading model organism for the study of vertebrate developmental biology and has begun to be used in studies of gastrointestinal congenital diseases. While the general morphology and development of the enteric nervous system (ENS) of the zebrafish are already known, specific details regarding the physiological function and morphological characteristics of enteric neurons is still incomplete.
Objective : The aim of the present study is to unravel the neurochemical coding of zebrafish enteric neurons, revealing specific subpopulations.
Methods : Using immunoenzymatic and multiple immunofluorescent staining methods on isolated intestines from adult and larval (72-96-120 hpf) zebrafish, we demonstrated and quantified the expression of different neurochemical markers representing presumptive excitatory, inhibitory and sensory innervation in the three functional intestinal segments.
Results : Three markers [tyrosine hydroxylase, vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating polypeptide (PACAP)] were only observed in enteric nerve fibres, while other markers [calretinin, calbindin, choline acetyltransferase (ChAT), serotonin (5-HT) and neuronal nitric oxide synthase (nNOS)] were also detected in neuronal cell bodies. In all segments of the adult intestine, ± 50% of the enteric neurons expressed calretinin, while ± 40% expressed calbindin, ± 40% ChAT and ± 20% nNOS. The proportion of 5-HT-positive neurons significantly and progressively decreased from the anterior part (± 23%) to the posterior part (± 11%) of the gut. No colocalization was observed between 5-HT and calretinin or calbindin, while all calbindin-positive neurons expressed calretinin. ChAT colocalized with calretinin and calbindin but not with 5-HT. The inhibitory neuropeptides, VIP and PACAP, were present from 72 hpf on in the middle and posterior part of the gut. Nitrergic neurons were also found from 72 hpf in these parts, while calretinin and calbindin were expressed in the midgut. From 96 hpf on the stimulatory neurotransmitter, 5-HT, was also expressed in the enteric nervous system.
Conclusion : The present results indicate that inhibitory neurons are the first to differentiate in the zebrafish ENS and that they play a significant role in the spontaneous motor activity of the gut observed between hatching (2-3 days post-fertilization (dpf)) and the onset of feeding (5-6 dpf). The results support also previous data that the ENS is well-developed before the start of feeding. In the adult intestine, the results are indicative of the presence of several subpopulations of enteric neurons, and of the existence of regional differences.

- B19 -

CONTRIBUTION OF HYPERGLYCEMIA, HO-1 EXPRESSION AND INFLAMMATION TO LOSS OF NNOS AND C-KIT EXPRESSION IN THE SPONTANEOUSLY DIABETIC BIOBREEDING-RAT. S. Kindt, W. Boesmans, T. Masaoka, P. Vanden Berghe, J. Tack. KULeuven.

Loss of expression of neuronal nitric oxide synthase (nNOS), as well as loss of interstitial cells of Cajal have been implicated in disordered motor control in animal models of type 1 diabetes. In NOD mice, loss of heme oxygenase-1 (HO-1) upregulation seems to lead to ICC loss through hyperglycemia-associated oxidative stress (Choi 2008). In the Biobreeding (BB) rat, spontaneous transmural inflammation was related to loss of nNOS expression in diabetes prone (BBDP) as compared to diabetes resistant animals (BBDR) (Kindt, DDW 2008). Our **aim** was to study the relationship between hyperglycemia, inflammation and HO-1 expression on one hand, and loss of nNOS and ICC on the other hand, in the BB-rat model.
Methods : After 16 weeks of hyperglycemia we analyzed the normalized mRNA expression of HO-1, nNOS, inducible NOS (iNOS) and the ICC marker c-KIT by real-time RT-PCR, and measured myeloperoxidase activity (MPO) in the proximal jejunum of 8 hyperglycemic BBDP (BBDP-H) rats. Results were compared to 8 sex- and age-matched normoglycemic BBDP (BBDP-N) and 8 BBDR rats. Data were analysed by ANOVA and correlation analysis.
Results : Intestinal inflammation was a feature of BBDP and not of BBDR rats (MPO 8.8 ± 2.0 vs. 2.6 ± 0.7 U/mg, $p = 0.007$). A significant decrease in nNOS mRNA expression was present in the BBDP-N and BBDP-H rats when compared to BBDR rats (0.70 ± 0.49 and 0.52 ± 0.38 vs. 2.61 ± 1.13 respectively, ANOVA $p = 0.02$). A significant inverse non-linear correlation was found between nNOS mRNA expression and iNOS mRNA expression (Spearman $r = -0.79$, $p < 0.0001$) as well as MPO concentration (Spearman $r = -0.57$, $p = 0.004$) in the BBDP rats. Across the groups, HO-1 mRNA expression was significantly linearly correlated with c-KIT mRNA expression (Pearson Rho = 0.92, $p < 0.0001$). Group differences in c-KIT mRNA expression did not reach statistical significance (6.38 ± 3.7 vs. 11.45 ± 3.53 vs. $6.80 \pm .17$ in BBDR, BBDP-N and BBDP-H respectively, NS). HO-1 expression was not correlated to MPO or to iNOS mRNA expression.
Conclusion : In the BB rat, loss of jejunal nNOS expression is correlated to the presence and severity of inflammation. In contrast, expression of the ICC marker c-KIT is independent from inflammatory markers, but is positively correlated to HO-1 expression. These observations suggest distinct pathophysiological mechanisms involved in the changes in neurotransmitter expression and loss of ICC observed in the BB rat model of Type 1 diabetes.

- B20 -

IMMUNOLOCALIZATION OF NOVEL ICC AND GIST MARKERS IN THE MOUSE ANTRUM. P. Gromova (1), P. Hague (1), S. Ralea (1), B.P. Rubin (2), C. Erneux (3), J.M. Vanderwinden (1). (1) ULB Brussels ; (2) Anatomic Pathology and Molecular Genetics, Cleveland Clinic, Lerner Research Institute and Taussig Cancer Center, Cleveland, OH, USA ; (3) IRIBHM, ULB Brussels.

Introduction : Interstitial Cells of Cajal (ICC) are tiny populations of mesenchymal cells located within the muscularis propria of the gastrointestinal tract, where they coordinate peristalsis through network formation and intrinsic pacemaking activity. Gastrointestinal stromal tumours (GIST) derive from ICC or their precursors. Oncogenic mutations of the receptor tyrosine kinase KIT are present in most GIST. KIT is the established marker for ICC and is required for their development and function. Additional markers to identify ICC and GIST would be valuable.
Material & methods : Here, we used IF to localize proteins previously identified by expression studies (1) in the gastric antrum in the K641E knock-in GIST murine model (2), expressing an oncogenic KIT mutant previously described in our laboratory (3).
Results : KIT K641E oncogenic mutant causes marked hyperplasia of KIT-ir cells in the antral musculature. Pde3a and Prkcq/Pkctheta were localized in KIT-ir ICC in WT and $KIT^{K641E:Neo/K641E:Neo}$ mice while Spry4 and Tpbg/5T4 were detected only in KIT-ir cells in $KIT^{K641E:Neo/K641E:Neo}$, but not in $KIT^{WT/WT}$ animals.
Conclusions : While Pde3a and Prkcq/Pkctheta belong to the expression profile of human GIST, they are also expressed in KIT + ICC in the normal mouse gut and thus could serve as novel ICC markers. Spry4 and Tpbg/5T4 represent novel markers for KIT + cells in oncogenic KIT mutants and are now being investigated in human GIST.
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- B21 -

IC CILIUM : IMMUNOFLUORESCENCE OF (IMMOTILE) CILIUM IN KIT-IR INTERSTITIAL CELLS OF CAJAL. S. Ralea, P. Hague, J.M. Vanderwinden. ULB.

Introduction : The immotile (primary) cilium is a unique cellular structure (1) which importance in signal transduction pathways crucial for embryological development (2), tissue differentiation (3) and diseases ("ciliopathies") (4) has been recently highlighted. The presence of a cilium in ICC, known from ultrastructural studies for decennia, has been recently revisited (5).
Objectives : To investigate for cilium in the mouse and rat GI tract using immunofluorescence (IF).
Methods : Double IF procedure was carried out on cryostat section of PAF fixed mouse and rat antrum, jejunum and colon for the ICC marker KIT(goat pAb) and one cilium marker : rabbit pAb ACIII(6) (on mouse&rat tissues),mouse mAb acetylated alpha-tubulin (7), mouse mAb LhS28 (8) (rat tissues only).
Results : ACIII-ir cilium, defined as a single, tiny (approx 0.5 micron diameter by 2 microns length), structure protruding from the perinuclear cytoplasm were scarce in ENS neurons in mouse and rat and in KIT-ir ICC in mouse antrum and jejunum – but absent in mouse colon and in rat gut. Acetylated alpha tubulin-ir and LhS28-ir proved disappointing, as both markers label countless fibrilary structures in nerve fibres closely adjacent to the KIT-ir ICC, precluding the identification of a single cilium.
Conclusion : ACIII-ir revealed the presence of primary cilium in KIT-ir ICC in the mouse proximal gut and in enteric neurons. Due to their tiny size and scarcity, high resolution imaging is mandatory. Although a mechanosensory role remains possible, the recent literature suggest additional essential functions for the primary cilium.
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JOINT MEETING

- D01 -

VISCERAL FAT AND INSULIN RESISTANCE ARE INDEPENDENTLY CORRELATED TO DEGREE OF LIVER STEATOSIS AND STAGE OF FIBROSIS IN A NON-SELECTIVE COHORT OF OBESE PATIENTS. S. Francque, A. Verrijken, I. Mertens, G. Hubens, E. Van Marck, P. Pelckmans, L. Van Gaal, P. Michielsen. UZ Antwerp.

Introduction : Non-Alcoholic Fatty Liver Disease (NAFLD) and the Metabolic Syndrome (MS) seem to be intimately linked. Clinical studies, however, suffer from important selection bias (e.g. elevated ALT or established liver involvement or bariatric surgery as selection criteria) or diagnostic inaccuracy (no histology).
Aims : To study in a prospective cohort of overweight patients without known liver involvement the presence of NAFLD/NASH and the relation between relevant clinical, metabolic and biochemical parameters and liver histology.
Patients and methods : Patients presenting to the obesity clinic underwent metabolic and liver assessment (blood analysis, Doppler-ultrasound, scintigraphy and aminopyrin breath test). If liver involvement was suspected, liver biopsy was proposed, preferentially transjugularly. Biopsy was scored according to the NASH Clinical Network Scoring System.
Results : 55 patients underwent a transjugular biopsy between September 2006 and May 2008. Mean age 45.5 ± 14.9 y ; mean BMI was 40.4 ± 7.2 kg/m^2 ; 34 were female (62%) ; 63% fulfilled the criteria of the MS. Seventeen (31%) had no steatosis, 18 (33%) stage 1, 14 (26%) stage 2 and 6 (10%) stage 3 steatosis. Seven (17%) met the criteria of NASH (NASH activity score e 5). Fibrosis stages were as follows : 32 (59%) stage 0, 13 (24%) stage 1, 3 (5%) stage 2, 4 (7%) stage 3 and 3 (5%) stage 4 (cirrhosis). Fifteen (27%) had portal hypertension (PHT), indicating serious impairment of liver blood flow. PHT was not related to fibrosis, as reported elsewhere. The degree of steatosis was significantly correlated to waist ($r = 0.46$, $p = 0.002$), waist-hip ratio (WHR) ($r = 0.45$, $p = 0.002$), fasting insulin ($r = 0.44$, $p = 0.003$), fasting c-peptide ($r = 0.45$, $p = 0.003$) and HOMA IR ($r = 0.50$, $p = 0.001$). After correction for waist, HOMA-IR remained significantly correlated to the degree of steatosis ($r = 0.41$, $p = 0.009$). The stage of fibrosis was significantly correlated to waist ($r = 0.53$, $p < 0.0001$), WHR ($r = 0.42$, $p = 0.007$), visceral fat (by CT) ($r = 0.37$, $p = 0.019$), fasting insulin ($r = 0.51$, $p = 0.001$), fasting c-peptide ($r = 0.58$, $p < 0.0001$) and HOMA IR ($r = 0.51$, $p = 0.001$). After correction for waist, HOMA IR remains significantly correlated with fibrosis($r = 0.33$,$p = 0.042$). Other parameters (e.g. ALT, GGT, ferritin) showed no significant correlation.
Conclusion : In this non-selective cohort of patients primarily presenting because of overweight (but not liver disease), NAFLD is prevalent but advanced fibrosis is infrequent. Visceral fat accumulation and insulin resistance are independently associated with both the degree of steatosis and fibrosis, underlining the close association between the MS and NAFLD/NASH.

- D02 -

ACCURACY OF CLINICAL SCORING SYSTEMS FOR FATTY LIVER AND ADVANCED LIVER FIBROSIS IN AN UNSELECTED OVERWEIGHT AND OBESE POPULATION. A. Verrijken, S. Francque, I. Mertens, P. Michielsen, L. Van Gaal. UZ Antwerp.

Introduction : Paralleling the increasing prevalence of obesity, diabetes, and the metabolic syndrome, nonalcoholic fatty liver disease (NAFLD) has become a common cause of chronic liver disease worldwide and can be associated with progressive fibrosis and cirrhosis. Clinical predictors of advanced NAFLD are needed to guide diagnostic evaluation and treatment. Existing scoring systems are mostly validated in patients with established liver disease. Their value in unselected patients remains to be established.
Methods : One score for fatty liver (Fatty Liver Index) and three scores for advanced fibrosis according to different parameters (BARD score, NAFLD Fibrosis Score, BAAT score) have been determined in an overweight and obese population who presented to the obesity clinic between August 2006 and July 2008, and who underwent a liver biopsy for a clinical suspicion of NAFLD.
Results : The cohort -156- patients was 66% female, mean age was 45 ± 12 y and mean BMI was 39.1 ± 6.6 kg/m^2. In 70.5% one or more of the liver tests were elevated. 86 (55.1%) patients did not have fibrosis, 51 (32.7%) had stage 1-2 fibrosis, and 19 (12.2%) had advanced (stage 3-4) fibrosis.

	Fatty Liver Index*	BARD score	NAFLD Fibrosis score**	BAAT score
Sensitivity	100.0%	36.8%	10.0%	62.5%
Specificity	-	62.0%	97.1%	37.1%
Positive Predictive Value	39.5%	11.9%	25.0%	25.5%
Negative Predictive Value	-	87.6%	91.8%	74.1%

*Fatty liver was compared to histology instead of ultrasound. Histology scores for steatosis 0-1 were compared to scores 2-3 (ultrasound reliable in detection of steatosis > 30%) ; **26.9% patients with indeterminate result.

Conclusion : Score systems for liver steatosis or fibrosis, often validated in highly selected populations, suffer from a low sensitivity and PPV in an unselected population of overweight patients and are therefore of little value in identifying patients with advanced disease. The NPV is, however, acceptable (except for BAAT), indicating their potential in a large epidemiological approach and in avoiding unnecessary liver biopsies.
This work is part of the project “Hepatic and adipose tissue and functions in the metabolic syndrome” (HEPADIP), which is supported by the European Commission as an Integrated Project under the 6th Framework Programme (Contract LSHM-CT-2005-018734).

- D03 -

DETERMINATION OF SEVERE FIBROSIS AND CIRRHOSIS IN ALCOHOLIC PATIENTS BY TRANSIENT ELASTOGRAPHY : A PROSPECTIVE COMPARISON WITH LIVER BIOPSY. F. Janssens, N. De Suray, Y. Horsmans, H. Piessevaux, P. De Timary, P. Stärkel. UCL Saint-Luc.

Background/aims : Transient elastography (TE, FibroScan®) is a non-invasive method to assess hepatic fibrosis by measuring liver stiffness. Cut-off values for different fibrosis stages have been validated for chronic hepatitis C (HCV). Whether these cut-offs apply to other liver diseases remains controversial. We evaluated the performance of TE in predicting severe fibrosis (^{3}F3) in alcoholic patients applying cut-off values validated for HCV.
Methods : 255 patients admitted for alcohol withdrawal between 01/2006 and 02/2008 were prospectively evaluated by TE and routine biochemistry allowing to calculate APRI and Forns score. Hepatic venous pressure gradient (HPVG) measurements and transjugular liver biopsy were proposed to patients classified as having severe fibrosis (^{3} F3) at TE. Results of TE, APRI and Forns were compared with liver biopsy specimens (^{3}15 mm ; ^{3} 6 portal tracts) using the Metavir classification. TE measurements were correlated to HPVGs.
Results : 239 patients successfully underwent TE of whom 72 had liver fibrosis scores ^{3} F3. 23 patients declined liver biopsy leaving a final study population of 49 patients. When compared with liver biopsy, 32 patients were classified correctly by TE (12 F3 ; 20 F4) whereas 17 (35%) differed by 2 fibrosis stages yielding a positive predictive value (PPV) of TE for liver fibrosis ^{3} F3 of 65%. Area under the receiver operating characteristic (AUROC) curves were 0.766 and 0.864 for severe fibrosis (^{3} F3) or cirrhosis (F4), respectively. Asignificant correlation was found between HPVGs and liver stiffness values at TE($r = 0.632$, $p < 0.001$). Sensitivity, specificity, PPV and NPV were63.4, 71.4, 92.9 and 25% for APRI > 1.5 and at 42.1, 85.7, 94.1 and 21.4% for Forns > 6.9, respectively. When modified cut-offs of 13.9 (F3) and 19.6 kPa (F4) were applied to our study population, sensitivity and specificity of TE reached 81% and 59% and 80% and 76%, respectively.
Conclusion : Currently validated cut off values are inadequate for predicting severe fibrosis and large prospective studies are required to validate new, likely higher cut-offs in alcoholic patients. Apri and Forns score performed reasonably well in predicting significant fibrosis in our alcoholic population.

- D04 -

UNIVERSAL HEPATOCYTE DIFFERENTIATION PROTOCOL FOR MULTIPOTENT ADULT PROGENITOR CELLS (MAPC), EMBRYONIC (ES) AND INDUCED PLURIPOTENT STEM CELLS (IPS). P. Roelandt (1), P. Sancho-Bru (1), K. Pauwelyn (1), K. Subramanian (2), B. Bose (1), T. Shimizu (1), K.Vanuytsel (1), W.S. Hu (2), F. Nevens (1), C. Verfaillie (1). (1) KULeuven, Belgium ; (2) University of Minesota, Mineapolis, USA.

Cultured hepatocytes are vital for drug development and pharmaceutical testing, bio-artificial liver devices, and cell therapy of liver diseases. One of the main drawbacks is the paucity of mature human hepatocytes. Hepatocytes differentiated from stem cells could be an alternative source. We hypothesized that differentiation of bona-fide hepatocytes will be better obtained by exposing pluripotent stem cells sequentially to the different growth factors present in embryonic liver development. Here we describe a multistep protocol for hepatocyte-like cells generation by exposing pluripotent stem cells to cytokines in a sequence that mimics liver development.
The liver differentiation protocol was optimized by using a first rat Multipotent Adult Progenitor Cell (MAPC) line : cells are sequentially exposed to Activin A and Wnt3a to induce primitive streak, mesendoderm and definitive endoderm (DE). We subsequently used FGF2 and BMP4, produced by the cardiac mesoderm to specify the DE to hepatic endoderm, and then FGF1, FGF4 and FGF8b, produced by the septum transversum mesenchyme induce hepatoblast induction. Final maturation was induced by addition of HGF and follistatin (to block biliary cell differentiation). High concentrations of dexamethasone were essential throughout the differentiation.
Real-time PCR and immunostaining showed expression of key genes of PS and DE after the first step. Hepatoblast and immature hepatocytes genes were observed after the third step, while genes expressed in mature hepatocytes were present at the end of the differentiation. Electronmicroscopy analysis of differentiated cells showed morphological characteristics of immature hepatocytes. Differentiated hepatocyte-like cells showed functional properties of mature hepatocytes, such as albumin secretion, glycogen storage, urea cycle, bilirubin glucuronidation and inducible cytochrome activity. With minor adjustments, the protocol could also be used for differentiation of several additional rat MAPC lines, 2 mouse MAPC lines, 2 human and 1 mouse embryonic stem cell, and 2 mouse induced pluripotent stem cell lines. This data demonstrates that a single robust protocol, modeled on embryonic liver development, induces hepatic differentiation from several pluripotent stem cell types and different species, generating cells with phenotypic and functional characteristics of small hepatocytes (akin to E14-17 in mouse or rat). This protocol is a first step for generating fully competent hepatocytes.

- D05 -

EVALUATION OF THE SAFETY AND EFFICACY OF DRUG ELUTING BEADS TRANS-ARTERIAL CHEMO-EMBOLISATION (DEB-TACE) COMPARED WITH CONVENTIONAL TACE FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA : A RETROSPECTIVE STUDY. V. Lannoy, P. Goffette, H. Piessevaux, J.F. Gigot, J. Lerut, Y. Horsmans, Z. Hassoun, P. Stärkel, I. Borbath. UCL Saint-Luc.

Background : Transarterial chemoembolization (TACE) is a validated treatment option for multifocal hepatocellular carcinoma (HCC). In an attempt to avoid systemic toxicity and increase local efficacy, microspheres loaded with doxorubicin, «Drug-eluting beads» (DEB), have been designed. No study compared this technique to conventional TACE. Aim : to compare two cohorts of patients, treated by conventional TACE (Conv-TACE) or by DEB-TACE, with regard to toxicity and efficacy.
Materials and methods : The files of 133 patients were analysed retrospectively. The first group (N = 71) was treated by conv-TACE during 2003 and 2004 and the second group (N = 62) by DEB-TACE in 2006 and 2007. Renal function, liver function (Child-Pugh) status and adverse events were analysed before and 4-6 weeks after each procedure. Therapeutic response was assessed 6 weeks after each TACE by MRI or CT-scan, according to EASL-modified RECIST criteria.
Results : The 2 cohorts were comparable in terms of age (70 vs 65 y), sex (72 vs 87% males), liver function (85 vs 75% Child-Pugh A, no Child-Pugh C), aetiology of cirrhosis (HCV, alcohol, HBV) and tumour status (median size 50 vs 48 mm for biggest lesion, unilobar lesions in 87 vs 82%). In the conv-TACE cohort, 16/71 patients (22%) experienced 18 episodes of severe toxicity (18/211 procedures, 8.5%) vs. 10/62 (16%) patients in the DEB-TACE cohort, with 12 severe complications (12/100 procedures). The most severe complications consisted of acalculous cholecystitis and pancreatitis ; no death occurred. Post-TACE syndrome, i.e. fever and/or pain, occurred with equal frequency in the 2 groups, 33% (conv) vs 43% (DEB) after the first TACE. Alopecia occurred in 3 patients in the conv-TACE group and not in the DEB-TACE group. A logistic regression model was build to assess causative factors for kidney failure after TACE in patients with normal renal function before treatment (n = 126). The baseline covariates, Child-Pugh status (A vs B, HR = 3.5), and creatinine (HR = 2.4 per 0.1 mg/dl increase) as well as the number of procedures (HR = 1.5 per procedure) were retained in the model, irrespectively of the treatment modality. Complete and partial necrosis was obtained in 72 vs 73% of lesions after 1 procedure in conv-TACE vs DEB-TACE respectively. Survival data will be available at the meeting.
Conclusions : In this retrospective study, DEB-TACE was found to be equally toxic and efficient as conv-TACE. For both techniques, caution is warranted in Child-Pugh B patients and in those with borderline normal renal function.

- D06 -

IMPACT OF GENOTYPE AND ETHNICITY ON THE PREVALENCE OF METABOLIC SYNDROME ASSOCIATED WITH CHRONIC HEPATITIS C. T. Serste, M. Nkuize, M. Van Gossum, M. Reynders, R. Scheen, J.P. Mulkay. ULB Saint Pierre.

Background & aim : HCV infection is associated with an increased risk of metabolic disorder especially in patients with genotype 1 and 4. Ethnical distribution in patients with Chronic Hepatitis C (CHC) is linked to the genotype. The aim of this study was to consider the eventual association between Metabolic Syndrome (MS), ethnicity and genotype in a large cohort of CHC patients.
Patients and methods *:* This study included all consecutive patients with CHC, followed up in our department of hepato-gastroenterology between January 2002 and September 2008. Demographic data and all components of the MS were collected.
Results : A total of 454 CHC patients were studied. Two hundred ninety-four had genotype 1/4 (64.8%), 160 (35.2%) patients had genotype 2/3. The prevalence of the MS was 11.5%.
Comparing patients according to their genotype, there were many Black Africans in the genotype 1/4 group (32.0% vs 1.2%, *Pd0.0001*). Genotypes 1/4 were significantly older (50.4 ± 12.8 vs 44.6 ± 10.6 years, *Pd0.001*). The prevalence of the MS was higher in patients with genotype 1/4 (11.7% vs 5.0%, *P = 0.03)*. Genotype 1/4 was associated independently with the presence of the MS (OR, 11.3 ; 95% CI : 1.4-89, *P = 0.02*).
Comparing patients according the presence of MS, patients with MS were older than those without (53.8 ± 11.6 vs 47.9 ± 12.5 years, respectively, *P* = .005). Genotypes 1/4 were found more frequently in patients with MS (96% vs 66.5%, respectively, *P* = 0.0001). There were many Black Africans in the MS group (48.0% vs 18.8%, *P* = 0.0001). Genotype 1/4 was the unique factor independently associated with the MS (OR, 14.68 ; 95% CI : 1.89-113.7, *P = 0.02*).
Conclusions : The prevalence of MS in Chronic Hepatitis C is independently associated with genotype ¼. Ethnicity is not independently linked to the MS.

Invited lecture

- D07 -

ANTIVIRAL RESISTANCE IN HBV and HCV. F. Zoulim. INSERM, Lyon, France.

In the past 10 years, significant progress has been achieved in the management of chronic hepatitis B with the successive development of six potent antiviral medications (lamivudine, adefovir dipivoxil, pegylated interferon alpha, entecavir, telbivudine and tenofovir). However, the clinical results of antiviral therapy have been limited by the emergence of antiviral drug resistance especially with the first generation of nucleoside analogs (lamivudine, adefovir and telbivudine). Furthermore, the unique mechanism of viral genome replication and persistence within infected cells is responsible for viral persistence even after prolonged therapy with the newer antivirals (entecavir and tenofovir). This is the major reason why life-long treatment is envisaged in the majority of patients, which may expose them to long-term risk of developing resistance. The understanding of the development of HBV drug resistance has allowed to significantly improve the management of antiviral resistance and to design better treatment strategies to prevent resistance. The current standard of care relies on treatment initiation with antivirals combining a strong antiviral potency and a high barrier to resistance. A precise virologic monitoring is required to measure antiviral efficacy, and to diagnose partial response or viral breathrough at an early stage. This allows to adapt antiviral treatment preferrably using an add-on strategy with a drug having a complementary cross-resistance profile. This strategy has been shown to be efficient in controling viral replication and preventing liver disease progression in the majority of patients. The future challenge will be to determine whether de novo combination of nucleoside analogs belonging to the new generation of drugs will provide an added benefit in terms of drug resistance and prolonged viral suppression. The identification of new antiviral targets will be important in that respect to develop more potent combination strategies aiming at HBsAg clearance. Chronic HCV infections mainly differs from HBV infection by the possibility to cure HCV infection with antiviral therapy. The current treatment of chronic hepatitis C relies on the combination of pegylated Interferon alpha and ribavirin. The sustained virologic response rate varies from 45% in genotype 1 infected patients to 90% in genotype 2/3 infected patients. Major research efforts have been made to develop new specific inhibitors of HCV infection targeting the viral protease or the viral polymerase to increase the response rate to therapy. It was shown that due to the HCV genome variability, drug resistant mutants are rapidly selected in vitro in the replicon system and in vivo in patients receiving monotherapy with these specific inhibitors in phase I/II trials. Therefore, these drugs have been further developed in combination with pegylated interferon alpha with or without ribavirin. It was shown with protease inhibitors that the triple combination with pegylated interferon and ribavirin was the best strategy to prevent the development of drug resistant mutants. Large phase III trials are ongoing with these drugs to determine the best treatment strategy to obtain the maximal antiviral efficacy and the best safety profile. Different classes of polymerase inhibitors are also in development including nucleoside analogs and non nucleoside inhibitors. Their development is also confronted to side effects (gastrointestinal and haemotological side effects) and to the development drug resistant mutants. Phase II trials are currenly ongoing. Other drugs targeting other steps of the viral life cycle are being investigated including inhibitors of cyclophilin (a cellular co-factor enhancing the activity of the viral polymerase), cell entry, viral morphogenesis, and immune modulators. In the near future, antiviral therapy of HCV infection will rely on the combination of a specific inhibitor plus pegylated interferon and ribavirin. Once several drugs with complementary cross-resistance profiles will have been evaluated, their combination will be investigated in clinical trials to determine whether or not interferon and ribavirin are mandatory in the treatment arsenal. In any case, the development of these antivirals should significantly increase the treatment success rate especially in the difficult to treat patients infected with genotype 1.

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- D08 -

THE VALUE OF FIBROSCAN IN PREDICTION OF VARICEAL BLEEDING RISK. I. Ratiu, D. Lazar, A. Goldis, M. Strain, A. Deleanu, R. Sirli, A. Tudora, I. Sporea. Timisoara, Romania.

Introduction : Fibroscan is a new noninvasive method able to evaluate the severity of fibrosis in patients with chronic liver diseases.
Aim : Purpose of this study was to determine if fibroscan values correlates with variceal bleeding risk
Material and method : A retrospective study on 149 cases of liver cirrhosis divided into 2 groups batch A with variceal hemorrhage (72 cases) and batch B without bleeding (77cases). We compared the fibroscan values between the two groups using unpaired t-test and we established a cut-off fibroscan value for the risk of bleeding using ROC curve.
Results : Comparing the value of fibroscan between batch A and B we have the following
Results : 41,07 + /- 21,21 in group A – patients with variceal hemorrhage versus 27,77 + /- 14,63 in group B – patients without bleeding p < 0,001 ES
Using ROC curve we establish a cut-off fibroscan value of 32 for the risk of bleeding in our study with a sensibility 58,44 and specificity 72,22. AUROC 0,695. p = 0,0001, + PV = 69,2, -PV = 61,9, CI 95% (0,614 to 0,768).
Conclusion : The differences between the two groups are ES in our study, therefore we can conclude that Fibroscan is a reliable noninvasive method for detection of variceal bleeding risk.

- D09 -

HEPATITIS C GENOTYPE 4 RESPONSE RATE TO PEGYLATED INTERFERON ALFA-2A AND RIBAVIRIN TREATMENT IN BELGIUM IS SIMILAR TO GENOTYPE 1. C. De Galocsy (1), L. Kaufman (2), S. Tomasovic (3), R. Brenard (4), F. D Heygere (5), J. Henrion (6), P. Langlet (7), M. Adler (8), J. Delwaide (9), F. Nevens (10). (1) HIS Bracops Hospital ; (2) Veeda Clinical Research.

Background and aims : Patients with genotype 4 (G4) chronic hepatitis C from Middle East respond better to treatment than genotype 1 (G1) patients. There are few data on the response rates to treatment of G4 patients living in Western Europe. Many G4 patients in Belgium originate from Central Africa, and their response to treatment seems lower.
Methods : We analysed the data from 2 randomized multicentric phase III studies conducted in Belgium, BerNar-1 and BerNar-2, comparing the sustained virological response (SVR) to pegylated interferon and ribavirin of 78 G4 patients (34 Caucasians, 44 Blacks) and 477 G1 patients (455 Caucasians, 12 Blacks), and assessing the predictors of SVR.
Results : Baseline characteristics of G4 and G1 patients were similar (mean age 46.4/ 46.5, male 46.2%/ 53.9%, mean BMI 25.8/25.4, mean viral load at screening 1590503 IU/ml/ 1587512 IU/ml, mean ALT at screening 76 IU/ml/ 90 IU/ml, clinical diabetes 6.4%/ 6.3%, naïve to treatment 96.2%/ 87.6%, relapsers to previous treatment 3.9%/ 9.7%, Metavir A0-1 72.4%/ 62.4%, Metavir F0-1 56.9%/ 46.8%, cirrhosis 19.5%/ 19.4%) except for race (G4 Blacks 44 = 56%, G1 Blacks 12 = 2.5%). Complete early virological response (cEVR) (< 600 IU/ml) was similar in G4 (73.2%) and G1 (68.1%). cEVR was also similar between Black and Caucasian G4 (70% vs 76.7%) and between Black and Caucasian G1 patients (63.6% vs 68%). Partial early virological response (> 2 log10 drop) was similar for G4 (72.1%) and G1 (71.3%). SVR was similar for G4 (51.3%) and G1 (51.8%). There was a trend for a higher SVR in Caucasians (G4 63.6%, G1 52.1%) than in Blacks (G4 40.9%, G1 33.3%). In multivariate analysis, the only predictors for SVR were the presence of cirrhosis, HCV viral load, age < 40 vs e40 yrs, and treatment status (relapsers vs naïve).
Conclusions : G4 patients in Belgium have the same SVR as G1 patients. As it is lower than the SVR described in Arab countries (and specially for Black G4 patients), the duration of treatment for G4 patients in Europe should not be shortened.

- D10 -

HCV AND LIVER TRANSPLANTATION : THE BENEFICIAL ROLE OF MIMIMAL TACROLIMUS (TAC) BASED IMMUNOSUPPRESSION. E. Bonaccorsi-Riani, N. Piette, O. Ciccarelli, B. Kabamba, Z. Hassoun, F. Roggen, C. De Reyck, C. Verbaandert, C. Sempoux, J. Rahier.

Aim : HCV allograft re-infection after Liver Transplantation (LT) is universal. The time-line of HCV re-infection in the immunocompromised host is more rapid and depends on several donor as well as recipient factors. Type of induction immunosuppression (IS) and (modality of) steroid use have both been said to induce a more aggressive recurrence (Rec). This impact has however till now never been studied in a well designed study.
Methods and material : During the period 2000-2005 35 HCV positive patients were included in a prospective, randomized, double-blind, placebo-controlled study comparing TAC-Placebo (PLAC) (n = 14) vs. TAC-short-term (3 mo) low-dose steroid (STER) (n = 21) use. PLAC and STER groups only differed in relation to cold ischemia time (p 0.03). All patients received identical peri-operative care, including peri-operative administration of 1gm of hydrocortisone. Routine liver biopsies were done at d7, 6 mo, 12 mo and then yearly. All biopsies were read blindly by three experienced pathologists. HCV recurrence and rejection were classified following Ludwig (taking into account portal (P), lobular (L) infiltration as well as degree of fibrosis (S) scored from 1 to 4-S1 : no fibrosis-S2 : beginning fibrosis-S3 : fibrosis-S4 : cirrhosis) and Banff scores. All patients had a complete follow-up of minimal 3 yrs.
Results : One, three and five year patient survival rates were 93 ; 93 and 78% in TAC-PLAC gr and 90 ; 71 and 65% in TAC-STER gr. One, three and five year graft survival rates were 93 ; 86 and 71% in the TAC-PLAC gr and 90 ; 65 and 60% in the TAC-STER gr (p 0.09). During the first three years one TAC-PLAC pt died due to recurrent hepatocellular cancer (HCCa) ; five TAC-STER gr 5 pts died due to HCV rec and one due to HCCa.

Outcome	TAC-PLAC (n = 14)	TAC-STER (n = 21)	
Pts with HCCancer	11	10	
Rejection therapy	1	2	
Antiviral (IFN)therapy*	1	4	
Histology Ludwig Score at one year			
Normal	0	3 (14.3%)	ns
Aspecific hepatitis	2 (14.3%)	0	ns
P1-3;L1;S1	7* (50%)	8*** (38.1%)	ns
P2;L1-2;S2	5 (35.7%)	5 (23.8%)	ns
P2;L1;S3	0	2** (23.8%)	p 0.03
P;L;S4	0	3	
Lethal cholestatic hepatitis	0	2	

At 3 years 5 (35%) of 14 TAC-PLAC survivors and 6 (40%) of 15 TAC-STER pts evolved towards S3-4 Ludwig score. During the fourth and fifth year of follow-up, one TAC-PLAC pt each died of de novo tumor at 45 mo and of HCV rec and B-lymphoma at 54 mo ; four TAC-STER pts in contrast died due to HCV rec at 49;73;76 and 79 mo. In total one (7%) of 14 TAC-PLAC pts and nine of 21 (43%)TAC-STER pts died of HCV recurrence (p 0.045).
Conclusion : Steroid-free, low-dose TAC IS has a major favourable impact on the early as well as long-term outcome of LT in HCV patients. Larger prospective, randomized, double-blind and placebo-controlled studies with long-term follow-up are mandatory to confirm the presented results and to improve the outlook of HCV liver recipients.

- D11 -

IMPROVED RESULTS FOR ADULT SPLIT LIVER TRANSPLANTATION WITH EXTENDED RIGHT GRAFTS.
M. Sainz-Barriga, E.L. Decoster, A. Geerts, I. Colle, H. Van Vlierberghe, I. Haentjens, L. Colenbie, S. De Sutter, J. Bontinck, B. Van Den Bossche, F. Berrevoet, B. de Hemptinne, R. Troisi, X. Rogiers. UZ Gent.

Background : Split liver transplantation is an efficient tool to increase the number of grafts available for transplantation. Children are benefiting most from this possibility while among some adult liver transplanters remain concerns that by splitting a liver, a good quality graft is turned into a marginal one. This concern is of utmost importance under the patient-oriented allocation system. We performed a retrospective review of our results with extended-right split grafts (eRLG) in liver transplantation under this allocation policy.
Methods : Between July 2001 and August 2008, 22 liver transplants using eRLG were performed in 21 adult patients. From 2007 on, we adopted a rapid technique minimizing warming of the graft during the ex-situ splitting.
Results : Several organs from donors not fulfilling the standard criteria for liver splitting were used : 5 donors (23%) with age > 50 years, 6 donors (27%) with a ICU stay longer than 7 days, and 9 donors (41%) with hemodynamic instability. Eighteen (82%) splitting procedures were carried ex-situ. Mean graft to recipient body weight ratio was 1.5 ± 0.3, mean cold ischemia time was of 579 ± 120 minutes. In 5 cases (23%) the organ was allocated primarily to the adult recipient. The main indications for transplantation were alcoholic cirrhosis 7 (32%), HCV related cirrhosis 6 (27%), HCC 3 (14%) and 4 (20%) were performed in high urgency patients. The median time on the waiting list was 130 days, range (1-628). The median MELD was 15, range (7-40). The median ICU time was 4 days, range (1-60), while the median in-hospital stay was 28 days, range (8-269). The median follow-up was 14 months, range (2-90). One patient (4.5%) was retransplanted due to PNF, 4 patients (18%) presented vascular complications and 2 (9%) presented biliary strictures. No early mortality (d3 months) was observed. Three-year patient and graft survival rates were 84% and 79% respectively. No graft was lost due to vascular or biliary complications neither related to high urgency transplantation. When dividing the patients prior or after 2007 (11 patients in each group), we observed a 100% patient and graft survival for eRLG liver transplantations performed after 2007. No significant differences were found between the studied parameters from donors or recipients in both groups.
Conclusions : This results are in line with the published series of split liver transplantation with eRLG. We performed eRLG transplantation from extended criteria donors and with the exception of one graft lost from a 66 years old donor that was retransplanted with success using another eRLG, no graft or patient loss were related to the eRLG. Provided careful selection, exceptions to classical donor criteria for splitting can be accepted with success, expanding the potential for this technique. Our results show that eRLG for adult recipients can be a good option, also in the high urgency setting. The significance of the improvement observed in the last 11 eRLG patients may be related to the learning curve in our centre and to the rapid ex-situ technique, longer follow-up and further experience are needed to confirm this result.

- D12 -

BEVACIZUMAB AND 5-FU + OXALIPLATIN OR IRINOTECAN AS NEOADJUVANT THERAPY FOR PATIENTS WITH NON RESECTABLE LIVER METASTASES.
B. Van Den Bossche, T. Boterbergh, S. Laurent, I. Dero, F. Berrevoet, M. Peeters, B. De Hemptinne, R. Troisi. UZ Gent.

Background : Patients with colorectal liver metastases (CRM) and initially non resectable metastases might benefit from preoperative chemotherapy (CTX) with the hope for disease resection. Bevacizumab (BV) seems to improve outcomes in patients with CRM but may induce chemotoxicity with increased perioperative morbidity. We retrospectively rewieved our experience with BV-based regimen.
Methods : From June 2007 to November 2008, 38 patients with non resectable CRM, received BV-based CTX regimen (Folfox/Folfiri). CTX was administered bi-weekly until 6 cycles. The sixth cycle did not include BV resulting in 5 w between the last administration and liver resection (LR).
Results : After a mean time of 6.6 ± 3.3 w with BV, 18/38 (47%) patients became resectable and underwent LR (objective response to neoadjuvant CTX on PET-CT or histology). Downstaging of CRM was observed in 33% of cases whereas 1 patient was considered not resectable at laparotomy. Seven patients (41%) underwent a major hepatectomy whereas minor and wedge resections were done in 7 and 3 other respectively. Neither increased per operative bleeding nor wound healing complication were recorded. One patient required blood transfusions. No postoperative mortality occurred and 2/17 (12%) major complications were recorded (intraabdominal abscess and postoperative bleeding). Radical resection (R0) was achieved in 12/17 (71%) patients. After a median FU of 5.75 m (range 1-18) all patients are alive with recurrence in five patients until now (only two of these were R0 resections).
Conclusions : Bevacizumab-based CTX regimen does not increase complication rate permitting to allow potentially curative liver resection in more than 47% of patients with initially non resectable CRM. A longer FU is needed to understand the real impact of this regimen on disease-free survival.

- D13 -

SURGICAL MANAGEMENT AND PROGNOSTIC FACTORS OF HILAR CHOLANGIOCARCINOMA (KLATSKIN TUMOR) : EXPERIENCE WITH 68 PATIENTS AT THE GHENT UNIVERSITY HOSPITAL.
R. Troisi (1), A. Sagnotta (2), S. Laurent (1), I. Dero (1), T. Bocchetti (2).

Background : Hilar cholangiocarcinoma is a rare tumor accounting for less than 1% of all malignancies. This study was conceived to assess multimodal treatment including surgical approach and to determine postoperative morbidity, mortality rate and prognostic factors for long-term survival.
Methods : From May 1992 to December 2006, 68 patients with a Klatskin tumor were evaluated in our institution. Clinicopathological data were analyzed and univariate and multivariate analyses carried out to determine significant prognostic factors affecting morbidity and mortality. Mean age was of 53.4 ± 12 years. M/F ratio was of 46/22.
Results : After a median FU of 28 months (1-84), 11/68(16%) of patients were non resectable (group A) and treated with palliative transtumoral stenting. The other 57 patients (group B) underwent surgery : n = 5 for Bysmuth type II ; n = 20 for type IIIa ; n = 23 for type IIIb and n = 9 for type IV. Median survival was of 6 months in non resected patients vs. 32 months in group B ($p = 0.001$). R0 resection was achieved in 41/57(72%) patients. Median survival was of 48 m in R0 vs. 10 m in R1-R2 resection ($p = 0.003$). In-hospital mortality was of 3.5%. Overall morbidity rate was of 35%. Factors related to a shorter survival were identified as : lymphatic and perineural invasiveness, R1-2 resection, AJCC stage. Overall 3 & 5 y patient survival was of 45% and 22% respectively.
Conclusions : Surgical approach for Klatskin tumor is the only chance for long-term survivals with acceptable surgical mortality rate. In our experience, radical oncological surgery was possible in more than 70% of cases leading to a significant survival. Perineural and lymphatic involvement combined to a R1-R2 resection correlated with shorter survival.

- D14 -

TUMOR RECURRENCE AFTER HEPATECTOMY FOR COLORECTAL LIVER METASTASES : THE NEED FOR NEW PREDICTIVE FACTORS ? V. Lucidi, A. Buggenhout, B. Nebbot, J.L. Van Laethem, V. Donckier. ULB Erasme.

Aim of the study : Constant progresses in the multimodal management of patients with colorectal liver metastases (CRLM) justify to regularly reevaluate the benefit of surgical resection and therefore, the value of predictive factors for selecting patients for surgery. We reviewed several established prognostic factors and scores in a recent series of patients operated for CRLM.
Methods : Sixty-one consecutive patients undergoing a first liver resection for CRLM were reviewed. Several established factors (primary and secondary tumor characteristics, biological, clinicopathological and operative data) were analyzed to assess their predictive values on operative morbidity and mortality and overall and disease free survivals. In addition, 2 prognostic scores, defined as score I (node positive primary tumor, disease free interval < 12 months, number of CRLM > 1, largest tumor > 5 cm and CEA > 200 ng/ml ; Fong *et al. Ann. Surg*., 1999, 230 : 309) and II (inflammatory response to tumor and number of CRLM > 8 ; Malik *et al. Ann. Surg*., 2007, 246 : 806) were evaluated. Univariate and multivariate comparisons were made using chi square or Fischer's exact tests and Cox model respectively. Predictive values of scores I and II were analyzed with Wilcoxon-Mann-Withney test.
Results : In the 61 patients, CRLM were synchronous in 70%, multiple in 63% and bilobar in 30% ; 84% of the patients received preoperative chemotherapy (response rate : 84%). Surgery consisted in major resections in 48% and in 2-steps hepatectomy in 12%. In 12% of the cases, a combined Radiofrequency was performed on additional non resectable lesion. Operative mortality and morbidity were 3 and 30% respectively. A largest tumor > 50 mm and a 2-steps hepatectomy were the only significant univariate prognostic factors for operative morbidity. Median follow-up was 15 months. One and 2-years overall and disease free survivals were 92 and 89% and 47 and 30% respectively. Positive lymph nodes at primary tumor, number of CRLM, bilobar distribution, increased CEA level and use of RF were significantly associated with tumor recurrence in univariate but not in multivariate analyses. Score I was found predictive of tumor recurrence, with a most discriminative score of 2 ($p = 0.001$). In contrast, no predictive value was found for score II.
Conclusion : In this recent series of patients operated for CRLM, none of the classical risk factors used to predict tumor recurrence after resection were found significant in multivariate analysis. Only clinical score I (Memorial Sloan-Kettering Cancer Center) was predictive. While the large majority of the patients received active neoadjuvant chemotherapy, a preoperative score > 2 remained associated with high short-term tumor recurrence rate, questioning the benefit of surgery and underlying the need to consider other adjuvant therapies and more sensitive biomarkers in these cases.

- D15 -

ENDOSCOPIC TREATMENT OF BILIARY COLICS AND PANCREATITIS DURING PREGNANCY : A SERIES OF 18 PATIENTS. W. Van Steenbergen. KULeuven Gasthuisberg.

Background and aims : The formation of gallbladder cholesterol stones and sludge is stimulated by female sex hormones. As such, biliary symptoms may occur during pregnancy. Because of pregnancy, clinicians are undecisive about their therapy. Therefore, patients often present with repeated attacks of colics and even with biliary pancreatitis. It is the aim to report our experience with endoscopic sphincterotomy in 18 pregnant women presenting with biliary symptoms.
Patients and methods : Between 12/2001 and 12/2008, 18 pregnant women, with a mean age of 32 ± 6 years (ranges 21-46 yrs), were referred for endoscopic treatment of symptomatic biliary stone disease. Pts presented during a first, second, third or fifth pregnancy in 7, 6, 3, and 2 cases, respectively. Five pts presented during the first trimester of pregnancy, 4 during the second, and 9 during the third trimester. All cases were admitted because of repeated biliary colics and in 4 patients, at least one episode of biliary pancreatitis had already occurred. None of the patients had adequately been treated prior to the endotherapy. On sonography, gallbladder stones and/or sludge were present in 17 cases, common bile duct stones in 4, and acalculous cholecystitis in 1. Sphincterotomy was carried out in all 18 patients, in the absence of a radiologic proof of common bile duct stones. In 3 cases, precut-sphincterotomy was necessary and in 6 patients, one or more stones were extracted from the bile duct. Hyperamylasemia after ERCP was defined as an elevation of the serum amylase level above the upper normal limit (100 IU/L). Late follow-up data were obtained by telephonic contact with the patients and their referring physicians.
Results : All 18 patients remained completely asymptomatic during further pregnancy. Fifteen patients had a normal delivery at 40 weeks ; one case with toxicosis was induced at 31 weeks, and 2 patients are still pregnant. Hyperamylasemia was observed in 8 patients after the procedure but a diagnosis of mild post-ERCP pancreatitis could only be made in 1 case. Eight pts received a cholecystectomy during further followup, but in only 3 cases CCE was performed because of recurrent symptoms. Ten patients had no CCE ; in six of them an ultrasound examination of the gallbladder was negative for stones 7 to 84 weeks after delivery.
Conclusions : Biliary colics and episodes of acute biliary pancreatitis during pregnancy can safely be managed by endoscopic sphincterotomy. This treatment leads to a complete absence of further biliary symptoms during pregnancy. After pregnancy, spontaneous disappearance of stones and sludge is frequently observed. A further decision toward cholecystectomy after pregnancy should mainly be based on recurrence of biliary symptoms, especially of acute cholecystitis.

- D16 -

CXCR4 EXPRESSION PREDICT EARLY RELAPSE AFTER ADJUVANT RADIOCHEMOTHERAPY IN PATIENTS WITH RESECTED PANCREATIC ADENOCARCINOMA. R. Marechal, P. Demetter, A. Berton, I. Salmon, J.L. Van Laethem. ULB Erasme.

Background : The chemokine receptor CXCR4 and the hypoxia inductible factor-1 alpha (HIF-1a) are implicated in pancreatic adenocarcinoma (PA) growth, dissemination and angiogenesis. These two protagonists are expected to play a key role in radiotherapy and chemotherapy failure in PA.
Methods : we conducted a retrospective study of patients undergoing curative surgery (R0 resection) for PA between 1998-2006 in one institution. All were treated with adjuvant radiochemotherapy (treatment group). The treatment group was case-matched with resected PA patients who did not receive any adjuvant therapy (control group). FFPE specimens were subjected to immunohistochemical analysis using tissue microarray and monoclonal antibodies against human CXCR4 and HIF-1a. Based on the intensity (I) and the extend (E) of staining, cases were stratified into those with high (E x I > 3) or low (E x I d 3) expression of CXCR4 and HIF-1a and the results were correlated with disease-free survival (DFS) and overall survival (OS).
Results : 31 PA patients (median age : 57 years, range : 39-76) were analysed in the treatment group and 30 patients (median age : 59 years, range : 38-81) in the control group. The two groups were well-matched in terms of age, tumor stage (T1-T2 *vs* T3-T4), tumor differentiation (poor *vs* well-moderate), lymph node status (N0 *vs* N +), lymphatic and vascular emboli. In multivariate analysis, CXCR4 expression appeared to be an independent predictor for DFS (low expression : median DFS = 19.1 months, 95% CI : 4.7-73.9 *vs* high expression : median DFS = 6.2 months, 95% CI : 2.8-10.7, p = 0.002) and OS (low expression : median OS = 65.5months, 95% CI : 43.9-87.1 *vs* high expression : median OS = 12.2 months, 95% CI : 7.9-16.4, p = 0.001) in the treatment group but not in the control group.
Conclusion : High CXCR4 expression in resected PA predict bad outcome after adjuvant radiochemotherapy.

- D17 -

PREDICTIVE VALUE OF C-REACTIVE PROTEIN LEVEL CHANGES ON THE LONG TERM OUTCOME OF INFLIXIMAB IN CROHN'S DISEASE. M. Jürgens (1), F. Schnitzler (1), K. Van Steen (2), J. Mahachie (2), V. Ballet (1), M. Noman (1), I. Hoffmann (1), G. Van Assche (2), P. Rutgeerts (1), S. Vermeire (1). (1) KULeuven Gasthuisberg ; (2) Montefiore Institute ULg.

Background & aims : Infliximab the chimeric monoclonal antibody to TNF is approved for treatment of moderate to severe Crohn's disease (CD) with 60-70% of patients reporting a clinical benefit both short and long term. We previously showed that loss of response necessitating dose increase and/or interval reduction is observed in 50% of patients over time, and in ± 20% this will lead to treatment discontinuation. Non-invasive markers may help to assess therapy outcome and/or need for treatment adjustment if proven accurate. C-reactive protein (CRP) is a reliable marker of inflammation in Crohn's disease. As nothing is known about the effects of CRP on long term outcome to IFX we analyzed the kinetics of CRP over time in a large referral cohort of CD patients treated with IFX.
Methods & patients : Serial CRP levels were studied in 547 CD patients with initial response to and treated long term with IFX (median follow up 4.9 yrs). CRP was measured before each IFX infusion and was correlated to long term clinical outcome and to need for dose increase and/or interval decrease (SPSS 15.0). A total of 7520 CRP values were available for analyses. Treatment adjustment was defined as successful, if patients continued therapy with IFX without need for other therapies or surgery.
Results : Of the total cohort, 273 patients (50.0%) showed sustained clinical benefit of therapy with IFX without need for dose adaptation, whereas 274 (50.0%) patients needed treatment adjustment for loss of response over time. There was no statistical difference between both groups in CRP before start of IFX, CRP measured after first IFX, nor the drop in CRP between both time points. In the 274 patients who needed therapy adjustment, the median CRP just before the adjustment was significantly increased (median 8.5 mg/L ; IQR 3.1-24.1) as compared to the CRP measured at week 4 (median 3.7 mg/L ; 1.5-10.5) ($p < 0.001$) but did not reach the pre-treatment levels (18.3 mg/L ; IQR 6.4-36.2 ; $p = 0.004$). In 127 (46.4%) patients, therapy adjustment was successful but in 153 (53.6%) patients, response could not be regained and was followed by discontinuation of IFX. The delta between CRP before and after therapy adjustment was not predictive of success of the treatment interventions (median 1.9 mg/L ; IQR -0.1-12.5 in the successful adjustments versus 2.2 mg/L, IQR 0-14.4 in the non-successful interventions respectively ($p = 0.66$).
Conclusion : CRP is a good non-invasive marker to follow up in patients with CD under IFX and may predict loss of response necessitating therapy adjustment. However, the change in CRP after dose increase did not predict the clinical outcome of dose adjustment in our cohort of patients.

- D18 -

SURGERY AND HYPERTHERMIC INTRAPERITONEAL CHEMOPERFUSION FOR PERITONEAL CARCINOMATOSIS FROM COLORECTAL CANCER : HOPE OR HYPE ? W. Ceelen, Y. Van Nieuwenhove, D. Vande Putte, M. Peeters, P. Pattyn. UZ Gent.

Introduction : cytoreductive surgery followed by hyperthermic intraperitoneal chemoperfusion (HIPEC) is considered the standard of care for peritoneal carcinomatosis (PC) originating from mucinous appendiceal tumours including the pseudomyxoma peritonei syndrome. We report our experience with cytoreduction followed by HIPEC in a cohort of patients with PC originating from non appendiceal colorectal cancer (CRC).
Methods : patients with isolated PC originating from CRC were elegible ; pseudomyxoma peritonei patients were excluded from the analysis. Cytoreductive surgery was followed by HIPEC with mitomycin C or oxaliplatin (460 mg/m^2). The extent of disease was scored using a scale ranging from 0-9, while completeness of cytoreduction (CC) was scored as complete (CC0), nearly complete (CC1, miliary residual disease) or incomplete (CC2, gross residual disease). Actuarial survival was estimated using the Kaplan Meier product limit method while the influence of covariates on survival was estimated using Cox regression.
Results : 89 patients (44% female, mean age 64 ± 1 years) were treated over 6 years. Mean anesthesia time was 9.6 ± 0.3 hours ; open perfusion (coliseum technique) was used in 61% of procedures. Macroscopically complete resection was achieved in 47% of patients. Postoperative 30 day mortality was 1.5% while major morbidity developed in 23% of patients. Morbidity was mainly related to the extent of surgery. Postoperative median stay was 17 days (range 10-82). The estimated overall survival probability was 30% at three years (median survival time 22 months). Survival was significantly better in CC0 patients (42 months) compared to CC1 (18 months) or CC2 (11 months). Survival was significantly worse in patients with ascites at surgery ($p < 0.01$) and in patients who did not receive adjuvant systemic chemotherapy ($p < 0.001$). The extent of disease score before surgery ($p = 0.34$), performance of a splenectomy ($p = 0.65$), and timing of PC (metachronous or synchronous, $p = 0.32$) were not significantly related to overall survival.
Conclusion : Cytoreduction followed by HIPEC offers a significant survival advantage in selected patients with PC originating from CRC with acceptable early toxicity. Survival is significantly associated with the completeness of resection, the presence of clinical ascites, and adjuvant chemotherapy.

- D19 -

THE POTENTIAL ROLE OF HENT1 AS A PREDICTIVE FACTOR IN ADVANCED CHOLANGIOCARCINOMA TREATED WITH GEMCITABINE. A RETROSPECTIVE ANALYSIS. L. Verbrugghe (1), R. Lai (2), C. Sempoux (1), J.F. Gigot (1), Y. Humblet (1), I. Borbath (1). (1) UCL Saint-Luc ; (2) Cross Cancer Institute, University of Alberta, Canada.

Background : Gemcitabine (GEM) is an accepted alternative to best supportive care in the treatment of advanced cholangiocarcinoma (CCK). The human equilibrative nucleoside transporter 1 (hENT1) is a ubiquitous protein and is the major means by which GEM enters human cells. A recent report demonstrated a significant correlation between tumoural expression of hENT1, assessed by immunohistochemistry, and survival after surgery and adjuvant GEM, in the treatment for pancreatic cancer (Farrell *et al*. *Gastroenterology*, 2008).
Objective : To evaluate the prognostic value of hENT1 expression in advanced CCK, treated with GEM.
Methods : From July 1998 to November 2007, 60 patients were reviewed, who had received GEM as first line chemotherapy for a locally advanced or metastatic CCK, excluding gallbladder. There were 30 males, with a median age of 62 years (range 43-80). Thirty patients had an extrahepatic (EH) CCK (Klatskin and distal tumours), 30 having an intrahepatic (IH) tumour. Immunohistochemistry was performed on paraffin embedded slides of 31 patients and scored as having no staining (negative) or low-high staining (positive) for hENT1, according to Farrell *et al*. Time to progression (TTP) and overall survival (OS) were compared for EH and IH CCK, as well as for positive and negative hENT1 tumours.
Results : For the whole population, the median TTP was 3 months and the median OS was 8 months. Toxicities were mild. EH CCK had a median TTP of 3 months and a median OS of 6.5 months, whereas IH CCK had a median of 5 months and a median OS of 10 months ($p = NS$). Twenty-two of the samples (74%) had a positive hENT1 immunostaining, the remaining being negative. The median TTP were 2 vs 4 months and the median OS was 4.5 vs 9.5 months for negative vs positive staining, respectively ($p = 0.002$). In IH CCK, we found a TTP of 1.5 vs 5 months ($p = 0.005$) and an OS of 4.5 vs 11 months ($p = 0.007$), for negative and positive hENT1 staining respectively. On the contrary, in EH CCK we found a TTP of 2 vs 2.5 months and an OS of 5 vs 8 months for negative and positive hENT1 staining respectively.
Conclusions : In line with previous studies, GEM showed acceptable efficacy in the treatment of advanced CCK. We found a strong correlation between hENT1 expression and OS or TTP, on the whole population and even more in intrahepatic CCK. These results suggest the potential predictive value of hENT1 in advanced CCK treated with GEM.

- D20 -

IMPAIRED BUTYRATE OXIDATION IS CLOSELY LINKED TO THE SULPHIDE DETOXIFICATION CAPACITY OF THE MUCOSA IN ULCERATIVE COLITIS. V. De Preter, I. Arijs, E. Houben, S. Vermeire, P. Rutgeerts, K. Verbeke. KULeuven Gasthuisberg.

Introduction : Butyrate, a colonic metabolite of carbohydrates, is considered as the major energy source for the colonic mucosa. Evidence from *in vitro* and *in vivo* studies indicates that the oxidation of butyrate is impaired in ulcerative colitis (UC). Hydrogen sulphide (H_2S) has been shown to inhibit the butyrate oxidation in colonocytes. Defective detoxification of sulphides leads to damage to the mucosa and has also been suggested to play a role in the aetiology of UC. The colonic mucosal thiosulfate sulfurtransferase (TST) enzyme has been shown to remove H_2S by conversion to the less toxic thiocyanate in the presence of cyanide. Until now, sulphide detoxification and butyrate metabolism have been investigated independently. The aim was to evaluate the relation between sulphide detoxification and butyrate metabolism in UC patients and controls.
Methods : Colonic mucosal biopsies were collected during endoscopy of 30 UC patients and of 17 control patients with normal coloscopy. The activity of UC was assessed using the endoscopic Mayo Score. TST activity was measured spectrophotometrically using Na-thiosulphate in the presence of cyanide (nmol thiocyanate produced/mg protein.min). Butyrate oxidation was measured by incubating biopsies with ^{14}C-labeled Na-butyrate and measuring the released $^{14}CO_2$ (nmol Na-butyrate metabolised/mg protein.h).
Results : Both butyrate oxidation and TST activity were significantly decreased in UC overall compared to controls ($p < 0.001$). Subsequent division of the UC patients into disease activity subgroups using endoscopic criteria, showed a significantly decreased butyrate oxidation and TST activity in the different subgroups as compared to controls (Table 1). Furthermore, a significant relationship was found between the TST activity and the butyrate oxidation in UC (Pearson $r = 0.754$, $p < 0.001$)

Table 1. — Butyrate oxidation and TST activity in UC compared to control colon (mean ± st dev)

	Butyrate oxidation	TST activity
Controls (n = 17)	23.0 ± 13.8	81.6 ± 24.1
UC (n = 33)	5.6 ± 5.2 [a]	32.5 ± 21.5 [a]
Mayo 0-1 (n = 12)	10.2 ± 5.3 [a]	50.8 ± 19.1 [a]
Mayo 2 (n = 5)	2.0 ± 1.4 [a,b]	26.3 ± 10.6 [a,b]
Mayo 3 (n = 13)	2.7 ± 1.2 [a,b]	18.1 ± 13.1 [a,b]

Significantly different from control colon (a) and quiescent UC (b) : $p < 0.01$.

Conclusions : Our study suggest that impaired butyrate oxidation is closely linked to the sulphide detoxification capacity of the mucosa in ulcerative colitis. The sulphide detoxification mechanism could explain the well-documented observation that cigarette smoking is beneficial in ulcerative colitis. Smoking increases blood cyanide levels and the colon has been shown to extract cyanide from blood. Hence, this cyanide could enhance the removal of thiosulphate from the colonic mucosa and thus facilitate the metabolism of sulphide to thiosulphate.

- D21 -

LIVER TRANSPLANTATION FROM DONATION AFTER CARDIAC DEATH (DCD) DONORS : BELGIAN EXPERIENCE 2002-2007. O. Detry (1), V. Donckier (2), V. Lucidi (2), D. Ysebaert (3), T. Chapelle (3), J. Lerut (4), O. Ciccarelli (4), J. Pirenne (5), D. Monbaliu (5), A. Deroover (1), P. Honoré (1), X. Rogiers (6), B. de Hemptinne (6), R. Troisi (6). (1) ULg, (2) ULB Erasme, (3) UZ Antwerpen, (4) UCL Saint-Luc, (5) KULeuven Gasthuisberg, (6) UZ Gent.

Introduction : DCD organ donors were recently proposed as a potential source of transplantable organs. All Belgian transplant centres developed an active program of DCD liver transplantation and participated to this study. We retrospectively reviewed the global Belgian experience in order to evaluate this experience and to analyse the posttransplant results in terms of patient and graft survivals, and in biliary complications.

Patients and methods : From 2003 to 2007, 58 DCD liver transplantations were performed in Belgium, 52 from Maastricht category III, 2 from category IV donors, and 4 after euthanasia. Amongst these 58 transplantations, 39 were performed with graft procured locally, and 14 from another Belgian centre and 5 from The Netherlands). Mean donor age was 44 years (range, 17-71). 32.7% were female. Mean donor BMI was 24.5. Mean ICU stay was 4.8 days (range, 0-19). Mean donor sodium level was 142.3 ± 0.8 mmol/L (mean ± SEM). Mean donor AST and GGT was 50.5 ± 5.7 U/L and 59.8 ± 12.1 U/L, respectively. Causes of brain lesions were trauma (23 cases), intracranial bleeding (17 cases) and ischemic (14 cases). All life support withdrawals were performed in the operating theatre. Mean delay between respiratory withdrawal and cardiac arrest was 14.7 min. Mean delay between respiratory withdrawal and aortic flush was 25 min. HTK was the most used preservation solution. Mean age of recipients was 55 years (range, 10-70). Mean MELD score at transplant was 15.8. Indication of liver transplantation was decompensated cirrhosis and/or hepatocarcinoma in most cases.

Results : Mean cold ischemia was 451 min (range, 148-770). Peak of transminases was 2,241 ± 338 UI/mL. Global patient survival was 91.3%, 81.2% and 68.1% at 1 month, 1 year and 2 years, respectively. Graft survival was 84.4%, 70.3% and 49.7% at 1 month, 1 year and 2 years, respectively. Causes of early mortality (n = 5) were operative death (n = 2), PNF, MOF and ARDS. Late deaths (n = 8) were due to accident (n = 2), malignant tumour (n = 5) and biliary sepsis. Two patients needed early retransplantation for PNF and hepatic artery thrombosis. Six patients needed later retransplantation for diffuse bile duct lesions. Eleven other patients developed biliary stenoses requiring endoscopy and/or surgery. In univariate analysis, significant donor factors for death were delay between respiratory arrest and cardiac arrest of more than 15 min, and cold ischemia of more than 6 hours. In the recipient factors, HU status of the recipient was the only significant risk factor for early death. Donor BMI > 25 was related to an icreased peak bilirubin.

Conclusion : DCD organ donors may be a source of viable liver grafts. However actual results are inferior to the results of liver transplantation from donors after brain death, and prognostic criteria should be evaluated to improve the results. Further experience is needed to determine these risk factors.

- D22 -

LONG-TERM SURVIVAL IN PATIENTS WITH NON SEVERE ALCOHOLIC HEPATITIS. D. Degré, A. Lemmers, T. Gustot, R. Maréchal, S. Evrard, M. Adler, O. Le Moine, J. Devière, C. Moreno. ULB Erasme.

Background : Severe alcoholic hepatitis (AH) defined by a modified discriminant function (mDF) score e 32 is associated with a high short and long-term mortality rate. The short-term survival of patients with non severe AH (mDF < 32) is much better but their long-term evolution is currently unknown. The aim of this study was to examine long-term survival and its predictive factors in patients with non severe AH.
Methods : we reviewed our database of patients who had liver biopsy, collected prospectively between 2003 and 2008. Diagnosis of AH was based on histological criteria.
Results : Among 311 patients with biopsy-proven alcoholic liver disease, 51 had non severe AH, including 40 with cirrhosis. These patients were admitted because of liver decompensation (n = 22) or abnormality of liver tests (n = 29). At baseline, patients' characteristics were : male gender : 66%, median age : 54 years (28-72), daily alcohol consumption : 30-80 g : 28.6%, > 80 g : 71.4%, liver decompensation at admission : 43.1% (encephalopathy : 40.9%, ascites : 63.6%, jaundice : 72.7%, variceal hemorrhage : 31.8%), infection : 26.2%, hepatic venous pressure gradient : 13 mmHg (1-27), prothrombin time : 64% (48-130), bilirubin : 2 mg/dL(0.3-8.2), creatinine : 0.7 mg/dL (0.3-1.3), albumin : 3.4 g/dL (2-5.90), AST : 69IU/L (25-423), ALT : 39IU/L (14-176), C-reactive protein : 1.8 mg/dL (0.16-11), Child-Pugh score : 8(5-11), MELD score : 12 (6-18) and mDF : 17.4 (0-31). The Kaplan-Meier 1-, 6, 12- and 24-months survival were respectively 97.7% (± 0.2), 90.4% (± 4.5),83.1% (± 5.8) and 71.6% (± 7.4). Causes of death were related to liver decompensation in 73% of the cases (sepsis with liver failure : 50%, liver failure : 37.5% and variceal hemorrhage : 12.5%). In univariate analysis, alcohol abstinence (p = 0.04), albumin (p = 0.014) bilirubin (p = 0.037), Child-Pugh score (p = 0.021), AST (p = 0.036) and MELD score (p = 0.005) were predictive factors of 2-years survival. In multivariate analysis, MELD score was the only independent prognostic variable (p = 0,001) with a best cut-off point of 12. Surprisinsly presence of cirrhosis and liver decompensation had no predictive value.
Conclusions : If the short-term survival of non severe AH patients is excellent, their long-term prognosis is worse. Patients with non severe AH and a MELD score greater than 12 have higher risk of 2-years mortality. Identifying this group of patients and defining a specific therapeutic strategy could be clinically relevant.

- D23 -

COMPARISON BETWEEN LAPAROSCOPIC, NOTES TRANSGASTRIC AND NOTES TRANSCOLONIC PERITONEAL EXPLORATION AND LAPAROSCOPIC ULTRASONOGRAPHY IN PIGS. J. Navez, R. Yeung, C. Remue, C. Descamps, B. Navez, J. Gigot, P. Stärkel, M. Philippe, A. Jouret-Mourin, C. Sempoux, P. Gianello, P. Deprez. UCL Saint-Luc.

Laparoscopic surgery is associated with reduced surgical trauma, and therefore with a less acute phase response, as compared with open surgery. We hypothesized that NOTES (Natural Orifice ranslumenal Endoscopic Surgery)procedures might also induce less acute phase response as compared with laparoscopy. The aim of our study was to compare the systemic production of cytokines and acute-phase reactants, the rate of intraperitoneal infection and inflammation in a randomised control trial of diagnostic laparoscopy (including laparoscopic ultrasonography, sinceithas been shown to improve the accuracy of resectability) and transgastric or transcolonic NOTES (including intraperitoneal endoscopic ultrasonography) in pigs.
Methods : 18 pigs divided in3 groups underwent eithera classic diagnostic laparoscopy with laparoscopic ultrasonography and peritoneal + liver biopsy or a transgastric or transcolonic NOTES laparoscopy with laparoscopic ultrasonography and peritoneal + liver biopsy. NOTES peritoneal access was performed with needle knife in the colon and needle knife + balloon dilation 15 mm in the stomach. Closure was either done by clipping (EZ-clip, Olympus) or by use of T-bars (Olympus). Inflammatory response were evaluated by sequential measurements of C-reactive protein levels, Interleukin-6 levels (ELISA), TNF-alpha levels (ELISA) and IL-6 mRNA levels (white blood cells) by real-time PCR. Bacteriologic sampling in the peritoneal fluid and pathology evaluation of the scars and visible lesions were performed during the procedure and at necropsy (at 10-14 days).
Results : Laparoscopic and Notes peritoneal access, US examination of all quadrants of abdomen, peritoneal and liver biopsy were possible and done in all pigs. Closure was successful in all pigs except 1 with a transcolonic approach who developed peritonitis and paracolic abscess. One pig in the NOTES transcolonic group died during without known cause (septic shock probably, 3 days after the procedure). No complication were seen when T-bars closure was performed during transgastric and transcolonic NOTES. Positive cultures were observed in 2 surgical pigs and in the pig who developed an abscess. No differences in CRP levels and interleukin measurements could be demonstrated between all groups.
Conclusion : No differences in the acute phase response could be seen between surgical and NOTES peritoneoscopy with ultrasonography. Closure of gastric wall was safer than closure of colon when clipping was performed. Closure with T-bars proved to be more efficient for both accesses.

- D24 -

CONVERSION FROM CALCINEURIN INHIBITOR THERAPY TO EVEROLIMUS IN LONG-TERM LIVER TRANSPLANT PATIENTS : RESULTS OF A PROSPECTIVE, RANDOMIZED, MULTICENTER TRIAL.
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Calcineurin inhibitors (CNIs) contribute to renal dysfunction following liver transplantation. This prospective, randomized, multicenter, six-month study (with an additional six months' follow-up) evaluated whether everolimus with CNI reduction or discontinuation would improve renal function in long-term liver transplant recipients experiencing CNI-related renal impairment. 145 maintenance liver transplant patients with CNI-related renal dysfunction (creatinine clearance [CrCl] 60 mL/min and 20mL/min [Cockcroft-Gault]) were randomized to start everolimus therapy with subsequent reduction or discontinuation of CNI (n = 72) or to continue receiving standard-exposure CNI (n = 73). At month 6, 80% of patients switched to everolimus had discontinued CNI. The mean change in CrCl did not differ significantly between groups (everolimus 1.0-10.2 mL/min, controls 2.3-7.8 mL/min ; p = 0.46). Among patients who continued everolimus as per protocol, the mean increase in CrCl was 2.1 mL/min (n = 53) and 3.8 mL/min (n = 38) at months 6 and 12, respectively, versus 2.4 mL/min (n = 68) and 3.5 mL/min in controls (n = 51). The high frequency of CNI dose reductions in controls (77% of patients) and the relatively long mean time post-transplant (> 3 years) are likely to have contributed to the small difference in CrCl. Biopsy-proven acute rejection occurred in 1.4% in each group, with no graft losses. Study drug discontinuation was higher in the everolimus group.
Conclusion : These data demonstrate that everolimus allows for discontinuation or major reduction of CNI exposure in liver allograft recipients suffering CNI-related renal dysfunction, without loss of immunosuppressive efficacy. Trials targeting earlier conversion post-transplantation are required to confirm the efficacy and safety of everolimus for improving renal function among liver transplant recipients.

- D25 -

ENDOSCOPIC EMR OR ESD IN LONG (L), EXTRA-LONG (XL) AND EXTRA-EXTRA-LONG (XXL) BARRETT'S ESOPHAGUS : DO WE REALLY NEED SURGERY OR ABLATION TECHNIQUES ? P. Deprez, T. Aouattah, R. Yeung, A. Jouret-Mourin, C. Sempoux, H. Piessevaux. UCL Saint-Luc.

Endoscopic resection (ER) of high-grade dysplasia (HGD) or intramucosal cancer (IMC) in Barrett's esophagus (BE) is now an effective and safe alternative to surgical treatment. Many experts still recommend surgery or a combination of resection and ablation in case of long Barrett. The aim of this study was to review our experience of endoscopic resection in long LBE (e3cm), extra-long XLBE (e5cm) and extraextra-long XXLBE (e10cm) Barrett's patients.
Methods : Patients with HGD or IMC with BE, without signs of submucosal infiltration or lymph node metastases on endoscopy or endosonography, treated by ER in a curative intent were included. Patients underwent ER sessions (cap EMR or ESD) either aiming at complete resection of BE or removal of neoplasia only (severe co-morbidity, anticoagulants, low grade dysplasia on pathological specimen, complications).
Results : 57 patients (mean age 53 [range 29-84], 53 M and 4W with HGD or IMC, treated between Jan 2001 and Oct 2008 were included, in a total cohort of 148 pts treated by ER for esophageal cancer (92 BE, 37 SCC, 14 cardia and misc.). Mean C and M (Prague classification) length of BE 3.9 [range 3-15] and 4.8 [3-15] 22 had LBE, 38 XLBE and 6 XXLBE. ER was performed by EMR by piecemeal (44 pts, 4.8 [1-13] pieces) or ESD (13, en block in all) in 1 to 5 sessions. Six pts staged T1sm2-3 on the pathology specimen or with R1 resection were referred for surgery with final diagnosis of HGD or mucosal cancer in 5 and T2 in 1 pt. Two of these patients had long ICU stays due to pulmonary and cardiovascular complications. Recurrence of HGD was seen in 5 pts (8.8%) at 9,12,12, 23 and 94 m and was retreated by EMR. Complete remission at last FUP (mean 37 months [3-94] was achieved in 51/51 pts (100%). In patients in whom complete removal of BE was attempted and without surgery, recurrence of visible BE mucosa (generally small islands < 10 mm) was observed in 8/33 (27%) and 4 (8%) patients had IM detected on the neo-z-line : complete eradication was therefore successful in 64% of pts (13L, 18 XL, 2XXL) . Complications occurred in 2 pts with delayed bleeding at 12 and 24 h treated with endoscopic techniques Symptomatic stenosis occurred in 14 pts (24%) and was effectively treated by endoscopic dilatations. XL and XXLBE treated with the aim of full BE removal were 'prophylactely' dilated starting 10 days after ER. One pt suffered perforation during dilation, and was treated with a covered plastic stent.
Conclusion : Long Barrett's esophagus with HGD/IMC can be effectively treated with extensive endoscopic removal even in lengths exceeding 5 to 10 cm. When complete resection of BE is achieved, neoplasia recurrence rate is lower than 9% during long term F-up, and is limited to the neo Z line without buried glands or recurrence seen in the resected area, even in XXL BE. The need for ablation techniques or surgery may therefore be questioned.

- D26 -

LOW MOLECULAR WEIGHT HEPARIN INHIBITS TUMOR ASSOCIATED ANGIOGENESIS IN VIVO.
I. Debergh, N. Van Damme, P. Pattyn, M. Peeters, W. Ceelen. UZ Gent.

Background : Recently, low molecular weight heparins (LMWH) were found to confer a survival advantage in advanced cancer patients. The exact mechanism underlying this observation is unclear, but may involve inhibition of tumor associated angiogenesis. We examined the effect of nadroparin on tumor associated angiogenesis in an in vivo window chamber assay.
Methods : Syrian golden hamsters were fitted with dorsal skinfold window chambers. Small (0.5-1 mm^3) fragments from A-Mel-3 or HAP-T1 tumors grown in donor animals were implanted in the chambers. Animals were treated with 10 daily injections of 0.07 ml saline or 200 IU of nadroparin. In vivo microscopy using FITC dextran was performed on day 0, day 3, day 6 and day 9 following tumor fragment implantation. Image analysis was performed to calculate changes in the number of microvessels per area (N), microvessel diameter (D), and vascular area fraction (VAF). Microvessel density (MVD ; vWF staining) and vascular maturation (pericyte coverage ; vWF/alpha-smooth muscle actin staining) were assessed histologically at the end of the 10-day treatment period. Data are expressed as mean ± SD or median (interquartile range). Differences were analyzed using the student t or Mann-Whitney rank sum test.
Results : In the control group (N = 23), a moderate increase in N was observed from 43.4 (32.7-68) to 46.6 (37.1-72.5) microvessels per cm^2 (P = 0.23) while D increased significantly from 12.9 ± 3 to 18.7 ± 5 μm (P < 0.001) between day 0 and 9. Vascular area fraction did not change significantly over this period.
In the nadroparin group (N = 23), however, N decreased from 132.4 ± 55 to 89.3 ± 55 microvessels per cm^2 (P = 0.138) and VAF decreased from 23.4 ± 4 to 15.7 ± 4% (P < 0.001) between day 0 and 9. Microvessel diameter increased in the nadroparin group from 17.2 ± 4 to 21.5 ± 6 μm (P = 0.047). Histologically, MVD was 11.1% (6.2-18.1) in the control group and 4.5% (1.8-9) in the nadroparin group (P < 0.001). Microvessel pericyte coverage was 73.6% (39.1-108.5) in the control group and 96.8% (74.3-126) in the nadroparin group (P = 0.012).
Conclusions : The LMWH nadroparin inhibits early tumor associated angiogenesis and affects microvessel maturation in vivo. Possibly, this mechanism explains the clinically observed antitumor effect of LMWH's in cancer patients.

- D27 -

INTESTINAL TRANSPLANTATION. AN ANALYSIS OF THE INTERNATIONAL INTESTINAL TRANSPLANT REGISTRY.
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Rationale : Intestinal Transplantation (ITx) is an alternative treatment to Total Parenteral Nutrition (TPN) in patients suffering from irreversible Short Bowel Syndrome (SBS). ITx remains a difficult procedure due to a high risk of immunological and infectious complications, and the need for profound immunosuppression (IS) and its side-effects. With the exception of a few large North-American centers, only small-size single-center ITx series have been reported and do not provide gastroenterologists in charge of SBS patients with a clear view of the current results and indications for ITx.
Aim : To collect evidence-based data on outcome of ITx worldwide.
Methods : ITx performed between 04/1985 and05/2007 were prospectively reported to a centralized database/registry. Demographics, indications, results and variables influencing outcome were retrospectively analysed.
Results : In the study period, 1720 ITx were performed in 1608 patients (112 reTx) in 69 ITx centers (only 3 did > 100 cases). 746 are isolated Intestine Tx (ITx) ; 594 Intestine-Liver Tx (ILTx) and 380 Multivisceral Tx (MVTx). 48% are female and 52% males. 60% are children and 40% adults. Indications to ITx are : SBS/TPN complications + severely altered quality of life. Etiology of SBS in children : gastroschisis (20%), motility (18%), volvulus (17%), congenital (14%), necrotizing enterocolitis (13%), various (18%). In adults : ischemia (24%), tumor (13%), crohn (12%), motility (10%), trauma (9%), volvulus (7%), various (25%). Induction IS is used in almost all cases but the type of induction differs (ATG, Anti-IL 2 R, Campath) without influence on outcome (p > 0.05). Maintenance Tacrolimus IS is superior to Cyclosporine (p < 0.001) and is used in most patients but recently Sirolimus has also been used (15%) (without difference on outcome-p > 0.05). Median hospital stay is 32 days (ITx) ; 60 days (LITx) and 39 days (MVTx). Of the 1720 Tx, 909 (56%) are currently alive. Longest survivor was Tx 18 years ago. Leading causes of death : multiorgan failure (60%), rejection (10%), lymphoma (8%), various (22%). Survival has improved in successive eras. In the last era (2005-2007), 2 year patient survival is 80% (ITx), 62% (ILTx) and 60% (MVTx) with a better survival for ITx (p = 0.001). Variables influencing outcome are : preTx status home *vs.* in hospital (p < 0.001) and rejection within 3 months (p = 0.024). Variables influencing outcome in 1 year survivors are : Tx type (ILTx do better than ITx and MVTx ; p < 0.001) and age (children do better *vs.* adults ; p = 0.012). In patients who survived > 6 months, 70% are nutritionally fully and 12% partially independent. Karnofsky performance score (surrogate of quality-of-life) is 90/100% in 70% of the patients. ITx activity has progressively increased over the years with now ~150 cases performed yearly.
Conclusion : With accumulated worldwide experience and optimization of antirejection and antiinfection protocols, the middle term results of ITx are now excellent. ITx has evolved into a life-saving (in addition to a quality-of-life restoring) procedure for SBS patients with severe TPN complications and should be considered *standard treatment* in these patients. Avoidance of rejection and transplanting patients before they become hospital-bound are pivotal in optimizing results. New strategies to reduce IS and better control rejection are necessary to perform ITx as a quality-of-life restoring procedure in patients free of severe TPN complications.

- D28 -

INFLIXIMAB DISCONTINUATION IN CROHN'S DISEASE PATIENTS IN STABLE REMISSION ON COMBINED THERAPY WITH IMMUNOSUPPRESSORS : A PROSPECTIVE ONGOING COHORT STUDY.

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Introduction : Infliximab (IFX) is an effective maintenance therapy in Crohn's disease (CD). The question of whether treatment with IFX can be safely interrupted after a period of prolonged remission is of great interest to patients and physicians.
Objectives : To asses the risk of relapse after IFX discontinuation in patients on combined maintenance therapy with immunosuppressors (IS) and to identify factors of relapse. A secondary objective was to assess response and tolerance to IFX re-treatment in relapsers.
Methods : Luminal CD patients treated for at least one year with scheduled IFX combined with IS and in stable remission without steroids for at least 6 months were prospectively recruited into the study. In all patients the following data were recorded at baseline (just before the last IFX infusion) : blood cell counts, CDAI, ileocolonoscopy with CDEIS evaluation, centralized evaluations of USCRP, fecal calprotectin, ATI and IFX through level. Patients were then followed up every two months. IS treatment was kept at a stable dose over the study period. Relapse was defined by a CDAI > 250 or a CDAI between 150 and 250 with a 70 points increase during two consecutive weeks. Demographic, clinical and biological factors at inclusion were explored for their potential association with time-to-relapse through log-rank method and hazard ratio (HR) were estimated through Cox model. In case of relapse, patients were retreated with IFX and both efficacy and tolerance of this re-treatment were evaluated.
Results : 115 patients were recruited in 20 GETAID centres between March 2006 and January 2008. Median duration of IFX and IS treatments were 2.2 years and 2.8 years, respectively. At inclusion, median CDAI and CDEIS were 37 and 0.7, respectively ; median USCRP, fecal calprotectin and IFX trough levels were 2.0 mg/l (n = 106), 51 microg/g (n = 85) and 3.8 microg/ml (n = 107), respectively. The reference date for this analysis was 15/4/08. After a median follow-up time of 12 months, 45 relapses have been observed. In univariate analysis, factors associated with the risk of relapse were current smoking, previous steroid treatment, lower haemoglobin, higher CDAI, CDEIS, USCRP and fecal calprotectin. In multivariate analyses, a model based on CDEIS (e2, HR = 3.0, P < 0.001) USCRP (e5 mg/l, HR = 3.8, P < 0.001), haemoglobin (d14.5 g/dl, HR = 4.7, P = 0.002) and IFX trough levels (e2microg/ml, HR = 2.9, P = 0.006) identified 4 subgroups of patients with increasing risk of relapse over time. Thirty seven relapsers are currently evaluable 4 weeks for after IFX re-infusion for response to IFX retreatment : 36/37 were in remission and none experienced significant acute or delayed reaction.
Conclusion : after a stable remission under combined IFX + IS therapy for at least one year, more than half of patients have not relapsed one year after IFX discontinuation. In relapsers, IFX re-treatment was well tolerated and induced remission in the short term. Updated results and prognostic models will be presented but a subgroup of patients with very low risk of relapse could be identified through a combination of biological and endoscopic markers. In these patients a treatment reduction could be discussed.

- D29 -

EFFICACY OF PRE-TREATMENT BIOPSIES IN PREDICTING FINAL HISTOPATHOLOGY OF ENDOSCOPICALLY RESECTED EARLY MALIGNANCIES IN THE UPPER GASTROINTESTINAL TRACT. R. Bisschops, G. De Hertogh, J. Beck, P. Nafteux, K. Geboes, A. Lerut. KU Leuven.

Endoscopic resection (ER) is an efficient and valuable treatment option for early mucosal well differentiated neoplastic lesions in the upper gastrointestinal tract (Pech 2008). Treatment algorithms are very often based on pathological findings from endoscopically obtained biopsies, however the diagnostic reproducibility of these are not as good as for ER specimens (Mino-Kenudson 2007). The current study aimed to correlate prospectively pre-ER pathological findings to the final pathological diagnosis obtained from the ER specimen(s). Methods : ERs from the upper GI tract were prospectively registered in a database between 2006 and November 2008. ERs were performed using the cap technique or a multiband mucosectomy device in the stomach and the esophagus, or by the lift and snare technique in the duodenum. The worst pathology known from biopsies from neoplastic lesions before ER was compared to the histology of the ER specimen. All biopsies and ER specimens were reviewed by at least two pathologists with specialized expertise in gastrointestinal pathology. Results : We studied the available histology of 100 consecutive ERs in the upper GI tract . ERs were performed in squamous esophagus (4), in Barrett's esophagus (63), in the cardia (6), in the stomach (19) and in the duodenum (8). The final diagnosis included : 4 squamous cancer of the esophagus, 1 columnar lined esophagus, 6 intestinal metaplasia (Barrett), 13 low grade and 13 high grade Barrett's dysplasia, 30 Barrett's adenocarcinomas, 4 adenocarcinomas in the cardia, 4 gastric adenocarcinomas, 12 gastric tubular adenomas with high(6) or low grade dysplasia(5) and 8 tubular adenomas in the duodenum (1 high grade). The overall accuracy of pre-ER biopsies in predicting final histology was 61%. 21% of the lesions were upgraded to a worse pathology. 16 of these 21 lesions were upgraded from low or high grade dysplasia to mucosal cancer or even submucosal cancer. The majority of these lesions (63%) were clearly visible as slightly elevated or depressed lesions : 7 type IIa, 1 type IIc, 2 type IIa-c and 1 type Is lesions. Remarkably, 5 pre-ER biopsies in Barrett's that were classified as high grade dysplasia with suspicion of carcinoma could be formally classified to either dysplasia (3) or carcinoma (2) after staging ER. Finally, 18% of the lesions were downstaged to a more benign final pathology. Conclusion : Endoscopically prelevated biopsies only moderately predict the final diagnosis after endoscopic resection. Reassuring histology in the presence of small visible early lesions should therefore be considered as an indication for staging ER to obtain a final histological diagnosis.

- D30 -

EFFECTS OF DELAYED INTRODUCTION OF CALCINEURIN INHIBITOR ON GFR IN LIVER TRANSPLANT : 12 MONTH DATA FROM A MULTI-CENTRE RANDOMISED CONTROLLED STUDY. J. Pirenne (1), N. Boon (2), I. Colle (3), O. Detry (4), J. Neuberger (5). (1) KULeuven ; (2) ULB Erasme ; (3) UZ Gent ; (4) ULg Sart Tilman ; (5) Queen Elizabeth Hospital, Birmingham, U.K.

Introduction : Following liver transplantation, late onset renal failure is a significant cause of morbidity and mortality. Interim results of a prospective, randomized, open-label study suggested that an IL2r antibody and mycophenolate mofetil (MMF) with delayed introduction of lower doses of tacrolimus in the immediate post-operative period would lead to less impairment of renal function.
Methods : In a prospective, open-label study of 12 months duration 525 patients undergoing first liver transplant were randomized to either A) tacrolimus (target trough blood level > 10 ng/ml) for the first month (n = 183) ; B) tacrolimus target level d8ng/ml and MMF 1 g bid IV until Day 5, then 1g bid PO (n = 170) ; C) daclizumab on Day 1 (2 mg/kg) and Day 7 (1 mg/kg), MMF as in B and tacrolimus (target level d8ng/ml) introduced on Day 5 (n = 172). Patients received corticosteroids according to local protocol. The primary end-point is change from baseline at week 52 of calculated glomerular filtration rate (cGFR).
Results : In an intention to treat analysis of available data the change from baseline in cGFR (ml/min) after 12 months on study was -23.94, -20.95 and -13.58 in A, B and C respectively ($p = 0.007$ A v C ; $p = 0.128$ A v B). In A, B and C respectively : mortality was 9.4%, 11.3% and 6.5%. Rejection requiring pulse immunosuppression therapy was seen in 24.3%, 26.8% and 16.7%.
Conclusion : MMF, IL2r blockade and delayed introduction of lower doses of tacrolimus leads to improved renal function at 12 months posttransplant and this without an increased frequency of rejection, graft loss or death. Because renal failure is a significant cause of morbidity and mortality, this strategy is likely to improve outcome/survival after liver transplantation.

- D31 -

AN EARLY DECISION FOR PTFE-COVERED-TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT IMPROVES SURVIVAL IN HIGH RISK CIRRHOTIC PATIENTS ADMITTED WITH AN ACUTE VARICEAL BLEEDING : RESULTS FROM AN INTERNATIONAL MULTI-CENTER RANDOMIZED CONTROLLED TRIAL.

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Background : Despite advances in management of acute variceal bleeding (AVB), Child-Pugh B-patients with ongoing bleeding at endoscopy, while under vasoactive drug regimen, or in Child-Pugh Class C, have a high risk of treatment failure and a poor prognosis despite the use of rescue TIPS. The present study aimed at evaluating if an early decision for PTFE-TIPS in such high-risk variceal bleeders could decrease the probability of treatment failure and mortality.
Methods : In 9 European centers, 63 high-risk cirrhotic patients with AVB, treated with vasoactive drugs and endoscopic band ligation (EBL), were centrally randomized within 24 hours after admission to receive either PTFE-TIPS, within the first 72 hours of admission (n = 32) or to continue standard medical-endoscopic management, followed after 3-5 days by nadolol/propanolol + isosorbidemononitrate + EBL (n = 31). In the medical/endoscopic arm, PTFE-TIPS was allowed for rescue therapy in case of failure of therapy. The main end-points analyzed were : 1) composite end-point : failure to control AVB or to prevent rebleeding requiring admission and transfusion ; 2) survival ; 3) safety and complications.
Results : There were no significant differences between the 2 groups in age, sex, etiology, Child-class and severity and treatment of the index bleed. Median follow-up was 16 months. Fourteen patients in medical-endoscopic versus 1 in the PTFE-TIPS-group reachted the composite endpoint ($P < 0.01$). One-year actuarial probability of remaining free of the composite endpoint was 50 vs 97% ($P < 0.001$). 7 patients of the medical-endoscopic group received a rescue PTFE-TIPS. Four additional patients had non-significant rebleeding during follow-up (3 in the medical-endoscopic arm vs 1 in the PTFE-TIPS-group). Sixteen patients died (12 in medical-endoscopic vs 4 in the PTFE-TIPS-group, $P < 0.02$). One year actuarial survival was 60% in the medical-endoscopic arm versus 86% in the PTFE-TIPS-group ($P < 0.01$). One-year actuarial probability of de novo arising or worsening ascites were 33% in the medical-endoscopic arm vs 13% in the PTFE-TIPS-group ($P = 0.11$), and that of encephalopathy was 40% versus 28% (NS). Most episodes of encephalopathy in the medical-endoscopic treatment arm were related to rebleeding/decompensation.
Conclusion : In high-risk cirrhotic patients admitted for AVB, early treatment with PTFE-TIPS is associated with significant, marked reductions in rebleeding and mortality.

- D32 -

SIGNIFICANT REDUCTION OF IRON NEED IN IBD PATIENTS AFTER INITIATION OF ANTI TNF TREATMENT.

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Background and aims : Approximately one third of IBD patients suffers from iron deficiency anemia, mainly due to gastrointestinal iron loss. Iron deficiency is commonly corrected with intravenous iron supplements. Anti-TNF therapy has been shown to induce mucosal healing in IBD and may therefore lead to a reduction in intestinal iron loss. We aimed to investigate whether the need for intravenous iron therapy can be reduced with anti-TNF treatment.
Methods : A series of charts of consecutive IBD patients with chronic need for IV iron supplements (iron sucrose (FeS), Venofer®) was studied. The need for FeS in order to keep the iron saturation index > 25% and serum ferritin levels > 40 ng/ml was recorded in the 6 months prior to the start of anti-TNF and in the 1st and 2nd 6 months thereafter.
Results : Eighteen patients (16 CD and 2 UC, 9 female, mean age 21 yrs) met the criteria for inclusion. In the 6 months prior to anti-TNF therapy (11 infliximab, 2 certolizumab pegol, 5 adalimumab), the need for FeS averaged 230 mg/month (IQR 75-300). During the first six months of anti-TNF therapy the need for FeS dropped significantly to 41.7 mg/month (IQR 0-212) ($p = 0.019$). During the second period of six months, no additional reduction was observed (mean 300 mg/month, IQR 0-900). Hemoglobin, ferritin and iron saturation levels for each period are shown below.

	- 6 months	Start of anti-TNF	+ 6 months	+ 12 months
Ferritin (ng/mL)	54 (12,75-93,5)	125 (66-245) (p = 0.06)	83 (38-359) (p = 0.53)	93 (55-227) (NS)
Iron saturation level (%)	11.5 (7.5-18.5)	18 (9-26) (p = 0.26)	16 (14.5-24) (p = 0.54)	13.5 (11.2-18.5) (NS)
Hemoglobin (Hb) (g/dL)	12.4 (11.3-12.8)	12.5 (11.4-13.7) (p = 0.38)	13.65 (13.0-14.7) (p = 0.01)	14 (12.6-14.6) (NS)
Monthly FeS need	230 mg (IQR 75-300)		41.7 mg (IQR 0-212.5) (p = 0.019)	41.67 (0-150) (NS)

Conclusion : FeS supplements before anti-TNF therapy replenished iron stores, but did not lead to an increased Hb level. After the administration of anti-TNF, Hb levels increased and the need for FeS therapy was significantly reduced. Mucosal healing most likely leads to a significant reduction in intestinal iron losses and anti TNF therapy stimulates erythropoiesis that is suppressed by circulating inflammatory mediators.

- D33 -

INCREASING INCIDENCE OF CLOSTRIDIUM DIFFICILE-ASSOCIATED DIARRHEA IN INFLAMMATORY BOWEL DISEASE. P. Bossuyt, J. Verhaegen, G. Van Assche, P. Rutgeerts, S. Vermeire. KULeuven.

Introduction : Over the last decade a rise in Clostridium difficile-associated diarrhea (CDAD) has been observed. A higher incidence of CDAD has also been suggested in patients with inflammatory bowel disease (IBD) and may be a challenging factor in the differential diagnosis of flares. It is unclear if the increase is caused by the enhanced use of immunosuppressive therapy in IBD.
Aim : We investigated if CDADis increasing in IBD and non-IBD patients and evaluated outcome and possible predisposing factors.
Methods : Through an electronic database of the Laboratory of Microbiology of our hospital (tertiary referral center), all stool samples from patients admitted for reason of diarrhea and hospitalized on gastroenterology wards between Jan 2000 and Jan 2008 were reviewed for diagnosis of CDAD (defined as diarrhea with positive A/B toxin). For analysis, we compared two periods of equal duration, 2000-2003 (period 1) and 2004-2007 (period 2).
Results : A total of 57 patients were admitted with CDAD, of whom 15 (26.3%) had concomitant IBD. A 3.75-fold increase in CDAD was observed from period 1 (n = 12) to period 2 (n = 45), irrespective of underlying IBD and with comparable total number of analysed stool samples, number of patients tested and number of stool samples per patient. There was no indication for an outbreak of ribotype 027. Non-IBD patients were significantly older (mean age 62.8 years) in comparison with IBD patients (42.5 years) (p = 0.001). Antibiotic use 3 months prior to the infection was reported in 35 patients (61.4%) and was higher in non-IBD (29/42 or 69%) than IBD patients (6/15 or 40%) (p = 0.047). Nine IBD patients were receiving concomitant immunomodulators, and this was not different from period 1 (2/3) to period 2 (7/12). None of the IBD patients had pseudomembranes on endoscopy. Most were treated with metronidazole or vancomycin and had a successful outcome. Most patients had a successful outcome and only one patient with ulcerative colitis needed semi-urgent colectomy. There were 2 deaths in the non-IBD group (one undefined systemic disease and one cardiac arrest). The duration of hospital stay was significantly lower in IBD patients (15.2 days) than non-IBD patients (27.7 days) (p < 0.001).
Conclusion : We observed a significant rise in CDAD in both IBD and non-IBD patients and this was not specifically related to ribotype 027. The outcome was favourable with only one IBD patient needing semi-urgent colectomy. Because C. difficile may both mimic and precipitate an IBD flare, it is essential that clinicians be vigilant to identify this complication and treat accordingly. The increasing use of immunosuppressives in IBD does not seem to influence the risk.

- D34 -

PSORIASIS AND ECZEMA SKIN LESIONS ASSOCIATED WITH TNF-BLOCKADE THERAPY IN IBD : NATURAL HISTORY AND CLINICAL CHARACTERISTICS. J.F. Rahier (1), S. Buche (2), L. Peyrin Biroulet (3), M. Lémann (4), M. Allez (4), J. Cosnes (5), Y. Bouhnik (6), E. Delaporte (2), J.F. Colombel (2). (1) UCL Mont Godinne, Belgium ; (2) CHU Lille, France ; (3) CHU Nancy.

Background : Among cutaneous adverse effects associated with tumor necrosis factor (TNF) ± inhibitors, psoriasis and eczema like lesions are the most frequent.
Aims & methods : Our aim was to assess clinical characteristics and outcome of skin disease in patients with IBD presenting with psoriasis and eczema like lesions induced by anti-TNF ± agents (infliximab, adalimumab and certolizumab). Cases were collected from 4 centers and systematically reviewed by one dermatologist and one gastro-enterologist.
Results : We identified 41 patients (35 Crohn's disease, 5 ulcerative colitis ; 34 women, 7 men ; mean age 27 years) with chronic inflammatory skin lesions (27 psoriasis and 14 eczema like lesions). 27 patients (65%) had a personal or familial history of skin diseases that was most often (16/27) an inflammatory skin disease. Eczema like lesions had variable localization whereas tinea amiantacea and flexural psoriasis were the most commonly seen psoriasis lesions. Skin lesions emerged while IBD was quiescent in 31 patients and were observed with any type of anti-TNF ± agents (32 infliximab, 6 adalimumab and 3 certolizumab). All patients were treated with topical corticosteroids and keratolytics. This resulted in partial or total remission in 28 patients and stable skin disease in 7. No follow-up was available in 6 patients. In patients with psoriasis, switching once or twice the anti-TNF ± was systematically associated with recurrence of the lesions (16/16). Switching was less frequent for eczema like lesions and the recurrence rate was lower (2/5). Definitive cessation of TNF ± inhibitors due to skin lesions was necessary in 7 patients.
Conclusion : In this cohort, inflammatory skin lesions induced by anti TNF ± inhibitors were characterized by (1) strong association with female gender, (2) high frequency of personal or familial history of skin disease, (3) absence of correlation with intestinal disease activity, (4) lack of improvement of psoriasis lesions after switching the anti-TNF ± and (5) need for anti-TNF ± cessation in 17% of patients.

- D35 -

LONG TERM OUTCOME OF PALLIATIVE TREATMENT WITH STENT AND/OR CHEMOTHERAPY FOR OBSTRUCTING CARCINOMA OF THE COLON. C. Breynaert, J. Vandervoort, M. De Man, P. Van Der spek, K. Hendrickx, J. Vanstiphout, L. Duville, F. Sermon, L. Lepoutre. Onze-Lieve-Vrouw Ziekenhuis, Aalst.

Background : Stenting can achieve long term palliation in patients with obstructing colon cancer who are at high risk for surgery. The role of palliation with stent insertion in patients who are at low operative risk is less clear. Complications, especially procedure- or chemotherapy-related perforation, remain a cause of concern.
Aim : The study aims to evaluate outcome, perforation rate and chemotherapy use in our patient population with obstructing colon cancer, treated with enteral stenting as definitive palliation.
Method : Patients who had a bridge-to-surgery or primary tumours, other than the colon, were excluded from this analysis.
All medical records of patients with obstructing colon carcinoma treated with enteral stent for palliation between June 1998 and June 2008 were retrospectively analysed for :
(1) Prognostic factors : age, alkaline phosphates, white blood cell count, performance status, number of metastatic sites, presence of peritoneal metastases.
(2) Stent failure and perforation.
(3) Chemotherapy : reason why treatment was withheld, duration of therapy, number of chemotherapy sessions and reason to stop the treatment.
(4) Survival (status December 1, 2008) : overall survival is calculated in different prognostic groups according to the Köhne's prediction model.
Results : From June 1998 until June 2008, 205 stents were placed for colon obstruction. Ninety-eight patients were identified as definitive palliative treatment with stent for obstructing colon cancer. Follow-up is available for 91 patients with a mean age of 73 (range 36-93,5). Overall survival is 7,7 months (1 day -32.3 months). The majority of patients had a poor prognosis : risk group based on clinical parameters following Köhne : high risk (20%), intermediate risk (40%) an low risk (40%). Two perforations were diagnosed. Fifteen patients (16%) died within 30 days after stent placement (mean age 81, rang 62-93,5) of whom 10 (66%) had an ECOG performance status of 2 or 3. Only a minority was procedure related. Fifty-five (60%) patients received chemotherapy for a mean period of 10,4 months, rang (1 week to

70 months). The delay between stenting and beginning of chemotherapy averaged 2 weeks. Survival in the chemotherapy group is 14,6 months (range 1.3 -72.9).
Conclusion : This large series shows that stenting is an efficient long term palliative treatment for patients with obstructing colon cancer with/without synchronous metastasis. In the elderly patient in poor general condition there is a higher mortality rate. This is mainly due to their critical illness, rather than procedure related. Most patients recover quickly after stenting allowing earlier administration of chemotherapy. The mean survival of patients treated with chemotherapy is more than one year. As in operated patients there is a subgroup of long-term survivors, suggesting that the presence of a stent in the colon does not have a negative impact on further disease course.

- D36 -

ENDOSCOPIC SUBMUCOSAL DISSECTION : PRELIMINARY RESULTS OF A EUROPEAN CENTER.
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Background and study aim : Endoscopic submucosal dissection (ESD) was first described in Japan for treatment of early gastric tumors by en-bloc resection. Many series from the Far East assessed and showed the feasability of ESD in the oesophagus and the colorectum. A few or no European studies have been published. So, we reported one of the first experience of ESD from an european center.
Patients and Methods : 26 consecutive patients were treated by ESD for gastric and rectal tumors between April 2007 and August 2008 at Paoli-Calmettes Institute of Marseille (France). The mean age of the patients was 65.5 ± 14,06 years (range 39-88). The efficacy, the feasability and the safety of this technique were assessed. The ESD procedure was carried out using Flex-Knife and IT-knife (Olympus Corporation) or a Safe-Knife (Life-Europe Company). As regards the gastric tumors, ESD was performed for 5 submucosal tumors, 2 large hyperplastic polyps and 4 early gastric cancers. All gastric tumors were previously assessed by endoscopic ultrasonography (EUS). The rectal lesions resected by ESD were 13 sessile polyps (size > 2 cm) classified usT1N0 by EUS and 2 neuroendocrine tumors.
Results : ESD was carried out in the rectum in 15 patients and the stomach in 11 patients respectively. The mean size of resected tumors was 27.69 ± 16.23 mm (32.6 ± 18.11 mm in the rectum and 21 ± 10.67 mm in the stomach). The en-bloc resection was possible in 18/26 patients (69.23%). The rates of en-bloc resection and en-bloc resection with tumor-free margins (R0) was 46,7% and 40% in the rectum and 100% and 90,9% in the stomach. The median operation time was 65 minutes (range 15-110). None of the patients needed blood transfusion or presented major bleeding during or after ESD. 2 perforations occured during the procedure after resection of large villous rectal lesions (size > 4 cm) and were managed conservatively. No reccurrence occured in patients with complete en-bloc dissection with a mean follow-up period of 7 months. 2 rectal recurrences were diagnosed 3 and 6 months after the endoscopic resection for lesions piece-meal resected and treated endoscopically. No recurrence occured in patients treated by ESD for gastric tumor with a mean follow-up period of 7 months (range 2-14).
Conclusion : ESD allowed an en-bloc resection in 69,23% of our patients. This technique seems to be more easily applicable for gastric tumors compared with rectal tumors.

- D37 -

ONE YEAR WEIGHT LOSS IN FIRST 21 PATIENTS TREATED WITH NEW TRANSORAL GASTRIC STAPLING SYSTEM. C. Moreno (1), G. Ojeda Valdes (2), L.F. Cuevas Herrera (2), J. Closset (1), A. Mehdi (1), P. Eisendrath (1), S. Dugardeyn (1), M. Baréa (1), M. Zalcman (1), O. Le Moine (1), J. Devière (1). (1) ULB Erasme, Brussels ; (2) Hospital Regional 1st Octubre, Mexico City, Mexico.

Background : The TOGA System is a new endoluminal stapling system for the treatment of obesity. Six month results for the first 21 patients treated were previously reported. We now report one year weight loss results for this group.
Methods : Patients were recruited based on established criteria for bariatric surgery. With patients under general anesthesia, the TOGA System was used to create a stapled restrictive sleeve along the lesser curve of the stomach. Patients were kept overnight for observation. Follow-up was completed at 1 week and 1, 3, 4, 5, and 6 months. Ethics committee approval was obtained to extend follow-up to 12 months, patients were re-consented and weights were collected.
Results : Twenty one patients were enrolled and treated. There were no significant adverse events. The most common procedure or device related AEs were vomiting, pain, nausea and dysphagia, which all resolved within 3 weeks. 7 patients received additional restrictions to reduce size of the sleeve outflow tract between 5 and 7 months after the initial treatment. At 12 month follow-up, weight was obtained for 20 patients. Mean weight loss was17.6 kgs. %EWL (ideal weight from Metlife) was 34.8% at 12 months, and %EBMIL (target BMI = 25) was 38.8% at 12 months.

	WL (lb)	WL (kg)	%EWL	%EBMIL	n
1 mo	17.6	8.0	16.2%	18.2%	21
3 mo	24.5	11.1	22.6%	25.3%	21
6 mo	26.5	12.1	24.4%	27.4%	21
12 mo	38.7	17.6	34.8%	38.8%	20

Conclusions : Despite improvements in safety of current surgical procedures for obesity, there exists interest in a less invasive treatment option. This first human study of the TOGA (transoral gastroplasty) procedure shows the procedure is preliminarily safe, and results in significant weight loss out to one year. These results should be confirmed in larger studies with longer follow-up.

- D38 -

TRANSORAL GASTROPLASTY (TOGA) FOR OBESITY : RESULTS OF SECOND PHASE MULTI-CENTER STUDY. C. Moreno (1), G. Ojeda Valdes (2), G. Costamagna (3), L.F. Cuevas Herrera (2), J. Closset (1), A. Mehdi (1), P. Eisendrath (1), S. Dugardeyn (1), M. Baréa (1), E. Coppens (1), O. Le Moine (1), J. Devière (1). (1) ULB Erasme, Brussels ; (2) Hospital Regional 1st Octubre, Mexico City, Mexico ; (3) Catholic University, Roma, Italy.

Background : Transoral gastric stapling is being evaluated as a less invasive restrictive procedure for morbid obesity. Results in the first 21 patients were reported previously (1). Device modifications were made to reduce procedure time and improve anatomic results. We report results of a second phase study conducted at 3 centers with the revised device.
Methods : Subjects met NIH criteria for surgery, were consented, and were treated with the TOGA System (Satiety, Inc.) from January 2007 to February 2008. Additional restrictions were allowed after 3 months. Follow-up was done at 1, 3, 6 and 12 months.
Results : 41 patients were enrolled (28 female). Mean age was 44.7 (21-60) and BMI was 42.7 (35.1-54.2). All procedures were completed uneventfully, except one patient with difficult anesthesia recovery. Mean procedure time was 94 min. Hospital stay was 1 night for all patients except the anesthesia AE who stayed 2 nights. Most common AEs were pain, gastric ulcer, asthenia, diarrhea, and nausea. One patient was lost to f/u at 1 month, and one exited at 6 months due to pregnancy. Six patients received additional restrictions at 4-5 months for insufficient weight loss. %EWL (Metlife) was 42.5% and %EBMIL (target BMI 25) was 49.0% at 12 months.

	BMI	WL (kg)	%EWL	%EBMIL	n
Baseline	42.7				41
1 mo	39.6	9.8	17.8%	20.0%	35
3 mo	37.5	16.1	28.7%	32.3%	30
6 mo	35.7	22.0	39.6%	44.1%	25
12 mo	32.6	20.0	42.5%	49.0%	7

Conclusions : This second phase study confirms safety of transoral gastroplasty with improved weight loss to 12 months. Procedure time was also significantly reduced.
Reference
1. Devière J. *et al*. Safety, feasibility and weight loss after transoral gastroplasty (TOGA) : first human multicenter study. *Surgical endoscopy*, 2008, 22 : 589-598.

- D39 -

IMPROVING PATIENT PROCESS AND CYCLE TIME DURING COLONOSCOPY WITH THE LEAN & SIX SIGMA METHODOLOGY. E. Raymakers, H. Piessevaux, B. Debande, O. Dewit, K. Azzouzi, M.A. Denis, D. Vandenbosch, I. Perez, Y. Mira, P. Deprez. UCL Saint-Luc.

Background and aims : Improving patient flow process and cycle times in an endoscopy unit is challenging but essential to improve patient's satisfaction, to reduce lead time of patients (duration of passage and stay of the patient in the unit), to improve endoscopy suites utilization and make best use of available resources. The aim of our work was to use the Lean & Six Sigma methodologies through the framework : "DMAIC - Define, Measure, Analyse, Improve, Control" to decrease patients cycle time and increase number of colonoscopy performed under anaesthesia without any change of available resources.
Methodology : To define and understand the patient care process in practice we used the tools of Process Map & Lead Time. Colonoscopy was chosen as the main objective since it is one of the most frequent endoscopy performed and involves anaesthesia. The patient profile was further defined as internal/external and with/without anaesthesia. Lead time included pre-exam (registration, preparation), exam (including sedation or anaesthesia mainly using IV propofol) and post-exam times (recovery, report and departure). Measures included statistical process control tools on patient lead time per profile and variability at each step of the process. The study took place in a tertiary academic hospital performing more than 12000 endoscopic procedures per year in 5 endoscopy suites, including 2100 colonoscopies. Measurements were done during 2 weeks and then discussed during workshops with the entire team (physicians, nurse and support staff, administration and finance, process engineer).
Results : 112 patients/colonoscopies were evaluated and total lead times for a colonoscopy with (n = 68) and without (n = 44) anaesthesia were 190 min [range 99-403] and 80 min [35-135], respectively. The differences were mainly due to the preparation step 23 min [0-95] with anaesthesia vs. 2 min [0-37] without and the post-exam period that included recovery until discharge with and without anaesthesia [126 min vs. 15], respectively. Occupation time of exam rooms was 46 vs. 40 min ($p < 0.015$) with and without anaesthesia, respectively. The key bottlenecks were further analysed and improvement opportunities are summarized in table. We have implemented them through small tests of change. This resulted in an increase of 7.6 to 9.7 colonoscopies per day, and of the ratio with/without anaesthesia of 55 to 62%.
Conclusions : The use of the Lean & Six Sigma methodologies, to process the patient's mapping and to measure the lead time, in order to identify bottlenecks and improvement opportunities, through a positive implication for patients, staff and the facilities, led to important changes in the unit organisation and efficiency with a significant decrease of colonoscopy cycle time.

	Baseline	Target
Punctuality for registration	20% regist. after appointment	0%
Start time of first exam	8h58	8h40
Variability for pre-exam (USL = upper specification limit)	USL at 95 min	USL at 50 min
Room occupancy		2008 vs. 2007 : + 28%

- D40 -

IMPACT OF REIMBURSEMENT POLICY IN BELGIUM ON THE REFERRAL PATTERN AND DIAGNOSTIC YIELD OF CAPSULE ENDOSCOPY. A SINGLE-CENTRE STUDY. S. De Rouck, P. Hindryckx, M. De Vos, D. De Looze. UZ Gent.

Background : From the 1st of July 2008 onwards capsule endoscopy (CE) is largely reimbursed for patients who are suffering from suspected obscure gastro-intestinal bleeding, unexplained by conventional upper and lower endoscopy. We hypothesize that the number of patients referred for CE is rising since that date, and that the number of relevant CE findings is decreasing simultaneously.
Methods : Three groups of patients referred for CE, within a time frame of 5 months, were compared : group "2006" (i.e. from January until May 2006), group "2008-before" (i.e. from February until June 2008) and group "2008-after" (i.e. from July until November 2008). Following characteristics were analysed : number of CE's, indication (iron deficiency anemia vs. overt bleeding), transfusion need of at least 2 units of packed cells, lowest haemoglobin level, relevant findings on CE.
Results : All patients were referred by gastro-enterologists after endoscopic work-up with at least 1 upper and 1 lower digestive endoscopy. More than 90% of referred patients came from non-academic centres. In table 1 the data are summarized :

	Group 2006	Group 2008-before	Group 2008-after
Number (Females)	17 (3F)	16 (9F)	28 (10F)
Mean age (y)	61,2	58,7	58,5
Iron-deficiency anemia - n	6	12	13
Overt bleeding (melena or red blood) - n	11	3	13
Other indications	0	1	2
Transfusion need - n (%)	14 (82%)*	10 (69%)	16 (57%)*
Lowest Hb level in g%	7,95	8,20	8,20
Relevant findings - n (%)	11 (65%)**	8 (50%)	9 (32%)*
	Active bleeding 0 Ulcerations 3 Angiodysplasia 2 Tumor 4 Other 2	Active bleeding 2 Ulcerations 2 Angiodysplasia 3 Tumor 1	Active bleeding 2 Ulcerations 2 Angiodysplasia 4 Ileïtis 1

* Transfusion need group 2006 vs. group 2008-after, p = 0,085.
** Relevant findings (diagnostic yield) group 2006 vs. group 2008-after, p = 0,035.

Discussion : Since the advent of capsule endoscopy its diagnostic yield is significantly diminished (halved) in 2 years time. Reimbursement of the capsule since the 1st of July 2008 may be considered an important factor to explain this. Although the current referral pattern is consistent with existing guidelines and Belgian reimbursement conditions, we have been observing some differences in patient selection since July 2008, with a trend towards less transfusion-dependent patients. We hypothesize that gastro-enterologists nowadays refer their patients with obscure gastro-intestinal bleeding earlier for CE, thus adding a group of less severe and spontaneously resolving obscure bleeders.

- D41 -

DUMPING SYNDROME FOLLOWING NISSEN FUNDOPLICATION IN CHILDREN. B. De Muynck, T. Bosmans, I. Hoffman. KULeuven Gasthuisberg.

Background and aims : Dumping syndrome is a well-recognized complication of gastric surgery in adults. In children, there have been increasing numbers of case reports following Nissen fundoplication, with a current prevalence of 10 to 30 percent. The aim of this study is to describe prevalence, symptoms, diagnosis, treatment and outcome of 190 children with antireflux surgery over a follow-period of 13 years.
Material and methods : We observed children who underwent Nissen fundoplication between January 1995 and August 2008. All surgical interventions were performed by three thoracic surgeons in our University Hospital. Children with symptoms suggestive of dumping syndrome were included and underwent further investigations. Presenting symptoms of dumping syndrome were pallor, diaphoresis, lethargy, nausea, failure to thrive, watery diarrhea and abdominal pain. The diagnosis was confirmed on an oral glucose tolerance test with ingestion of 1.75 gr/kg glucose orally and measurements of plasma glucose levels after 0', 30', 60', 120' and 180'. Hyperglycemia was defined as any glucose level above 166, 151, 130 and 122 mg/dl capillary blood at 30, 60, 120 and 180 minutes respectively. Hypoglycemia was defined as capillary blood glucose below 50 mg/dl at any time during the test. The caloric intake and subdivision in protein, carbohydrates and fat intake was calculated. The standard treatment was diet, consisting of a balanced intake of 10-12% protein, 45% carbohydrates and 45% fat.
Results : Hundred ninety patients (122 boys, mean age 6,7 years) underwent anti-reflux surgery. Twenty patients (14 boys, mean age 4.9 years) or 11% had symptoms suggestive of dumping syndrome. The most common postprandial symptoms were abdominal pain (11/20), diarrhea (10/20), nausea (9/20), pallor (9/20). The mean interval to reporting of dumping symptoms was 24 months (range 2 _ 168 months). An oral glucose tolerance test was performed in 18 patients. Sixteen children had abnormal results. Hypoglycemia (minimum 28 mg/dL) was found in 2 patients, 2 patients had a positive test due to combined hypo- and hyperglycemic values, 12 patients had significant hyperglycemia (maximum 486 mg/dL) during the test. Two tests were normal but the children nevertheless had symptoms suggestive of dumping during the test. Measurements of caloric intake were performed in 18 patients. The mean caloric intake was 1336 kcal daily, consisting of average 14% protein (min 11%, max 19%), 49% carbohydrates (min 38%, max 60%) and 38% fat (min 29%, max 46%). All except one child were succesfully treated with anti-dumping diet. One patient had good clinical improvement on Acarbose (3,7 mg/kg daily).
Conclusions : Dumping syndrome is a complication of anti-reflux surgery, with a prevalence of 10% in our study. Following fundoplication, strict follow-up is mandatory, because growth and quality of life are compromised in children with dumping syndrome. Abdominal pain, diarrhea, nausea and pallor are the most presenting symptoms. An oral glucose tolerance test is a simple and inexpensive diagnostic. The diet has proven to be a very effective form of treatment in children.

- D42 -

EFFICACY AND SAFETY OF 12-WEEK TREATMENT WITH PRUCALOPRIDE IN PATIENTS WITH SEVERE CHRONIC CONSTIPATION : COMBINED RESULTS OF 3 IDENTICAL, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIALS. J. Tack (1), G. Beyens (2), R. Kerstens (2), L. Vandeplassche (2). (1) KULeuven ; (2) Movetis NV, Turnhout.

Objective : To evaluate the combined efficacy and safety results of a 12 weeks treatment regimen with 2 and 4 mg of prucalopride (PRU) of 3 identical, pivotal, double-blind, randomized, parallel-group, placebo (PLA)-controlled phase III trials in patients with chronic constipation (CC).
Methods : The data of the three trials, each with a PLA, PRU 2 mg and PRU 4 mg treatment arm, were combined. A drug-free run-in phase was followed by a 12-week treatment phase. The primary efficacy parameter was the % of patients with an average of e3 spontaneous complete bowel movements (SCBM) per week over the 12 week treatment period. The main secondary efficacy parameter was the% of patients with an average of e1 SCBM per week over the 12 week treatment period. Adverse events (AEs), vital signs, laboratory tests and ECG were assessed at baseline and during treatment.
Results : The % of patients with an average of e3 SCBM or e1 SCBM per week over the 12 week treatment are presented.

Time-point	Placebo N = 645		PRU 2 mg N = 640		PRU 4 mg N = 639	
	N	n (%)	N	n (%)	N	n (%)
Run-in	643	4 (0.6)	638	5 (0.8)	639	8 (1.3)
Weeks 1-12 ^{3}3SCBM/week	645	73 (11.3)	640	151 (23.6)***	639	158 (24.7)***
Weeks 1-12 ^{3}1SCBM/week	630	155 (24.6)	612	264 (43.1)***	593	279 (47.0)***

*** $p < 0.001$ vs. placebo (pairwise comparison).

Both doses of prucalopride were well tolerated, and the most frequently reported treatment-related AEs (headache, abdominal pain, nausea, and diarrhoea) occurred mainly on the first day of treatment. The rates of occurrence of these AE's after Day 1 were similar between PLA and PRU. No clinically relevant differences were observed between PRU and PLA in laboratory parameters, vital signs or ECG values during the trial period.
Conclusions : Treatment during 12 weeks with PRU 2 mg and 4 mg significantly improves bowel function and is safe and well tolerated by patients with CC.

- D43 -

Accuracy of a simplified IBS self-report questionnaire compared to the Rome III criteria in distinguishing IBS from organic disease. S. Van Gool (1), H. Piessevaux (2), B. Fischler (3), J. Tack (1). (1) KULeuven ; (2) UCL Saint Luc ; (3) ULB St Pierre.

Background : In a recent epidemiological study, we observed a major discrepancy between the incidence of IBS based on Rome 3 criteria or on self-report after reading a simple description (long-standing discomfort with changes in stool pattern ; 14 vs. 19%).
Our **aim** was to study the performance of the Rome 3 and self-report definitions in patients undergoing colonoscopy for abdominal pain/discomfort.
Methods : Consecutive patients referred for first-time colonoscopy with abdominal symptoms filled out questionnaires to collect demographics, Rome 3 criteria, response to simplified IBS description, and the presence of alarm symptoms or a family history of inflammatory bowel disease (IBD) or colon cancer.
Results : Out of 247 patients (mean age 54.9 ± 0.9, 115 men), 23% met the Rome 3 criteria for IBS. Compared to Rome 3-negative patients, they were significantly younger (46 ± 2 vs. 57 ± 1 yrs, $p < 0.0001$), more likely to be female (69 vs. 49%, $p < 0.05$) and more likely to report alarm symptoms (73 vs. 54%, $p < 0.05$). Both groups did not differ in positive family history (18 vs. 25%, NS) or relevant organic disease (IBD, cancer, large polyps) during colonoscopy (12 vs. 13%, NS). Self-reported IBS according to the simplified description occurred in 105 patients (42%). They were significantly younger (52 ± 2 vs. 57 ± 1 yrs, $p < 0.05$), more likely to report alarm symptoms (72 vs. 49%, $p < 0.001$) and more likely to be female (57 vs. 47%, $p < 0.05$). No difference was found in positive family history (24 vs. 27%, NS), or relevant organic disease (10 vs. 14%, NS) between both groups. Using Rome criteria and negative endoscopy as gold standard, the simplified IBS-description had 90% sensitivity, 67% specificity, 48% positive, and 96% negative predictive value for Rome 3 IBS. The IBS group identified by a simplified questionnaire did not differ from the Rome 3 IBS group in terms of family history (18 vs. 24%, NS), alarm symptoms (73 vs. 72%, NS), or organic findings during endoscopy (10 vs. 12%, NS). The discrepancies between self-reported IBS and Rome 3 IBS were mainly attributable to time or frequency restraints of the Rome 3 definition (not present > 6 months in 37% ; frequency < 3 days/month in 21%) or lack of relief by defecation (26%).
Conclusion : Similar to Rome 3 criteria, a simplified IBS-description identifies a group of patients with bowel symptoms who are younger, predominantly female, with a low prevalence of significant organic disease during colonoscopy. Alarm symptoms are not helpful in distinguishing organic from functional colonic disorders. Rome 3 criteria, which identify approximately half of these patients, underestimate the prevalence of IBS in this population.

- D44 -

LARYNGEAL NEUROPATHY IN PATIENTS WITH PERSISTING GLOBUS SENSATION. J. Arts, L. Holvoet, R. Bisschops, P. Caenepeel, D. Dewulf, F. Bruyninck, E. Dejaeghere, D. Sifrim, J. Janssens, J. Tack. KULeuven.

Background : Globus, defined as a feeling of a lump in the throat, is a frequently occurring symptom of unknown etiology. Pathophysiological mechanisms that have been implicated include gastroesophageal reflux disease (GERD), oesophageal motor disorders, overactive cricopharyngeal muscle, psychological factors, gastric islet patches in the proximal esophagus and deformations of the cervical spine. We recently observed that some patients report a unilateral location of the globus sensation.
The **aim** of the present study therefore was to analyse the results of esophageal testing and electromyographic (EMG) examination of the larynx in globus patients seen at a Neurogastroenterology outpatient clinic.
Methods : From the charts of consecutive patients, we extracted data on upper gastrointestinal endoscopy, esophageal manometry and pH monitoring, fluoromanometric examination of swallowing, and laryngeal EMG. EMG was measured on the nervus recurrens and laryngeus. Numbers are shown as mean ± SD.
Results : A total of 40 patients (mean age 52 ± 13 ; 26 females) with persisting globus were studied. A unilateral location of the globus sensation was reported by 26%, while the majority (74%) reported bilateral symptoms. All patients had received prior empirical PPI therapy without symptom relief. Esophageal pH monitoring off PPI was pathological in 36% of the patients. Esophageal manometry was normal in 60% of the patients, with hypocontractility in 15%, nutcracker esophagus in 5% and non-specific motor disorders in 20%. Fluoromanometric swallowing examination was normal in 44%. Increased resting pressure of the upper esophageal sphincter was found in 43% of the patients (186 ± 46 mmHg) and incomplete relaxation was found in 6%. Laryngeal EMG was pathological in 34 patients (85%), with increased latency times > 2.9 msec of right or left recurrent or laryngeal nerves. Increased latencies were found bilaterally in 18 patients (43%), on the left hand side only in 15 patients (38%) and on the right hand side only in 1 patient (3%). Concordance between the site of symptoms and of increased latencies was poor (53%). The occurrence of laryngeal neuropathy could not be explained by the patients' past medical history, including infections, generalised neuropathy, radio- or chemotherapy.
Conclusion : Patients with globus sensation have a very high prevalence of bilateral or left-sided laryngeal neuropathy. Laryngeal neuropathy seems to occur independently from the presence of GERD or abnormal esophageal motility. Further studies will be required to investigate whether laryngeal neuropathy is a marker for globus and wheter a causal relationship exists between neuropathy and symptoms.

- D45 -

ENDOSCOPIC TREATMENT OF PREMALIGNANT LESIONS AND EARLY OESOPHAGEAL CANCER : SAFETY AND EFFICACY IN AN EARLY TRAINING STAGE. R. Bisschops, A. Lerut, I. Demedts, G. De Hertogh, P. Nafteux, K. Geboes, P. Rutgeerts. KULeuven.

Background : Endoscopic resection (ER) has become the treatment of choice for early mucosal well differentiated oesophageal cancer (Pech *et al*. 2008). Proper training is required to engage in this field of endoscopy and little is known about the risks of the procedure in the hands of an endoscopist starting to perform these procedures.
Aim of this study was to assess prospectively the safety and efficacy of ER during the learning curve of endoscopic resection in a centre introducing this treatment.
Methods : All procedures were performed by one single endoscopist who received hands-on a training by endoscopists with longstanding experience in endoscopic resection. ER was performed using the cap technique or a multi band mucosectomy device (Duette). All procedures were prospectively registered for staging outcome, complications, complete remission and long term follow-up.
Results : 80 ERs were performed in the oesophagus (8 in squamous epithelium, 6 in the cardia, 66 in Barrett's oesophagus) in 62 patients (42 male, mean age 66). Final diagnosis was : papilloma (1), granular cell tumour (2) squamous cell cancer (5), columnar lined oesophagus (1), intestinal metaplasia (7) low grade dysplasia (16), high grade dysplasia (14) and adenocarcinoma (34). Mild bleeding that could be controlled during the procedure occurred in 38% of the procedures. There were no delayed or clinically significant bleedings (no blood transfusion). ER was complicated with a perforation in 1 patient (1.3%) which was managed conservatively. Stenosis occurred in 8 patients (11%) (only requiring dilation in 1 patient (1.3%)). Of the 62 patients 2 were treated out of clinical protocol and in total 14 (22%) of the patients had to be referred for additional oesopgahectomy. Nine (15%) were referred for surgery after staging ER (submucosal (sm)and/or blood vessel invasion or poor differentiation (n = 8), technical failure(n = 1)). An additional 5 patients (incomplete removal of carcinoma (n = 4), and 1 sm tumour recurrence after 12 months follow-up) underwent surgery during follow-up. 7 patients are still under treatment. In total 88% (39/44) of the patients treated with curative intent achieved complete remission.
Conclusion : Provided proper hands-on training, efficacy and complications of endoscopic resections during the learning curve of this procedure is comparable to those reported in other series by experienced interventional endoscopists.

- D46 -

POSTOPERATIVE COMPLICATIONS AFTER TRANSTHORACIC ESOPHAGECTOMY FOR ESOPHAGEAL AND GASTROESOPHAGEAL JUNCTION (GEJ) CANCER ARE NEGATIVELY AFFECTING ONSET OF EARLY RECURRENCE. T. Lerut, J. Moons, W. Coosemans, P. De Leyn, H. De Caluwé, G. Decker, D. Van Raemdonck, P. Nafteux. KULeuven Gasthuisberg.

It is hypothesized that complications after esophagectomy for cancer because of negative interference with the immune system may have a negative effect on recurrence and its timing and/or for the same reason on onset of metachronous malignancy.
Aim : To assess the impact of complications, using the Clavien classification, on recurrence and its timing and/or on onset of metachronous malignancy.
Methods : An analysis was performed on 150 consecutive patients operated on with curative intent between January 2005 and May 2006. Five R_2-resections and seven patients with synchronous tumours were excluded. Complications were divided according to the Clavien classification* : no complications (Grade 0), complications requiring drugs (e.g. antibiotics) or blood transfusion (Grade 2), complications requiring interventional treatment (Grade 3), life threatening complications requiring ICU admittance (Grade 4) or postoperative mortality (Grade 5).
Results : Mean age was 63.1 years, male-female ratio was 4 :1 ; 76.1% of the patients underwent primary surgery, 23.9% induction therapy, R_0-resection rate was 92.8%. Adenocarcinoma was found in 75%.
Clavien classification : Grade 0 : 29.7%, Grade 2 : 35.5%, Grade 3 : 17.4%, Grade 4 : 15.9%, Grade 5 (postoperative mortality) : 1.4%.
Ten patients developed recurrence within 6 months adding up to 29, 39, 42 within 12, 18, 24 months, totalling up to 50 at 3 years. Metachronous malignancy occurred in 4 patients at 1 year totalling up to 7 patients at 3 years.
Univariate analysis withheld complications, LN-status, number of positive nodes, extracapsular node involvement (EC), pStage, pT, and R_1-status as factors negatively influencing occurrence of recurrence.
In the multivariate model complications, EC, R_1-status were independent negative factors. Cox-regression analysis indentified R_1-status, EC and complications as significant determinators for the timing of recurrence.
In neither univariate nor multivariate analysis the onset of metachronous malignancy was correlated to complications.
Conclusions : This study suggests a correlation between complications and onset of early recurrence and its timing but no correlation with onset of metachronous malignancy.
Clavien classification, beside R_1-status and EC lymph node involvement, appears to be useful as prognostic indicator for both onset of early recurrence and its timing.
Achieving esophagectomy without postoperative complications is of utmost importance also for oncologic reasons given its negative potential on early oncologic outcome.
Reference
1. Dindo D., Demartines N., Clavien P.A. Classification of surgical complications : a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann. Surg*., 2004 Aug, 240 (2) : 205-13.

- D47 -

COMPLETE PATHOLOGICAL RESPONSE AFTER NEOADJUVANT CHEMORADIOTHERAPY FOR STAGE II AND III RECTAL ADENOCARCINOMA PREDICTS EXCELLENT LONG-TERM OUTCOME. A. Wolthuis, F. Penninckx, K. Haustermans, E. Van Cutsem, A. Dhoore. KULeuven.

Introduction : Neoadjuvant chemoradiotherapy is standard of care in patients with clinical stage II and III rectal cancer to improve local control after TME-surgery. The impact on ultimate pathological staging is variable and the aim of this study is to evaluate long-term disease control in patients who had a complete pathological response.
Methods : All consecutive patients surgically treated for mid and distal rectal adenocarcinoma after neoadjuvant treatment were identified from a prospectively collected database. Patient demographics included age, sex and body mass index (BMI). Rectal tumors were staged by CT-scan and endorectal ultrasonography and stage II and III tumors received neoadjuvant chemoradiotherapy according to a standardized protocol. Radical surgery was performed after an interval from 6 to 8 weeks. Final pathology was copied from the pathology report. Only patients with a complete pathological response were eligible for inclusion. Survival analyses were performed using the Kaplan-Meier method.
Results : Between january 2000 and december 2007 a total number of 332 patients received a TME after neoadjuvant therapy. Sixty-one patients (18.4%) had a complete pathological response. Mean age for this selected group of patients was 63 years old and 59% of the patients were males. Mean BMI was 25,3 kg/m^2. At a median follow-up of 55.7 months (range : 11.6-104.3 months) a 5-year survival rate of 97.7% and a disease free survival of 92.1% was noted. Recurrence occurred in 4 patients (6.6%) after a mean follow-up of 19.7 months. One patient developed locoregional recurrent disease and died after 29.6 months. Another patient died from pulmonary and adrenal metastatic disease after 89.2 months.
Conclusion : Despite the initial tumor stage, those patients who have a complete pathological response have an excellent chance of longevity.

- D48a -

COMPARISON OF THE COLONIC METABOLITE FINGERPRINT IN CHRONIC KIDNEY DISEASE WITH HEALTHY STATE. V. De Preter (1), B. Meijers (2), B. Bammens (2), P. Rutgeerts (1), P. Evenepoel (2), K. Verbeke (1). (1) KULeuven ; (2) KULeuven Gasthuisberg.

Introduction : Chronic kidney disease (CKD) is characterised by changes in biochemical and physiological functions. Although the exact pathogenic mechanisms for these changes are still largely unknown, it is accepted that there is a progressive retention of some compounds that are normally excreted by the kidney. An important subgroup of retention solutes originates from protein fermentation in the colon. Accumulation of fermentation metabolites in CKD is associated with further deterioration of renal function, increased overall mortality and increased incidence of cardiovascular disease. While colonic fermentation solutes clearly affect the progression and prognosis of CKD, it is unclear whether CKD itself interferes with colonic protein fermentation. The aim of this study was to compare the faecal volatile compounds (VOC) fingerprint between CKD patients and healthy subjects.
Methods : Faecal samples were obtained from 55 healthy subjects and 12 CKD patients. A purge-and-trap sample preparation system, coupled on line to a GC-MS (time-of-flight) was applied to analyse the VOC. AMDIS software was applied to extract purified mass spectra from overlapping components. Cluster analysis was used to compare the metabolic profiles. Statistics were done using SPSS 15.0.
Results : A total of 237 different VOC were identified in the faecal samples with an average of 62 ± 8 VOC per control and 51 ± 4 per CKD patient. Five VOC were found in all analyzed samples : acetone, benzaldehyde, 4-methyl phenol, 2-methyl propanal and toluene. VOC fingerprints clearly clustered in 2 groups, discriminating between CKD and controls. Subsequently, we identified twelve VOC, which significantly differed between healthy subjects and CKD patients.

Differences in % occurrence of VOC controls and CKD patients

VOC	Healthy	CKD	p-value (X^2)
1-Pentanol	4%	58%	< 0.001
2-Methyl-2-propen-1-ol acetate	0%	50%	< 0.001
4-Carene	0%	42%	< 0.001
Benzene, 1,2,3-trimethyl	96%	42%	< 0.001
Benzeneacetaldehyde	64%	0%	0.015
Cyclohexane	11%	75%	< 0.001
Cyclohexene, 1-methyl-4-(1-methylethylidene	67%	0%	0.005
Cyclopropanecarboxaldehyde	5%	40%	0.009
Furan, 2-methyl	15%	92%	< 0.001
Hexane	69%	0%	0.003
Limonene	96%	50%	0.002
Oxepine, 2,7-dimethyl	68%	0%	< 0.001

Conclusions : We report the first analysis of fermentation metabolites in a cohort of CKD patients versus healthy volunteers. These findings suggest that CKD interferes with bacterial colonic protein fermentation. In turn, retained protein fermentation metabolites adversely affect outcomes of CKD patients. Combined, these data suggest a complex colo-renal interplay which might provide new therapeutic targets for CKD.

- D48b -

BILIARY COMPLICATIONS IN LIVING DONOR LIVER TRANSPLANTATION AN INVARIABLE AND SIGNIFICANT MORBIDITY IRRESPECTIVE OF THE OPERATIVE APPROACH. COMPARISON BETWEEN TWO DIFFERENT INSTITUTIONAL EXPERIENCES. R. Troisi (1), R. Montalti (2), A. Lauterio (3), A. Slim (3), A. Giacomoni (3), L. De Carlis (3), B. de Hemptinne (1). (1) UZ Gent ; (2) Modena Hospital, Italy ; (3) Niguarda Hospital, Milano, Italy.

Background : Biliary complications (BC) in adult-to-adult living donor liver transplantation (LDLT) are the major cause of morbidity affecting early and long-term outcomes. We analyzed retrospectively the incidence, risk factors and overall morbidity of 115 consecutive LDLT comparing two different surgical approaches.
Materials and methods : Clinical data of 115 consecutive LDLT from Ghent University Hospital (Center 1) and Niguarda Hospital Milan (Center 2) performed during September 1999 and November 2007 were evaluated. Recipient's M/F ratio was 70/45 and the median age was of 54 y (range 19-66). Pre and perioperative differences were as follows : Cholangio-CT for donor evaluation, intraoperative cholangiography (IOC), cutting ducts after parenchyma transection, HTK perfusion, single stitches anastomosis (Center A) ; Cholangio MRCP for donor evaluation, no IOC, cutting before parenchyma transection, Celsior perfusion and running/single stitches anastomosis (Center 2). Ductoplasty for joining 2 single apart ducts in one anastomosis were identically performed with absorbable suture, duct-to-duct anastomosis preferred when possible and external biliary drainage routinely applied.
Results : Following a median FU of 46 m (range 12-106 m) a total of 38/115 (33%) patients experienced early (leaks) and 26/115 (22%) late (stenosis) BC (p = ns for Center 1 vs. Center 2). Ductoplasty were significantly associated with early and late BC (p = 0,007 for Center 1 and 0,049 for Center 2). Univariate analysis identified ductoplasty as significant factors for early BC and this was confirmed by the multivariate Log. Reg. Ductoplasty and small ducts (< 4 mm) were significantly associated to late BC at the univariate analysis and the multivariate confirmed ductoplasty as independent risk factors. According to this experience 5/115 (4,3%) grafts were lost due to BC. Overall survival was of 70% and 64% at 5-y in patients with and w/o BC (p = ns).
Conclusions : BC represents a significant source of morbidity potentially leading to graft loss in LDLT. Avoidance of ductoplasty, irrespective of surgical approach, may significantly lower this incidence. Efforts to improve results are warranted.

- D49 -

MARGINAL ULCERS AFTER LAPAROSCOPIC GASTRIC BYPASS : A RETROSPECTIVE STUDY OF 348 PATIENTS. L. Kohnen, P. Tromme, P. Honoré, J. De Flines, J. Belaiche, M. Meurisse, A. De Roover. ULg Sart-Tilman.

Background : Marginal ulcer (MU) is a well-recognized complication after Laparoscopic Roux-en-Y gastric bypass (LRYGB). It is diagnosed in 1% to 16% of patients in the literature. We reviewed the incidence, presentation and outcome of MU in consecutive patients undergoing LRYGB.
Methods : A total of 348 LRYGB were performed during a 4-year period. We systematically used a gastrojejunal hand-sewn anastomosis. During this period, an endoscopic evaluation was performed before surgery for each patient and after surgery if indicated.
Results : The mean follow-up, mean age and mean BMI was respectively 22 months, 42 years and 43. The mortality rate was 0%. Overall incidence of gastrojejunal anastomotic fistulae was 0%.The mean hospital stay was 4 days but since 1 year, 87% of the patients leave the hospital within 3 days and 60% within 2 days.
Four patients (1.1%) presented an early (d 30 days) MU. All were successfully medically treated. Late (> 30 days) MU occur in 34 patients (9.7%). 23 were medically treated by PPI (6.6%) and 11 were finally surgically treated after failed medical treatment (3.1%).
5 patients presented perforated MU with acute abdominal pain (1.4%). The median time to perforation after the LRYGB was 5.5 months (range : 4-8 m). The treatment was made by open surgery in all cases. 6 patients (1.7%) presented chronic MU which finally benefits of laparoscopic MU resection. Recidivant MU after surgery occur in 1 patient.
20 patients (59%) presented at least 1 risk factor of MU : nonsteroidal anti-inflammatory drugs (NSAIDS) in 5 patients (14.7%), smoking in 15 patients (44%), history of ulcus in 6 (17.6%) and Helicobacter pylori presence in 5 (14.7%).
Conclusion : MU is an underreported complication after LRYGB. The real incidence appears to be significant (> 9%). Medical treatment of MU is unsuccessful in 32% and emergency surgery occur in 1.4% for acute perforated MU. NSAIDS, smoking, history of ulcus and Helicobacter pylori seems to be risk factor. Further studies are needed to identify the exact physiopathology of MU.

- D50 -

THE ROLE OF BODY MASS INDEX AND WAIST CIRCUMFERENCE IN DETERMINING REFLUX PATTERN AND PROXIMAL EXTENT OF REFLUX EVENTS. E. Scarpellini, K. Blondeau, R. Vos, J. Tack. KULeuven.

Background : Epidemiological studies established that symptoms and lesions of gastroesophageal reflux disease (GERD) are associated to increasing body weight, and especially increased waist circumference (IWC). Proximal extent of the refluxate has been implicated in the occurrence of symptoms during reflux events. It is unclear whether IWC, through higher intra-abdominal pressure, is associated with a higher proximal extent of reflux events.
Aims : To evaluate the impact of body mass index (BMI) and waist circumference on the characteristics of reflux events.
Methods : Nineteen GERD patients underwent measurement of body mass index (BMI), waist circumference and ambulatory 24h Multichannel intraluminal impedance-pH (MII-pH) monitoring. Composition (liquid, air, acid or weakly acidic) and proximal extent of reflux events were determined from MII-pH. Data (mean ± SEM) were compared by student's t-test.
Results : Six patients were studied on and 13 off PPI. Overweight patients (BMI > 25, n = 12) had a higher number of reflux events (76 ± 14 vs. 37 ± 5, $p < 0.05$), especially liquid (43.6 ± 15.3 vs. 6.1 ± 1.5, $p < 0.05$) and upright (69.9 ± 13.8 vs 34.3 ± 4.2, $p < 0.05$) reflux events, compared to those without overweight. Acid exposure did not differ significantly between both groups (4.9 ± 2.1 vs. 2.1 ± 1.1%, NS). The number of reflux events with extent to 15 cm above the lower esophageal sphincter (LES) did not differ significantly (25.9 ± 4.7 vs. 13.9 ± 4.9, NS). Patients with IWC (> 85 cm, n = 14) had a higher number of reflux events (72.1 ± 12.4 vs. 33.0 ± 5.9, $p = 0.01$), especially liquid events (38.6 ± 13.4 vs. 5.2 ± 1.7, $p = 0.03$), non-acid events (47 ± 11.4 vs. 20.6 ± 5.8, $p = 0.05$), compared to those without IWC. The increase was found for upright (65.9 ± 12.0 vs. 31.4 ± 5.3, $p = 0.02$) as well as supine (6.3 ± 1.6 vs. 1.6 ± 0.7, $p = 0.02$) reflux events. Acid exposure did not differ significantly between both groups (4.5 ± 1.8 vs. 2.0 ± 1.4%, NS). The number of reflux events with extent to 15 cm above LES was significantly higher in those with IWC (26.3 ± 4.1 vs. 8 ± 4.3, $p = 0.01$). Patients with IWC had a higher number of weakly-acidic reflux-related cough events (1.4 ± 0.7 vs. 0 ± 0, $p < 0.05$). The mean symptom index for weakly acidic reflux-related symptoms was significantly higher in those with IWC (27 ± 10 vs. 0 ± 0, $p < 0.05$). Most of these differences were preserved when the patients on PPI were excluded from the analysis.
Conclusions : A higher BMI is associated with a higher number of upright liquid reflux events. An IWC is associated with a higher number of liquid and non-acid reflux events. In subjects with IWC, proximal extent of the refluxate is higher and associated with symptoms related to weakly acidic reflux, like cough.

- D51 -

TOLERANCE OF SELECTIVE AND CONVENTIONAL CHEMOEMBOLIZATION FOR HEPATOCELLULAR CARCINOMA. T. Sersté (1), V. Ozenne (2), M.P. Vuilerme (2), O. Farges (2), D.C. Valla (2), V. Vilgrain (2), F. Degos (2). (1) CHU Saint Pierre, Brussels ; (2) Hôpital Beaujon, Clichy, France.

Background/Aim : The treatment of reference for unresectable hepatocellular carcinoma (HCC) is transarterial chemoembolization (TACE). There is increasing evidence that a better tumour necrosis is obtained with selective than with conventional TACE. The aim of this study was to assess whether selective TACE using distal catheterisation of the feeding artery of the tumour was associated with fewer side effects than conventional TACE.
Methods : 96 patients with cirrhosis who underwent TACE for HCC between January 2004 and November 2007 were retrospectively studied. According to the modality for the administration of TACE, patients were classified in two groups : patients with conventional TACE (common or right hepatic artery embolization) and patients with selective TACE. Post procedural biochemical tests and components of the post-chemoembolization syndrome (associating abdominal pain, fever and nausea) where systematically studied.
Results : 54 patients underwent conventional TACE while 42 patients underwent selective TACE. At univariate analysis, the incidence of fever was significantly higher in the conventional group and the duration of fever was longer. Evolution of transaminases, bilirubin plama levels and prothrombin index after TACE was similar in both groups. At multivariate analysis, the unique factor associated with the occurrence of fever was the conventional procedure.
Conclusion : Features of post-chemoembolization syndrome are less severe and less common with selective than with conventional TACE. Both procedures have a similar impact on liver function.

- D52 -

LIVER TRANSPLANTATION OUTCOME AND MELD ALLOCATION SYSTEM. M. Sainz-Barriga, E.L. Decoster, F. Goudsmedt, K. Boterbergh, B. Van Den Bossche, F. Berrevoet, A. Geerts, I. Colle, H. Van Vlierberghe, X. Rogiers, B. De Hemptinne, R. Troisi. UZ Gent.

Background : Since 2007 the MELD allocation system is used in Eurotransplant. Under this allocation system the patients with higher MELD are given priority to receive a liver graft. Higher MELD predicts a reduced expectancy of life while waiting for a suitable organ to be transplanted but contradictory results are reported regarding its association with outcome after transplantation. We performed a retrospective review of our results to address this issue.
Methods : Between January 2007 and November 2008, 102 liver transplantations were considered for analysis. The patients were divided into two groups, inferior to MELD score 30 and equal or superior to MELD score 30.
Results : Ninety-one adult patients were transplanted using 6 living donor grafts, 12 split liver grafts, 4 donors after cardiac-death, and 69 whole liver grafts from brain-death donors. Eleven paediatric patients were transplanted using 10 split liver grafts and a living donor liver graft. Mean MELD of the entire series was 24.6 ± 8.6 showing a Gaussian distribution. Seventy five patients were included in the MELD < 30 group (mean MELD 21 ± 5.9), and 27 in the > 30 MELD group (mean MELD 36 ± 3.7). Twelve patients (44.4%) were transplanted in high-urgency (HU) status in the higher-MELD group compared to 2 patients (3%) in the lower-MELD group ($p < 0.001$). The median time in the waiting list was shorter in the higher-MELD group : 5 days, range (1-730) vs. 89 days, range (1-981), $p = 0.01$. The median ICU time of the patients was longer in the higher-MELD group : 10 days, range (1-60) vs. 4 days, range (1-93), $p = 0.01$. The median in-hospital stay, although longer in the higher-MELD group, did not differ significantly : 28 days, range (1-269) vs. 19 days, range 84-761 in the lower-MELD group, $p = 0.8$. The median follow-up was 10.2 months, range (0.03-23). Two patients (7.4%) in the higher-MELD group and 11 patients (14.7%) in the lower-MELD group presented vascular complications ($p = 0.5$). One patient (4%) in higher-MELD group and 15 patients (20%) in the lower-MELD group presented biliary complications ($p = 0.09$). One year graft survival rates were 96% and 91% for higher-MELD and lower-MELD groups respectively, ($p = 0.5$). One year patient survival rates were 81% and 82% for higher-MELD and lower-MELD groups respectively, ($p = 0.4$). Ten patients died on the waiting list during the study period, four of them with MELD higher than 30.
Conclusions : Patients with higher MELD scores received a liver graft sooner than patients with lower MELD. The ICU stay of patients transplanted in the higher-MELD score group was longer, evidencing the more complex management of this patients. The MELD score superior or equal to 30 did not worsen outcome in our experience.

- D53 -

ENDOLUMINAL FUNDOPLICATION (ESOPHYX®) IN GERD PATIENTS REFRACTORY TO PPI THERAPY EFFECT ON SYMPTOMS, PPI USE AND DISTENSIBILITY. J. Arts, L. Holvoet, R. Bisschops, P. Caenepeel, D. Dewulf, R. Vos, T. Degreef, D. Sifrim, T. Lerut, P. Rutgeerts, J. Tack. KULeuven.

Proton pump inhibitor (PPI) therapy is the standard of care in gastroesophageal reflux disease (GERD) patients, while a surgical fundoplication is proposed for poor responders to PPI or those who desire not to be PPI-dependent. Endoscopic anti-reflux procedures, including the Endoluminal Fundoplication (EF), are being evaluated for their potential to relieve symptoms in GERD.
Our **aim** was to evaluate EF in patients with persisting symptoms in spite of adequate PPI therapy.
Methods : GERD patients with persisting typical or atypical reflux symptoms during PPI therapy, and a proven temporal association on impedance/pH monitoring, were recruited for this pilot trial. Exclusion criteria were age < 18, high grade esophagitis including Barrett's and hiatal hernia > 3 cm. Symptoms were evaluated off PPI before, 2 and 4 months after the EF. Assessments at baseline and after 2 months included 24 hour pH/impedance monitoring off PPI, esophageal manometry and measurement of the distensibility of the lower esophageal sphincter (LES). PPIs were stopped at 4 weeks, and started as prn after 2 months. All data (mean ± SD) were analyzed by Student's t test.
Results : 10 patients (8 men, mean age 37.5 ± 10.4) were recruited. Under complete sedation a mean of 11.5 ± 2.9 fasteners were applied during a mean procedure time of 52 ± 6 min. Transient throat ache, retrosternal pain and minor bleeding were the only adverse events. EF significantly improved GERD symptoms off PPI at 2 months (13.5 ± 7.4 vs. 5.0 ± 4.8, $p < 0.01$) and on PPI at 4 months (9.0 ± 4.9 vs. 2.0 ± 4.8 ; $p < 0.01$), and was associated with decreased PPI need (14.0 ± 7.3 vs. 11.5 ± 5.1 units/2 months, $p < 0.05$). LES pressure (14.0 ± 12.3 vs. 17.0 ± 7.8 mmHg, NS) and pH/impedance monitoring at 2 months were not significantly altered (% time pH < 4 : 9.2 ± 9.1 vs. 8.2 ± 6.1%, NS ; no change in weakly acidic reflux events). There was a tendency towards decreased distensibility of the LES (8.8 ± 3.3 vs. 3.5 ± 1.4 ml/mmHg, $p = 0.08$).
Conclusion : The present uncontrolled trial of EF in refractory GERD suggests symptomatic improvement. We found no differences in objective reflux or LES pressure, but distensibility of the gastroesophageal junction tended to be decreased. These findings need confirmation in a controlled study.

- D54 -

COMPLETION RATE OF CONVENTIONAL COLONOSCOPY : REASONS FOR FAILURE AND ALTERNATIVES AFTER UNSUCCESSFUL INTUBATION OF THE CECUM. N. Messaoudi, T. Moreels, P. Pelckmans. UZ Antwerp.

Background : An essential quality indicator for colonoscopy is the ability to intubate and visualize the cecum. Colonoscopy completion rates vary substantially and guidelines suggest a cecal intubation rate of ~ 90% to be up to standard.
Aims & methods : We retrospectively analyzed colonoscopy completion rates in our academic endoscopy unit and aimed to investigate reasons for failure and alternative investigations in case of an incomplete conventional colonoscopy. From January 1 to December 31, 2006, a total of 1617 colonoscopies were performed with conventional 130 cm long colonoscopes. In 76% of the procedures conscious sedation with midazolam and fentanyl was used, 20% was performed under general anesthesia and in the remaining 4% no sedation was administered. Patients with prior colon surgery (n = 116), as well as those with no or poor bowel preparation (n = 295) were excluded.
Results : Overall colonoscopy completion rate was 85%. Differences in success rates among endoscopists were noted (range 75% to 100%) and cecal intubation rates were lower in colonoscopies performed by assistants in training (81%) as compared to experienced endoscopists (87%). Most frequent reasons for failure of cecal intubation were redundant colon (30%), sigmoid looping (16%), patient's discomfort (16%), postoperative bowel fixation (11%) and diverticulosis (10%) and the most difficult segment to surpass in the colon was the hepatic flexure. Colonoscopies performed under general anesthesia had a significantly higher success rate (93%) as compared to other types of sedation (83%). Female gender and older age were significantly related to lower levels of completion. Alternative investigations after incomplete conventional colonoscopy were virtual CT colonography (17%), colonoscopy under general anesthesia (8%), use of a gastroscope (8%) double-balloon enteroscope (6%), redo colonoscopy (2%) and barium enema (1%). Finally, an adenoma detection rate of 31% and a rate of serious complications of 0,17% was noted.
Conclusions : In our academic endoscopy unit, 15% of conventional colonoscopies were incomplete, which is in accordance with the data reported in the literature. Known factors to decrease completion rates were : technical experience, patients' gender and age, type of sedation. This study identified redundant colon, sigmoid looping and patient's discomfort as the main reasons for failure. In addition the hepatic flexure was the most difficult colon segment to surpass. Several alternatives are available to visualize the entire colon after incomplete conventional colonoscopy. These retrospective findings have implications for practice and teaching and are useful in targeted quality improvement programs for colonoscopy.

- D55 -

THE FIRST PROSPECTIVE ENDOSCOPIC EXPERIENCE WITH THE EPTFE-COVERED VIABIL STENT IN PATIENTS WITH A DISTAL MALIGNANT BILIARY STENOSIS. W. Van Steenbergen. KULeuven Gasthuisberg.

Background and aims : Endoscopic insertion of a biliary stent is standard practice in the palliative treatment in patients with distal malignant biliary obstruction. Plastic, uncovered metallic as well as covered metallic stents are available, but all types of stents are characterized by their own advantages and disadvantages. The ePTFE-covered Gore Viabil stent is designed to minimize bacterial adherence and dislocation, and to prevent tumor ingrowth. Experience with this stent is mainly limited to the percutaneous approach. It is the aim of the present prospective pilot study to report our experience with the endoscopic application of this stent in patients with distal malignant biliary obstructions.
Patients and methods : Eleven patients with an inoperable biliopancreatic malignancy, presenting with obstructive jaundice by a stenosis of the distal and/or middle third of the bile duct, without apparent metastatic disease, and with an Eastern Cooperative Oncology Group (ECOG)score of 0 to 1, were included. All patients received an ePTFE-covered Viabil stent of 10 mm diameter, and with transmural side-holes in the proximal part of the stent. Primary endpoints were stent patency and patient survival, as expressed as the time intervals in days between stent insertion and a well-documented stent obstruction and death, respectively.
Results : No complications occurred after stent insertion and a signifant drop in bilirubin was already found after 24-48 hours. Overall median patient survival was 220 days ; 9 patients died free of jaundice from non-stent related causes. Stent dysfunction occurred in 3/11 patients and always resulted from massive stone impaction of the stent. Stent patency was 80% at 3 months and at 6 months, and 60% at 9 months. In the 10 patients who died, lifetime palliation was 70%.
Conclusions : The endoscopic insertion of a Gore Viabil stent in patients with a malignant distal biliary stenosis is safe and effective in the treatment of cholestatic symptoms. This type of stent is not free of stent dysfunction, and endoscopic reinterventions were necessary in 3 out of 11 treated patients. Stent dysfunction always resulted from stone/sludge impaction, although the covering of the stent has been developed to minimize bacterial adherence and sludge formation. Further studies seem warranted with regard to the conditions and mechanisms of sludge formation in this covered type of stent, in order to further improve stent patency rates.

- D56 -

DIFFERENTIAL GENE EXPRESSION PROFILE OF THE LIVER IN A 24-HOUR PORCINE MODEL OF FLUID-RESUSCITATED FECAL PERITONITIS. H. Van Malenstein, J. Wauters, P. Van Hummelen, H. Cauwenberghs, K. Reynders, M. Van Wambeke, L. Langouche, A. Wilmer, J. Van Pelt. KULeuven Gasthuisberg.

Introduction : The molecular and pathophysiological mechanisms underlying sepsis-related liver dysfunction are not yet fully understood. To elucidate the pathways involved we investigated gene expression by microarray in a clinically relevant porcine model of fluid-resuscitated septic shock.
Methods : Anesthetized and ventilated pigs (40 ± 3 kg) were randomly assigned to septic shock by fecal peritonitis (S, n = 6) or control (C, n = 6). In vivo liver samples were collected at baseline (BL) and 24h for analysis of mRNA expression by Affimetrix microarray (S : n = 2, C : n = 2). Changes in gene expression (ratio in base 2) between BL and 24 h and at 24 h were analyzed by GeneMath 3.5. To identify the affected molecular pathways, gene expression data were mapped on a pathway database using MapFinder 2.1.
Results : Septic pigs developed a normotensive, hyperdynamic circulation. In sepsis 2495 genes had significantly changed (> 2-fold up or < 0.5-fold down) between 24h and BL versus only 1955 genes in the control group. After pathway mapping, we identified pathways of inflammation, apoptosis and cell death being upregulated in sepsis, which were not altered in controls ($p < 0.05$). 24 h after induction, sepsis had limited effect on expression of mitochondrial genes. Detoxification by P450 cytochrome enzymes was upregulated in controls while septic pigs showed no change or even downregulation ($p < 0.05$).
Conclusion : Fluid-resuscitated sepsis is accompanied by an upregulation of inflammation, apoptosis and cell death, while hepatic detoxification is suppressed.

- D57 -

CIGARETTE SMOKE INDUCES APOPTOSIS IN THE FOLLICLE-ASSOCIATED EPITHELIUM OF MURINE PEYER'S PATCHES. S. Verschuere (1), P. Vlummens (1), P. Verbrugghe (2), K. Bracke (3), I. Rottiers (1), G. Brusselle (3), C. Cuvelier (1). (1) UZ Gent ; (2) University of Western Australia, Perth, Australia ; (3) U Gent.

Background : Recently, cigarette smoking has been associated with the development of several auto-immune diseases, including rheumatoid arthritis and Crohn's disease. The cellular and molecular mechanisms through which cigarette smoking predisposes to Crohn's diseaseare unknown. Cigarette smoke-induced apoptosis is described in several organs in *in vivo* and *in vitro* models of chronic smoke exposure, and might play a role in the pathogenesis of several smoke-associated diseases. The aim of this study was to quantify apoptosis in normal Follicle-Associated Epithelium (FAE) of murine Peyer's patches and compare this to apoptosis ratesin the FAE of smoking mice.
Methods : C57BL/6 male mice were exposed to cigarette smoke for 24 weeks (chronic exposure) while a control group was exposed to air during the same period. After 24 weeks the mice were sacrificed and Peyer's patches were dissected for histology. Immunohistochemistry for active caspase-3 was performed on paraffin-embedded tissue sections of 11 Peyer's patches of smoking animals and 11 Peyer's patches of controls. To compare apoptotic activity between smokers and controls, the apoptotic index (number ofapoptotic cells per 100 cells) in the FAE was calculated. An unpaired student T-test was applied to evaluate statistic significance of the observed values.
Results : A statistically significant increase in apoptosis of FAE cells was observed in smoking mice compared to air-exposed mice ($P = 0.002$). In the FAE of smoking animals, the mean apoptotic index was 1.82 (95% CI 1.42-2.23), whereas the mean apoptotic index in the FAE ofnon-smoking animals was 0.92 (95% CI 0.64-1.12). In both groups, mostapoptoticcells were seen at the apex of the FAE.
Conclusion : We quantified the rate of apoptosis in the FAE of Peyer's patches of smoking mice and their non-smoking siblings by caspase-3 immunohistochemical staining. An increased apoptotic index in the FAE of smoking animals was observed. Our results demonstrate that cigarette smoke induces a significant increase of apoptosis in the FAE of murine Peyer's patches and may point to a role for smoking in the pathogenesis of intestinal inflammation. Further investigation needs to clarify whether this increase in apoptosis influences normal function of the FAE.

- D58 -

POOLED PAC-SYM AND PAC-QOL RESULTS FROM 3 IDENTICAL, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE III TRIALS WITH PRUCALOPRIDE IN PATIENTS WITH SEVERE CHONIC CONSTIPATION. J. Tack (1), J. Ausma (2), R. Kerstens (2), L. Vandeplassche (2). (1) KULeuven ; (2) Movetis NV, Turnhout.

Objective : To determine the effect of prucalopride (PRU) on symptom improvement and constipation specific quality of life (QOL) in patients with severe chronic constipation.
Methods : Data of 3 identical pivotal phase III trials were combined. Each with 3 parallel treatment groups and treatment duration of 12 weeks : placebo, PRU 2 mg and PRU 4 mg. The patient assessment of constipation symptoms (PAC-SYM) questionnaire consists of 3 subscales : abdominal - (AS), stool - (SS), and rectal symptoms (RS) over a 2 week recall period. Disease-related QOL was assessed using the validated PAC-QOL self-report questionnaire with four subscales : physical discomfort, psychosocial discomfort, worries and concerns, and satisfaction. Both PAC-SYM and PAC-QOL items were rated on a 5-point Likert scale (0 = absent - 4 = very severe) at baseline, week 4 and week 12.
Results : Average changes from baseline showed statistically significant improvements compared to placebo on PAC-SYM for both PRU 2 mg and 4 mg at both week 4 and week 12. At the recommended dose of PRU 2 mg 33.2% of patients had an increase e1 point in overall PAC-SYM score compared to 21.5% at placebo after 12 weeks treatment. The most marked effect of PRU was observed on the subscales AS and SS. The proportion of patients with an improvement e1 point on overall PAC-QOL scores and on the satisfaction subscale were statistically significant higher for both the PRU 2 and 4 mg groups compared to the placebo group at weeks 4 and 12. This also holds for most of the other PAC-QOL subscales. After 12 weeks of treatment, the proportion for overall PAC-QOL was 36.5% on the recommended dose of PRU 2 mg compared to 18.6% on placebo. For the satisfaction subscale this was 44.0% on PRU 2 mg compared to 22.2% on placebo, representing an improvement in satisfaction of more than 20% after 12 weeks treatment.
Conclusions : Prucalopride alleviates symptoms in patients with severe chronic constipation and improves their satisfaction and constipation related QOL.

- D59 -

LONG-TERM OUTCOME OF TRANSCATHETER EMBOLOTHERAPY FOR ACUTE LOWER GASTROINTESTINAL HEMORRHAGE. G. Maleux, F. Roeflaer, S. Heye, J. Vandersmissen, A.S. Vliegen, I. Demedts, A. Wilmer. KULeuven.

Objectives : to assess the safety, short and long-term efficacy and durability of transcatheter embolization for lower gastrointestinal hemorrhage and to analyse the overall survival of the embolized patients.
Materials and methods : Between January 1997 and January 2008, 122 patients were referred for angiographic evaluation to control major lower gastrointestinal hemorrhage. 43 patients (35,3%) presented with angiographic signs of contrast extravasation. In 39 patients (26 men, 13 women, mean age 67.7 years) a transcatheter embolization was performed to stop the bleeding.
Results : In all 39 patients no contrast extravasation could be depicted on completion angiography after embolization. Rebleeding occurred in 8 patients (20%), in 6 of them within the first 30 days after embolization. Ischemic intestinal complications requiring surgery occurred in 4 patients (10%) within 24 hours after embolization. Long-term follow-up depicted an estimated survival after 1, 3 and 5 years of respectively 70.6%, 56.5% and 50.8%.
Conclusion : Transcatheter embolotherapy to treat lower gastrointestinal bleeding is very effective, with a relatively low rebleeding and ischemic complication rate, mostly occurring within the first month after the embolization. Long-term follow-up shows a very low late re-bleeding rate and half of the embolized patients survive more than 5 years.

- D60 -

LARGE SPECTRUM OF LIVER VASCULAR LESIONS INCLUDING HIGH PREVALENCE OF FOCAL NODULAR HYPERPLASIA IN PATIENTS WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA. THE BASL REGISTRY BASED ON 28 PATIENTS. R. Brenard (1), X. Chapaux (1), P. Deltenre (2), J. Henrion (2), S. De Maeght (2), Y. Horsmans (3), I. Borbath (3), A. Leenaerts (4), J. Van Cauter (4), S. Francque (5), T. Sersté (6), C. Moreno (7), P. Mengeot (1), J. Lerut (3), C. Sempoux (3). (1) Hôpital St Joseph Gilly ; (2) Hôpital de Jolimont, La Louvière ; (3) UCL Saint-Luc ; (4) CHU Charleroi ; (5) UZ Antwerp ; (6) CHU Saint Pierre ; (7) ULB Erasme.

Rendu Osler disease or hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by arteriovenous malformations, telangiectasia and aneurysms. The prevalence of patients with liver involvement ranges from 8 to 31% but raises to 79% when screening is performed in asymptomatic patients with suspected HHT. Under the aegis of the Belgian Association of the Study of the Liver Belgian, a registry of HHT patients and liver involvement was initiated in 2005, to evaluate the clinical, radiological and pathological presentation of these patients. We report the data of this registry based on 28 patients (18 women and 10 men). Twenty-one patients (75%) were asymptomatic. Within the 7 symptomatic patients (25%), 4 suffered from high output cardiac failure ; 2 died before liver transplantation and 1 was transplanted. Three patients developed symptomatic biliary disease ; 2 were transplanted and 1 was listed. Intrahepatic shunts and a large hepatic artery (6 to 14 mm, mean : 9.3 mm) were found in all patients and are characteristic of liver involvement. We observed a high prevalence (44%) of asymptomatic hepatic tumours with radiological and histological characteristics of focal nodular hyperplasia (FNH) for the majority of these tumours. The histological examination of the 3 explanted livers revealed the coexistence of a large spectrum of hepatic vascular lesions including intrahepatic shunts, FNH, nodular regenerative hyperplasia, sinusoidal dilatation and ischemic cholangiopathy. All of these lesions should be diagnosed early to avoid invasive procedures even if a liver biopsy was performed in 5 of our patients without complications. The liver biopsy led to the diagnosis of HHT in only 1 patient. In conclusion, liver involvement in HHT is characterized by a high prevalence of FNH and a large spectrum of vascular lesions such as intrahepatic shunts, nodular regenerative hyperplasia, sinusoidal dilatation and ischemic cholangiopathy which may coexist simultaneously in a same patient.

- D61 -

COMPARISON OF EPINEPHRINE INJECTION ALONE AND EPINEPHRINE ASSOCIATED WITH BIPOLAR COAGULATION IN HEMORRAGIC GASTRO-DUODENAL ULCER. A. Frère, R. Delrez, M. Lebas, B. Delhougne, M. Dartevelle, C. Gillard, F. Croës, D. Dresse, A. Denoël, C. Brixko, J. Deflandre. CHR Citadelle, Liège.

Background : Hemorragic gastro-duodenal ulcer (GDU) with active bleeding or with non-bleeding visible vessels may benefit from endoscopic treatment. Various endoscopic treatements have been used but few prospective comparative trials have been published. Some studies have shown that combination therapy using injection of epinephrine and thermo-coagulation is more efficient than single endoscopic injection or medical therapy.
Aims : To compare the efficacy and safety of epinephrine injection alone or asociated with contact bipolar coagulation in bleeding GDU, performed by an eight gastroenterologists team in the real live.
Methods : From august 1st-2004 to november 30- 2007, 128 patients with actively bleeding GDU or with non-bleeding visible vessels (Forrest 1A, 1B, 2A) were prospectively randomized to epinephrine injection alone (group 1) or associated with bipolar coagulation (group 2). All patients received perfusion of pantoprazole, 8 mg/h. after a bolus of 80 mg. Primary efficacy endpoints were rebleeding assessed by repeated endoscopy performed after 24 h and 72 h, clinical outcome, need of further endoscopic treatment and need of surgery. Secondary effcacy endpoints were total volume of blood transfusion and mortality rate.
Results : Initial hemostasis was succesful in all patients but 2, in each group. Therefore, endoscopic treatment failed in 12/65 (17,2%) in group 1 but in 9/63 (14,3%) in group 2. Eleven patients required a second endoscopic treatment, 2 patients required urgent surgery and 1radiological embolisation, in group 1. Nine patients, in group 2, required a second endoscopic treatment and only one required urgent surgery. The mean volume of blood transfusion was 826 (+ /- 882) ml, in group 1 and 784 (+ /- 809) ml, in goup 2. Nineteen patients died before being discharged : 10 in group 1, 9 in group 2. None of these results were stastically significant.
Conclusions : In our experience, combined endoscopic treament with epinephrine associated with bipolar coagulation can be used by a large endoscipal team in the real live without serious side effect. With this combined treament we observed a positive trend in terms ofneed of blood transfuson, rebleeding preventionand requirement of urgent radiological or surgical procedure.

- D62 -

COLONOSCOPY IN DAILY PRACTICE : A QUALITY ASSESSMENT STUDY. E. Vanderstraeten, P. Burvenich, K. Rasquin, E. Monsaert. Maria Middelares Gent.

In our department, a prospective quality assessment study was performed about colonoscopy. Between February and July 2007, data were collected from all consecutive colonoscopies, concerning epidemiology, indication, procedure and diagnostic value.
737 colonoscopies (of which 114 were combined with a gastroscopy during the same procedure) were performed by 5 endoscopists during this 6 months period. All patients were sedated with intravenous Propofol by the anaesthesiologistduring the endoscopic procedures. The two most common indications for colonoscopy were a change in bowel habits and RBPA (together 36.5% of all indications).
The caecum was reached in 97% of cases ; our polypectomy rate (number of patients with polypectomy / total number of colonoscopies) was 30% (94 non-adenomatous polyps ; 215 adenomas with low grade dysplasia ; 29 adenomas with high grade dysplasia or carcinoma in situ) ; 35 colorectal carcinomas were detected. Our median “caecum to rectum time” including time for polypectomy was 5 minutes (range 1-30 minutes ; 51% of all cases above the target of 6 minutes). We had 5 postpolypectomy bleedings (0.6%) of which 1 patient needed operation, and 1 perforation (0.14%). Microscopic colitis was detected in 21% of cases with chronic diarrhea.
We found no correlation between the “appropriateness of colonoscopy” by EPAGE (European Panel of Appropriateness of Gastro-intestinal Endoscopy) and diagnostic value, nor between indication by a gastro-enterologist versus a general practitioner and diagnostic value of the endoscopic procedure.
As a consequence of this quality study, we will closely follow up our complication rate, and further focus on our “caecum to rectum time”, standardize our protocol for describing polyps and endoscopic procedure, and facilitate the capturing of endoscopic pictures.

- D63 -

FECAL TRANSPLANTATION FOR RECURRENT CLOSTRIDIUM DIFFICILE COLITIS, AN UNDERUSED TREATMENT MODALITY. S. Naegels, I. Ruytjens, L. Terriere, G. Blinder, J. Holvoet, R. Hellemans. Middelheim Hospital ZNA Antwerp.

Fecal bacteriotherapy (the administration of feces from a healthy donor through naso-enteric tube, retention enema or colonoscopy) has been used in the treatment of recurrent or fulminant Clostridium difficile colitis. However much prejudice still exists about this treatment.
We successfully treated a 59-year-old woman who presented with four episodes of clinically and endoscopically severe Clostridium difficile colitis with a fecal transplant from a first degree relative (her brother). Previous episodes were treated with vancomycin (first attack) and vancomycin tapering/pulsed scheme (second and third episode). Each time only a few days after stopping treatment the patient relapsed. After seven days of oral vancomycin 4×500 mg/day for the fourth episode her symptoms had only improved moderately. The patient then consented to a fecal transplant. Vancomycin was stopped the day before fecal bacteriotherapy. Her brother was screened for transmittable diseases and donated stools on five consecutive days. The material was immediately mixed with + /-300 cc normal saline and then centrifugated. The first day she received a polyethylene glycol bowel prep after which the supernatant of the fecal material was instilled in the terminal ileum and throughout the colon through the channel of a colonoscope. The four following days colonoscopy was repeated without bowel prep and supernatant was infused into the right colon. Within 24 hours the patient felt better with disappearance of her diarrhea and at endoscopy there was marked improvement of the colitis with visible formation of solid stools and almost complete normalisation of the colonic mucosa on day 5 (on day 1 there were still extensive areas of colitis with focal ulcers and erythema). The patient was discharged on day 6 and is still without symptoms after 4 months (while before she always relapsed within one week of stopping vancomycin). Stool cultures and toxin assay for Clostridium were negative on week 1-2-3-4-5-9 of follow up.
To our knowledge no other cases of successful fecal transplantation for recurrent Clostridium colitis have been reported in Belgium. However we strongly believe this treatment modality should be seriously considered when one is confronted with a second or third recurrence or in the case of fulminant colitis resistant to metronidazole and vancomycin as a last alternative before colectomy. Our case also suggests that administrating the supernatant after centrifugation of the fecal material can be sufficient.

- D64 -

ENDOSCOPIC REMOVAL OF DYSFUNCTIONNING RINGS OR BANDS AFTER VERTICAL BANDED GASTROPLASTY. D. Blero (1), P. Eisendrath (2), A. Vandermeeren (3), O. Le Moine (4), J. Devière (5). ULB Erasme.

Background : Intragastric band migration or dysfunction are common long-term complications of both vertical banded gastroplasty (VBG) and adjustable gastric banding (Lap-Band) which classically require surgical treatment.
Objective : In this series, we describe the endoscopic removal of partially eroded lap-bands or silastic rings as well as non eroded dysfunctionning rings after VBG.
Design : Case series.
Setting : A tertiary-care European academic center.
Patients : 13 patients were treated, 3 patients with eroded Lap-Band, 3 with eroded silastic rings and 7 patients with refractory outlet stoma stenosis after VBG.
Interventions : Endoscopic removal was performed within one or two sessions, according to the presence and extent of band erosion at presentation, including optional placement of a self expandable plastic stent (SEPS) across the band, followed about one month later by (stent and) and extraction with transsection if needed using a wire-cutting system.
Results : No complication was noted. A failure was due to huge adhesions formation around a Lap-band on the lesser curve of the stomach and the left liver lobe. 12/13 endoscopic removals were successful in 1 (n = 2) and 2 (n = 10) sessions.
Conclusions : Endoscopic removal of dysfunctionning bands or rings is safe and feasible by the use of a one or two steps endoscopic procedure.

- D65 -

MULTICHANNEL INTRALUMINAL IMPEDANCE PH-MONITORING IN THE MANAGEMENT OF GLOBUS PHARYNGEUS. S. Vanden Branden (1), B. Roosens (1), J. Hulstaert (2), S. Vandebosch (1), D. Urbain (1). (1) UZ Brussel ; (2) AZ Jan Portaels Vilvoorde.

Introduction : Globus is defined as a physical sensation of a lump in the throat. It is a common symptom that often prompts consultation with an otolaryngologist or a gastro-enterologist. The exact etiology is unknown but recent literature gives a great attention to reflux as a possible cause. Globus often persists despite proton pump inhibitor (PPI) therapy. The role of nonacid reflux has never been evaluated. Ambulatory 24 h multichannel esophageal impedance pH-monitoring (pH/MII) allows to detect acid and weakly-acid reflux events, as well as alkaline reflux.This technology also improves diagnostic accuracy for describing upper esophageal reflux events, bolus clearance time and acid clearance time. Our goal was to use pH/MII to determine reflux characteristics in patients with globus off PPI therapy at different levels of the esophagus and to evaluate reflux-symptom correlation.
Methods : Eighteen adults with globus symptoms without heartburn underwent pH/MII off PPI therapy. Sensors were placed 5 cm above lower esophageal sphincter (LES), 15 cm above LES and at the upper esophageal sphincter (UES). The symptom index and symptom sensitivity index were calculated.
Results : We detected in the group of patients a total of 980 reflux episodes at the distal probe, 415 reflux episodes at 15 cm and 50 reflux episodes reached at the UES.Of these episodes, 37% were acid, 60% were weakly-acidic, 3% were alkaline. Of all the globus symptoms, 35% are related to reflux detected by pH/MII, mostly weakly acidic.
Conclusions : In adults with globus symptoms off PPI therapy, weakly acidic refluxes are detected in about one third of the cases. However, we infrequently detected reflux episodes reaching the UES suggesting that other mechanisms play a role in globus symptoms.

- D66 -

THE IMPACT OF PREOPERATIVE HEPATIC HYDROTHORAX ON THE OUTCOME OF ADULT LIVER TRANSPLANTATION. T. Serste (1), C. Moreno (2), C. Francoz (3), W. Abdel Razek (3), C. Paugam (3), J. Belghiti (3), F. Durand (3). (1) ULB Saint Pierre ; (2) ULB Erasme ; (3) Hôpital Beaujon, Clichy, France.

Introduction : Hepatic hydrothorax is an uncommon complication of end-stage liver disease. It is generally admitted that orthotopic liver transplantation (OLT) is the best treatment option in this situation. The impact of preoperative hepatic hydrothorax on post transplant course has not been clearly investigated.
Patients and methods : We retrospectively reviewed 273 adult patients who underwent OLT between January 2002 and December 2005. Eleven patients with preoperative hepatic hydrothorax were identified (Group 1). Pretransplant data, postoperative complications and survival were compared with two control groups of 11 patients each, matched for age, gender, year of transplant and severity of cirrhosis : Group 2 included patients with tensed ascites but no hepatic hydrothorax. Group 3 included patients without ascites.
Results : Pretransplant patient's characteristics were not different between the 3 groups, except for the presence of tense ascites (absent in Group 3). No significant differences in the duration of mechanical ventilation, intensive care unit (ICU) stay and in-hospital stay were observed between the 3 groups. Neither there were significant differences in terms of incidence of sepsis and early post operative death. However, the cause of death was different. Three patients in the Group 1 died due to infectious pneumonia, while none of the patients in Groups 2 and 3 died from this complication.
Conclusions : Liver transplantation is a good therapeutic option for cirrhotic patients with hepatic hydrothorax. The presence of preoperative hepatic hydrothorax does not imply more postoperative complications. However, the outcome of postoperative pneumonia seems to be potentially worse than in patients without hepatic hydrothorax.

- D67 -

MARGINAL ULCER AFTER ROUX-EN-Y GASTRIC BYPASS. B. Strubbe (1), M. Cabooter (1), B. Dillemans (1). (1) AZ St-Jan Bruges.

Background/Aim : A marginal ulcer is a well-known complication after Roux-en-Y gastric bypass (RYGB). The aim of this retrospective, non-randomized study was to assess the incidence and the outcome of marginal ulcers after RYGB.
Methods : Between January 2005 and May 2007, 1,405 patients underwent a RYGB. 1,105 patients were included in the study and the follow-up ranged from 3 to 31months postoperatively. A standard preoperative endoscopy or Helicobacter pylori testing was not systematically carried out.
Results : In 92 (8.3%) of 1,104 patients in whom a RYGB was performed, an endoscopy was achieved based on upper gastro-intestinal symptoms. In 54 (4.9%) patients a marginal ulcer was diagnosed. There was a complicated ulcer in 14 patients (26%) (2 perforations, 5 bleedings, 7 stenoses), 6 of them (11%) requiring surgical operation (2 perforations, 1 bleeding, 3 refractory symptoms). After healing of the ulcer under high dose of PPI, still 11 patients (20%) had a recurrence after mean follow up of 15.6months.
Conclusions : There was an incidence of 4.9% of marginal ulcer after RYGB. We conclude that marginal ulcer after RYGB represents a clinical important problem, because of the high incidence, the complexity, and the tendency to recur. A vigorous treatment with high-dose PPI is required. Although the pathogenetic role of Helicobacter pylori infection is still unknown, preoperative detection and treatment is advised.

- D68 -

SHORT AND MID-TERM FOLLOW UP OF 10 PATIENTS WITH EOSINOPHILIC OESOPHAGITIS. A CLINICAL, HISTOLOGICAL AND ENDOSCOPIC STUDY. J.-F. Rahier (1), J.-P. Martinet (1), A. Badaoui (1), A. Bourgeois (2), O. Borgniet (3), M. Delos (1), T. De Ronde (1). (1) UCL Mont Godinne ; (2) Clinique Saint Luc Bouge ; (3) CHR Dinant.

Background : Eosinophilic esophagitis is a clinicopathological entity predominantly found in adults presenting with upper gastrointestinal (GI) symptoms, mainly dysphagia, food bolus impaction and GERD-like symptoms refractory to medical treatment. Various endoscopic features have been described. A dense eosinophilic infiltration of the esophageal epithelium (> 20 eos/HPF) is the histological sign required for diagnosis. Topical fluticasone is the standard treatment. Little is known about the natural history of the disease.
Aims : The aim of the study was to assess the clinical, histological and endoscopic evolution of the disease.
Methods : We included 10 adult patients with eosinophilic esophagitis (7 men and 3 women ; mean age 40 years) whose diagnosis had been made more than 3 months before study. Patients had been treated with topical corticosteroids (n = 3), proton pump inhibitors (PPIs) (n = 3) or both (n = 3).One patient remained untreated. After a mean of 27 months (range 3-108), patients underwent a comprehensive examination including a questionnaire (n = 10) and an endoscopic evaluation with proximal and distal esophageal biopsies (n = 9).
Results : Corticosteroids were well tolerated and dramatically improved quality of life in 5 patients. Treatment was unsuccessful and early abandoned in 1 patient. Cessation of corticosteroids was associated with recurrence of upper GI symptoms in 5 patients with a highly variable delay (range 2-400 weeks). In this group, the use of PPI did not affect the frequency and severity of symptoms. Among patients treated with PPI alone (n = 3), 2 remained long term treated and asymptomatic. In one third of patients (3/9), endoscopic features were identical to previous findings. Eosinophilic epithelial infiltration persisted in 7 patients, most of them (6/7) being symptomatic. Patients with normal histology (n = 2) had no symptoms. During follow up, none of the patients took medical advice despite symptoms. One patient self-treated with a second course of corticosteroids and improved rapidly.
Conclusion : Eosinophilic esophagitis is a chronic inflammatory disorder of the esophagus. In our cohort, (1) recurrence of upper GI symptoms was frequent, (2) macroscopic aspects of the esophagus was modified in 2/3, (3) histological abnormalities persisted in most patients and (4) patients seemed to be undertreated.

- D69 -

MDM2 AMPLIFICATION IN COLORECTAL CARCINOMAS. S. Laurent, P. Pattyn, M. Peeters, P. Pauwels. UZ Gent.

p53 is mutated in 40% of colorectal cancers, demonstrating the crucial role of p53 in tumor suppression. Importantly, p53 inactivation can also result from the amplification/overexpression of its inhibitor MDM2. We studied MDM2 amplification in colon cancer.
MDM2 oncoprotein is an E3 ubiquitin ligase required to maintain the p53 protein at low levels.
In the literature, no much is known about a potential role of MDM2 in colorectal cancers. In fact, a paper in Nature Review Cancer (2006) cited that no MDM2 amplification could be found in colorectal cancers.
In our study, we examined 10 colon cancers for MDM2 amplification by Fluorescence In Situ Hybridisation (FISH). 5 cases clearly showed amplification, with sometimes cluster formation. These findings can have important therapeutic consequences, since MDM2 antagonists are in development (Nutlin 3a etc.)
In conclusion : MDM2 amplification clearly exists in colorectal cancers. This finding can have therapeutic potential.

- D70 -

LAPAROSCOPIC MANAGEMENT OF COLONOSCOPIC PERFORATIONS. L. Bouffioux (1), C. Coimbra (1), A.C. Lespagnard (1), D. Dresse (2), O. Detry (1), A. De Roover (1), P. Honore (1), J. Belaiche (1), A. Denoel (2), J. Deflandre (2). (1) ULg Sart Tilman ; (2) CHR CITADELLE, Liège.

Background : The gold standard surgical treatment of colonoscopy perforations remains laparotomy with or without ostomy. Laparoscopic management is a recent approach, only described in small series.
Objective : We hypothesised that laparoscopic treatment of iatrogenic colon perforation would result in equal therapeutic efficacy, less morbidity, decreased length of stay, and overall better short-term outcome compared to open methods.
Methods : We retrospectively reviewed the records of patients with iatrogenic colonoscopic perforations between 1980 and 2008 in two different centers. The patients' demographic data, perforation location, therapy and outcome were recorded.
Results : A total of 43 iatrogenic perforations were identified in 22 men and 21 women (median age : 66.5 y). All but one were managed operatively (19 laparoscopy, 23 laparotomy). The sigmoid colon was the most frequent site of perforation (65.1%). Patients underwent primary repair (52.4%), resection with primary anastomosis (26.2%), or fecal diversion (21.4%). Patients diagnosed within 24 hours (76,2%) were more likely to have minimal peritoneal contamination (30 patients vs 2 ; $P = 0.01$) and to undergo a laparoscopic approach with primary repair or resection with anastomosis. Patients diagnosed after 24 hours (23,8%) were more likely to have fecal contamination or purulent peritonitis (8 patients vs 3 ; $P = 0.01$) and to undergo ostomy by laparotomy. Global morbidity and mortality were 31% and 7.1%, respectively. Three of the laparoscopic procedures had to be converted in laparotomy because of the length of the injury (1 case) and the fragility of the tissues (2 cases). Overall patients who underwent laparoscopic repair had shorter length of stay (mean 10.18 vs 16,65 days ; $P = 0.01$), lower morbidity (12.5% versus 43.4%, $P = 0.02$) and mortality (0 patient vs 2). Postoperative complications were associated with older age, corticosteroid use and delay between perforation and surgery.
Conclusions : Laparoscopic repair of iatrogenic colonic perforations in experienced hands is a viable alternative to the open approach, and may reduce morbidity.

- D71 -

UPPER GASTROINTESTINAL ENDOSCOPY AND HELICOBACTER PYLORI IN HIV INFECTED PATIENTS : A PROSPECTIVE STUDY. M. Nkuize, S. Dewit, V. Muls, R.S. Ntounda, A. Sarafidis, J.P. Mulkay, H. Tibary, M. Buset. ULB Saint-Pierre.

Aim : More than 90% of HIV infected patients develop upper gastrointestinal (UGI) symptoms. Various diseases are involved, including Helicobacter pylori (Hp) infection. We aimed to evaluate : firstly the prevalence of Hp infection among all HIV infected patients who undergo UGI endoscopy, and secondly endoscopic observations according to CD4 count and Hp status.
Methods : In a prospective database, we included any HIV infected patient who underwent UGI endoscopy for the first time from January 2004 until December 2007. The parameters studied are : demographics, immunity and viral load, endoscopic and histological observations.
Results : Among 159 patients included, 42 (26.41%) were Hp positive. We considered a CD4 threshold of more or less than 200 cells/mm^3. When CD4 > 200, we observed a statistically significantly higher rate of Hp infection ($p = 0.029$), normal UGI endoscopy ($p = 0.03$), histological reflux oesophagitis ($p = 0.0002$), and a significantly lower rate of oesophageal candidasis ($p < 0.001$) and use of opportunistic prophylaxis ($p = 0.0001$). There was no statistically significant difference in gender, tobacco habits, alcohol intake, antiretroviral therapy, endoscopic reflux oesophagitis, gastric and duodenal ulcer. HIV-Hp co infection is associated with duodenal ulcer ($p = 0.01$; OR 9.341 (1.626-53.672) and female gender ($p = 0.04$; OR 0.393 (0.161-0.959).
Conclusion : The prevalence of HP among HIV infected patients undergoing UGI endoscopy is around 26.5% in our population. The prevalence of HP infection and histological reflux oesophagitis may be increased when immunity is improved. Our results show that HIV-HP co infection is associated with duodenal ulcer and perhaps with female gender.

- D72 -

PREVALENCE OF SCHISTOSOMA MANSONI INFECTION AMONG PATIENTS VISTING AGARO HEALTH CENTRE, JIMMA ZONE, SOUTH WEST ETHIOPA. Z. Mekonnen (1), Y. Hussen (1), S. Suleman (1), V.M. Eric (2), S. Chatterjee (2). (1) Jimma University, Ethiopia ; (2) U Antwerp, Belgium.

There is a long history of research and control in the field of liver schistosomiasis that has resulted in major scientific and public health accomplishments. They include the role of liver immunologic regulation in chronic infections, the association of helminth infections with Th1-regulating Th2-type immune responses and the critical role of interleukin-13 in liver fibrogenesis.
S.mansoni infection rates of above 60% in Ethiopian school children have been reported from highly endemic communities from Tigray, and from a number of other communities in different part of Ethiopia. At Agaro teaching health centre, Jimma zone, Ethiopia, a lab based cross sectional study using formal-ether concentration technique was carried out in April 2007, in which from a sample size of 113 persons, the overall prevalence of infection was 12.38% with 24.8% women and 9.6% men patients. At the same time, four different species of soil transmitted helimnthis namely Tricuris tricuria (24.6%), Hookworm (19.3%), Ascaris lumbricoid (17.4%)and H.nana (8.8%) were identified. Similar study conducted in Jimma specialized hospital (2006), indicated 15.27% prevalence of S.mansoni infections in 203 patients. The most affected age group in this studywas 11-15 years, and female patients appearedaffected more.
A number of cases of liver cancer, colorectal, giant follicular lymphoma and some other cancers have been reported in association with schistosomiasis infections. A coordinated effort that combines epidemiological, malacological surveys, parasitological and liver ultrasonographic examinations shall be implemented to determine the magnitude of morbidity and severity of this diseases and to take appropriate measure against its control. This work was supported by research and publication office of Jimma University, Jimma, Ethiopia.

- D73 -

TO EVALUATE THE EFFECTIVENESS OF THE FLAVONOID FRACTION OF MICRONISED IN PATIENTS WITH HEMORRHOIDAL DISEASE UNDERGOING INSTRUMENTAL THERAPY. C. Quintela, M.J. Bettencourt, J. Barbosa, A. David Marques. CHLC – HSAC.

Introduction : The hemorrhoidal disease can manifest itself, most often by rectal bleeding and / or prolapse. The piles are to be classified grade I to IV. Instrumental therapy instrumental is applied grade II and III and the more frequent complications are pain, rectal bleeding, burning and itching. The fraction of flavonoid micronised (FFM) as 90% of micronized diosmin and 10% of flavonoids (hesperidin) that improves the tone venous and lymphatic drainage, reduces permeability protecting the capillary microcirculatory of inflammatory processes.
Aim : The main objective of this study is to understand whether there is the advantage of associating FFM instrumental to the therapy. Evaluate the efficacy and safety of therapy associated with FFM .
Methods : The prospective study included 108 patients (n = 108) with hemorrhoidal disease, 78 men and 30 women, 66 with grade II and 42 Grade III. They were treated instrumentally (elastic band and polidocanol 1% - 0.5 cc in the pedicle). Group 1 with FFM, 3 g/d (3 days) and 1.5 g/d for 1 month (n = 54). Group 2 without FFM (n = 54). The groups were evaluated at 2, 4, 8.12 weeks and the signs and symptoms evaluated on a scale of 1 to 4 (1 – absent and 4 – severe). We excluded patients who were immunosuppressive therapy, oral anticoagulants, aspirin, other NSAIDs and antiplatelet with diabetes, coagulopathy, chronic liver disease and heart disease, local infection, immunodeficiency, inflammatory bowel disease, pregnant.
Results : Group 1 there were 39 men and 15 women ; 30 with grade II and 24 Grade III. In that group, 3 patients had complications (5.5%) – prolapse and bleeding. In group 2, 39 men and 15 women, 36 with grade II and 18 grade III. Within this group, 10 patients had complications (18.5%) – prolapse, bleeding and pain.
Conclusions : The FFM with the instrumental therapy reduces the complications of around 13%. For this reason, we believe that there is a great benefit to associate with the usual therapy.

- D74 -

TOPICAL DILTIAZEM AS TREATMENT OF CHOISE TO ANAL FISSURE. C. Quintela, M.J. Bettencourt, A. David Marques. CHLC – HSAC.

Introduction : The anal fissure is a wound burden. The poor irrigation local as well as the contracture perpetuates the process. The therapeutic effective has past by obtaining the relaxation of sphincter either by a mechanical or chemical process.
Aim : Evaluate the effectiveness of topical diltiazem under healing and symptomatic relief in anal fissure
Methods : Open prospective study. Were included 138 patients, 60 males and 78 female with anal fissure at 6 or 12 h, aged between 22 and 70 years, with average age of 42. 6 years. Patients presenting constipation were treated with fibres having been excluded those who didn´t improve. Were excluded under 18, pregnant women, with inflammatory bowel disease, prior anal surgery, allergic to diltiazem, with diarrhoea. Also excluded were cases where there was suspected of infection and / or Neoplasia. The diltiazem was applied in the form of gel merging 2%, having been used as the KY excipient® gel. Gel was applied by digitally in morning and night from 8 to 12 weeks. Checks were made in accordance with the analogue linear scale to 0,2, 4, 8 and 12 weeks of therapy. The symptoms were classified on a scale of the 4. The healing was evaluated at 8 and 12 weeks. Were posted the effects of toxico. All patients had pain level 4 in accordance with the score.
Results : All patients completed the treatment effects accessories. In relation to pain, to 2 weeks 66 patients (47.8%) had zero score, 36 (26%) had score 1 and the other (26.2%) score 2. To 4 weeks 102 (74%) had score 0, 12 (8.7%) score 2 and 24 (17.3%) score 3. The rectal bleeding disappeared to 4 weeks in 114 patients (82.6%). 114 (74%) patients had healing of fissure to 4 weeks. At 8 weeks 24 (17.3%) patients retained fissure with score 2 pain. From these 6 (4.3%) were operated and 18 (13%) were subjected to toxin botulinum injection.
Conclusion : The diltiazem under topical in the concentrations proposals is effective and safe in therapeutic of anal fissure chronic symptomatic. Is an important weapon therapy as first option in the approach of the patient with this type of disease.

- D75 -

RADIOFREQUENCY ABLATION VERSUS RESECTION FOR RESECTABLE COLORECTAL LIVER METASTASES : TIME FOR A RANDOMISED TRIAL ? AN UPDATE. S. Mulier (1), T. Ruers (2), J. Jamart (3), L. Michel (3), G. Marchal (4), Y. Ni (4). (1) CHIREC, Brussels ; (2) Antoni van Leeuwenhoek Hospital, Amsterdam ; (3) University Hospital Mont-Godinne, (4) KULeuven Gasthuisberg.

Background : Resection is the gold standard for the treatment of resectable colorectal metastases. Nevertheless, many centers are currently performing RFA for this controversial indication. The aim of the study was to update and critically review the oncological evidence in favour of and against the use of RFA for resectable CRLM in general and in favour of and against conducting a randomised trial in a selected subgroup of patients.
Methods : An exhaustive review was carried out of publications up till July 15th 2008.
Results : Local recurrence rate after resection of CRLM is 1.2%-10.4%. Local recurrence rate after open RFA (laparotomy) of CRLM Ł 3 cm is 2%. Local recurrence rates however are much higher (up till 66.7%)for larger tumours and for the percutaneous and laparoscopic route. RFA and resection induce profoundly different biological effects, which may influence survival.
Conclusions : Local recurrence rate after open RFA for CRLM < 3 cm seems to be equivalent to resection. A randomised trialis justified in this subgroup of patients. A randomised trial is currently not justified for larger tumours nor for percutaneous or laparoscopic RFA, since local recurrence rates in these groups are too high.

- D76 -

UTILIZATION OF HERBAL ANTIMALARIAL REMEDIES AS ALTERNATIVE AMONG ASSENDABO INHABITANTS (SOUTHWEST ETHIOPIA). S. Suleman (1), Z. Mekonnen (1), G. Tilahun (1), E. Van Marck (2), S. Chatterjee (2). (1) Jimma University, Ethiopia, (2) UA Antwerp, Belgium.

Malaria affects 300-500 million people each year, resulting in over 1 million deaths. Particularly, falciparum malaria mimics other diseases with its varied presentations that should be considered during the differential diagnosis of acute illnesses, like hepatorenal syndrome, hepatic failure, and acute hepatitis. Malaria involvement of different organs and tissues/cells is well known with its specific histopathological changes. Thus, effective antimalarial therapy should be in place not only to reduce mortality and morbidity of malaria, but also to reduce the risk of parasite resistance to the drugs. The currently used drugs often present adverse side effects as their metabolism involves the liver.Yet not many new drugs have been developed to tackle malaria, and alternative modes of malaria treatment should be assessed and included in the national malaria treatment guidelines.
To assess the current utilization of traditional herbal medicines in the management of malaria among inhabitants of Assendabo town (Jimma, Ethiopia), a cross-sectional study was done on randomly selected inhabitants of Assendabo town and its surrounding villages using structured questionnaires.
Among the 300 respondents interviewed, 86 (28.7%) reported perceived illness within two weeks of recall of which 41 (47.7%) used traditional medicine and home made remedies. This study has documented 13 different types of plants traditionally used to treat malaria. *Ajuga remota (*Armagusa) and *Moringa olifera* (birbira) were among the plants and different literatures support the findings that the documented medicinal plants have active principles against Plasmodium species.
Traditional herbal remedies could be treatment alternatives if supported by scientific evidences and standardized as to the way they are utilized in the community. However, they might bring about serious toxicities leading to organ damages unless controlled and integrated into clinical medicine. Although this sstudy has documented considerable proportion of herbal remedies, the authors would like to recommend further studies like *in vitro* and *in vivo* activity tests, clinical trails and organ toxicities (e.g. liver) of the herbal drugs.

- D77 -

LESS IS MORE : FUTURE OR STEPSTONE TO NOTES ? K. Fierens, K. Van Renterghem, D. Van De putte, W. Ceelen, Y. Van Nieuwenhove, P. Pattyn. UZ Gent.

LESS (LaparoEndoscopic Single Site) surgery is anacronymreferring to laparoscopic surgery through one single incision. Together with NOTES (Natural Orifice Transluminal Endoscopic Surgery) it is the next step in minimally invasive surgery. While NOTES offers the possibility of scarless surgery without any extra abdominal incision, these types of procedures are not yet commonly accepted because of the unfamiliarity of most surgeons with flexible endoscopes and most gastroenterologists with intraperitoneal anatomy. At this moment there is a need for the appropriate tools to offer maximal safety for NOTES procedures. The procedures used in LESS surgery are similar tocommonlaparoscopic interventions and can be considered as a stepstone towards less invasive surgery or even NOTES. We present a video of a delayed appendectomy, performed through a single incision using a Triport™ device.Small series ofadvanced urological and abdominal operations, have already been published on this subject, indicating its technical feasibility. The actual benefits of LESS however will need to be investigated in clinical studies.

- D78 -

UNUSUAL EVOLUTION OF A REFRACTORY CELIAC DISEASE, TYPE 1, AFTER 18 YEARS OF GLUTEN-FREE DIET. CASE REPORT AND REVIEW OF THE LITERATURE. P. Gruselle (1), P. Ooghe (1), P. Delree (2), N. Nagy (1). (1) Hopital Vesale Montigny Le Tilleul ; (2) IPG Gosselies.

Case report : the diagnosis of celiac disease (CD) is made in 1988 in a 49 years old lady with longstanding history of severe diarrhea, sideropenic anemia (hemoglobine 9.7) and weight loss ; with total villous atrophy of duodenal mucosa, crypt hypertrophy and lymphoplasmacytic infiltrate of the chorion. Steatorrhea : 44.1 gr/day ; serum antigliadine antibody : + 1/80 ; calcemia : 7.4mg/ dl. Coloscopic biopsies reveals a collagenous colitis.
A gluten-free diet provides a quick clinical and biological response and negativation of antigliadine antibody ; but with fast reappearance of diarrhea with steatorrhea. Two years later, endoscopy shows always a atrophic-looking duodenal mucosa with subtotal atrophy ; despite a full compliance to the gluten-free diet and negative celiac serology ; with no sign of pancreatic insufficiency, lactose intolerance, intestinal bacterial overgrowth&The colonic biopsies shows a normalised sub-epithelial collagen layer.
Evolution : persistance of diarrhea with steatorrhea ; subtotal duodenal mucosal atrophy after nine years of treatment (1997) – severe fluctuant neutropenia (Nadir : 392/microliter) with final diagnosis, after full evaluation, of Paroxysmal Nocturnal Hemoglobinuria (MIRL and DAF tests).
After 18 years of evolution : suspicion of EATL or enteropathy -associated T-cell lymphoma (loss of weight, asthenia, severed diarrhea) not confirmed (duodenal biopsy almost normalised, no aberrant T-cell population, no clonal TCR gene rearrangement _ no malignancy at abdominal scanner and small bowel enteroclysis). At this time, ileo-coloscopy shows a total VILLOUS ATROPHY of ileal mucosa with excess of IEL (40%) .
A treatment with ENTOCORT (Budesonide) 9 mg/ day gives a impressive result with immediate cessation of the diarrhea and a gain of 8 kgs. A control with ileoscopy and capsule endoscopy is provided in the future.
Discussion : this very unusual case (the first one, to the best of our knowledge*) illustrates a very long evolution of refractory celiac disease type 1, with very late recovery of the duodenal mucosa but persistance of total ileal mucosal atrophy, with no demonstrated occult lymphoma ; and finally clinical response to Budesonide.
(*no prior history of CD and no biopsies in the case n° 7 of Muhammad A., *J. Clin. Gastroenterol.*, 2008, 42 : 980-983).

- D79 -

A RARE CASE OF NEONATAL CHOLESTASIS WITH NORMAL GAMMA GT. B. De Muynck (1), J. Merckx (1), P. Witters (1), M. Lodeweyckx (2), D. Cassiman (1), J. Breckpot (1), T. Roskams (1), M.A. Morren (1), I. Hoffman (1). (1) KULeuven.

A 6 week-old Moroccan girl was referred because of prolonged neonatal cholestasis. The parents originate from two neighbouring villages, but frank consanguinity was excluded. There was no history of neonatal cholestasis in either family. The patient has 2 older sibs. She had a normal newborn screening result.
On clinical examination there was an extremely dry, jaundiced skin and scalp hypotrichosis without dysmorphy or hepatosplenomegaly.
On laboratory analysis, there was conjugated hyperbilirubinemia (6.22 mg/dL(nl < 1.0)), raised transaminase levels (AST 635 U/L(nl < 32), ALT 698 U/L(nl < 31), high serum bile acids (126 μmol/L(nl < 6)) with normal gamma glutamyl transferases (GGT 59 μmol/L(nl < 218)), normal albumin levels and prothrombin time. An obstructive cause of cholestasis, neonatal infections, viral hepatitis, a broad range of metabolic and endocrine diseases were excluded.
A liver biopsy showed a panlobular chronic cholestasis with delayed maturation and irregular bile ducts without giant cell hepatitis. Immunohistochemistry revealed cytoplasmic and canalicular staining of the bile salt export pump as seen in chronic cholestasis .On electron-microscopy there was cholestatic parenchyma without Byler bile or other pathognomonic abnormalities.
This work-up virtually excluded traditional causes of neonatal cholestasis with raised GGT and those with normal GGT : progressive familial intrahepatic cholestasis type 1 (PFIC 1 : no giant cell hepatitis, no Byler bile), PFIC2 (BSEP staining) and bile acid synthesis defects (measurable bile acid levels, no giant cell hepatitis).
The association of Neonatal Ichthyosis, Sclerosing CHolangitis (NISCH syndrome) and alopecia was first described in 2002 by Baala *et al*. It is caused by a loss of function mutation in the gene encoding the tight junction protein claudin-1. Tight junctions form a physical barrier that separates bile from plasma in the liver and is important in normal skin. DNA sequencing in our patient revealed a homozygous deletion in exon 1 of the CLDN1 gene (200-201 delTT), which confirms the diagnosis of the NISCH syndrome.
Currently only five patients are reported, of which four in two consanguineous marriages of Moroccan origin with the same mutation as found in our patient. Variable penetrance of hepatic disease in the NISCH syndrome has already been shown, ranging from total regression of cholestasis to liver failure necessitating liver transplantation.
Supportive treatment was started with ursodeoxycholic acid, rifampicin, fat-soluble vitamins and skin ointments.
On follow-up there was the persistence of the ichthyotic state, whereas the cholestasis regressed to normal at the age of 9 months. The girl is thriving well, with weigth and length on the 75th percentile and normal psychomotor development.

- D80 -

INFLAMMATORY SYNDROME ASSOCIATED WITH A LIVER MASS. S. Vanden Branden (1), D. Urbain (1), K. Vanhove (1), A. Hoorens (1), T. Roskams (2), H. Reynaert (1). (1) UZ Brussel ; (2) KULeuven.

A 27-year-old Caucasian woman presented with episodes of fever and severe pain in the right hemi-abdomen. Two months earlier, she was admitted to the hospital with the diagnosis of a community acquired pneumonia. She had no other medical history. The patient had been on oral contraceptives for the past 12 years, but did not take any other medication. On physical examination there was tenderness of the right upper quadrant. She had a fever of 38°C, but the remainder of the clinical examination was normal. Laboratory tests showed an inflammatory syndrome including CRP : 150,8 mg/dl ; fibrinogen : 1012 mg/dl ; ERS : 120 mm/h and leukocytosis : 12000/mm^3. Liver function tests revealed high levels of alkaline phosphatase (184 U/l) and ^{3}-glutamyltransferse (73 U/l). Bilirubin, ALT and AST were normal. PET-FDG scan showed a pathological hot spot in the right liver lobe. Therefore, liver imaging studies were performed. Ultrasound demonstrated a large nodule and three additional smaller nodules in the right liver lobe. MRI revealed a large nodule in segment 6 (38 mm across) and 6 additional subcentrimetic nodules in the right lobe. A guided liver biopsy was performed and the diagnosis of an inflammatory telangiectatic adenoma was made. A partial hepatectomy of segment 5 and 6 was performed. Recovery was uneventful. After surgical treatment the inflammatory syndrome disappeared and liver function tests normalized. This has been demonstrated previously after surgical removal of inflammatory telangiectatic adenomas. Definitive pathological examination confirmed the diagnosis of an inflammatory telangiectatic adenoma. Moreover, on the resection specimen multiple small steatotic adenomata were identified. Recently, a comprehensive analysis using histological and immunohistochemical features enabled the identification of 4 hepatocellular adenoma subtypes : steatotic adenomas with mutation of the HNF1 ± gene, non-steatotic adenomas with mutation of the b catenin gene and adenomas without either mutation, constituted with or without inflammatory lesions. Using this classification, our patient had an inflammatory telangiectatic adenoma as well as multiple steatotic adenomas.
In conclusion, we present a very rare case of a systemic inflammatory syndrome caused by an inflammatory telangiectatic adenoma, successfully treated with surgery.

- D81 -

THORACOLAPAROSCOPIC IVOR LEWIS ESOPHAGOGASTRECTOMY WITH A HANDSEWN ANASTOMOSIS IN PRONE POSITION. G.B. Cadière (1), A. Rajan (2), G. Dapri (1), L. Fodderie (2), J. Himpens (1). (1) ULB Saint-Pierre ; (2) CHIREC, Brussels.

Background : With increasing enthusiasm for minimally invasive esophagectomy, a laparoscopic and thoracoscopic Ivor Lewis esophagogastrectomy with intrathoracic anastomosis is performed when at all possible. Circular stapler is usually used in order to create the intrathoracic anastomosis. We report a completely thoracoscopic handsewn double-layer esophagogastrostomy, realized with the patient in prone position, during a thoracolaparoscopic Ivor Lewis esophagogastrectomy.
Method : A 51 years-old man consulted for complete dysphagia associated to weight loss. A barium swallow evidenced a sliding hiatal hernia and a lumen defect of the lower third of the esophagus. Gastroscopy showed the presence of a suspect lesion at 30 cm. Endoscopic ultrasound evidenced a 35 × 16 mm lesion, with irregular margins, and the absence of mediastinal lymph nodes (stage : T2N0). Biopsy showed characteristics of adenocarcinoma. CT-scan confirmed both the presence of the esophageal mass and the absence of lymph nodes. General anaesthesia and double-lumen endotracheal tube intubation were used. First the patient was placed in supine position, and 5 abdominal trocars were placed. Celiac lymphadenectomy started with skeletonization of the hepatic artery until the root of left gastric artery was reached. The left gastric artery and vein were sectioned. A wide Kocher maneuver as well as pyloroplasty were performed. The distal esophagus was dissected up until the level of the inferior pulmonary vein. Polar gastrectomy was performed by multiple applications of a linear stapler blue load, from the crow's foot medially to the greater curve laterally. The upper part of the gastric remnant was anchored to a penrose and advanced through the hiatus into the right chest. Subsequently the patient was placed in prone position. Three trocars (two 5-mm and one 10-mm) were placed on the posterior axillary line in the 5th, 7th, and 9th right intercostal space. The middle and lower esophagus were dissected. Mediastinal lymphadenectomy with en-bloc resection of the left inferior mediastinal pleura was performed. The azygos vein was ligated and sectioned. The mid-esophagus was transected by scissors just at the level of the azygos vein, and the stomach was well placed into the chest. A completely thoracoscopic handsewn double-layer anastomosis was performed using PDS 2/0 (external layer) and Maxon 3/0 (internal layer) running sutures. A chest tube was left in the pleural cavity. Finally the patient was re-placed in supine position in order to retrieve the specimen in a plastic bag through a suprapubic incision. The intraabdominal stomach was fixed to the hiatus, and a drain was left through the latter.
Results : Total operative time was 340 minutes and blood loss was 150 ml for laparoscopy and 20 ml for thoracoscopy. The patient had an uneventful recovery ; the gastrograffin swallow on 4th postoperative day showed a good passage through the anastomosis and absence of leak. The patient was discharged on the 6th postoperative day. Pathologic report confirmed the adenocarcinoma of the esophagus (stage : pT2bN1Mx).
Conclusions : Thoracoscopy in prone position permits to surgeon to operate in an ergonomic position, and to perform a completely thoracoscopic handsewn anastomosis, without selective lung desufflation. Thanks to this anastomosis the

risk of postoperative leak can be reduced, and the hospital stay and patient's comfort appeared improved.

- D82 -

COPPER METABOLISM : WILSON DISEASE AND OTHERS. X. Stéphenne, L. Beauport, X. Schlogel, N. Revencu, E. Sokal. UCL Saint-Luc.

We report the case of a 16-years old adolescent affected by an attenuated form of the Menkes disease : the occipital horn syndrome. The boy presented in early infancy with feeding and behavioural disorders, and was diagnosed as moderate autism. He was followed clinically by neuro-paediatricians. At the age of 15-years, he presented again with an expressionless face, thick hair, skin hyper-laxity and rigid walk. Wilson disease was considered as a possible aetiology to his neurological problems. Serum ceruloplasmin was decreased to 10 mg/dl and serum copper to 39 μg/dl. Twenty-four h urinary copper excretion was 18 μg/dl and increased under D-Penicillamine challenge test up to 1023 μg/dl. Kayser-Fleisher ring was absent. Nuclear magnetic resonance imaging of the brain did not detect central grey nuclei injury. A liver biopsy was performed, and liver copper tissue was not elevated (20 μg/g dried tissue (nl < 50)). Furthermore, no mutations were found in the ATP7B gene.
Additional investigations demonstrated calcifications at the site of attachment of the trapezius and sterno-cleido-mastoid muscles to the occipital bone (called occipital horns). Bladder diverticula were also present in our patient, confirming further the diagnosis of occipital horn syndrome (OHS).
In the family screening, 2 of 3 patient brothers had a similar biochemical phenotype, but without clinical symptoms. OHS is a late-onset form of the severe neonatal Menkes disease, causing early infancy death. Menkes disease is due to defect in intracellular copper deficiency and is linked to mutations of a gene localized on chromosome X, ATP7A, coding for an intracellular copper-transporting protein. The resulting free copper deficiency alters the functioning of copper-dependent enzymes, thereby causing the symptoms of the disorder.
We conclude that the occipital horn syndrome, although rare, must enter in the differential diagnosis of Wilson disease, as the blood biochemical results may be confounding. Tissue copper determination is essential to differentiate the two diseases, and undue copper chelating treatment might be inappropriate.

- D83 -

MODIFIED MULTIVISCERAL TRANSPLANTATION INCLUDING SMALL BOWEL, LIVER AND PANCREAS IN AN 8-MONTH-OLD CHILD WITH CONGENITAL SHORT BOWEL SYNDROME. F. Goudsmedt, A. Verryckt, F. Berrevoet, R. Troisi, A. De Jaeger, X. Rogiers, B. de Hemptinne. UZ Gent.

Congenital short bowel syndrome is a rare condition of newborns associated with a high mortality. An eight months old boy, diagnosed with congenital short bowel syndrome with needfor longterm total parenteral nutrition (TPN), was admitted at the pediatric intensive care unit (PICU) for acute liver failure due to prolonged usage of TPN. The day after admission a combined liver-small intestine-pancreas transplant with double-loop ileostomy was performed. Six days postoperatively oral feeding was initiated and lead to good production of stool ; Two months after transplantation the boy could leave the hospital in good condition with a stable growth curve and good gain of weight. No episodes of rejection occurred. The double-loop iliostoma was closed after 6 months. The patient was followed for 2 years at the pediatric policlinic. In this period the child had 1 event of RSV bronchiolitis, 2 common colds and an episode of severe dehydratation due to vomiting and diarrhea, which required admission at the pediatric ward.
This is a case report of a successful modified multivisceral transplant including small bowel, liver and pancreas without any major complication resulting in a good general condition of a young child.

- D84 -

ABSENCE OF PLACENTAL GROWTH FACTOR AGGRAVATES DSS-INDUCED ACUTE COLONIC INJURY.
P. Hindryckx (1), A. Waeytens (1), D. Laukens (1), H. Peeters (1), J. Vanhuysse (2), P. Carmeliet (3), M. De Vos (1). (1) Gent ; (2) Brugge ; (3) Leuven.

Background : Angiogenesis has recently been described as a component in inflammatory bowel disease (IBD) pathogenesis. The vascular endothelial growth factor (VEGF) homologue placental growth factor (PlGF) establishes its angiogenic capacity during pathophysiological conditions. The role of PlGF in experimental colitis has never been investigated.
Aim : To investigate the role of PlGF in murine dextran sulphate sodium (DSS) colitis.
Methods : Acute DSS colitis was induced in PlGF knock-out (-/-) and wild-type (WT) mice. Disease activity was calculated during the course of the experiment. Mice were sacrificed at several timepoints. Colonic injury was evaluated by colon length, intestinal epithelial apoptosis (TUNEL assay) and histological score. PlGF and VEGF were measured in distal colonic lysates by ELISA. Mucosal vascularization was quantified by computerized morphometric analysis of CD31 stained distal colonic sections. Intestinal hypoxia was visualized by pimonidazole staining and semi-quantitatively analysed by western blot for hypoxia inducible factor alpha (HIF-1 ±).
Results : During DSS colitis, PlGF-/- mice showed significantly increased disease activity ($P < 0,001$), colonic shortening ($P = 0,049$), colonic epithelial apoptosis ($P = 0,025$) and histological damage score ($P = 0,036$) compared to WT mice. DSS colitis was associated with a significant increase of PlGF (in WT mice) and VEGF (both in WT and PlGF-/- mice) in distal colonic tissue. Despite similar VEGF levels were reached in WT and PlGF -/- mice, the latter showed significantly less mucosal angiogenesis after DSS administration (mean vascular density : $P = 0,046$, mean vessel diameter : $P = 0,027$). This was associated with increased tissue pimonidazole uptake and accumulation of HIF-1 ±.
Conclusion : Knock-out of placental growth factor strongly blocks angiogenesis during an acute mucosal injury associated with increased colonic hypoxia, and results in a worsening of the disease course.

- E02 -

EDUCATIONAL ASPECTS TO OPTIMIZE TRANSITIONAL PROCESS. I. Aujoulat. UCLouvain.

At least 12% of adolescents live with a chronic condition (1). In this population, the transition from childhood to adulthood is a complex process, which needs to be adequately supported by the health-care system, in order to support optimal development and quality of life, and to prevent non-adherence and medical complications. There is growing evidence of a risk of non-adherent behaviours in adolescents and young adults when they transfer from pediatric to adult-oriented care. However is it adolescence and/or transition that contribute to non-adherence ? Both the medical and developmental needs of the patients need to be addressed, in order to support their emerging capacity to self-manage their condition and life, which is the ultimate goal of the transitional process. The transfer from pediatric to adult-oriented care is only one aspect of the transitional process. Another important aspect of this process is the transfer of the responsibility for treatment from the caregivers to the young patients. These two aspects of the transitional process should not be considered as two separate tasks but should be articulated into a coherent and well coordinated patient education programme instead (2). In order to be effective, such a programme should involve not only the patients, but also their parents and the health care teams, and address not only condition and treatment specific issues, but also common concerns of young people and general health education issues (3).

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- E07 -

LIVING WITH CELIAC DISEASE : QUALITY OF LIFE AS ASSESSED BY PARENTS AND CHILDREN/ ADOLESCENTS. A.Vanoppen, B. Gers, P. Alliet. Virga Jesse Ziekenhuis Hasselt.

Introduction : Celiac disease is a chronic disorder, which can influence all aspects of life. Nowadays, health-related and disease-related quality of life is considered as an important issue in chronic diseases.

Aim of this study : To evaluate health-related and disease-related quality of life in children (6-12 years) and adolescents (13-18 years) with celiac disease by specific questionnaires and to relate it to the view of their parents. To check IgA endomysium antibodies in serum.

Results : 48 patients were asked to participate in the study. 37 of them (25 girls) agreed to fill in both questionnaires, which means a response rate of 77%. 25 of them (18 girls) had an age between 6-12 years. 29/37 patients completed the food diaries, of which 20 (12/25 children) were useful for further analysis.

Scores on emotional, social, familial and physical well-being were in general good. Children with celiac disease however obtained higher scores than adolescents. Girls had higher scores than boys. The scores of the parents were in the same range as the ones of their children. In the disease-related questionnaire, the overall scores were much lower than in the health-related questionnaire. Girls had in general a better score than boys. Adolescents did communicate better about their disease than children. Parents scored the disease-related quality of life lower than their children, as well in the younger as in the older age group. Endomysium antibodies were negative in 35/37 patients (94.6%).

Conclusion : Health-related quality of life in celiac children on a gluten free diet is good. Disease- related quality of life however is lower. Psychological support might be useful to be considered.

- E09 -

TEN YEARS OUTCOME ANALYSIS OF ISOLATED AND COMBINED LIVER TRANSPLANTATION IN CHILDREN LISTED FOR ELECTIVE AND HIGH URGENT TRANSPLANTATION. SINGLE INSTITUTIONAL EXPERIENCE. T. Couvreur, T. Sablon, M. Van Winckel, R. De Bruyne, S. Van Biervliet, A. De Jaeger L. Colenbie, F. Berrevoet, R. Troisi, X. Rogiers, B. De Hemptinne. UZ Gent.

Introduction : Survival after liver transplantation has improved significantly over the last decade with pediatric recipients faring better than adults. We retrospectively reviewed our series during the last 10 years taking into account elective and urgent isolated or combined liver transplants in children.
Methods : From January 1999 to November 2008, a total of 40 children received 47 transplantations : 27 (57%) were split grafts, 3 (7%) were reduced and 17 (36%) were whole grafts. Mean age was 4,5 years old (SD = 5,3 y). Patients who needed multiple transplantations were considered as one patient. Thirty children were transplanted in the elective setting, 10 in the HU. Indications in the HU setting were acute fulminant liver failure of unknown origin (45%), alfa 1 antitrypsine deficiency (19%) and others as Hepatitis A, depakine-intoxication, veno-occlusive disease or neonatal cholestasis (each accounting for 9%). Transplantations in the elective group were mainly performed because of metabolic disorders (31%) or biliary atresia (22.5%). Two children received combined liver/kidney transplantation ; one received liver/small bowel/pancreas transplantation. Using a Kaplan-Meier statistical method, we defined an actuarial curve which was based on the variables survival and mortality rate between children listed as HU or as elective candidates. Differences in survival were calculated by log-rank test.
Results : After a median follow-up of 47 months (range 1-134), 3/10 (30%) children transplanted in the HU setting died (graft failure in 2 cases and an incurable cerebral mitochondrial disease in the other) whereas only one death (3%) occurred in the elective setting (graft failure). Since 2000 all of the children listed as elective candidates survived. The overall actuarial survival rate was of 90% (97% Elective vs. 70% HU ; p = 0,065).
Conclusions : The results provided by this study are encouraging despite the small patient population compared to the average survival of 70% recorded in the literature. Considering the broad spectrum of pathologies, we can now look back on a successful survival rate in children after 17 years experience in liver transplantation.

- E10 -

DOUBLE-BALLOON ENTEROSCOPY IN PEDIATRIC PATIENTS : EXPERIENCE OF TWO UNIVERSITY HOSPITAL ENDOSCOPY UNITS. T.G. Moreels (1), P.B. Mensink (2), E.J. Kuipers (2), J.C. Escher (2), P.A. Pelckmans (1). (1) UZ Antwerp, Belgium ; (2) Erasmus Medical Center Rotterdam, The Netherlands.

Introduction : Double-balloon enteroscopy (DBE) enables complete endoscopic intubation of the gastrointestinal (GI) tract. Both Fujinon EN-450P5 pediatric and EN-450T5 treatment endoscopes are available, differing in outer diameter and diameter of the accessory channel.
Aim : We evaluated feasibility and diagnostic yield of DBE in a pediatric population with suspected small bowel pathology.
Methods : Retrospectively, all DBE procedures in pediatric patients (age < 18 years) were evaluated, using the DBE database of both university hospitals.
Results : From March 2005 until April 2008, DBE was performed in 14 pediatric patients : 7 males, mean age 13 (7-17) years. Indications were suspected Crohn's disease (n = 6, 43%), anemia (n = 3, 22%), protein loosing enteropathy (n = 2, 14%), unexplained abdominal complaints (n = 2, 14%) and Peutz-Jeghers syndrome (n = 1, 7%). In 6 (43%) patients small bowel diagnostics were performed prior to DBE : 4 wireless capsule enteroscopies, 1 MRI enteroclysis and 1 Meckel scintigraphy. In 3 (50%) patients these investigations revealed abnormalities. DBE was performed under general anesthesia in 13 (93%) and fluoroscopy was used in 4 (29%) procedures. Both proximal and distal procedures were performed in 11 (79%) patients, and in 3 (21%) patients only a proximal procedure was performed. Complete small bowel visualization was achieved in 1 (9%) patient (with a combined procedure). The EN-450P5 endoscope was used for the majority of procedures, whereas the EN-450T5 endoscope was only used for 3 older children (> 15 years). In 13 (93%) patients abnormal findings were detected with DBE : 6 Crohn's disease, 2 intestinal lymphangiectasia, 2 postoperative small bowel adhesions, 1 paralytic ileus, 1 NSAID-induced jejunal erosions and 1 jejunal polyp. Mucosal biopsies were taken during 8 (57%) procedures. One polypectomy was performed in a patient with Peutz-Jeghers syndrome. No complications occurred during or after the DBE procedures.
Conclusions : DBE is feasible and safe in pediatric patients. Similar outcome results as compared to the adult population are obtained. Preferably, the EN-450P5 pediatric endoscope is used because of its smaller diameter. Since DBE is an invasive procedure, it is best performed under general anesthesia and the use of fluoroscopy should be limited because of radiation exposure. At this moment no guidelines are available regarding the use of DBE in pediatric patients.

- E11 -

PEDIATRIC INTERVENTION STUDY WITH A PROBIOTIC MIXTURE (BACILAC FORTE®) IN ACUTE GASTROENTERITIS. G. Veereman (1), S. Staelens (1), J. Wijffels (2). (1) UA Antwerp, Queen Paola Children's Hospital ; (2) Belgium Network of Open Source Analytical Consultants, Brussels.

The aim of this study was to evaluate the acceptability, safety & effect on diarrhea and accompanying symptoms of a probiotic mixture (Bacilac Forte) compared to placebo in children with a clinical diagnosis of mild acute gastroenteritis. The active product contained 3, 3 10 9CFU pro capsule of a probiotic mixture :20% Lactobacilus rhamnosus, 20% L acidophilus, 20% L casei, 20% L plantarum, 20% Bifidobacterium infantis. Study design was prospective DBPC in a primary care setting. IRB approval was obtained and subjects were included after parental consent. Otherwise healthy eutrophic children ages 1 mth-5 yrs, consulting their GP b/o liquid stools more than 3 tid for less than 48 h with dehydration less than 9% were included. Exclusion criteria were exposure to antibiotics or probiotics within 4 wks, bloody diarrhea & fever. Subjects received active or placebo tid. Parents kept a diary until D9 rating acceptability of the product, various parameters and any adverse event. Subjects were re-examined between D7-9. In total 48 subjects enrolled, 47 were analyzed (22 in the intervention group), 53% were girls, 91% Caucasian, age was 24 + -15 mths, with no differences between groups. Average stool frequency was 5/d, 45% had cramps, 21% regurgitation, 45% vomiting and 23% upper respiratory symptoms. Statistical analysis used GLMM and non-parametric tests. Based on diary there was no difference in duration of diarrhea and stool frequency (although a sharper decrease in the active group) but stool consistency returned to normal more rapidly (D3) in the active group ($p = 0.0205$). Also stools were more soft and formed over the observation period in the active group whereas subjects in the placebo group showed more variation (more hard and watery stools). During the course of the study there were no differences in cramps, regurgitation, vomiting, upper respiratory symptoms and appetite between groups. Acceptability of the capsules was rated easy by over 70% on all occasions for both groups, Tolererance was excellent : crying, flatulence, sleep and general behavior were rated similarly. Two adverse events (AE) were recorded in the intervention group : 1 otitis media and 1 gastroenteritis with dehydration necessitating hospitalization (frequency of AE not different for both groups by fisher exact $p = 0.203$). In conclusion : tid administration of the probiotic mixture Bacilac Forte to young children with mild gastro-enteritis was safe, well tolerated and led to more rapid normalization of stool consistency with overall more formed and soft stools.

- E12 -

PROFILE OF BELGIAN PEDIATRIC CROHN'S DISEASE SUBJECTS (2) : SNAP SHOT AT DIAGNOSIS. M. Rogalidou (1), I. Hoffman (2), T. Mahler (1), S. Staelens (1), S. Van Biervliet (3), M. Scaillon (4), P. Bontems (4), I. Paquot (5), F. Bury (5), S. Colinet (5), W. Arts (6), B. Hauser (7), F. Smets (8), E. Socal (8), P. Alliet (9), E. Janssens (9), O. Bauraind (10), I. Etienne (11), G. Veereman (1). (1) UA Antwerp, Queen Paola Children's Hospital ; (2) UZ Gasthuisberg, Leuven ; (3) UZ Gent ; (4) ULB Reine Fabiola.

The IBD working group of Bespghan° recently established a registry of Belgian pediatric CD with ulterior goal to study demographical data, treatment and disease course over 5 years. We here report data on the first 100 patients at the time of diagnosis. Diagnosis was made within 12 wks of symptoms in 52.7% & within 21 wks in 75%. Mean age at diagnosis was 11.7 + 2.7 yrs corresponding with mean bone age 11.6 + 3.3 yrs. The mean z-score for height was -1.1 + 1.3. Pubertal stages were 56.8% Tanner stage I and 21.6% stage II. Concomitant conditions (mostly autoimmune type) and extra intestinal symptoms were observed in 4.4% and 26.4% of patients respectively. PCDAI (total) was retrieved from 58 charts : 3 scored less than 10, 18 had mild to moderate disease (PCDAI 10-30) and 37 rated severe (> 30). The PCDAI at diagnosis was ccorrelated negatively with birth weight, ($p = 0.0473$) and birth height ($p = 0.0407$) not with age at presentation or symptom duration. Diagnosis was based on endoscopy and histology in 87.7% (in accordance with Porto criteria[1]), 97% had (ileo)colonoscopy, 68.7% OGD, 1% sigmoidoscopy. Imaging studies : 61.6% of patients had abdominal US, 25.3% small bowel enteroclysis, 14.1% CT and 9.1% MRI. White cell scan was used in 2% and small bowel capsule in none. Based on endoscopic exploration the most frequently involved intestinal area was the ileocolon (52%). Histological diagnosis was possible in 72.7% patients undergoing OGD. Initial therapeutic approach was monotherapy with steroids in 4%, 5ASA in 9.5%, antibiotics in 1.4%. Combination of steroids with azathioprine was prescribed in 36.5%, with MTX in none, with 5-ASA in 27% and with antibiotics in 10.8%. Enteral nutrition was used in 9.5% and always in combination with drugs. In conclusion : At diagnosis children presented with extensive involvement of the GI tract but the small bowel remains mostly unexplored. The severity of disease at presentation may be related to intrauterine growth. Data collection on disease progression and therapy is being conducted.
Bespghan : Belgian Society for Pediatric Gastroenterology Hepatology and Nutrition
1. Porto criteria JPGN 41 ; 2005.

- E13 -

OESOPHAGEAL EOSINOPHILIC INFILTRATION : MEANING AND EVOLUTION IN PAEDIATRICS.
L. Muyshont, P. Bontems, A. Salame, C. Deprez, S. Cadranel, M. Scaillon. ULB Reine Fabiola.

Eosinophils are usually not present in the normal esophagus and an eosinophilic infiltration (EI), always abnormal, is most often due to gastro-oesophageal reflux. The presence of an EI e 20 / field (f) associated to a non-response to the appropriate anti-reflux therapy suggests Eosinophilic esophagitis (EE) and represents its main criterion.
Aim : To evaluate the differential diagnosis between peptic esophagitis and EE and the possible evolution of EI.
Methods : The clinical data of 3552 patients (P) in whom esophageal biopsies were taken during the period 2000-2006 were revised and those corresponding biopsies presenting an EI reviewed using a 40 x magnification.
Results : Out the 88/3552 P (2,5%) who presented with an EI e5 / f, 45 (51%) had an EI between 5 -19 / f (G1) and 43 (49%) an EI e 20/f (G2). The two groups differ for age (median 8 y in G1 vs 7 y in G2) and for endoscopic findings ($p = 0.05$) : normal (40% in G1 vs 9% in G2) On the contrary symptoms as well as pH-metry recordings are similar in the two groups : abdominal pain 55% vs 46%, vomiting 38% vs 40%, allergy 25% vs 36% and abnormal pH-metry in 15/18 P in G1 (= G1a) vs 10/18P in G2 (= G2a). After adequate anti-reflux therapy, the disappearance of the EI was observed in 10 P G1a as well as a normal pH-metry was recorded in 8 P G2a. However in these 8 P the disappearance of EI was observed only in 2P but an EI e 20/f persisted in the remaining 4 P. Despite a normal pH-metry, 8P of G2 were treated with PPIs. A follow up, available in 6/8 P, showed a decrease of EI in 2 P and a persistence of an EI e 20 / f in 4P. In 4/8 P defined as EE with an EI e 20/f therapy consisted in an antiallergic diet combined with topic steroids and decrease of EI was observed in all 4P.In 16/88P, a previous earlier (1 month to 6 years, med 18 months) normal oesophageal biopsy evolved into an EI (8P G1 and 8P G2).
Conclusions : The presence of an EI may vary upon time and a gastro-oesophageal reflux should always be ruled out. An EI e 20 / f suggest EE. Although usually unsuccessful, an anti-reflux therapy is nonetheless indicated, even in case of a normal pH-metry. Careful follow-up of the evolution of eosinophilic infiltration is not only justified but strongly advised.

- E14 -

PEDIATRIC INTESTINAL TRANSPLANTATION.
J. Pirenne (1), D. Monbaliu (1), W. Coosemans (1), R. Aerts (1), T. Darius (1), L. Dedrye (1), M. Miserez (1), A. Van Den Bosch (1), R. Hierner (1), P. Schlesser (2), P. Ferdinande (1), L. Desmet (1), D. Vlasselaers (1), I. Hoffman (1), R. Lombaerts (1). (1) KULeuven ; (2) Montegnée / Rocourt Hospital.

Introduction : Intestinal transplantation (Itx) has evolved from an experimental procedure into a surgical/medical curative (albeit high risk) treatment in children suffering from irreversible bowel failure and life-threatening complications while under total parenteral nutrition (TPN).
Patients : In a series of 9 consecutive Itx performed at our center, 2 were pediatric cases and are reported herein.
Case 1. BA is a 3-year-old boy suffering from a postoperative volvulus leading to complete small bowel necrosis/resection. He developed liver failure due to the combined effects of a macrophage activation syndrome and TPN/bowel insufficiency. He received an *en bloc* multivisceral graft including *liver/duodenum/pancreatic head/entire small bowel*. Arterial inflow to the graft is *via* an interposition donor aortic tube including the celiac trunk and the superior mesenteric artery and anastomosed end-to-side to the recipient aorta. Venous drainage is *via* a piggy-back anastomosis of the graft suprahepatic vena cava to the native hepatic veins. Native portal vein is anastomosed to the graft infrahepatic vena cava. Intestinal continuity is restored proximally and distally and a distal loop ileostomy is constructed allowing endoscopic access to – and biopsies of – the graft. Immunosuppression consisted of anti IL 2 receptor blockade, tacrolimus, azathioprine, and low dose steroids. He presented a complicated postoperative course mainly due to the impossibility to close the abdominal wall primarily (as the consequence of a massive reduction of the abdominal domain/capacity in a multioperated child). This was treated by a combination of vacuum assisted closure (V.A.C.®) therapy, wound manager, reoperations for partial fistula resection, and eventually skin grafting. He is now 4 years post-transplant doing well, nutritionally independent, rejection-free, growing well and leading a near-to-normal life pattern under low immunosuppression.
Case 2. GC is a 8-year-old girl with complete resection of small and large bowel and multiple reoperations following necrotizing enterocolitis in the setting of prematurity, and colitis ulcerosa. She also developed micronodular cirrhosis probably vascular in origin and without clear TPN-associated cholestatis. To prevent the difficulty of abdominal closure encountered in case 1, extra skin development was obtained by placement of 2 large subcutaneous osmotic (self-expanding) skin expanders at her right epigastrium (440 cc) and right abdominal flank (660 cc). She received an*en bloc liver/duodenum/entire pancreas/entire small bowel/right large bowel* graft. Surgical technique was similar to case 1. Because of previous skin expansion, skin could be closed primarily and no reintervention was necessary (except for the scheduled closure of the distal loop ileostomy at 2 months post-transplant). She is doing well rejection-free 4 months post-transplant under tacrolimus and low dose steroids.
In conclusion, these 2 cases of pediatric Itx (of a series of 9 consecutive Itx) demonstrate that the procedure is life-saving and life quality-restoring and therefore should be considered early in children suffering of irreversible small bowel failure and associated life-threatening complications (e.g. liver failure in these 2 cases).

- E15 -

THE ADDITIONAL VALUE OF MULTICHANNEL INTRALUMINAL IMPEDANCE IN THE DETECTION OF GASTROESOPHAGEAL REFLUX IN CHILDREN WITH PULMONARY SYMPTOMS. K. Cosyns, J. Tack, I. Hoffman. KULeuven Gasthuisberg.

Multichannel intraluminal impedance (MII) is a new technique for evaluating gastroesophageal reflux (GER), depending on changes in the resistance by the presence of liquid or gas bolus inside the esophageal lumen. Its use in combination with pH-metry has been proved to allow an accurate measurement of gastroesophageal reflux at all pH-levels, both acidic and non-acidic. As GER-type symptoms often persist despite adequate antiacid treatment, it has been suggested that these symptoms might be due to non-acid reflux. Moreover in patients with atypical GER with pulmonary symptoms the role of non-acid reflux is not yet determined.
The **aim** of our study is to assess the additional yield of combined pH-metry and MII in children with typical GER symptoms and GER related pulmonary symptoms.
Methods : In 19 patients between 2 and 16 year of age, 24-hour pH-metry and MII was performed. All 19 patients were symptomatic : 11 patients suffered from gastrointestinal symptoms (pyrosis), 8 patients had pulmonary symptoms (frequent pulmonary infections, chronic cough, bronchiectasis) and 3 patients had a combination of both. In the patient group with gastrointestinal symptoms 5 already underwent Nissen fundoplication, without complete resolution of the symptoms. 3 patients received proton pump inhibitors at the time of the investigation. To determine an abnormal pH-metry we used the known reference values expressed in% of time of pH under 4 (considered abnormal if > 6% during daytime, > 4% during nighttime and > 4% in total). We evaluated the cutoffs for total MII reflux episodes at multiple steps of 10 to establish a cutoff level, which would distinguish typical GER from atypical GER with related pulmonary symptoms.
Results : We observed 6 (31%) patients with an abnormal pH-metry. Four of them belonged to the group with gastrointestinal symptoms. Five of them had acid reflux during the day (6.8%-17.7%, mean 11.4%), 1 had also acid reflux during the night (5.3%). Only 2/8 of the patients with pulmonary symptoms had significant acid reflux episodes on standard pH-metry (mean total 5.6%). MII showed high numbers of total reflux episodes in 5 (26%) of the patients. One patient had gastrointestinal symptoms and 4 had pulmonary symptoms. When a cutoff value of 40 is used, MII showed more abnormal results in the patient group with pulmonary symptoms, 4/8 compared to the patients with gastrointestinal symptoms 1/11.
Conclusion : In infants and children combined pH-MII monitoring increases the likelyhood of demonstrating GER, both acid and non-acid as compared with pH-metry alone. In our study we detected a high prevalence of non-acid reflux in pediatric patients with pulmonary symptoms who had a normal pH-metry. Especially in these population of patients MII seems to have an important additional yield when a cutoff value of 40 is used. To further determine correct reference values for MII in infants and children larger studies are necessary.

- E16 -

PROFILE OF BELGIAN PEDIATRIC CROHN'S DISEASE SUBJECTS : DEMOGRAPHY AND BACKGROUND OF THE FIRST 100 PATIENTS. M. Rogalidou (1), I. Hoffman (2), T. Mahler (1), S. Staelens (1), S. Van Biervliet (3), M. Scaillon (4), P. Bontems (4), I. Paquot (5), F. Bury (5), S. Colinet (5), W. Arts (6), B. Hauser (7), F. Smets (8), E. Socal (8), P. Alliet (9), E. Janssens (9), O. Bauraind (10), I. Etienne (11), G. Veereman (1). (1) Pediatric Gastroenterology & Nutrition, Queen Paola Children's Hospital ZNA Antwerp ; (2) UZ Gasthuisberg, Leuven ; (3) UZ Gent ; (4) University Children's Hospital Queen Fabiola, Brussels ; (5) CHC Clinique de l'Esperance, Liege ; (6) ZOL, Genk ; (7) UZ VUB, Brussels ; (8) UCL Saint-Luc, Brussels ; (9) Virga Jesse Hospital, Hasselt ; (10) Clinique Saint-Pierre, Ottignies, [11] CHR de la Citadelle, Liège.

Recent reports on pediatric IBD patients show that early onset Crohn's disease (CD) is characterized by a phenotype with extensive, severe and complicated course (1,2). Epidemiological observations seek to identify factors and parameters that may influence the evolution and prognosis of CD. The IBD working group of Bespghan° recently established a registry of Belgian pediatric CD with ulterior goal to study demographical data, treatment and disease course over 5 years. IRB approval was obtained from all participating centers. Both parents consented and subjects gave assent prior to inclusion. We report on the background and history of the first 100 inclusions. The group counts 60 boys and 40 girls, 92 are Caucasian. At the time of registration patients belong to the following age groups : 3 are 2-5yrs old, 15 are 6-11 yrs old and 82 are 12-18 yrs old. The youngest patients (< 12 yrs) are mostly boys (13 vs 5 girls). Historical data were obtained by retrospective chart analysis and by additional questioning. Birth history reveals that 85% were delivered by vaginal route, mean gestational age was 38.4 wks + 2.3, and 19 patients were premature. The mean birth weight was 3.3 kg + 0.6 and height 49.8 cm + 3.0. Data on neonatal feeding are available from 77 infants : 67.6% were breastfed for a mean duration of 11.4 wks. The majority (60%) of formula used was cow's milk based. There was a suspicion of food allergy in 20.9% infants (33.3% to cow's milk). In their prior history 18.5% needed dietary restrictions and 38.5% underwent surgery. During the last 3 months prior to diagnosis 18.3% took antibiotics, 17.3% suffered a minor infectious episode, 21.4% experienced a major stressful event.
Family history was positive for IBD (CD and Ulcerative colitis) in 52.3% and a 1st degree relative in 15.4% or a 2nd degree relative in 27.7% with CD. Family history for autoimmune diseases (exc diabetes & hepatitis) was positive in 49.2%. Smoking occurred in 12 families but no patient was active smoker.

In conclusion : in the Belgian pediatric CD population prior disease history appears unremarkable. Positive family history for IBD & autoimmune diseases is common. Further study will compare phenotypes with disease course and factors that may influence severity and prognosis.
Bespghan° : Belgian Society for Pediatric Gastroenterology Hepatology and Nutrition.

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Belgian HP

Invited lecture

- H01 -

HELICOBACTER PYLORI AND OBESITY / BARIATRIC SURGERY. **R. Ntounda (1), M. Nkuize (1), V. Muls (1), J.P. Mulkay (1), R. Saidi (1), G. Dapri (1), G.B. Cadiere (1), A. Burette (2), M. Buset (1). (1) ULB Saint-Pierre ; (2) Clinique de la Basilique.**

Background : Obesity is a major health problem, particularly in developed countries, which contributes to increased morbidity and mortality and to a variety of diseases. Surgical therapies offer the best hope for substantial and sustained weight loss in the extremely obese. One of the most effective bariatric operations is the Roux-en-Y Gastric Bypass (RYGB), which leads to an average weight loss of 65-70% of the excess within 1-2 years after surgery and a sustained weight loss of 50-55% of the excess at 15 years. Long-term mortality after gastric bypass surgery is significantly reduced, particularly death from diabetes, heart disease, and cancer. This operation renders a portion of the gastrointestinal tract inaccessible to endoscopic screening and treatment for ulcer disease or cancer. For this reason, many clinicians consider it important to screen bariatric patients for *Helicobacter pylori* infection (*H. pylori*) preoperatively and to attempt to eradicate infection if present. Others claim logistical and economical arguments against routine screening and treatment. The prevalence of *H. pylori* infection varies from 25-50% in developed countries to 70-90% in the third world. *H. pylori* infection is present in 30-67% of patients scheduled for bariatric surgery, and preoperative testing in these patients may be useful.

Helicobacter pylori and obesity : The incidence of *H. pylori* infection varies depending on age, socio-economic status, and ethnicity, and reported rates are likely to mirror the population at a specific institution. Alterations in immune function associated with obesity have been hypothesized to increase infection rates. The relation between *H. pylori* and obesity is very controversial. Studies on prevalence of *H. pylori* infection in morbid obese patients compared with a normal weight population resulted in conflicting results with some finding higher prevalence, others lower or comparable incidences of *H. pylori* gastritis. The major cause of these differences seems to be due to methodological problems : small sample sizes, different definition of obesity and selection of patients, various detecting methods of *H. pylori* infection, no control group, and lack of consideration of confounding factors are among the reasons to explain the discrepant results. Blaser and Atherton assumed that *H. pylori*-induced persistent and uncontrolled gastric inflammation would lead to deregulation of appetite and calorie homeostasis through its effect on the expression of gut hormones such as ghrelin (3,10,11). Many studies have provided evidence that nutritional status and gastric and plasma ghrelin dynamics are altered in response to *H. pylori* infection. Also, in a randomised study, eighty non-diabetic naive *H. pylori*-positive patients were divided into two groups according to their BMI : normal BMI and overweight/obese BMI e 25. Successful eradication was observed in 85.4% of the normal BMI group compared with 55% of the overweight/obese group ($p < 0.005$; OR : 4.77 ; 95% IC : 1.64-13.87).

Is endoscopy necessary before and after bariatric surgery ? : The rationale for performing upper gastrointestinal (UGI) endoscopy before bariatric surgery is to detect and/or treat lesions that might potentially affect the type of surgery performed, cause complications in the immediate postoperative period, or result in symptoms after surgery. The value of a routine endoscopy prior to bariatric surgery in asymptomatic patients remains controversial because of the lack of observational studies in this group. Guidelines from outside the United States recommend preoperative UGI endoscopy in all patients before bariatric surgery, regardless of the presence or absence of symptoms. In some studies asymptomatic patients with *H. pylori* gastritis, scheduled for an RYGB, where significantly more likely to have an abnormal endoscopy than negative (94% vs. 51%). The infected patients were also more likely to develop postoperative marginal ulcers.

Recent advancements in small-bowel double-balloon enteroscopy have allowed us to evaluate mucosal alterations in the excluded stomach. Safatle-Ribeiro *et al.* have recently reported the utility of double balloon enteroscopy and documented the *H. pylori* status in the excluded stomach. Lord *et al.* reported one case of adenocarcinoma in the remnant stomach 13 years after RYGB. Three authors using double-balloon enteroscopy, described metaplasia, atrophy, and erosive and hemorrhagic gastritis in the remnant stomach several months after surgery. Perforated peptic ulcer was also reported in 11 cases in the distal stomach.

Gastrointestinal complications of bariatric surgery : Bariatric operations present the best answer not only to the main disease, but also to comorbidities. On the other hand, bariatric surgery is not free of risk. Although perioperative mortality of bariatric surgery is less than 1%, usually due to anastomotic leaks with peritonitis or pulmonary embolism, non-lethal complications are frequent. Early complications of gastric bypass surgery are acute gastric distension secondary to edema and obstruction at the enteroenterostomy, which can be treated with radiographic-guided percutaneous gastrostomy or reoperation with tube gastrostomy. Late complications are associated with marginal ulcer. These ulcers are thought to be due to a combination of preserved acid secretion in the pouch, tension from the Roux limb, ischemia from the operation, nonsteroidal anti-inflammatory drug (NSAID) use, staple-line disruption, the presence of staples or suture material (foreign body reactions), and *H. pylori* infection. Other complications are stomal stenosis,

gastrointestinal bleeding, internal hernia, dumping syndrome/diarrhea, cholelithiasis, GERD, fistula, bezoars, band slippage and erosion.

Why patients infected with *H pylori* should be treated before bariatric surgery ?

1- Eradication should decrease the risk of gastro duodenal ulcer disease in the remnant stomach, and thus decrease early postoperative symptoms attributable to ulcers in the patients as well as ulcer complications in the future.

2- there may also lower the incidence of postoperative marginal ulcers as observed in patients who had screening and preoperative *H. pylori* eradication, compared with patients who did not undergo screening .

3- in patients with non-ulcer dyspepsia, there is evidence of moderate benefit of *H. pylori* eradication.

4- *H. pylori* is a class I carcinogen in the development of gastric cancer with odds ratios of 2.0 to 5.9 and case reports describe gastric cancer in patients after gastric bypass.

5- there is a lack of correlation between symptoms and endoscopic findings.

6- In RYGB, the by-passed gastric and duodenal mucosa are no longer within the reach of standard endoscopes post-operatively, making it more difficult to treat lesions that could have been diagnosed preoperatively (21).

Conclusion : Obesity and its complications have reached epidemic proportions, increasing the amount of obesity surgery procedures. Some of these operations make a portion of the gastrointestinal tract unavailable for traditional endoscopic screening. Routine use of preoperative upper-endoscopy already revealed significant pathology in many patients before laparoscopic gastric bypass. Recent advancements in small-bowel double-balloon enteroscopy have allowed us to evaluate mucosal alterations in the excluded stomach and detect abnormalities including cancer. Therefore, all patients who test positive for *H. pylori* gastritis preoperatively should be treated with proton pump inhibitors and antibiotics, and should only be admitted to surgery if tested negative post treatment with urease breath test or stool test e 4 weeks.

Invited lecture

- H02 -

PATHOLOGY OF HELICOBACTER SUIS IN RODENT MODELS. B. Flahou, F. Haesebrouck, R. Ducatelle. U Gent.

Recent investigations have indicated that '*Helicobacter heilmannii'* type 1 is the most prevalent gastric non-*H. pylori Helicobacter* species in man. It has been shown to be identical to *H. suis*, a *Helicobacter* species colonizing the stomach of more than 60% of slaughter pigs. This bacterium has only recently been isolated *in vitro* from the stomach mucosa of slaughter pigs. To evaluate the pathogenicity of this micro-organism, mice of two strains (Balb/c and C57BL/6) and Mongolian gerbils were inoculated intragastrically with an *in vitro* cultured strain of *H. suis*. Animals were sacrificed at 3 weeks, 9 weeks and 8 months p.i.. All infected animals were positive in PCR. In gerbils, immunohistochemical staining showed bacteria mainly in the antrum. In the fundus, bacteria were exclusively found near the forestomach/ stomach transition zone. In both mice strains, bacteria colonized all stomach regions. At all timepoints, most gerbils showed a marked lymphocytic infiltration in the antrum, becoming worse in course of time and often present as lymphoid follicle-like structures with expanding germinal centers. This inflammation was accompanied by a mild loss of parietal cells and destruction of the normal tissue structure. Inflammation could also be seen at the forestomach/ stomach transition zone. At 3 weeks p.i., mice showed a minimal inflammatory response, whereas at 8 months p.i., most mice showed a moderate to marked lymphocytic infiltration, limited to the fundus. In infected mice, a higher proliferation rate of mucosal epithelial cells was detected when compared to non-infected mice.

H. suis is highly related to *H. felis*, which mainly colonizes the stomach of cats and dogs, but can also be found in the stomach of humans infected with a non-*H. pylori Helicobacter* species. In the gerbil model, *H. suis* induces a more severe inflammation but a less severe loss of epithelial cells, as compared to *H. felis*. This could indicate that these highly related species may also cause different pathological changes in humans.

- H03 -

TWENTY YEARS (1988-2007) SURVEY OF H. PYLORI EPIDEMIOLOGY AND RESISTANCE IN BELGIUM.
V.Y. Miendje Deyi. ULB Brugman.

From January 1988 to December 2007, 52566 gastric biopsies were cultured in CHU-Brugmann's bacteriology lab serving several centres. The specimens from antrum and corpus were taken in the course of 32037 endoscopies in 22612 patients (77% adults, 23% children) aged from 1 to 99 years old.
The yearly evolution of the prevalence of *H. pylori* infection means by culture was assessed according to patients age and ethnical backgrounds. Yearly evolution of primary & secondary resistances for commonly used antibiotics (macrolides, imidazoles, quinolones, amoxicillin and tetracycline) was also analysed.
The annual proportion of infected patients decreased gradually from 43.4% in 1988 to 29% in 2007. Significant differences were observed between ethnic groups with lowest infection rate among Northern Europeans patients. Surprisingly, no trend of decline in the prevalence of *H. pylori* infection was observed over the years for the North Africans children under 9 years old.
No resistance was observed for amoxicillin. Tetracycline resistance was very rare (0.1%). Evolution of primary and secondary resistances to others antibiotics are represented in the following table, respectively for children and adults patients. The first years of the study are compared to the last ones.

		Macrolides (CL)		Imidazoles (MZ)		Quinolones (FQ)		Dual R (CL/MZ)		Multi R (CL/MZ/FQ)	
		1ary R	2ary R	1ary R	2ary R	1ary R	2ary R	1ary R	2ary R	1ary R	2ary R
Children	88-91	7%	9%	10%	93%	0%	0%	2%	0%	0%	0%
	04-07	10%	25%	19%	39%	0%	2%	3%	16%	0%	0%
Adults	88-91	5%	25%	29%	83%	5%	0%	2%	14%	0%	0%
	04-07	15%	54%	37%	49%	16%	39%	6%	29%	2%	20%

This survey points out the variability of the prevalence of *H. pylori* infection among persons living in the same geographic area. The increasing resistance to macrolides and quinolones is also highlighted.

- H04 -

EPIDEMIOLOGICAL TRANSITION FROM H. PYLORI TO NSAIDS/ASA IN ASSOCIATION WITH ULCER IN BELGIUM (1989-2008) : A 20 YEARS PROSPECTIVE LONGITUDINAL STUDY (1989-2008). A. Burette (1), Q. Marcelis (2), C. De Prez (3), J. Vanderpas (3), V.Y. Miendje (3). (1) CHIREC / La Basilique - E. Cavell ; (2) ULB ; (3) ULB Brugmann.

Aim of the study : The aim of this study was to document the evolution of relative proportion of *H. pylori (Hp)* infection and non steroid anti-inflammatory drug (NSAIDs) or acetylsalicylic (ASA) intake in the aetiology of gastric (GU), duodenal (DU) or combined gastro-duodenal ulcers (GDU) in our population during the last 20 years.
Method : This prospective, longitudinal, observational study included all dyspeptic patients attending the endoscopy clinic in one single centre (e 80% out patients) which were documented to have duodenal ulcer (DU), gastric ulcer (GU) or gastric + duodenal ulcer (GDU). Ulcer was defined as a loss of mucosal integrity of e 5 mm. Pyloric ring ulcers were classified as duodenal ulcers. Detailed demographic, clinical and diagnostic data were prospectively recorded including, NSAIDs or ASA intake, smoking or alcohol intake, past history and co-morbidities. Exclusion criteria were previous gastric surgery, malignant ulcer and Cameron ulcers. The use of antibiotics or PPI (within the last 3 weeks) was also evaluated by a specific questionnaire.
At endoscopy biopsy specimens from the antrum and the corpus were obtained for rapid urease test (RUT), culture and histology. In case of discordance or absence of evidence of *Hp* infection on the initial evaluation/biopsy based testing, a further control by serology testing ± C^{13}-UBT was performed in most cases. Also, patients having received antibiotic (whatever the reason), bismuth, anti-H2 or PPI-based therapy within the last 3 weeks were tested by serology and retested later e 4 weeks after completion of PPI therapy by the combination of the biopsy based tests or C^{13}-UBT. The same attitude was adopted in case of bleeding if initial check-up was negative for *Hp*. Comparison of frequencies (Chi-square tests, ^{2}2) was performed with EpiInfo software from Centers of Disease Control and Prevention, Atlanta.
Results : 1339 patients were evaluated from 1989 to 2008, and the distribution of duodenal ulcer (59.60%, 798 cases), gastric ulcer (34.95%, 468 cases) or gastric + duodenal ulcer (73 cases, 5.45%) did not change significantly over the entire period (^{2}2 test : not significant).
The results of the univariate analysis are presented in the following table (^{2}2, Chi-square test for linear trend and level of statistical probability). NS = not significant (Prob > 0.10)

Table : Evolution of the percentages of *Helicobacter pylori* (HP +) infection or of non steroid anti-inflammatory drug or acetylsalicylic intake (NSAIDs/ASA +) in four periods

Period : (Total Nb of GDU)	1989 to 1993 (N = 524)	1994 to 1998 (N = 386)	1999 to 2003 (N = 263)	2004 to 2008 (N = 166)	^{2}2	Probability
Hp + NSAIDs/ASA-	64.89% (340)	50.26% (194)	52.47% (138)	44.38% (75)	24.98	< 0,0001
Hp + NSAIDs/ASA +	13.93% (73)	18.39% (71)	15.59% (41)	20.12% (34)	2.75	NS
Hp-NSAIDs/ASA +	13.17% (69)	22.80% (88)	25.48% (67)	21.30% (36)	13.31	0.0003
Hp-NSAIDs/ASA-	8.02% (42)	8.55% (33)	6.46% (17)	12.65% (21)	0.983	NS

The number of cases decreased markedly by two thirds during the four periods, i.e. a significantly greater decrease (P < 0.001) than the population screened which was progressively reduced to 50% of the initial population (1989-93 vs 2004-08). The evolution of respective proportions of ulcers due to *Hp* decreased significantly in mirror of the increase of the respective proportions of ulcers associated with NSAIDs and/or ASA intake. This evolution was clinically relevant and affected ± one fifth of the total of the total number of patients with GD ulcers. Neither the percentage of *Hp* + ve patients and NSAID intake (Hp + NSAIDs/ASA +), neither the percentage of ulcers in patients not exposed to both factors (Hp-NSAIDs/ASA-) did change significantly during these 20 years.
Conclusion : This study confirms the declining incidence and prevalence of peptic ulcer reported in developed countries that paralleled the falling prevalence of *Hp* infection as a significant diminution of the number of GDU was observed during the study period (p < 0.001). The decrease is principally due to a change in ulcers associated with *Hp* infection. This resulted in a relative increase in the proportion of ulcers due to NSAIDs and/or ASA intake. In our population, the absolute number and the relative proportion of non-*Hp*, non-NSAID/ASA remains stable.

- H05 -

A PROSPECTIVE EVALUATION OF RESISTANCE FOR H. PYLORI IN BELGIUM. S. Vandebosch, V. Mattens, F. Mana. UZ Brussels.

Background : *Helicobacter pylori* (*H. pylori*) is a Gram-negative bacterium that colonizes the stomach. Although most infected individuals have no symptoms, *H. pylori* gastritis is strongly linked to the development of duodenal and gastric ulcer disease, gastric cancer and MALTomas.
The route of transmission is unknown. Parents and siblings seem to play a primary role in transmission. Infection is typically acquired in early childhood and occurs more frequently in developing countries than in industrialized countries.
The standard first-line therapy is a one week triple therapy consisting of amoxicillin, clarithromycin and a proton pump inhibitor. However, an increasing number of infected individuals are found to harbour antibiotic-resistant bacteria resulting in initial treatment failure. The third Maastricht Consensus Report states that effective treatment should achieve an intention-to-treat eradication rate of over 80% and therefore proposed to adapt the treatment to the prevalence of antimicrobial resistance in the region.
Aim of the study : In Belgium results about resistance are scarce.
The primary aim of this prospective study is to evaluate the resistance pattern for *H. pylori* infection in the area of Brussels, Belgium and to investigate if differences exist depending on the region where one is born.
Patients and methods : In every patient, more than 14 years old, presenting for an endoscopy and in whom *H. pylori* infection is suspected, 2 biopsies were taken at the antrum, 2 at the corpus and 1 at the angulus for anatomopathological examination (histochemistry) and 1 at the corpus and antrum for culture. Each patient was given a questionnaire about age, birthplace and origin.
Differences between groups were statistically assessed by Anova analyses.
Results : *H. pylori* infection was suspected in 192 patients. Eighty one patients (42%) were of Belgian origin, 21 (11%) were of foreign origin but born in Belgium and 90 patients (47%) were born in a foreign country (99% developing countries). A positive culture and histochemistry was found in 70 patients (36%). In 7 patients (3,6%) histochemistry was positive with negative culture and in 16 patients (8,3%) culture was overgrown by other bacteria. The group of 93 patients with positive histochemistry contained respectively 17.2% Belgians and 82.8% patients of foreign origin. The risk of finding *H. pylori* in foreigners proved to be significantly higher than in Belgians ($p = 0.000$).
In the group with positive culture 10 patients (14%) are of Belgian origin, 10 patients (14%) were what we call “new Belgians” (born in Belgium but of foreign origin), and 50 patients (72%) were immigrants. The resistance pattern for *H. pylori* was as follows : 100% sensitivity to amoxicillin, 14% resistance to clarithromycin, 28% resistance to metronidazole and 26% resistance to ciprofloxacin. Double resistance was found in 11.4% and tripple resistance in 4.3%.
Details of these results in our specific subgroups, showed in the Belgian group 40% resistance to clarithromycin as well as 40% to metronidazole and 50% to ciprofloxacin. For the “new Belgians” we found a 20% resistance to clarithromycin, 30% to metronidazole and 10% to ciprofloxacin, and in the “immigrant” group 8% resistance to clarithromycin, 26% to metronidazole and 24% to ciprofloxacin.
We compared the resistance patterns in the Belgian group with the 2 other groups of foreign origin, since we presumed that patients of the “new Belgians” group got infected by there immigrant-parents. No significant difference was found between the groups using Anova analyses.
Conclusion : Concerning the resistance to metronidazole and clarithromycine, the resistance patterns found in our small group of patients was comparable with older Belgian studies. Resistance to amoxicillin is still not existing. The low resistance to claritromycine permits the continuation of the classical triple therapy. Resistance to ciprofloxacine, a rescue antibiotic, is increasing and the highest in Europe, limiting its use.
A high number of false negatives or overgrowth cultures were found in our study, showing the difficulties of culture.
More patients of foreign origin were tested and found positive for *H. pylori* gastritis, although general numbers of endoscopy performed in our hospital show a majority of native Belgian patients (72%) compared to patients of foreign origin (28%). This is due to a higher suspicion of *H. pylori* infection on endoscopy in the latter group and the known higher infection rates in patients from developing countries.
Although the native Belgians seem to have more resistance to all of the antibiotics tested, we could not demonstrate a statistically significant difference between the two groups. A larger study group is required to confirm these trends.

- H06 -

SEQUENTIAL THERAPY VS. STANDARD TRIPLE THERAPIES FOR HELICOBACTER PYLORI INFECTION.

P. Bontems (1), N. Kalach (2), G. Oderda (3), L. Muyshont (1), A. Salame (1), L. Waroquier (1), Y. Miendje Deyi (4), S. Cadranel (1), M. Scaillon (1). (1) Queen Fabiola Children Hospital, Brussels ; (2) Clinique St Antoine, Lille, France ; (3) University of Piemonte, Novara, Italy ; (4) ULB Brugmann.

Aim : To assess the eradication rate of Helicobacter pylori (H. pylori) infection in children using a sequential treatment regimen compared with the classical Omeprazole-containing triple therapy. The secondary objective was to evaluate the impact of the antimicrobial susceptibility of H. pylori strains on the eradication rates.
Methods : Prospective, open-label study, multi-center study. Children with non-ulcer dyspeptic manifestations undergoing upper GI endoscopy were included if H. pylori infection was proven by histology and culture. Children having received proton pump inhibitor, H2-blockers or antibiotics during the 4 weeks preceding endoscopy were excluded. Children received randomly either a 10-days sequential treatment comprising omeprazole with amoxicillin (AMO) during 5 days and omeprazole, clarithromycin (CLA) and metronidazole (MET) for the remaining 5 days or a 7-days treatment, comprising omeprazole with AMO and CLA in case of H. pylori strains susceptible to CLA or MET in case of resistance to CLA. H. pylori eradication was assessed by 13C urea breath test (UBT) at least 8 weeks after completion of the treatment, considered successful if the UBT was negative.
Results : From October 2007 to September 2008, 98 children were included (58 female/42 male, median age 11y range 1,5 to 17). Age and sex distribution was similar between groups but resistance rate was higher in the sequential group. Eradication was achieved in 74 children out of 88 who returned for a follow-up test. The intention-to-treat eradication rate (ITT) was thus 76% (sequential 41/55 = 75%, triple therapy 33/43 = 77%) and the per-protocol cure rate (PP) 84% (sequential 41/49 = 84%, triple therapy 33/39 = 85%). In case of CLA resistance, ITT eradication rate was 9/14 (sequential 7/12 = 58%, triple therapy 2/2) and PP 9/13 (sequential 7/11 = 64%, triple therapy 2/2). In case of MET resistance, ITT eradication rate was 13/18 (sequential 9/13 = 69%, triple therapy 4/5) and PP 13/15 (sequential 9/10 = 90%, triple therapy 4/5). Both treatment were well tolerated.
Conclusion : Sequential treatment seems highly effective for eradicating H. pylori, with similar or higher eradication rate than with triple therapy prescribed in accordance with antimicrobial susceptibility testing. However, in case of CLA resistance the cure rate is decreased. Sequential treatment may be used as a first line therapy if antimicrobial susceptibility testing is not available in population with CLA resistance not exceeding 20%.

IBD

Invited lecture

- I01 -

NEW TRENDS IN EPIDEMIOLOGY OF PAEDIATRIC IBD. G. Veereman. Queen Paola Children's Hospital ZNA Antwerp.

Patients with Crohn's Disease (CD) first manifest their symptoms during childhood in 25% of cases. Concurrently, the age at which the first symptoms may appear, is decreasing to around 5 years of age. The incidence of CD has been increasing in children of all ages. Although the incidence of CD is lower in children than in adults, there has been a rapid rise over the last few decades, which now seems to be stabilizing. In the UK, the incidence of CD rose from 1.3 per 100,000 between 1983 and 1988 to 3.11 per 100,000 between 1989 and 1993, a threefold increase over 10 years. More recent epidemiological data indicates that the incidence of CD is now approximately 5.0 per 100,000 in the North American and European paediatric populations, with 25% to 30% of all CD patients being diagnosed before age 20 years. A slightly higher incidence of 5.2 per 100,000 children per year has been observed in children younger than 16 years with IBD in the UK and the Republic of Ireland. In Belgium the population under 18 years is 2 344 140. Assuming an incidence of 5.2 per 100,000 children per year, the incidence of new CD cases per year could be as high as 115. However, the prevalence of pediatric CD, their presentation, mode of care and follow-up is unknown in our country. Thus, the incidence of CD in children is rising alarmingly in westernized societies. Although the disease is similar to that in adults, there are clinical consequences unique to children, most importantly growth retardation and delayed sexual development. Consequently, it is important to diagnose and treat paediatric CD early. The aim of treatment should be to not only maintain symptomatic remission, but also to impact the course of disease. Therapeutic modalities have been recently expanded by the development of biological therapy.

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- I02 -

PRIORITIZATION OF MUCOSAL PROTEASE/PROTEASE INHIBITOR CANDIDATE GENES IN INFLAMMATORY BOWEL DISEASE THROUGH A SYSTEMATIC REVIEW ANALYSIS APPROACH. I. Cleynen (1), C. Mendes (2), T. Bekkering (1), E. Kellen (1), P. Juni (2), P. Rutgeerts (1), S. Vermeire (1), D. Lottaz (2). (1) KULeuven, Belgium ; (2) University of Bern, Switzerland.

Introduction : Inflammatory bowel disease (IBD) is an epithelial barrier disease characterised by chronic mucosal inflammation of the gastrointestinal tract. Proteases and protease inhibitors (P/PIs) affect one or several components that contribute to mucosal barrier integrity, and may thus be important players in IBD pathophysiology. According to the MEROPS database, the human genome encodes 641 and 150 known protease and protease inhibitor genes respectively.

Aims : The aim of this research, which fits in an FP7 European project (IBDase), was to identify from the extensive list of P/PIs those with highest possible impact on susceptibility and pathogenetic mechanisms in IBD for subsequent genetic and functional studies.

Materials and methods : We used a systematic review analysis approach containing two parts : a survey of human genetic studies performed in IBD and of P/PI gene variants, and the revision of studies on P/PI expression in the intestine in humans and animal models. The PubMed database was searched with relevant (MESH) terms for title/abstract fields. Potentially relevant references were retrieved to be screened on full text. Details of study design and quality, patient characteristics and results were entered in a database. Critical genomic regions associated with IBD, and Crohn's disease or ulcerative colitis specifically, were extracted, reflecting particular study characteristics. Scored summary tables were generated based on the total number of studies either reporting on the up or downregulation of a specific P/PI in IBD, or its presence in a critical genomic region.

Results : The initial search for genetic studies generated 1514 hits. 174 were included based on title and abstract, 63 on full text. Among the top-scored genes are several P/PI genes that have not yet been studied in IBD, as genes belonging to the ubiquitinase family of proteases and kalikreine-related peptidases. The initial search for expression studies generated 161 hits, 105 were included based on title and abstract, 70 on full text. Results clearly showed that expression studies were published preferentially for certain P/PI genes and/or families, for instance the matrix metalloproteinases and their inhibitors. Although some P/PIs came out of both genetic and expression analyses, most were different. P/PI expression regulation is thus not obviously linked to genetic association of polymorphisms in IBD.

Conclusion : The systematic review approach enabled us to prioritize P/PI candidate genes which will now become subject of genetic and functional studies in the context of the multidisciplinary effort of this European consortium. Novel pathogenetic insight can be expected.

- I03 -

EVIDENCE FOR SIGNIFICANT OVERLAP BETWEEN COMMON RISK VARIANTS FOR CROHN'S DISEASE AND ANKYLOSING SPONDYLITIS. M. Georges (1), D. Laukens (2), C. Libioulle (1), C. Sandor (1), M. Mni (1), B. Vander Cruyssen (2), H. Peeters (2), D. Elewaut (2), M. De Vos (2). (1) ULg, (2) UZ Gent.

Objectives : A large multicenter genome-wide association scan for Crohn's Disease (CD) has recently reported 40 CD susceptibility loci, including 29 novel ones (19 significant and 10 putative). To gain insight into the genetic overlap between CD and ankylosing spondylitis (AS), these markers were tested for association in AS patients.
Methods : The Illumina Golden Gate assay was used to genotype 182 AS patients for SNP markers corresponding to 39 of these CD-markers (excluding *NOD2*). Individual marker allele frequencies were compared between cases and previously described controls from the multicenter study. Joint p-value distributions compared with expectations in the absence of true alternative hypotheses.
Results : Two previously established associations, namely with the MHC and IL23R loci, were confirmed. In addition, rs2872507, which maps to a locus associated with asthma, showed a significant association with AS ($p = 0.03$). The distribution of p-values for the remaining 36 SNPs was significantly skewed towards low p-values unless the top 5 ranked SNPs (ORMDL, *NKX2-3, PTPN2*, *ICOSLG* and *MST1)* were excluded from the analysis.
Conclusions : Association analysis using risk variants for CD lead to the identification of a new risk variant associated with AS, in addition to the confirmation of 2 known and the detection of 5 potentially relevant associations contributing to common susceptibility of CD and AS.

- I04 -

SEROREACTIVITY TO FAECALIBACTERIUM PRAUSNITZII AND THE PRESENCE OF THIS MICRO-ORGANISM IN FECES OF CROHN'S DISEASE PATIENTS AND CONTROLS. N. Vermeulen, M. Joossens, S. Vermeire, P. Rutgeerts, X. Bossuyt. KULeuven.

Introduction : Crohn's disease (CD) and ulcerative colitis (UC) are considered to originate from an aberrant immune response towards commensal bacteria in the gut in genetically predisposed patients. An imbalance of beneficial and detrimental commensal organisms, called dysbiosis, has been observed in IBD patients. Up to 80% of IBD patients show reactivity to bacterial components in their serum. A reduction of Faecalibacterium prausnitzii has recently been reported in CD patients.
Aim : We evaluated whether patients with IBD show seroreactivity to F. prausnitzii and whether this reactivity is related to the presence of F. prausnitzii in fecal samples.
Methods : We separated a total protein extract of F. prausnitzii on 2D gel elektrophoresis and blotted serum of 4 UC patients, 8 CD patients and 8 healthy controls. We also examined DGGE profiles of fecal bacteria in 4 CD patients and 4 age and sex matched controls. We identified a band on DGGE gel as F. prausnitzii by sequencing.
Results : We found seroreactivity against several proteins in UC patients, CD patients, and healthy controls. No clear differences could be observed between the different groups, except for one prominent spot which was detected on western blots in 3 of 4 UC patients and in 6 of 8 CD patients. A very faint spot was observed in 1 of 8 healthy controls. F. prausnitzii was present in feces of 3 of the 4 CD patients and in all healthy controls. Interestingly, the same patient which lacked the band corresponding to F. prausnitzii on DGGE also lacked the prominent spot on western blot analysis.
Conclusion : Blotting of serum against a protein extract of F. prausnitzii revealed strong seroreactivity against one protein in 9 of 12 patients with IBD which was not found in healthy control sera. Absence of this seroreactivity in one patient with Crohn's disease correlated with absence of the bacterium on DGGE analysis of the fecal flora. Identification of this protein might allow to understand why this bacterium exhibiting anti-inflammatory effects is found in reduced amounts in the feces of patients with IBD.
https://secure.scholarone.com/user_images/ddw2009/1546/593425/FigDDW2009.jpg

Invited lecture

- I06 -

NEW ECCO CONSENSUS ON EXTRAINTESTINAL MANIFESTATIONS OF CROHN'S DISEASE. M. De Vos. UZGent.

About one third of IBD patients develop extraintestinal manifestations (EIM) involving articular, bone, skin, eyes, liver and thromboembolic symptoms. Statements about characteristics and treatment were published in 2006 (1) but very recently revised by ECCO working party and presented at the ECCO meeting in February in Hamburg.
Recent data emphasised the lack of consistent prospective data involving the participation of specialists as rheumatologists, dermatologists.. permitting a well circumscribed definition.
Although in many cases evolution parallels the response to treatment initiated for the intestinal inflammation, in other cases specific treatment needs to be initiated as NSAIDs for articular manifestations, biphosphanates for osteopenia, ursodeoxycholic acid for liver disorders and anticoagulants for thromboembolic manifestations. Recent studies in similar diseases supported the central role of inflammatory cytokines principally TNF alpha and the dramatic response to anti-TNF treatment. However prospective controlled studies evaluating the effect of the drugs on EIM in IBD are lacking and need to be considered in future trials with other new drugs.

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- I07 -

SCHISTOSOMA MANSONI PROTEINS ATTENUATE MURINE EXPERIMENTAL COLITIS : DETERMINATION OF T CELL CYTOKINES TO UNRAVEL IMMUNOLOGICAL PATHWAYS. N. Ruyssers (1), B. De Winter (1), J. De Man (1), A. Loukas (2), J. Weinstock (3), P. Pelckmans (1), T. Moreels (3). (1) UZ Antwerp, (2) Queensland Institute of Medical Research, Brisbane, Australia, (3) Tufts New England Medical Centre, Boston, USA.

Background : The aim of this study was to unravel underlying immunological pathways by which *Schistosoma mansoni* soluble worm proteins (SmSWP) improve the healing process of 2,4,6 trinitrobenzene sulfonic acid (TNBS) colitis in mice.
Methods : Male Swiss OF1 mice were divided in 4 groups : Control + phosphate buffer, TNBS + PBS, TNBS + 25 ¼ g SmSWP (n = 4-7). Colitis was induced by intrarectal administration of 10 mg TNBS in 30% ethanol. Control mice were given saline intrarectally. Six hours after TNBS or saline injection mice were treated i.p. with 25 ¼ g SmSWP or PBS. Three days later, mice were sacrificed and T cells were isolated from colon, mesenteric lymph nodes (MLN) and spleen. Focussing on the balance between Th1, Th2, Th17 and Treg cells in these tissues, we measured the relative mRNA expression of IFN-^{3} and IL-12, IL-4 and IL-5, IL-17, IL-10 and TGF-^{2} by real-time RT-PCR with control-PBS group as calibrator.
Results : Induction of colitis significantly increased the relative expression of IFN-^{3} mRNA locally in the colon from 1.0 ± 0.0 in controls to 11.2 ± 3.6 in TNBS-PBS mice, while treatment of colitis with SmSWP reversed IFN-^{3} expression to 2.1 ± 0.6. Treatment of control and TNBS-colitis mice with SmSWP significantly downregulated IL-17 expression in colonic T cells and in MLN T whereas it significantly increased the relative mRNA expression of IL-10 (1.8 ± 0.3 in control + SmSWP and 1.6 ± 0.2 in TNBS + SmSWP) and TGF-^{2} (1.4 ± 0.2 in control + SmSWP and 1.3 ± 0.2 in TNBS + SmSWP) in colonic T cells. This SmSWP-induced upregulation of Treg cytokines was not observed in MLN and spleen T cells, where we could demonstrate a TNBS-induced upregulation of IL-10. We did not find a significant Th2 cytokine response in any of the tissue types.
Conclusions : Our results suggest that the beneficial effect of *Schistosoma mansoni* proteins on TNBS colitis is linked to stimulation of regulatory T cells and suppression of proinflammatory T cells. Furthermore, we provide evidence for the importance of the recently discovered Th17 pathway that is influenced by SmSWP treatment.

- I08 -

SURGICAL CLOSURE OF CROHN'S RECTOVAGINAL FISTULAS IN THE ERA OF BIOLOGICAL THERAPIES. G. Van Assche, S. Vermeire, P. Rutgeerts, C. Ruffolo, F. Penninckx. KULeuven.

Purpose : The treatment of rectovaginal fistulas remains challenging.
The study aims to review early and late results of surgery for rectovaginal fistulas and to assess the effect of anti-TNF treatment on primary healing.
Methods : Fifty-two patients who underwent surgery for a rectovaginal fistula between January 1993 and December 2006 were identified using a prospectively collected database. Outcome analysis was performed in February 2008.
Data was collected on the surgical procedures attempted in order to achieve fistula closure.
A sub-analysis was conducted on 23 patients (23/52 = 44.2%) who received anti-TNF treatment.
Results : Fistula closure was achieved in 80.7% (CI : 67.1%-89.1%) of patients.
The primary and secondary surgical success rates were 55.8% and 56.5%, respectively.
Primary healing was not significantly increased,66.7%, CI :41.0%-86.7% in patients who received anti-TNF treatment, compared to 56%, CI 37.9%-72.8% in patients who had not received a biological .
Duration of disease ($p = 0.037$) and previous colitis ($p < 0.001$) influence primary healing. T
he median follow-up time was 109 months (range 24-180). Late recurrence occurred in 4 patients (9.5% of patients).
Conclusions : The majority of patients will achieve closure of Crohn's rectovaginal fistula following surgery.
Preoperative anti-TNF treatment showed a trend to increased primary healing.

- I09 -

INCREASED INTESTINAL VEGF EXPRESSION AND MUCOSAL VASCULARIZATION IN PATIENTS WITH SPONDYLARTHROPATHY. P. Hindryckx, G. Serry, D. Laukens, H. Peeters, M. De Vos. UZ Gent.

Background : Chronic intestinal inflammation observed in about a quarter of patients with spondylarthropathy (SpA) is linked to Crohn's disease (CD). Increased expression of Vascular Endothelial Growth Factor (VEGF) and intestinal mucosal angiogenesis has been described in Crohn's disease, in correlation with disease activity.
Study aim : To investigate VEGF expression and mucosal angiogenesis in SpA patients with or without subclinical gut inflammation.
Methods : Sections of paraffin-embedded ileal and colonic biopsies from healthy controls (HC) (negative control, number of biopsies (N) = 20), Crohn patients (positive control, N = 20), SpA patients without gut inflammation (N = 30) and SpA patients with subclinical gut inflammation (N = 28) were immunostained for VEGF and for CD31 and blindly scored by computerized morphometric analysis using specialized software.
Results : Both intestinal VEGF activity and mucosal vascular density (MVD) were higher in SpA patients versus healthy controls, independent of the presence of chronic gut inflammation (HC vs SpA patients without gut inflammation : $P < 0,05$ and $P < 0,01$ respectively for VEGF and MVD ; HC vs SpA patients with chronic gut inflammation : $P < 0,001$ both for VEGF and MVD). Nevertheless, presence of subclinical chronic gut inflammation was associated with much stronger intestinal VEGF expression and mucosal vascularization, resembling the pattern of Crohn's disease (SpA patients with gut inflammation vs SpA patients without gut inflammation : $P < 0,001$ for VEGF ; $P < 0,01$ for MVD). Unlike in Crohn patients however, the high VEGF activity and MVD observed in SpA patients with chronic gut inflammation was not clearly dependent on the presence of active inflammation in the biopsy (epithelial infiltration of polymorphonuclear cells, ulcerations, granulomas).
Conclusion : A pro-angiogenic intestinal phenotype is observed in SpA patients which, unlike in Crohn's disease, is not clearly dependent of active gut inflammation.

SBNC – VVKVM

- N02 -

NUTRITIONAL ASSESSMENT OF PATIENTS UNDERGOING ELECTIVE COLORECTAL RESECTION FOR CANCER. L. Moerman, E. De Waele, S. Mattens, B. De Waele, G. Delvaux. UZ Brussels.

Colorectal cancer (CRC) patients are not known to have important food intake impairment and therefore are seldom nutritionally screened. A large multicenter study involving 997 CRC patients, however, identified a body weight loss > 10% as an independent risk factor of post-operative mortality (*Arch Surg*, *2005, 278-283*). The aim of the study presented here was to estimate the degree of malnutrition in patients undergoing elective colectomy for CRC in our institution.

Methods : In this preliminary report on 45 CRC patients the following data were prospectively recorded : age, gender, height, body weight (BW), body mass index (BMI), unintentional BW loss during the last 6 months, nutritional screening using the NRS 2002 Score, estimated daily energy and protein requirements, serum albumin level, tumor location, preoperative radiotherapy, type of surgical procedure and eventual histological tumor differentiation and stage. Postoperatively, BW and serum albumin concentration were determined on a weekly basis, and at discharge from hospital.

Results : *Preoperative nutritional status* : 27 patients (60%) had < 5% weight loss during the 6 months preceding surgery, 16 patients (36%) had 5-10% weight loss and 2 patients (4%) had > 10% weight loss. The serum albumin concentration was 3.9 ± 0.4 g/dL and the median NRS score was 3 (range 2_4). *Postoperative nutritional status*. None of the patients was able to maintain the preoperative weight and 23 patients had lost < 5% BW, 19 patients had lost between 5 and 10% BW and 3 patients had lost > 10% BW at discharge. The mean albumin concentrations after 1 week and at discharge were 3.2 ± 0.4 and 2.9 ± 0.4 g/dL, respectively. The overall weight loss since the beginning of disease was < 5% in 7 patients (16%), 5_10% in 20 patients (44%) and > 10% in 18 patients (40%).

Conclusion : A number of CRC patients had already a variable degree of preoperative malnutrition, but the nutritional status of all patients included in this study deteriorated during their hospital stay, resulting in a final loss of more than 10% of the pre-illness body weight in 40% of the patients. Since malnutrition represents an unfavorable prognostic factor in the management of CRC, routine preoperative nutritional screening, followed by individualized nutritional care in those at risk, seems of cardinal importance.

- N03 -

WEIGHT LOSS AS THE MOST POWERFUL PREDICTOR OF POSTOPERATIVE MORTALITY AND MORBIDITY AFTER SURGERY FOR HEAD AND NECK CANCER. L. Pire. ULg Sart Tilmant.

The present study aimed at retrospectively evaluating the incidence of postoperative complications in consecutive patients who underwent surgery for head and neck cancer (HNC) between 2002 and 2004, and to compare the incidence of complications according to demographic criteria, the extension of the tumor (TNM classification), the type and characteristics of surgery, any adjunctive therapy, the preoperative percentage of weight loss (WL), body mass index (BMI) and nutrition risk score (NRS).

Patients and methods : One hundred and four patients affected by HNC admitted for head and neck surgery were considered included. All-cause postoperative complications and mortality rates were recorded and the risk factors were compared by uni- and multivariate analyses.

Results : Among the 104 patients, 54% had a NRS score > 3, 39% had a WL > 5%. Postoperative complications occurred in 40% and the postoperative mortality was 14%. The rate of postoperative complications did not differ between patients with NRS < or > 3. The rate of infectious complications and the mean length of stay were higher in patients with WL > 5% than in those without WL. By multivariate analysis, WL > 5% was found as the strongest predictor for infectious complications and mortality (both $p < .05$).

Conclusion : The present study confirm that a preoperative WL > 5% is associated with an higher likelihood of complicated postoperative course (1,2). These patients are the most likely to benefit from perioperative nutrition support (3).

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- N04 -

HOME CARE AND CARE HOMES : STRATEGY FOR IMPLEMENTING ADEQUATE NUTRITION. REPORT OF THE COUNCIL OF EUROPE (4/12/08). M. Arvanitakis (1), P. Coppens (2), L. Doughan (3), A. Van Gossum (1). (1) ULB Erasme ; (2) EAS ; (3) CD-P-SC.

Malnutrition is frequent in elderly patients residing in care homes and home care. Consequences of this state are significant. In particularly, malnutrition may prolong the patients' hospital stay, increase the complication rate up to 20 times and increase death rates compared to well-nourished patients with the same diseases.
Screening with various tools is the first step in assessing and treating malnutrition. A multidisciplinary approach is important to successfully tackle malnutrition on multiple levels : the patients, the health care workers (medical and non-medical), the public, policy makers and society stakeholders.
A series of recommendation has been proposed in order to act on different levels. With regard to prevalence and causes, recommendations include promoting better nutrition, oral hygiene and avoiding social isolation, as well as encouraging family-style meals in care homes. More specifically for older individuals, it is important to preserve autonomy by offering adapted housings and catering services. Concerning the impact of malnutrition on health care costs, recommendations include enabling smooth transitions from hospital to other settings, expanding reimbursement for dietary counselling, supplemental foods and artificial nutrition. Continuous educational programs in clinical nutrition may enhance awareness in health care professionals involved in nutritional care of individuals. More specifically, dieticians should have a central role in this domain. Public health messages concerning malnutrition and its consequences must reach all individuals. Finally, government policies should include assigning political responsibility for elderly people and putting malnutrition at the political agenda.

- N07 -

THE ROLE OF THE NUTRITION NURSE IN THE TEACHING AND FOLLOW-UP OF PATIENTS ON LONG-TERM HOME PARENTERAL NUTRITION. A. Ballarin. ULB Erasme.

A programme of Home Parenteral Nutrition (HPN) has been initiated in Erasme Hospital in 1987. During the last 20 years, about 130 patients were discharged home on HPN. Globally, 60% of these patients suffered from short bowel related to benign diseases and the others were cancer patients who were enable to be fed either orally or enterally.
Altogether this experience includes 100000 days of HPN. Since 6 years a specialized nutrition clinical nurse (NCN) has been dedicated to this programme. The NCN is a member of the Nutrition team that also includes physicians, dieteticians and pharmacists.
The role of the NCN is of primary importance regarding HPN. The major tasks are as follows :
– Information to the patient and relatives.
– Teaching the patient for HPN handling.
– Close contact with the physicians and pharmacist.
– Creation of documents for teaching and follow-up.
– Logistic organisation at home.
– Contact with community nurses if needed.
– Contact with the general practionner.
– Care about legal document for reimbursement.
– Follow up of the patient.

iN the field of HPN, the NCN is a "liaison" nurse who coordinates all the care givers.
The main objectives are to provide the best quality care, to improve the quality of life of this patient and to be cost-effective in avoiding rehospitalisation.

- N08 -

THE SUPPLEMENTATION OF 10G/DAY ARABINOXYLO-OLIGOSACCHARIDES, A NEWLY PROPOSED PREBIOTIC, TO THE DIET OF HEALTHY SUBJECTS IS WELL TOLERATED. L. Cloetens, W. Broekaert, C. Courtin, J. Delcour, P. Rutgeerts, K. Verbeke. LfoRCe.

Introduction : Arabinoxylo-oligosaccharides (AXOS) have recently been proposed as a new class of prebiotics. AXOS are obtained by enzymatic degradation of arabinoxylan, a major hemicellulose component of cell walls in cereals. Previously, a stimulation of faecal bifidobacteria was observed after administration of 2.2 g and 5.0 g/day AXOS to healthy subjects. In this study, we tested the tolerance of healthy subjects to AXOS.
Materials and methods : Twenty healthy subjects participated to this randomised, placebo-controlled cross-over study. They received AXOS (2×5 g/day) or placebo (= maltodextrin) for 3 weeks with a wash-out period of 4 weeks in between. Before and immediately after each intake period, blood samples were obtained to determine haematological parameters, clinical chemistry parameters (enzymes, proteins, minerals, vitamin A and E, glucose) and blood lipids. The subjects graded the following 7 symptoms : gastrointestinal discomfort which diminishes after defaecation, gastrointestinal pain which diminishes after defaecation, gastrointestinal discomfort concomitant with constipation or diarrhoea, gastrointestinal pain concomitant with constipation or diarrhoea, flatulence, cramps and bloating. The severity of the symptoms was graded on a 4-step scale ranging from no (0), mild (1), moderate (2) to severe (3) symptoms. A total score was calculated as the mean of the individual symptom scores.
Results : AXOS or placebo treatment did not affect most of the haematological and clinical chemistry parameters. Small, yet significant, changes in the levels of haematocrit, RBC, LDH, total protein and potassium were found after the intake of AXOS. After the intake of placebo, the levels of mean percentage haemoglobin/erythrocyte, platelet count, LDH and albumin were significantly different. However, these outcomes were not considered important since they were slight and remained within the normal range. Total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol content were not influenced.
After intervention with AXOS, the total symptom score was slightly increased. Particularly, the flatulence score was significantly more increased after AXOS than after placebo ($p = 0.026$). Yet, mean scores for all individual symptoms were lower than 1 and thus, graded as mild.
Conclusion : Administration of 10g/day AXOS to healthy subjects was well tolerated.

- N09 -

THE INCREASED LEVEL OF BIFIDOBACTERIA AND A BENEFICIALLY MODULATED COLONIC METABOLIC ACTIVITY SUGGEST THE PREBIOTIC POTENTIAL OF ARABINOXYLO-OLIGOSACCHARIDES IN HEALTHY SUBJECTS. L. Cloetens (1), Y. Delaedt (2), W. Broekaert (1), C. Courtin (1), F. Ollevier (2), J. Delcour (1), P. Rutgeerts), K. Verbeke (1). (1) LfoRCe ; (2) KULeuven.

Introduction : Arabinoxylo-oligosaccharides (AXOS) are a degradation product of the cereal fibre arabinoxlyan. In this study, we investigated their prebiotic potential in healthy humans. We measured their effects on faecal total bifidobacteria and B adolescentis and their influence on parameters of colonic metabolism during and after the intervention. Ammonia metabolism was analysed using the biomarker lactose-[^{15}N, ^{15}N_]-ureide (^{15}N-LU) and urinary phenolic excretion was determined as a measure of protein fermentation.
Materials and methods : Twenty healthy subjects participated to this placebo-controlled, cross-over study. They received AXOS (10 g/day) or placebo (= maltodextrin) for 3 weeks with a wash-out period of 4 weeks in between. Before and immediately after each intake period, all subjects consumed a test meal (without substrate) labelled with ^{15}N-LU. Thereafter, urine (48 h) and faeces (72 h) was collected. Ten subjects also performed an extra test after 2 weeks intake (during intervention). Faecal microbiota (log/g dry faeces) were measured using real-time PCR whereas the ^{15}N enrichment (% of administered dose) in the samples was measured by combustion-IRMS. Stimulation in microbial activity after prebiotic intake is reflected in a larger fraction of ^{15}N-NH_3 being taken up by the bacteria and excreted in faeces and a smaller fraction of the label being excreted in urine. Urinary phenolics (mg/24 h) were assessed by GC-MS.
Results : The level of bifidobacteria was significantly increased during (+ log1.1, $p = 0.028$) and after intake of AXOS (+ log0.8, $p = 0.012$). After the intake period with placebo, bifidobacteria were increased as well, yet to a lower extent (+ log0.4, $p = 0.008$). The level of B adolescentis was significantly increased after intake of AXOS (+ log0.7, $p = 0.013$). During AXOS and placebo intake, urinary ^{15}N-excretion was significantly decreased (-11%, $p = 0.015$ and -5%, $p = 0.015$, respectively) whereas faecal ^{15}N-excretion increased (+ 3%, not significant and + 8%, $p = 0.036$, respectively). After the intake period with AXOS and placebo, no changes in ^{15}N-excretion were seen. Urinary p-cresol excretion was significantly decreased during intake of AXOS ($p = 0.011$) and tended to decrease after the intake period. Phenol excretion remained unchanged. Placebo did not influence p-cresol and phenol excretion.
Conclusion : At a dose of 10 g/day, AXOS exert prebiotic properties in healthy humans. Changes in composition of microbiota were related to changes in metabolic activity. However, changes in metabolic activity were more pronounced during than immediately after the intervention, suggesting that the actual fermentation of the carbohydrate is an important factor.

BGDO

Invited lecture

- O01 -

TREATMENT OF CANCER OF THE ESOPHAGUS AND GASTROESOPHAGEAL JUNCTION : LESSONS OF 2000 ESOPHAGECTOMIES. A. Lerut. KULeuven Gasthuisberg.

Between 1975 and June 2007 two thousand esophagectomies for cancer of the esophagus and gastroesophageal junction (GEJ) were performed in a single centre.
Over this period of 32 years a gradual increase of adenocarcinoma was seen being over 70% of all esophagectomies nowadays. Over time the overall 5-year cancer specific survival increased from 29.2% to 47.3% and the overall 10-year cancer specific survival from 24.8% to 34.1%. This impressive improvement of outcome is multifactorial. Through better surgical techniques and improved perioperative management postoperative mortality after primary surgery decreased from 11.9% in the seventies to 1.9% in the period 2005-2007. However postoperative mortality after induction radiochemotherapy, although showing a similar trend remained higher being 61.9% in the period 2005-2007.
Better selection and improved staging through introduction of EUS, high resolution CT and PET scan have resulted in a sharp decrease of exploratory thoracotomies which are currently well below 5%. Nevertheless accuracy of clinical staging in particular of the T and N factor remains suboptimal with under- and overstaging in approximately 25% of the patients.
Refinement of surgical techniques in particular the introduction of en bloc resection and more extensive lymphadenectomy substantially improved R0 resection rate from 79% in the first cohort of 500 patients up to 91.6% in the last cohort of 500 patients.
As a result primary surgery in advanced pathologic stage III cancer today offers 5-year cancer specific survival figures of over 25% and if the number of involved lymph nodes is limited to ≤ 6 and located in the peritumoral area the 5-year survival reaches nearly 40%.
However despite these improved outcome figures the majority of patients presenting with advanced stage carcinoma will die mostly from systemic recurrent disease.
In an effort to improve the outcome of these patients multimodality regimens have been introduced. But the results of the available trials remain conflicting and until now no clear advantage has been shown as to the overall 5-year survival figures. It appears that only complete responders, in our experience 25%-30% of these patients seem to benefit the non responders paying the price as they appear to have a worse outcome. Tumor markers for responses prediction unfortunately are still lacking.
Also adjuvant therapy is lacking evidence of offering a substantial survival benefit.
As to the early carcinoma (T1) endoluminal resection is rapidly gaining importance for T1a tumors. For T1b tumors because of the risk of lymph node involvement, reaching 30% in our series, esophagectomy combined with proper lymphadenectomy remains the gold standard.
Conclusion : The results of surgical treatment of cancer of the esophagus and GEJ has substantially improved over the last 3 decades. These improvements are mainly due to epidemiological and diagnostic aspects, a better selection of patients, better surgical technique and perioperative management and in carefully selected patients multimodality regimens.

- O02 -

SMALL-CELL CARCINOMA OF THE OESOPHAGUS : A MULTICENTRE, RETROSPECTIVE STUDY OF THE RARE CANCER NETWORK. B. Vos (1), T. Rozema (2), R. Miller (3), A. Hendlisz (1), J.-L. Van Laethem (4), K. Khanfir (5), D. Weber (6), P. Van Houtte (1). (1) ULB Institut Bordet ; (2) UMC St. Radboud, Nijmegen, Nederland ; (3) Mayo Clinic, Rochester, Minnesota, U.S.A. ; (4) ULB Erasme ; (5) Hôpital de Sion, Sion, Switzerland ; (6) Hôpitaux Universitaires de Genève, Switzerland.

Background : Small cell carcinoma of the oesophagus (SCCO) is a rare and aggressive malignant tumour with a poor prognosis. The aims of this retrospective study were to analyse the epidemiology, clinical characteristics and treatment outcomes of this patients.
Methods : Between 1994 and 2004, 24 patients with SCCO from several centres were reviewed for data on demographics, presenting symptoms, diagnosis, disease stage, type of treatment and outcome.
Results : SCCO occurs in the sixth decade : median age (IQR) : 65 (59-69) years with a male predominance (63%). All patients were Caucasian. The most common complaining symptoms were rapidly progressive dysphagia (79%), weight loss (54%) and retrosternal/epigastric pain (46%). The tumour arises primarily in the middle (52%) or in the lower (35%) third of the oesophagus. History of tobacco and alcohol exposure was present in 90% and 70% of case, respectively. Paraneoplasic syndromes were not observed. Extensive disease was present in 13 cases (54%) at initial diagnosis. In histology, SCCO was pure, differentiated, in 54% of cases (13/24), and pure, undifferentiated/anaplastic, in 38% of cases (9/24). Mixed tumours with squamous differentiation were present in 8% of cases (2/24). In treatment, six patients (25%) received only symptomatic management. In treated patients (18/24), six received chemotherapy alone and twelve were treated by a combination of chemotherapy with local treatment (surgery or radiation therapy). The overall median survival (IQR) was 11 (8-20) months for all 24 patients. Chemotherapy increased the survival compared to symptomatic management in extensive disease (median survival (IQR) : 9.5 (6-14) vs. 6 (4-7) months, $p = 0.05$). In limited disease, concurrent chemo-radiotherapy was more effective than non-concurrent treatment (median survival (IQR) : 36 (14-93) vs. 11 (9-15) months, $p = 0.044$). All cases of a complete response (8 cases) to treatment were observed in limited disease SCCO and seven of them received chemotherapy in association to local therapy. Four of eight complete responders were alive more than two years after treatment.
Conclusions : Chemotherapy is the cornerstone of treatment of SCCO in all stage. Concurrent chemo-radiotherapy should be considered as the standard treatment for limited disease SCCO.

- O03 -

PHASE II STUDY OF PREOPERATIVE HELICAL TOMOTHERAPY FOR RECTAL CANCER. M. De Ridder (1), B. Engels (1), H. Everaert (1), A. Hoorens (1), A. Sermeus (1), K. Tournel (1), D. Verellen (1), Y. Van Nieuwenhove (2), B. Op De Beeck (3), H. Versmessen (1), G. Storme (1). (1) UZ Brussel ; (2) UZ Gent ; (3) UZA.

Purpose : Preoperative chemoradiotherapy is standard of care for locally advanced rectal cancer according the PROCARE guidelines. However, adding concomitant chemotherapy to preoperative radiotherapy does not improve survival or the incidence of distant metastasis, and is associated with considerable grade 3 + toxic effects (1,2). The Tomotherapy Hi·ART II system is dedicated to intensity-modulated and image-guided radiotherapy. These technological evolutions allow increasing the dose to the tumor and decreasing the volume of healthy tissue that is irradiated (3,4). The aim of this study is to explore the efficacy and toxicity profile of helical Tomotherapy in the preoperative treatment of rectal cancer.
Patients and methods : This interim analysis reports the first 74 patients. A dose of 46 Gy, in daily fractions of 2 Gy, was delivered to the presacral space and the perineum if an abdominoperineal resection was deemed necessary. No concomitant chemotherapy was administered, but the dose of radiation was increased by a simultaneous integrated boost to 55.2 Gy, when the circumferential resection margin (CRM) was less than 2 mm. Acute toxicity was evaluated weekly. The response was determined by measuring the metabolic tumor volume prior to and five weeks after the end of radiotherapy by FDG-PET or PET-CT.
Results : 31 patients presented with a T2 or good T3 tumor (CRM e 2 mm) and entered the no boost group, 43 patients presented with a bad T3 (CRM < 2mm) or T4 tumor on MRI and entered the boost group. Lymph node metastases were suspected in 85% of patients. Median age was 69 years (range 32 _ 85). One patient, in the no-boost group, developed grade 3 enteritis. No other grade 3 + acute toxicities were observed. 9, 7, 24 and 1 patient developed acute grade 2 gastrointestinal, urinary, dermatologic and gynecologic toxic effects respectively. The mean decrease in metabolic volume was $61 \pm 30\%$ in the no boost group, compared to $76 \pm 27\%$ in the boost group ($p = 0,04$). An abdominoperineal resection was deemed necessary for 31 patients before radiotherapy. 8 of these patients could benefit from sphincter sparing surgery. 6 patients developed an anastomotic leak within 30 days post surgery, 7 patients had an R1 resection. With a mean follow-up of 12 months, no local relapses were observed.

Conclusions : Helical tomotherapy reduces acute toxicity in the preoperative radiotherapy of rectal cancer. A simultaneous integrated radiation boost increases the metabolic response, without excessive toxicity. This approach will be compared to chemoradiation in a randomized multicenter phase III trial.

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- O04 -

SIGMOID COLON CANCER WITH METASTASIS : RESECTION OF THE PRIMARY TUMOUR FOLLOWED BY PALLIATIVE CHEMOTHERAPY VERSUS PALLIATIVE CHEMOTHERAPY ALONE. S. Van Gool (1), V. Moons (2), P. Christiaens (2), G. Van Olmen (2), G. Dhaens (2). (1) KULeuven Gasthuisberg ; (2) Imeldaziekenhuis, Bonheiden.

Background : One third of patients diagnosed with colorectal carcinoma presents with liver metastasis. It is unknown whether these patients benefit from primary tumour resection prior to palliative chemotherapy.
Aim : We performed a single centre retrospective study comparing the characteristics and outcome of patients with cancer of the sigmoid colon and metastasis treated with resection of the primary tumour followed by palliative chemotherapy versus palliative chemotherapy alone.
Patients and methods : Using anatomopathological reports and subsequently medical files 34 patients meeting the criteria diagnosed between June, 2003 and September, 2007 were identified. Patients with and without primary resection were compared for patient characteristics, duration and type of chemotherapy, survival, obstruction/stenosis, and established prognostic factors of metastatic colon cancer (ECOG-score, number and location of metastasis, LDH, alkaline phosphatase, blood cell counts, weight loss, albumin, CEA, CRP, AST, ALT, bilirubin, PT, gamma GT). Statistical analysis was performed with Fisher's exact test, unpaired t-test and Mann-Whitney-test.
Results : 13 patients (8 m/5 f, mean age 63.85) were treated with surgery followed by palliative chemotherapy, while 21 patients (16 m/5 f, mean age 70.43) were initially treated with palliative chemotherapy. Patients in the first group were overall younger (mean age 63.85 + /- 2.47 yrs (SEM) versus 70.43 + /- 2.02 yrs, $p = 0.049$). Before the start of therapy there was no difference in obstructive symptoms among the two groups ($p = 0.1164$). During follow-up 7 of the 21 patients treated with primary palliative chemotherapy developed obstruction, which was treated with endoscopic stenting ($n = 3$), stenting followed by resection ($n = 1$) or argon plasma coagulation ($n = 1$), or surgical resection ($n = 2$). None of the patients who underwent primary surgery developed local recurrence or obstructive symptoms. The patients treated with surgery followed by palliative chemotherapy survived significantly longer (mean 21.4 + /- 1.99 versus 10.71 + /- 1.69 months after diagnosis, $p = 0.0072$), and received chemotherapy for a longer period of time (16.23 + /- 2.44 versus 8.86 + /- 1.24 months, $p = 0.054$). Of the prognostic markers alkaline phosphatase (mean 127.31 versus 227.95 U/l, $p = 0.0179$), haemoglobin (mean 14.62 versus 12.34 g/l, p d 0.001), CEA (mean 310 versus 1956 ng/ml, $p = 0.066$) and ECOG ($p = 0.0286$) were more favourable in patients treated with surgery first.
Conclusion : In this survey patients who underwent initial resection tended to be younger and fitter. Overall, they could received more chemotherapy and survived longer. Patients who did not have surgery developed local problems quite frequently needing further intervention. Patients in decent physical condition should preferably undergo surgery prior to initiation of chemotherapy .

- O05 -

MAJOR HEPATECTOMIES INCREASES THE RISK OF MORBIDITY AND MORTALITY IN SIMULTANEOUS RESECTION OF SYNCHRONOUS LIVER METASTASES AND COLORECTAL CANCER. B. Van Den Bossche, K. Boterbergh, S. Laurent, I. Dero, F. Berrevoet, M. Peeters, B. De Hemptinne, R. Troisi. UZ Gent.

Background : Resection of the rectal primary neoplasm with synchronous liver metastases (LM) is warranted, because this is the only strategy with curative potential. Combined resection remains controversial because of the risk of morbidity. We reviewed our experience of combined colorectal and LM surgery in patients in stage IV of colorectal cancer (CRC).
Methods : From October 1995 to July 2008, 12 (0.1%) out of 1155 liver resections were combined to the resection of primary CRC. Mean patients age was of 58.2 ± 12.4 y. Indications for combined surgery was mainly due to logistic and technical reasons.
Results : Mean size of CRM was of 4.9 ± 4.6 cm. Type of liver resection consisted on 6 (46.2%) minor, 5 (38.5%) major hepatectomies and 2 atypical resections. Length of hospital stay was 20.5 ± 12.6 days. Six patients (50%) had a major complication consisting in : liver insufficiency with encephalopathy (n = 1) ; liver absces(n = 1) ; ARDS (n = 1) ; pneumonia (n = 1). In 2 patients with intraabdominal abscesses a dehiscence of colorectal anastomosis was recorded requiring surgical exploration and diverting stoma. When comparing morbidity between major and minor hepatectomies we could withdraw, respectively 4 severe (67%), 2 mild (33%) complications and 2severe (33%), 4 mild (67%) complications ($p = 0.24$; Fisher's exact). After a median FU of 21.5 m (range 1-82m) eight patients deceased, nearly all (88%) due to progression of malignancy. One patient was lost in follow up and three patients are disease-free.
Conclusions : Simultaneous liver and colorectal resection in patients with stage IV CRC is feasible but patients undergoing major hepatectomies are at risk of severe morbidity potentially leading to increased mortality rate. Patient's medical condition, age, extent of primary and metastatic disease should all influence the choice of the appropriate strategy.

- O06 -

DEFINING THE OPTIMAL THERAPY SEQUENCE IN SYNCHRONOUS RESECTABLE LIVER METASTASES FROM COLORECTAL CANCER : A DECISION ANALYSIS APPROACH. E. Van Dessel, K. Fierens, P. Pattyn, Y. Van Nieuwenhove, M. Peeters.

Introduction : Approximately 20% of colorectal cancer CRC) patients present with potentially resectable liver metastatic disease. When a major hepatectomy is required, simultaneous resection is generally discouraged because of significant potential morbidity. Traditionally, patients undergo resection of the primary tumor first. Recently, the 'liver first' approach has been advocated consisting of upfront chemotherapy followed by liver resection, ultimately followed by resection of the primary. Since no comparative trials have been performed, a formal decision analysis may support clinical decision making.
Methods : a decision tree was constructed consisting of two therapy sequences : a) colectomy, chemotherapy, hepatectomy and b) chemotherapy, hepatectomy, and colectomy. Transition probabilities including the risk of death from complications or disease progression were obtained for each step from literature data and entered into a decision model. Sensitivity analysis was performed to evaluate the model's validity under a variety of assumptions. Calculations were done using TreeAge Pro software.
Results : After a large number of simulated trials, the global overall survival (median) was 38 months. The results of the decision analysis demonstrate that the 'liver first' approach (chemotherapy followed by hepatectomy followed by colectomy) is associated with a better outcome (5 year survival 42% versus 29%, $P < 0.01$). The model proved to be sensitive to operative mortality, but not to chemotherapy related mortality.
Conclusion : The results of this decision analysis suggest that, in patients with synchronous resectable colorectal liver metastases, the 'liver first' approach results in a superior outcome. Randomized trials will be needed to confirm the results of this simulation based outcome.

PATHOLOGY & BGDO

- P01 -

AGGRESSIVE SYSTEMIC MASTOCYTOSIS : ABOUT A CASE OF INTESTINAL PRESENTATION. D. Bafort (1), C. Sempoux (1), Ph. Bohon (2), I. Théate (1), A. Jouret-Mourin (1). (1) UCL Saint-Luc ; (2) Hôpital de Fourmies, France.

A 43-year-old woman was referred for watery diarrhoea and abdominal pain of 3-month duration. No other symptom or sign were identified at the initial history-taken and clinical examination. Endoscopic investigations of both upper and lower digestive tracts were performed, with no macroscopic abnormalities. Biopsies of the duodenal and ileal mucosae showed shortening of the villi, with increased infiltration of lymphocytes, plasmocytes, and eosinophils. In addition, numerous small atypical clear cells were identified as degranulated mast cells only by immunostaining for CD117 and tryptase. Complementary clinical and biological investigations, and particularly the mention of past *urticaria pigmentosa*, further supported the diagnosis of aggressive systemic mastocytosis (SM).
SM is a rare entity, which only represents 10% of all mastocytosis. The diagnosis is frequently difficult, and the evolution may be aggressive and life-threatening. SM is characterized by mast cells proliferation in skin, lymph nodes and parenchymal organs. Typical symptoms include pruritus, flushes, asthma, headache and malabsorption. In case of intestinal involvement, endoscopic investigations may show mucosal changes, such as nodularities. The difficulty of diagnosis relies on the identification of degranulated atypical mast cells. Therefore, immunostaining for CD117 and tryptase should be performed in the diagnostic procedure of undetermined malabsorption and diarrhoea.

- P02 -

EVALUATION OF THE USE OF INTRALESIONAL CIDOFOVIR IN THE TREATMENT OF INTRA-ANAL CONDYLOMATA ACUMINATA WITH DYSPLASIA. S. Struyf (1), G. De Hertogh (2), G. Coremans (2), K. Geboes (2). (1) KULeuven, (2) UZLeuven.

Introduction : Anogenital infections with human papillomavirus (HPV) are among the most common sexually transmitted diseases. The incidence of squamous cell cancer (SCC) developing from HPV-related anal condylomata acuminata with dysplasia is increasing in homosexual men. Anal warts are classically treated with coagulation, but this therapy is associated with much patient discomfort and frequent recurrence of the lesions. Cidofovir is an acyclic nucleoside phosphonate analogue used for the treatment of cytomegalovirus retinitis in AIDS patients. In pilot studies, topical application of cidofovir in patients with anal condylomata proved effective (1).
Aims & methods : The aim of our study was to evaluate the effectiveness of repeated intralesional injection of cidofovir in anal condylomata with histologically proven dysplasia.
Seven homosexual men presenting with internal anal warts with dysplasia were treated with repeated intralesional injections of 5-25 mg cidofovir on a monthly basis, followed by coagulation for lesions that did not clear. Biopsies taken before the first application of cidofovir were evaluated microscopically for the presence and grade of dysplasia using a 3-tiered classification system (anal intra-epithelial neoplasia, AIN grade I, II or III). These data were compared with results obtained from the last follow-up biopsy in every patient. Biopsies were evaluated on haematoxylin-eosin stained slides, and with immunohistochemical stains for an HPV capside protein and for Ki-67, a proliferation marker.
Results : Five patients entered the study with AIN grade II, 2 with grade I. All patients received 3 or 4 cidofovir injections and 2 were afterwards treated with coagulations. The treatment duration ranged between 6 and 30 months (mean : 17 months). Four patients are currently still under treatment. The last follow-up biopsy showed no dysplasia in 2 patients, AIN grade I in 4 and AIN grade II in 1. There was an increased HPV capside protein expression in 1 patient, and a decreased expression of Ki-67 protein in another patient.
Conclusion : The combination therapy of intralesional cidofovir injection followed by coagulation for internal anal warts with dysplasia was successful : a decrease in the grade of dysplasia was seen in 4 out of 7 patients. There was no disease progression and no patient developed SCC under treatment. On the other hand, dysplasia was not eliminated by cidofovir treatment per se : this is to be expected, as it is the morphological equivalent of accumulated genetic defects. Cidofovir can be a good alternative for the current treatment of multiple, painful coagulation sessions with high levels of disease recurrence.
Reference
1. *Dis. Colon Rectum*, 2003, 46 : 1103.

- P03 -

COMPARATIVE EXPRESSION OF GLYPICAN 3 ONCOFETAL PROTEIN ON HUMAN HEPATOCELLULAR CARCINOMA CELLS AND THEIR TUMOURS IN NUDE MICE. J. Abarca-Quinones, H. Pelerin, M. Dorvillius, N. Van Hul, F. Jamar, Y. Horsmans, B. Van Beers, I. Leclercq. UCLouvain.

The Glypican 3 oncofetal protein (GPC3), a heparan sulfate proteoglycan anchored to the membrane, is expressed at a markedly elevated level in Hepatocellular carcinoma (HCC). GPC3 expression is considered a specific marker for HCC, but not all HCC re-express this oncofetal protein. Also, some immunoreactivity can be detected in a small subset of cirrhotic and/or dysplastic nodules. The aim of the present study was to investigate GPC3 expression in human hepatocellular carcinoma cell lines (HepG2, Hep3B and HuH7 cells) and their xenografts tumours in nude mice by Western Bloting (WB) and Immunocyto/histochemistry (IMCH), using a monoclonal antibody against GPC3 (Clone 1G12 from BioMosaics-Canada). As demonstrated by WB analysis of cell lysates, GPC3 expression varies depending of the cell line considered, and decreases after a serial cellular passages. HepG2 and Hep3B cells showed a high GPC3 expression. The IMCH analysis of these cells detected a membrane and cytoplasmic localisation of antigen, similar to observed in clinical HCC. By contrast, GPC3 expression by HuH7 cells is weak. The percentage of GPC3 expressing cells was 85, 70 and 10% in HepG2, Hep3B and HuH7 cells, respectively. Injection of each of the 3 cell lines into in nude mice, leads to the development of subcutaneous tumours. Morphologically, those tumours ressemble HCC types encountered in clinical practice. The tumours obtained presented a solid and/or trabecular patterns ; the mitosis were numerous and often atypical. Some areas of necrosis as well apoptotic cells were observed. The HepG2 tumour xenograft was characterised by a rich vascular pattern. The IMCH tumour analysis showed a strong membrane and cytoplasmic expression in HepG2 and Hep3B tumour xenografts with some patchy negative foci. In accordance with low percentage of GPC3 expressing cells in culture, the HuH7 xenograft tumours showed a few positive cells with intra-cytoplasmic granular patterns of GPC3. In HepG2 xenografts, preliminary in vivo experiments using I125-labelled Anti GPC3 antibody showed an accumulation of the antibody in tumour, blood, lung and skin. GPC3 represent an attractive target for HCC, imaging, drug development and immunotherapy. Our results indicate that HepG2 and Hep3B, but not HuH7, cells can be utilised for in vitro and in vivo GPC3 studies and to test and validate, as preclinical studies, the potential of GPC3 antibodies for targeted recognition of GPC3 expressing cells.

- P04 -

THE ANGIOSTATIC CHEMOKINE PLATELET FACTOR-4 VARIANT (PF-4VAR/CXCL4L1) IS EXPRESSED IN HUMAN GASTROINTESTINAL CANCER. H. Verbeke (1), S. Struyf (1), G. De Hertogh (2), J. Van Damme (1), K. Geboes (2). (1) KULeuven, (2) UZLeuven.

Background : Chemokines are important regulators of tumor progression through regulation of angiogenesis, chemotaxis of leukocytes, stimulation of tumor proliferation and metastasis. The chemokine family can be subdivided in two major subclasses, CXC and CC chemokines, depending on whether the first two cysteine residues are separated by an amino acid (CXC) or are adjacent (CC). Angiogenesis is a complex process influenced by angiogenic and angiostatic CXC chemokines, such as stromal cell derived factor-1 (SDF-1/CXCL12) and PF-4var/CXCL4L1, respectively.
Aim : The aim of the present study was to examine the expression of angiogenic and angiostatic molecules in human cancer.
Material and methods : Immunohistochemistry for PF-4var, SDF-1 and vascular endothelial growth factor (VEGF) was performed on surgical samples from3 controls and from 49 patients operated for cancer i.e. 14 oesophageal squamous cell carcinoma (SCC), 15 oesophageal adenocarcinoma (AC) and 20 colon AC.
Results : Angiostatic PF-4var was strongly detected in colorectal cancer, whereas the expression in oesophageal cancers was rather weak. Staining for angiogenic SDF-1 was almost negative in oesophageal SCC whereas a more intense and frequent staining was observed in AC of the oesophagus and colon. Staining for VEGF was moderately to strongly positive in all three types of cancer examined, although it was less prominent in oesophageal AC.
Conclusion : This study demonstrates the expression of an angiogenic as well as an angiostatic chemokine in epithelial tumors. The heterogenous expression of chemokines within the tumor and between the different cases, but also between the different tumor types may indicate a distinct role of the various chemokines in the complexity of tumor development.

- P05 -

EMR FOR SUPERFICIAL ESOPHAGEAL NEOPLASMS : A COMPARISON OF DIAGNOSIS ON PRE-EMR BIOPSIES AND EMR SPECIMENS. J. Beck, K. Geboes, G. De Hertogh, P. Van Eyken, R. Bisschops. UZ Leuven.

Background : Diagnosis and staging of superficial esophageal neoplasia is difficult on pinch biopsies, even when obtained by chromo-endoscopy. Endoscopic mucosal resection (EMR) is an adequate staging and therapeutic procedure in this setting. Moreover, EMR samples contain a large amount of tissue and allow the evaluation of mucosal landmarks. EMR may therefore also improve diagnostic accuracy. The aim of our study was to evaluate diagnostic consistency between pre-EMR pinch biopsies and subsequent EMR samples.
Design : We retrospectively reviewed a consecutive series of 55 patients who underwent EMR for superficial esophageal neoplasia diagnosed on pinch biopsies obtained with (n = 24) or without chromo-endoscopy. The mean number of endoscopies before EMR was 2,1 and the mean number of biopsies per endoscopy was 7,0. Diagnoses included : adenocarcinoma (n = 20), high-grade dysplasia (HGD, n = 25), low-grade dysplasia (LGD, n = 2), squamous cell carcinoma (SCC, n = 5), and benign lesions (n = 3).
Results : There was a diagnostic discrepancy between pre-EMR pinch biopsies and EMR samples in 6 patients (10%). In 4 of these, biopsies were obtained by chromo-endoscopy. The EMR samples of these 6 patients showed less severe lesions than the pre-EMR biopsies. One SCC on pinch biopsy showed LGD on EMR and 5 Barrett's HGDs on pinch biopsy showed LGD in 1 and no neoplasia in 4 EMR samples. Re-evaluation of the pre-EMR biopsies led to a revision of the diagnosis in 3 cases of Barrett's HGD. Two of them were reclassified as 'basal crypt dysplasia-like atypia' and one as reactive atypia in an ulcer margin.
Conclusion : In general, evaluation of pre-EMR esophageal pinch biopsies and subsequent EMR samples delivers identical diagnoses. Inconsistencies may arise by sampling error or faulty diagnosis on the pre-EMR biopsies. Especially basal crypt dysplasia-like atypia in Barrett's esophagus seems to be a challenging lesion, the precise nature of which is moreover still controversial. It might be missed on pinch biopsies and it is also unclear whether this lesion is visible on chromo-endoscopy given its location underneath the mucosal surface.

- P06 -

MDM2 OVEREXPRESSION IS ASSOCIATED WITH RESTRICTED PATTERN OF P53 OVEREXPRESSION AND INVERSELY CORRELATES WITH TP53 MUTATION IN COLORECTAL CANCER. C. Nyiraneza, C. Sempoux, P. Camby, A. Kartheuser, R. Detr, K. Dahan. UCLouvain.

Purpose : The majority of *TP53* alterations are point mutations usually associated with a stabilization of the mutant p53 protein and its overexpression. Contrasting with negative and strong diffuse p53 immunohistochemical expression profiles, we identified a particular p53 immunostaining pattern, that we called 'restricted pattern of p53 overexpression', significantly associated with microsatellite instability (MSI) positive colorectal cancers (CRCs), but also found in a subset of microsatellite stable CRCs, and characterized by a low rate of *TP53* mutation. This study was undertaken to investigate the possible mechanisms leading to p53 protein stabilization in CRCs.
Patient and methods : In 77 consecutive CRCs, with complete data on MSI-status and DNA mismatch repair system (MMR) deficiency or sufficiency, we correlated p53 immunohistochemistry (IHC) and *TP53* mutational status with IHC of proteins involved in p53 regulation feedback loop : MDM2, LATS2, PLK1, and $p21^{WAF/Cip1}$.
Results : MDM2 protein accumulation was significantly more frequently observed in tumors with restricted pattern of p53 overexpression (27/32, 84%), than in p53 negative (2/11, 18% $P = 0.000146$), and p53 diffuse (11/34, 32% $P = 0.000019$) immunostained tumors. These tumors also showed normal $p21^{WAF/Cip1}$ protein expression (30/32, 94%), whereas a significant decrease/or an absence of $p21^{WAF/Cip1}$ protein expression was observed in tumors with p53 negative (6/11, 55% $P = 0.001654$), and with p53 diffuse (16/34, 47% $P = 0.000223$) immunostaining. PLK1 protein overexpression was detected in 78% of tumors (60/77), and no significant difference was observed among the 3 groups of p53 expression patterns ($P = 0.821808$). A significant decrease/or an absence of LATS2 protein cytoplasmic expression was observed in 30% (23/77) of all CRCs, but no significant difference was observed among the 3 groups of p53 immunostaing patterns ($P = 0.9$). *TP53* mutation was detected in 38 (38/77, 49%) tumors, and an inverse relationship was observed between MDM2 protein overexpression and *TP53* mutations ($P < 0.0001$), while low/or negative $p21^{WAF/Cip1}$ expression was significantly associated with *TP53* mutations ($P < 0.0001$). By contrast, no significant association between *TP53* gene mutational status and PLK1 overexpression, and loss of cytoplasmic LATS2 expression was observed.
Conclusion : This study confirmed that with regardless of MSI-status, colorectal cancers exhibiting the restricted pattern of p53 overexpression form a distinct tumor entity. We hypothesized that MDM2 overexpression, and normal $p21^{WAF/Cip1}$ protein expression might contribute to the favorable prognosis in these patients.

- P07 -

A CARCINOSARCOMA OF THE RECTUM, AN UNCOMMON TUMOUR, COMPOSED OF TWO RELATIVELY RARE NEOPLASTIC COMPONENTS. M. Abdul-Hamid, R. Riedl, I. Van Den Bosch, F. Bakers, A. Oosterkamp, A. Driessen. University Hospital Maastricht, The Netherlands.

Colorectal cancer is the third most common cancer in men and women, representing 12% of all cancers. The most common histologic type is the adenocarcinoma with a prevalence of upto 90% of all colorectal tumours. Our case is a 82-year old female, presenting with an extremely rare variant of colorectal cancer. Endoscopy, performed because of rectal blood loss, revealed a large ulcerated tumour of the rectum. Despite adjuvant radiochemotherapy, clinical examinations showed a locally advanced stage tumour (T4N1) for which patient underwent surgery. Microscopy of the resection specimen demonstrated a deeply infiltrating tumour with a heterogeneous growth pattern. The main component was epithelial of origin, consisting predominantly of solid nests with central necrosis. Immunohistochemistry revealed that this part consisted of large and small neuro-endocrine cells (CD56 + , synaptophysin +). The minor part were spindle cells with a pleiomorphic appearance, imbedded partially in an eosinophylic matrix, resembling osteoid. This part, which did not express a cytokeratin marker, was mesenchymal of origin, expressing only vimentin. This marker was not present in the epithelial component. Based on the morphology and the immunohistochemical examination the tumour was diagnosed as carcinosarcoma, consisting of a mixed type neuro-endocrine carcinoma and a mesenchymal tumour, resembling an osteosarcoma.
A carcinosarcoma is an extremely rare tumour of the gastro-intestinal tract, most commonly found in the oesophagus. In contrast to a sarcomatoid carcinoma or spindle cell carcinoma the mesenchymal component, which may correspond to a chondro-, osteo- or rhabdomyosarcoma, shows no expression of an epithelial marker. This mesenchymal component is commonly associated with a classical adenocarcinoma. In our case a mixed type neuro-endocrine tumour was found. Carcinosarcomas have a very poor prognosis, as they have an aggressive behaviour with a rapid growth and a high incidence of local recurrence and distant spread.

- P08 -

USEFULNESS OF P53 AND P504S (RACEMASE) FOR THE DIAGNOSIS OF BARRETT'S INTRAEPITHELIAL NEOPLASIA. K. Ho Minh duc, C. Sempoux, H. Piessevaux, P.H. Deprez, A. Jouret-Mourin. UCL Saint-Luc.

Barrett's esophagus is an established risk factor of oesophageal adenocarcinoma. The detection of intraepithelial neoplasia (IEN) is currently regarded as the gold standard for identifying patients at risk for neoplastic transformation. However there is a large inter-observer variability in diagnosing IEN. The aim of this study was to analyse the usefulness of p53 and p504S (racemase) to improve the diagnosis procedure. A series of 50 cases including 27 biopsies and 23 mucosectomies, with 17 non or indeterminate IEN (ND/IND), 10 low-grade (LG) and 19 high-grade (HG) IEN, and 4 intramucosal adenocarcinoma (AD) was reviewed by one resident and two experienced gastro-intestinal pathologists. p53 and p504S immunostainings were performed with clone D07 (Biocare Medical) and clone 13 H4 (Dako) antibodies, respectively. The p53 staining was characterized by a strong nuclear signal, whereas p504S reactivity showed a faint, granular, cytoplasmic staining. The degree of staining in all cases was evaluated according to the following scale : 0 (0% of positive cells) ; 1 + (a significant signal in few scattered cells) ; 2 + (cluster and > 10% of positive cells).

		N		p53		p504S	
		(-)	(+)	(+ +)	(-)	(+)	(+ +)
ND/IND	17	10	6	1	13	4	0
LG	10	0	3	7	5	4	1
HG	19	0	3	16	5	4	10
AD	4	1	0	3	2	0	2

In conclusion, the sensitivity of p504S for the detection of LG and HG was 50% and 74%, respectively, whereas all LG and HG cases were significantly positive for p53. However, the specificity was poor for both immunoreactive markers. Consequently, the strong nuclear immunoreactivity of p53 might be helpful in orienting and confirming a diagnosis of IEN, in contrast to the discrete granular cytoplasmic expression of p504S.

- P09 -

CONFOCAL LASER ENDOMICROSCOPY OF COLORECTAL NEOPLASIA : A SYSTEMATIC ANALYSIS BY THE PATHOLOGIST. A.S. Van Rompuy, K. Geboes, G. De Hertogh, P. Rutgeerts, R. Bisschops. KULeuven.

Confocal endomicroscopy can be used by experienced and well trained endoscopists to discriminate between benign and neoplastic lesions in the colon (Kiesslich 2004). In the stomach pathologists are able to predict final histology based on CLE images of sufficient quality (Kitabatake 2006).
Aim : We wanted to have CLE images of the colon systematically assessed by pathologists for different architectural characteristics and correlate this to the final histological diagnosis.
Methods : 36 patients referred for colonoscopy (surveillance, familial risk, follow-up of colorectal cancer) were prospectively included in the study. Polyps were targeted by CLE (Pentax-Optiscan after IV fluorescein injection). Normal colonic sites were targeted ad random. Biopsies from the lesions were obtained routinely and targeted to the CLE imaging site. The images were analysed by two pathologists independently using a series of parameters including : number of crypts per image, intercryptal distance, crypt diameter, crypt shape, epithelial cell shape, presence of goblet cells and the vascular pattern. Non quantitative items were scored qualitatively (0 absent, 0.5 partial, 1 discrete, 2 moderate, 3 high). A mean score for each parameter was calculated. CLE images were compared with histology.
Results : Images of 17 tubular adenomas, 9 hyperplastic polyps and 10 normal colonic sites were analysed.The number of crypts was higher in adenomas (9.53) in comparison to normal mucosa (8 + /-2) and hyperplastic polyps (7.8). In normal mucosa, the internal diameter of the crypts is lower (87 ± 12 μm) compared with hyperplastic (106 μm) and adenomatous polyps (141 μm). Intercryptal distance and crypt diameter are constant in normal mucosa, but highly variable in hyperplastic and adenomatous polyps. Goblet cells are abundantly present in normal mucosa and hyperplastic polyps. The number of goblet cells is decreased in adenomas. Vascular and stromal features were only assessable in 50% of images. Analysis of confocal images matches routine histology very well. Conclusion : These preliminary data confirm that in vivo taken CLE images can be used by pathologists in the differential diagnosis of classical polyps. Further controlled, prospectively collected data are needed to evaluate sensitivity and specificity.

Invited lecture

- P10 -

COLORECTAL CANCER, KRAS AND THE PATHOLOGIST. J. Van Krieken. Nijmegen, The Netherlands.

The optimal use of EGFR-targeted therapies for patients with colorectal cancer requires accurate *KRAS* mutation testing. Testing for *KRAS* mutations generally comprises three stages : 1) referral for *KRAS* mutation testing ; 2) selection of the tissue block containing the tumor area of interest ; and 3) DNA extraction and *KRAS* mutation analysis. In the current clinical setting, colorectal cancer patients are not routinely screened for *KRAS* mutation status. Pathologists test for *KRAS* mutations only upon the specific request of a clinician. Clinicians, in turn, request *KRAS* genotyping only if the test results are intended to guide decisions on patient management. These practices might not be sufficient for optimal patient care. The process of requesting *KRAS* status testing, finding the original tissue block and reporting the test results is cumbersome, time-consuming and prone to errors. Therefore, routine mutation testing at the time of initial diagnosis of stage II and III tumors should be considered.
The pathologist plays a central role in *KRAS* mutation testing. First, the pathologist is responsible for choosing the most appropriate tissue block to be tested. Second, the pathologist should ensure that the tissue block selected for *KRAS* genotyping contains sufficient quantity of invasive tumor cells needed for analysis. Finally, the pathologist is responsible for documentation, which should include results from HE staining analysis as well as from *KRAS* mutation testing, and for preparation of the pathology report. If the testing is performed by a reference laboratory, the pathologist should integrate the test results into the pathology report.
Based on current knowledge, the most appropriate material for *KRAS* mutation testing is primary tumor tissue. This type of material is commonly archived and thus accessible, and typically contains sufficient amount of invasive carcinoma cells required for *KRAS* mutation testing. If an endoscopic biopsy of the primary tumor is performed, it is important that the material obtained contains adequate amount of adenocarcinoma cells in the area identified.
However, it is estimated that 20% of the target patient population will present with metastatic disease and will not have archival material from the primary tumor. This poses an important challenge for the pathologist in the selection of appropriate material for *KRAS* mutation testing. In this situation, the panel recommends that *KRAS* mutation testing is performed using material from the metastatic tumor, for example from resected liver metastases or positive

lymph nodes. The pathologist must ensure that the metastatic tissue block contains adequate amount of adenocarcinoma cells.
To address the need for standardized *KRAS* mutation testing methods and procedures in colorectal carcinoma, two working groups of the European Society of Pathology (ESP), the Diseases of the Digestive Tract ESP Working Group and the Molecular Pathology ESP Working Group, convened an expert panel to develop guideline recommendations and a proposal for a European QA program for *KRAS* mutation testing.
To support these proposed initiatives, the European QA program intends to establish and maintain a website (http://esp-pathology.org) that will provide the latest recommendations as well as potentially an overview of validated laboratory methods, standardized operating procedures, and accreditation criteria relevant for *KRAS* mutation testing.
As our understanding of the genetics and molecular biology of colorectal cancer advances, other parameters will hopefully be identified as predictors of treatment outcome. Presently, *KRAS* mutation status must be considered in the appropriate therapeutic context for each patient. The guideline recommendations and European QA program proposed here for *KRAS* mutation testing will help to ensure that all patients who may or may not benefit from EGFR-targeted therapies are identified in a timely and consistent manner. Although the proposed QA program is intended for the standardization of *KRAS* mutation testing methods and procedures, this expert panel is of the opinion that such a program can potentially be adapted to incorporate other predictive biomarkers in colorectal cancer as they become available.
The fundamental initiatives of the proposed European QA program are as follows :
1) The European QA program for *KRAS* mutation testing aims to provide timely, standardized, evidence-based guidelines for the performance of a diagnostic test for *KRAS* mutations on colorectal tumor tissues.
2) The European QA program intends to collaborate with existing regional and/or national QA programs to develop strategies and standardized procedures that help to ensure optimal performance, interpretation and reporting of *KRAS* mutation analysis. To achieve this, the European QA program will provide administrative and logistic support and networking opportunities for the development and implementation of standardized operating procedures and QA criteria for proficiency testing and competency assessments. The European QA program will also coordinate accreditation of participating laboratories at the European and regional level.
3) The European QA program will facilitate the administrative process and reimbursement discussions in each country in the European Union by providing the necessary documents and QA schemes for implementation and performance of diagnostic tests for *KRAS* mutation analysis.

- P11 -

PRIMARY SMALL-CELL CARCINOMA OF THE PANCREAS : AN ANALYSIS OF 23 CASES REPORTED IN THE LITERATURE. B. Vos, A. Awada, A. Hendlisz. Institut Bordet Brussels.

Background : Small cell carcinoma of the pancreas (SCCP) is a rare and aggressive tumour with a poor prognosis. SCCP is only reported as case reports in the literature. The aims of this study were to analyse the epidemiology, clinical characteristics and treatment outcomes of this patients.
Methods : Forty-two cases of SCCP have been reported in the English-language literature since 1973. Twenty-three cases with complete data from eighteen papers were analyzed. Data were reviewed for data on demographics, presenting symptoms, diagnosis, disease stage, type of treatment and outcome.
Results : SCCP occurs in the sixth decade : median age (range) : 62 (37-75) years with a male predominance (74%). The most common complaining symptoms were abdominal pain (62%), weight loss (52%), jaundice (33%). The tumour usually had a singly location (76%) and occurs especially in the head of the pancreas (56%). Extensive disease was present in 91% of cases at initial diagnosis. Serum concentrations of neuron-specific enolase (NSE) were increase in the four cases where values were specified and decrease with treatment. In histology, SCCP were pure in 79% of cases. In treatment, nine patients (39%) received symptomatic management. In treated patients (14/23), eight received chemotherapy alone and four were treated by a combination of chemotherapy with local treatment (surgery or radiation therapy). Surgery alone was reported in two cases. The overall median survival was 3 months (range, 0.5-50 months). There is a significant difference of survival between symptomatic management versus treated patients with median survivals of 1 month (range, 0.5-2) versus 6 months (range, 2-50) respectively ($p < 0.001$). This difference was significant for all therapeutic options : Chemotherapy alone ($p = 0.001$), surgery alone ($p = 0.029$), and chemotherapy associated with local treatment (surgery or external radiation therapy, $p = 0.011$). There was no significant difference in survival between chemotherapy alone (8 cases) vs. chemotherapy associated with local treatment (3 cases) ($p = 0.464$).
Conclusion : Serum NSE is an important tumour marker for diagnosis and/or assessment of treatment effect in patients with SCCP. Chemotherapy is currently considered as the cornerstone of management for patients with SCCP. The role of local treatment combined to chemotherapy remains unclear due to the small number of cases.

- P12 -

THE RAS INHIBITOR FARNESYLTHIOSALICYLIC ACID INHIBITS THE GROWTH OF HEPATOCARCINOMA CELL LINES IN VITRO AND IN VIVO. N. Charette, V. Lannoy, C. De Saeger, I. Leclercq, Y. Horsmans, P. Stärkel. UCL Saint-Luc.

Background and aims : Ras activation has been shown to be a frequent event in hepatocarcinoma (HCC). Its downstream targets, the raf/MEK/ERK and PI3K/AKT/mTOR pathways, are currently considered as promising therapeutic targets. Farnesylthiosalicylic acid (FTS) inhibits ras by dislodging its activated form from its membrane docking sites. The aim of this study was to evaluate the effect of FTS in two HCC cell lines, Huh7 and HepG2, and to examine its impact on cell proliferation, apoptosis, and signaling through ERK and AKT-mTOR.
Methods : Cells were cultured for up to 5 days in culture medium containing dimethylsulfoxyde (solvent) or FTS at concentrations ranging from 50 to 150 μM. Cell number, viability and proliferation were assessed by standard cell count and colorimetric WST-1 and BrdU incorporation assays, respectively. Apoptosis induction was evaluated by a caspase 3/7 activity assay after 24 hours of treatment. ERK, phospho-Erk, AKT, phospho-AKT, p70 and phospho-p70 (mTOR activation) were determined by immunoblotting. Finally, HepG2 cells were subcutaneously implanted in nude mice. FTS (10 mg/kg) was administered intraperitoneally daily when tumors became palpable. Response to treatment was assessed by tumor weight at sacrifice compared with solvent-treated controls.
Results : FTS induced a significant time- and dose-dependent decrease in the number of viable cells in both cell lines. A dose-dependent decrease in BrdU incorporation occurred before the effect on cell number became apparent. In addition, an increase in caspase 3/7 activity was observed after 24 hours of treatment. In FTS-treated cells, ERK phosphorylation decreased in both cell lines and a decrease in AKT phosphorylation was observed in Huh7 cells. p70 phosphorylation did not change in both FTS-treated cell lines. Finally, FTS treatment in xenografted mice for 10 days reduced tumor growth by 55% compared with untreated controls.
Conclusion : FTS inhibits proliferation in a dose-dependent manner and rapidly induces apoptosis in HepG2 and Huh7 cells. This effect is associated with disruption of ERK and possibly AKT signalling, but not mTOR activation. Moreover, FTS treatment in vivo inhibits tumor growth in a HepG2 xenograft model.
Acknowledgments : This work was supported by grants from the Fondation Saint-Luc and the FSR, Belgium.

- P13 -

DIFFUSE PERITONEAL LYMPHOMATOSIS IN A PATIENT WITH CROHN'S DISEASE TREATED WITH IMMUNOSUPPRESSIVE THERAPY. E. Willems, G. De Hertogh, F. Claus, D. Vanbeckevoort, K. Van Pelt. KULeuven.

Case report : A 30 year old man known with a 15-year history of Crohn's disease presents at the emergency department with progressive pain in the right lower quadrant, anorexia, nausea and weight loss. He had been previously treated with Infliximab (Remicade) and Azathioprine (Imuran) (between 2001 and 2007) and is currently medicated with Adalimumab (Humira). Laboratory work-up reveals a microcytic anemia, elevated CRP (6 $\times$ ULN) and increased LDH levels (11 $\times$ ULN). A contrast enhanced abdominal CT reveals diffuse tumoral thickening of the peritoneum and a mass at the ileocolic junction. Liver and spleen are enlarged, and there are signs of intestinal obstruction.
An exploratory laparoscopy reveals multiple soft, friable peritoneal lesions. The biopsies show a rather cohesively growing infiltrate of tumor cells with large, vesicular, often slightly eccentric nuclei with prominent central nucleoli and ample amphopilic cytoplasm. Immunophenotyping was positive for LCA, CD79a, PAX5, CD138 and negative for CD10, CD19, CD20, CD30, Bcl6, ALK, KSHV. The proliferation index was 90% (Ki-67 staining). In-situ hybridization for Epstein-Barr virus RNA was negative. The differential diagnosis was diffuse large B cell lymphoma, immunoblastic DD plasmablastic type. Chemotherapy was initiated, but the patient died 9 months later.
Discussion : Plasmablastic lymphoma (PBL) is an aggressive neoplasm, considered a rare variant of diffuse large B cell lymphoma. It has typically been reported in association with HIV infection, showing predilection for oral mucosa and the jaw. Only one other case of PBL in a patient with Crohn's disease and treatment with infliximab has been reported. This case is the first report of PBL presenting as aggressive peritoneal lymphomatosis.
This patient has at least two risk factors for developing lymphoma : (1) the increased frequency of lymphoma in the setting of Crohn's disease, and (2) the longterm immunosuppressive treatment with Azathioprine and anti-TNF agents. Although the impact of each risk factor alone may be small, the overall effects may be additive, if not synergistic. Therefore, risk assessment of developing lymphoma is important in patients with Crohn's disease, in particular when treated with anti-TNF agents.

- P14 -

PET EVALUATION OF ANTI-VEGF TREATMENT IN COMBINATION WITH CHEMOTHERAPY IN MCRC. N. Van Damme, C. Van De Wiele, W. Ceelen, S. Laurent, I. Dero, K. Geboes, B. Lambert, P. Smeets, M. Peeters. UZ Gent.

Background : Bevacizumab, a recombinant humanized monoclonal antibody that binds and neutralizes vascular endothelial growth factor, prolongs overall survival and progression-free survival when added to standard chemotherapy in metastatic colorectal cancer (CRC) patients.
Aim : The purpose of the study was to explore the feasibility and the possible role of ^{18}F-FDG-PET as response predictor for treatment with bevacizumab in liver metastatic colorectal cancer patients.
Patients and methods : Twelve chemo-naive patients (7 women and 5 men), with histologically confirmed diagnosis of metastatic CRC, were treated with chemotherapy and bevacizumab. Bevacizumab (Avastin®) was administered IV at a fixed dose of 5 mg/kg. ^{18}F-FDG-PET scans were performed before treatment and 8 days after cycle 4. Patients received 4 MBq/kg body weight of ^{18}F-FDG intravenously followed by 250 ml of sodium chloride and 20 mg of furosemide. Image acquisition with the use of an integrated PET-CTscanner (Philips Gemini PET-CT, Philips Medical Systems) started 60 minutes after injection. FDG-PET scans were read by a nuclear medicine physician, blinded to the results of clinical parameters. The most representative liver lesions were used as target lesions. PET results were quantified by calculating the mean standard uptake value (SUV).
Results : Twenty-five out of 30 representative liver metastases showed a decline in SUV (mean D 57%, range 14-82%) between the first and second FDG-PET scan. One lesion had the same mean SUV, while mean SUV increased in the other 4 liver lesions (mean D 15%, range 2.3-67%).
Conclusion : From the present data we can conclude that ^{18}F-FDG-PET scan is feasible. In order to know whether the change in SUV can be used as a reliable response predictor, the results of PET analysis need to be correlated with the CT imaging results according to RECIST criteria. These results will be presented at the meeting.
This study was supported by an unrestricted research grant from Roche.

Invited lecture

- P16 -

HOW CLOSE TO THE PATHOLOGIST CAN BE THE RADIOLOGIST FOR THE LOCAL STAGING OF COLO-RECTAL CANCER ? A STORY OF OUR DAILY COLLABORATION. E. Danse (1), C. Sempoux (1), J. Jamart (2), A. Jouret-Mourin (1). (1) UCL Saint-Luc ; (2) UCL Mont Godinne.

Context : One of the reasons for the better care of patients with colo-rectal cancer is the optimisation of the collaboration between the different specialities concerned by this disease. The multidisciplinary teams for discussion of digestive cancer have lead to a daily confrontation between imaging findings and pathological results.
The aim of this lecture is to present the current status of the capabilities and limits of radiology for the local staging for colon and rectal cancer, on the basis of the literature and our local experience.
For colon cancer, chest and abdominal CT is the procedure of choice for the TNM staging. The accuracy of CT for the prediction of the T stage is ranged from 40 to 95%. For the N stage, the accuracy ranges from 19 to 84%. Other local findings participating to the local stage of the colonic cancer indicating vascular permeation, have been recently evaluated with an accuracy of 70%.
For rectal cancer, MRI has the place of choice but CT is also to be considered as an alternative method participating to the T & N stages of the disease ; both techniques are also used for the prediction of the circumferential resection margin. For MR, the prediction of the T stage is noted between 39 to 78%. The accurate MR prediction of the N stage is noted between 55 to 74%. For CT, the prediction of the T stage has an accuracy of 33 to 86%. For the N stage, CT is associated with an accuracy of 50 to 81%. For the CRM, the literature is only giving results for MR, with an accuracy of 67 to 93%. In patients with preoperative radio-chemotherapy, the respective performances of CT and MRI done just before surgery are showing a low concordance with pathology both for T stage (concordance CT/pathology : 44% and MRI/Pathology :39%), for N stage (concordance CT : 50%, MRI : 55%), and for the CRM (concordance CT : 46%, MRI :70.5%). Additionnaly, a downstaging of the rectal cancer is noted after radio-chemotherapy with CT in 61% and with MRI in 83%.
Our present conclusion is that cross sectional imaging can be helpful for the prediction of the local stage of a colorectal cancer, with better results for the rectum. Downstaging of the cancer is also better assessed with MRI than with CT. But at this time, radiological findings are not enough similar to final pathologic results. Efforts have to be done in order to identify more imaging findings helping the clinicians to adapt the presurgical therapy.

- P17 -

UNUSUAL FEATURES IN A LIVER BIOPSY LEADING TO AN UNSUSPECTED DIAGNOSIS. C. Fervaille (1), R. Brenard (2), A. Jouret-Mourin (1), I. Borbath (3), P.H. Deprez (1), B. Van Beers (1), X. Chapaux (2), J. Rahier (1), C. Sempoux (1). (1) UCL Saint-Luc ; (2) Hôpital St-Joseph, Gilly.

A routine check-up performed in a 45-year-old man revealed an isolated elevation of gGT. Clinical examination was normal as well as all other biological tests. At liver ultrasound, several non specific bilobar nodular lesions were found, ill-delimited and hypervascularized at MRI. To rule out malignancy, a liver biopsy was decided. The liver parenchyma had a normal architecture, with regular liver cell plates but exhibited dilated vascular channels. In addition, within the portal tracts, we observed direct connections between the arteriole and the venule, without intervening capillaries. No tumoral lesion was found. Combined with the radiological findings, these histological features lead to propose a diagnosis of Rendu Osler disease. Investigating then the patient's past medical history in details, he mentioned epistaxis in childhood and gastrointestinal bleeding and epistaxis in his father. Genetic investigations confirmed later the diagnosis (ALK1 mutation). Rendu Osler disease or hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by arteriovenous malformations, telangiectasia and aneurysms affecting mucosa, skin, lungs, brain and gastrointestinal tract. Liver involvement is frequent but asymptomatic in the majority of the patients. It consists in extensive intrahepatic vascular malformations associated with blood shunting. Other lesions such as focal nodular hyperplasia, parenchymal perfusion disorders and biliary disease are also described but less frequently. If symptomatic, morbidity and mortality can be substantial. Indeed, patients with HHT may develop high output cardiac failure, portal hypertension or peribiliary necrosis leading to liver transplantation.

- P18 -

ICONOGRAPHY OF LIVER LESIONS OF PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA. X. Chapaux, R. Brenard. Hôpital St-Joseph (GHC), Gilly.

Background : Knowledge of the radiological presentation of liver lesions of patients with hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber) is needed in order to avoid useless invasive procedures in unsuspected cases.
Our **aim** is to to describe radiological frequent findings in those patients ; signs of increased hepatic blood flow, vascular hepatic lesions, Focal Nodular Hyperplasia, and two classical complications, biliary ischemia due to blood flow stealing and heart failure related to intrahepatic shunts. We'll speak of the place of the different radiological modalities in the diagnosis.

- P19 -

DOUBLE GRANULAR CELL TUMOR OF THE DISTAL OESOPHAGUS. G. De Hertogh, J. Coolen, G. Vanneste, R. Vanslembrouck, D. Vanbeckevoort. UZ Leuven.

Clinical data : Patient X, a 52 yrs old male, presented in our hospital with worsening dysphagia due to a growing submucosal tumor in the distal oesophagus. The patient was a smoker (100 pack yrs) and consumed 6 units alcohol per day. His medical history was blank. At echo-endoscopy, 2 distinct submucosal tumors of 15 and 20 mm diameter were seen between 39 and 42 cm from the incisors. One of the lesions had doubled in size compared with previous year's evaluation. No submucosal rim could be distinguished beyond the largest lesion.
Radiology : A combined FDG-PET/CT-scan was performed. The PET-scan showed a diffuse, low-level accumulation of tracer substance in the oesophagus, without clear FDG-avid focal lesions. There was also a faint signal infracarinal. At whole-body CT-scan, the distal oesophagus was slightly and asymmetrically thickened to the right. No suspicious lymph nodes or focal lesions suggestive for metastases were seen.
Pathology : The patient underwent a subtotal oesophagectomy plus partial gastrectomy. Macroscopically, two small submucosal nodules with intact, whitish overlying mucosa were seen just proximal to the oesophagogastric junction. Microscopically, these lesions were well-delineated and consisted of nests of large, polygonal cells with an eosinophilic, faintly granular cytoplasm and small, mildly pleiomorphic nuclei. Mitotic figures were not seen. The largest lesion abutted to the muscularis propria. A PAS-stain and an immunohistochemical stain for S100-protein were positive in the tumor cells. Twenty-two lymph nodes were retrieved and all were negative.
Conclusion : The final diagnosis was double granular cell tumor of the distal oesophagus. The patient is recovering well from his operation.

- P20 -

ABOUT AN UNCOMMON CAUSE OF INTESTINAL MECHANICAL OBSTRUCTION. E. Danse, C. Sempoux, A. Jouret-Mourin, J. Donnez. UCL St Luc.

The authors report a case of a 40 year old woman admitted for progressive upper abdominal disconfort and for whom the final diagnosis of small bowel endometriosis was suggested with sonography and furtherly confirmed with surgical and pathological findings. The first investigation was a gastroscopy showing an extrinsic mass compressing the duodenal outlet. An abdominal CT investigation was then required and showed signs for small bowel mechanical obstruction with a transitional zone on the right side of the epigastric area at the place of an ill-defined mesenteric mass. Sonography (US) was also performed, after CT, to precise the nature of this mass. US contributed to the detection of a nodular centimetric lesion coming from the mesenteric folds and penetrating the deep layers of the small bowel, at the level of the transition between the distended and the collapsed bowel loops. Intestinal endometriosis was suspected on the basis of sonography. Surgery was then performed on the same day and the pathological findings confirmed the diagnosis of bowel wall endometriosis.
The presentation of this case is the opportunity to review the imaging findings suggestive of this disease, and to correlate these findings with pathological data.

- P21 -

MULTIFOCAL EPITHELIOID ANGIOSARCOMA OF THE SMALL BOWEL. G. De Hertogh, R. De Wyngaert, R. Vanslembrouck, E. Wisanto, D. Vanbeckevoort. UZ Leuven.

Clinical data : Patient X, a 70 yr old male, presented with persistent transfusion-dependent anemia after coronary artery bypass graft surgery. The patient quit smoking since long and underwent an appendectomy for appendicitis decades ago. An upper and lower gastrointestinal endoscopy were negative. A video capsule enteroscopy showed two malignant-looking, actively bleeding polypoid lesions in the mid-jejunum and proximal ileum.
Radiology : A CT-scan was performed. The two lesions were observed in their predicted location. The jejunal tumor could not be delineated from an adjacent bowel segment. Multiple mesenteric adenopathies were present, with the largest close to the ileal tumor. Multiple irregular masses suspicious for metastases were seen in the liver and spleen. The most probable diagnosis was neuro-endocrine tumor, with adenocarcinoma, GIST, lymphoma or metastatic tumors as alternatives.
Pathology : The patient underwent a double segmental enterectomy. Macroscopically, two polypoid tumors were seen which penetrated deeply in the bowel wall. Microscopically, these lesions were poorly circumscribed and consisted of sheets of plump, epithelioid cells with considerable nuclear pleiomorphism and many mitotic figures (up to 12/10 HPFs). In between these cells, numerous erythrocytes were present. The following immunohistochemical stains were performed and were all negative : cytokeratin, EMA, chromogranin, calretinin, LCA, CD68, CD138, alpha-SMA, CD34, c-kit, S100, HMB-45 and melan A. Only a vimentin stain was positive. Ultimately, a factor VIII-related antigen and CD31-stain were performed, which were clearly positive.
Conclusion : The final diagnosis was multifocal epithelioid angiosarcoma of the small bowel. The patient is currently receiving chemotherapy.

- P22 -

COMBINED HEPATOCELLULAR AND CHOLANGIOCELLULAR CARCINOMA WITH RADIOLOGICAL CHARACTERISTICS OF FOCAL NODULAR HYPERPLASIA. I. Willekens (1), A. Hoorens (1), B. Op De beeck (2), C. Geers (1), F. Vandenbroucke (1), J. De Mey (1). (1) UZ Brussel ; (2) UZ Antwerpen.

A 47-year-old woman presented with a 2 month history of general illness, a little weight loss and a palpable mass in the right flank. She had been hepatitis B positive for more than 15 years. Alpha-fetoprotein levels were elevated.
Abdominal ultrasound and computed tomography (CT) were performed in another hospital. Ultrasound revealed a tumour in the liver. The CT scan showed a heterogeneous contrast-enhanced mass in segment 5 and 6 of the liver. A subsequent MRI of the abdomen demonstrated a focal liver lesion in the right lobe, homogenous hypointense on T1 and lightly hyperintense on T2 with a central scar. The dynamic contrast-enhancement established a relatively hypervascular nature with a late enhancement of the central scar. The contrast-enhancement and morphologies revealed a focal nodular hyperplasia. A right hepatectomy was performed. A white firm tumoral mass of 8.5 cm in diameter and 1 satellite nodule of 1 cm were identified during macroscopic assessment. Microscopy showed a combined hepatocellular and cholangiocellular carcinoma in a non-cirrhotic liver.
Combined hepatocellular and cholangiocellular carcinoma is a rare tumour type containing unequivocal elements of both hepatocellular carcinoma and cholangiocarcinoma that are intimately mixed. It shows similar geographical distribution differences and similar age and sex distribution as for hepatocellular carcinoma. Some authors have reported a worse prognosis when compared to patients with hepatocellular carcinoma alone.

- P23 -

NSAID-INDUCED DIAPHRAGM DISEASE OF THE RIGHT COLON : REPORT OF A CASE AND REVIEW OF THE LITERATURE. E. Vanderlinden, A. Cheragwandi, F. Mana, F. Vandenbroucke, G. Delvaux, A. Hoorens. UZ Brussel.

A 43-year-old woman presented with iron deficiency anaemia. She described a 2.5 year history of crampy abdominal pain and anorexia with 20-kg weight loss over 6 months. There was no diarrhoea. Although colonoscopy revealed stenosis of the right colon, a biopsy demonstrated only slight mucosal fibrosis without inflammation. Abdominal CT and double contrast colonic radiography were suggestive for Crohn's disease : multiple stenotic lesions of the terminal ileum and right colon, prestenotic dilatations, and an inflammatory appearance of the caecum. The descending and sigmoid colon displayed diverticular disease. Mesalazin therapy was started. During subsequent explorative laparotomy no stenotic lesion was observed exteriorly. Colonoscopy 1 month later showed 5 consecutive non-inflammatory stenotic lesions. The 2 most proximal stenoses were very narrow with pinpoint lumina. Despite dilatation no passage was possible. Together with a daily intake of NSAIDs for 3 years, these findings were diagnostic of NSAID-induced diaphragm disease. Symptoms improved with corticotherapy, but recurred when the dosage was lowered. Following a second unsuccessful attempt to dilate the stenotic lesions 2 months later, the patient opted for surgical therapy. An extended right hemicolectomy was performed. Pathological examination of the right colon revealed typical NSAID-induced diaphragm disease with stenosis by mucosal fibrosis and hyperplastic splayed muscularis mucosae extending into the lamina propria and submucosa. Although this is a well described entity, diagnosis of this condition is commonly delayed. It was originally reported as a disease of the small intestine, but now often presents with right sided colonic strictures as a consequence of the use of enterocoated and slow-release NSAID preparations.

- P24 -

INTRADUCTAL DEVELOPMENT OF PANCREATIC NEUROENDOCRINE TUMOURS. A. Mathieu, M.A. Bali, N. Nagy, J. Closset, A. Negulescu, M. Delhaye, C. Matos, P. Demetter. ULB Erasme.

Intraductal growth of endocrine tumours of the pancreas is rare and only 5 cases have been described in literature. We report two cases of endocrine tumours of the pancreas, in men of 48 and 68-years-old, respectively. The lesions were discovered following episodes of acute pancreatitis. An abdominal computerized tomography (CT) scan was performed in the first case and revealed a richly vascularized mass of the pancreatic body associated with an enlarged upstream main pancreatic duct, compatible with a neuroendocrine tumour. In the second case an endoscopic ultrasonography was performed and showed a cystic lesion compatible with a mucinous cystadenoma. Magnetic resonance cholangiopancreatography (MRCP), T2-weighted technique, revealed in the two cases a markedly distended main pancreatic duct above a mural nodule suggestive of intraductal papillary mucinous tumour (IPMT). A gadolinium enhancement was observed suggesting the possibility of invasive carcinoma. Fine needle aspirations guided by endoscopic ultrasonography were performed and revealed in the two cases the presence of endocrine cells. The two patients were treated by surgical resection. Microscopically, the lesions consisted in grade II endocrine tumours according to Rindi's classification, showing intraductal development. The lesions expressed neuroendocrine markers. A liver metastasis was observed in the second case. Although extremely rare, pancreatic neuroendocrine tumours with intraductal growth must be included in the differential diagnosis of IPMT by MRCP.

- P25 -

UNUSUAL PRESENTATION OF AN INSULINOMA. P. Meunier, J.F. Monville, I. Scagnol, E. Hamoir. University Hospital ULg.

The authors report the case of a middle aged woman with a past history of psychiatric troubles (several hospitalizations) until one of her doctors mentionned the possibility of an organic hyperinsulinism.
All explorations didn't show, however, any trouble except repetitive hypoglycemias, and the diagnosis of nesidioblastosis was evoked.
The situation evolved satisfactorily under appropriate treatment until a recent deep "hypoglycemic crisis".
The biological laboratory tests emphasized deep reversible hypoglycemias and inappropriate insulin secretion.
Echoendoscopy and MR identified a pancreatic headtumor of 2 cm in diameter which was punctured (cytology).
The cytologic sample was compatible with a neuroendocrine tumor. This has been confirmed at the pathologic analysis (insulinoma).
This case gives us the opportunity
– to consider briefly the diagnosis progression,
– to emphasize the role of imaging in the evaluation of these patients.

Invited lecture

- P26 -

RADIOLOGICAL-PATHOLOGICAL CORRELATIONS IN THE STAGING OF CHRONIC LIVER DISEASE. J. Cobbold. Imperial College London, London, UK.

Liver fibrosis is a characteristic histopathological lesion of chronic liver disease, described as a semi-quantitative categorical variable. Histological assessment of liver biopsy is the reference standard for the assessment of chronic liver disease, but is subject to sampling variability, inter- and intra- observer variation, and risk of adverse events.
Imaging technologies have the potential to examine different features of chronic liver disease non-invasively, including : hepatic and extra-hepatic macroscopic morphological changes ; signs of portal hypertension ; vascular changes ; liver stiffness ; indices of inflammation and cell turnover and quantification of hepatic lipid. A number of studies have demonstrated correlation between imaging-derived indices and fibrosis stage. Approaches to the assessment of each of these features in published studies will be discussed, with particular emphasis on : ultrasound indices, including transient elastography and contrast-enhanced ultrasound ; magnetic resonance techniques, such as magnetic resonance spectroscopy ; diffusion-weighted imaging and elastography.
Correlation between imaging techniques and fibrosis in well-characterised patient cohorts has been inferred as measurement of fibrosis. For example, early studies of transient elastography in chronic liver disease demonstrated a clear correlation between liver stiffness and fibrosis stage. However, subsequent studies showed strong relationships with hepatic inflammation and portal hypertension, with possible effects of hepatic steatosis. The emerging picture is of a physical parameter influenced by a number of biological processes, the most dominant of which depends on the disease or patient cohort in question.
Radiological techniques have the potential to provide comprehensive characterisation of chronic liver disease beyond fibrosis staging. However, attention must be paid to the biological processes affecting the measured physical parameters. Head-to-head studies of multiple techniques, including incorporation of serum markers, may guide development of complementary combinations of tests. Ultimately, longitudinal and interventional studies will be required to validate these techniques for use clinically and in the development of novel therapeutic strategies.

- P27 -

INTRADUCTAL PAPILLARY MUCINOUS NEOPLASM OF THE PANCREAS : RADIOLOGICAL-PATHOLOGICAL CORRELATION. B. Op De beeck, G. Roeyen, M. Luijks, T. Moreels, A. Snoeckx, M. Spinhoven. U.Z. Antwerpen.

Learning objectives : To illustrate the spectrum of radiological-pathological findings of IPMN. To discuss the radiological classification and the impact on the therapeutic management. To focus on the signs of invasive and noninvasive IPMNs.
Background : IPMN is a grossly visible, mucin-producing epithelial neoplasm that predominantly grows within the main pancreatic duct or one of its branches. IPMNs account for 3 to 5% of all pancreatic neoplasms, and 20% of all cysic neoplasms of the pancreas. It is a slow growing tumour with a nonspecific and indolent nature of the symptoms.
Imaging findings : IPMNs are divided in 3 types : branch duct (commonest), main duct and mixed type. General signs are : diffuse dilatation of the MPD or lobulated multicystic dilatation of the branch ducts seen in an older male population group. Specific findings are : bulging of the papilla, intraductal papillary projections, and communication between the MPD and a cystic lesion. Signs of suspected malignancy are : solid mass, mural nodules, peripancreatic extension, MPD dilatation > 6 mm, cyst size > 30 mm. The main duct and mixed duct type have a higher prevalence of malignancy. Therefore, resection is recommended in this group, as long as the patient is a good surgical candidate with a reasonable life expectancy.
Conclusion : IPMN is one of the more exciting pancreatic entities to be characterized in the past few decades. Slow, noninvasive growth provides an opportunity to surgically resect these neoplasms while they are still curable. Recognition of the imaging features of IPMN may help correct diagnosis and treatment planning.

- P28 -

A CASE OF GLUCAGONOMA : CORRELATION BETWEEN CLINICAL, RADIOLOGICAL AND HISTOLOGICAL FINDINGS. A. Van Landeghem, P. Smeets, J. Van Huysse, J. Verstraete. UZ Gent.

Clinical history : A 44-year-old woman presented with an intermittent dermatosis of the upper and lower extremities, perioral and vaginal region, a burning mouth and watery diarrhea. The symptoms had been present for several years. Laboratory findings revealed an elevated level of glucagon. Glucose and HbA1c were normal. CT-imaging and somatostatin receptor imaging was performed.
Imaging findings : CT revealed a large, heterogenous mass in the body and tail of the pancreas with cystic components.There was noevidence of locoregional spread or distant metastases.
SPECT images acquired 24 hours following injection of a diagnostic tracer activity of Indium-111 labeled octreotide showed significant uptake by the lesion in the pancreas.
Based on the CT findings, octreotidscan,clinical history and hormone analysis, the diagnosis of glucagonoma was made, which was confirmed pathologically.
Pancreatic islet cell tumors (ICTs) are rae neuroendocrine neoplams that can be associated with genetic syndromes such as multiple endocrine neoplasia type 1 (MEN 1), von Hippel-Lindau disease, neurofibromatosis type 1, and tuberous sclerosis. ICTs account for approximately 1.3% of cancers arising in the pancreas. In the past, they have been categorized as either functioning or non-functioning. Howevere, because all of the neoplasms are hormonally active, it is more accurate to classify them as either syndromic or nonsyndromic. ICTs are named according to the active hormone they produce : insulinoma, gastrinoma, VIPoma, glucagonoma, somatostatinoma and ACTH-producing tumors. Syndromic tumors usually manifest at a relatively small size owing to the symptoms produced by the associated hormone production. Nonsyndromic ICTs are larger at initial presentation. Larger tumors more commonly demonstrate cystic changes, necrosis, calcification, local invasion and distant metastases. The two most commonn types are insulinoma and gastrinoma. The glucagonoma is more rare with a prevalence estimated at 1/20 000 000. Glucagonomas are slow-growing neoplasms with non specific symptoms at the beginning. They are frequently malignant, with approximately 60% of patients presenting with liver metastases. Most of the lesions are relatively large and occur in the body or tail of the pancreas. patients present with a typical rash called necrolytic migratory erythema, which usually affects the genitals. Patients may also experience stomatitis, diarrhea, anemia, weight loss, depression and deep vein thrombosis. This paraneoplastic syndrome is termed the 4D syndrome" (dermatosis, depression, diarrhea and deep vein thrombosis) and is pathognomonic. The treatment for patients with syndromic ICTs and no metastases consists ot total surgical resection. CT is the primary imaging modality and plays an important role in the diagnosis, staging and surgical planning of ICTs. Because ICts are often vascular, they can appear well enhanced at arterial imaging. The hypervascularity of ICTs is a key feature that helps differentiate it from other pancratic neoplasms. Intravenous contrast material is also essential for detection of vascular encasement and liver metastases.

- P29 -

UNUSUAL PRESENTATION OF LIVER ADENOMA : CASE REPORT. P. Sierens (1). (1) UZ Brussel. P. Sierens, F. Vandenbroucke, A. Hoorens, B. op de Beeck, J. de Mey.

A 28-year-old Caucasian female was referred to the radiology department with complaints of headache and night sweats after an episode of left upper lobe pneumonia, labs showed persistent elevation of CRP and anaemia without clear-cut explanation.
Thoracic CT showed besides the clearing of the pulmonary consolidation a number of focal liver lesions.
In the further workout contrast-enhanced CT scan with CT fluoroscopy guided biopsy, PET-CT scan and MR imaging with liver specific contrast agent were performed.
The diagnosis of hepatic adenomatosis was confirmed at pathology after partial hepatectomy.
Based on the findings in this patient, we discuss the different imaging features of multiple adenoma : non-fatty adenoma, fat-containing lesion and teleangiectatic inflammatory adenoma.

- P30 -

GROOVE'S PANCREATITIS AND CYSTIC DYSTROPHY OF THE DUODENAL WALL : RARE CAUSE OF SEVERE EPIGASTRIC PAIN. A. Snoeckx, G. Van Den Eynden, B. Op De Beeck, T. Chapelle, I. Brosius, P. Parizel. UA Antwerp.

Case 1 is a 62-year-old man who presented with a one-year-history of multiple episodes of severe epigastric pain. Extensive investigations could not reveal any cause. During the last episode discrete inflammatory abnormalities in the duodenal groove were noted. Control abdominal CT-scan and MRI 6 months later showed multiple cystic lesions in the duodenal wall. Diagnosis of groove's pancreatitis with cystic dystrophy of the duodenal wall was made. Histopathologic examination after whipple-procedure confirmed the presence of multiple cystic lesions in the duodenal wall with fistulization to the pancreatic head.
Case 2 is a 63-year-old man who also presented with a long-standing history of multiple episodes of epigastric pain. During cholecystectomy extensive inflammation of the duodenal wall was noted and confirmed at histopathology. Follow-up CT-scan 3 months later showed small cystic lesions in the duodenal wall, suggestive of cystic dystrophy.
Cystic dystrophy of the duodenal wall is a rare lesion with multiple cysts located in the thickened submucosal or muscular layer. Lesions are induced by inflammation of ectopic pancreatic tissue in the duodenal wall. The cause is still unknown. Clinically this entity presents as groove's pancreatitis, which is a segmental pancreatitis that affects the groove between the head of the pancreas, the duodenum and common bile duct. In this presentation we want to discuss the imaging findings on CT and MRI with histopathologic correlation and review of literature findings. Whereas CT can easily demonstrate a thickened duodenal wall, MRI is the modality of choice to demonstrate the cystic changes. Histopathologic confirmation is rare since it requires invasive surgery. Knowledge of both entities is important since it may mimic other cystic processes and malignancy. Inflammatory changes in the duodenal groove should alert the radiologist to the diagnosis of groove pancreatitis.

- P31 -

DIFFUSION MAGNETIC RESONANCE IMAGING AS SURROGATE MARKER TO ANTI-VEGF TREATMENT RESPONSE IN MCRC ? P. Smeets, N. Van Damme, W. Ceelen, C. Vande Walle, C. Van De Wiele, I. Dero, K. Geboes, S. Laurent, M. Peeters. UZ Gent.

Background : In patients with liver metastasis arising from colorectal cancer, hepatic resection offers the best change on long-term survival. Magnetic resonance imaging (MRI) is an attractive modality for evaluating anti-angiogenic cancer therapies. Additionally, diffusion-weighted MRI (DW-MRI) provides unique information related to tumor cellularity and integrity of cell membranes and thus may be sensitive to changes in the tumor microenvironment that occur after treatment.
Aim : The purpose of the study was to evaluate the use of DW-MRI as a tool to evaluate response to induction treatment with bevacizumab and chemotherapy in metastatic colorectal cancer patients.
Patients and methods : Twelve chemo-naive patients (7 women and 5 men), with histologically confirmed diagnosis of metastatic CRC, were treated with chemotherapy and bevacizumab. Bevacizumab (Avastin®) was administered IV at a fixed dose of 5 mg/kg. Standard MRI and DW-MRI were performed using a 1.5-T superconducting magnet (Magnetom Avanto, Siemens, Germany) with a phased-array body coil. MRI scans were performed before and 8 days after 1 cycle of therapy. Signal intensities at different diffusion gradients (b50, b400, b800) and Apparent Diffusion Coefficients (ADC) of the metastatic lesions were calculated. Normal liver parenchyma and muscle tissue were measured as a reference.
Results : Preliminary results show a change in ADC values measured at different locations in the liver metastases before versus 1 cycle of therapy.
Discussion : These promising results point out that DW-MRI may prove to be a reliable tool to evaluate anti-angiogenic cancer therapy in mCRC. However, these findings need to be confirmed in a larger patient group.
This study was supported by an unrestricted research grant from Roche.

- P32 -

COMPARISON OF TWO CONTRAST AGENTS FOR DCE-MRI EVALUATION OF ANTI-VEGF TREATMENT IN COMBINATION WITH CHEMOTHERAPY IN M CRC. P. Smeets, N. Van Damme, W. Ceelen, C. Vande Walle, C. Van De Wwiele, I. Dero, K. Geboes, S. Laurent, M. Peeters. UZ Gent.

Background : Magnetic resonance imaging (MRI) is an attractive modality for evaluating anti-angiogenic cancer therapies. Additionally, Dynamic Contrast Enhanced-MRI (DCE-MRI), with a rapid acquisition of images before and after intravenous contrast media administration, can be used to assess changes in tumor vasculature in response to angiogenic inhibitors.
Aim : The purpose of the study was to compare DCE-MRI with Gadofosveset trisodium (Vasovist®, Bayer Schering Pharma, BSP) and Gadopentetate dimeglumine (Gd-DTPA, Magnevist®, BSP) contrast agents in the assessment of response to induction treatment with bevacizumab and chemotherapy in metastatic colorectal cancer patients.
Patients and methods : Twelve chemo-naive patients (7 women and 5 men), with histologically confirmed diagnosis of metastatic CRC, were treated with chemotherapy and bevacizumab. Bevacizumab (Avastin®) was administered IV at a fixed dose of 5 mg/kg. DCE-MRI was performed using a 1.5-T superconducting magnet (Magnetom Avanto, Siemens, Germany) with a phased-array body coil. DCE-MRI scans were performed before treatment and 8 days after 1 cycle of therapy. One or two days after the DCE-MRI with Gd-DTPA the same MRI scan was performed with Gadofosveset trisodium. Pharmacokinetic parameters (K^{trans},V_e,V_p) were obtained presuming the Tofts-Kermode compartment model analysis. The area under the enhancement curve (AUC) was also calculated.
Results : Preliminary results from the analysis of the scans performed with Gd-DTPA showed a decrease inK^{trans} and AUC in responders. The pharmacokinetic parameters calculated from the scans obtained with Gadofosveset trisodium were not consistent at first analysis.
Discussion : DCE-MRI with Gd-DTPA is a powerful tool to evaluate therapy response in this setting. Gadofosveset trisodium is a blood pool agent because of its macro-molecular behavior due to its binding to serum albumin upon IV administration. The results obtained with Gadofosveset trisodium are confusing, probably due to the fact that upon fist pass through the liver the binding to albumin is not yet complete.
This study was supported by an unrestricted research grant from Roche.

SIX SOCIETIES SYMPOSIUM

- S01-

COLORECTAL CANCER PRECURSORS AND NON-POLYPOID NEOPLASTIC LESIONS. R. Lambert. Lyon, France.

Premalignant, or malignant, neoplastic lesions of the colon and rectum are called superficial when their extension in the depth of the bowel wall is limited to the mucosa or the submucosa. Until recently, they were considered to develop through the unique adenomatous polyp -carcinoma sequence described by Morson in 1974 and the sequence of mutations initiated with *APC* inactivation, *KRAS* mutation and *TP53* inactivation described by Fearon and Vogelstein in 1988. During the last two decades these theories were challenged by :
1 - the recent introduction of video-colonoscopes and a dramatic progress occurring in the endoscopic description : high resolution images, chromoendoscopy, magnification endoscopy, and other enhancements, have increased our ability to analyze the surface architecture and histology of neoplastic lesions.
2 - the demonstration that serrated lesions establish a bridge between non-neoplastic hyperplastic polyps and neoplastic lesions.
3 - the molecular approaches based on microdissection and DNA sequencing with Polymerase Chain Reaction, which further complicated the conceptual framework of distinct colon cancer subclasses, pathways and genetic profiles.
A revision of the classification of neoplasia in the colorectal mucosa is now justified. The gross morphology of superficial neoplastic lesions, includes polypoid and nonpolypoid subtypes described in the Paris classification. A Workshop was recently held in Kyoto on the role of non-polypoid lesions. The polypoid subtypes, classified as slightly elevated (IIa), flat (IIb) or slightly depressed (IIc), are easily missed during endoscopy if chromoscopy is not used ; however it is estimated that near to 50% of advanced tumors develop from those nonpolypoid precursors.
The Paris endoscopic classification of superficial neoplastic lesions : esophagus, stomach, and colon : November 30 to December 1, 2002. *Gastrointest. Endosc.*, 2003, 58 (6 Suppl) : S3-43.
Kudo S., Lambert R., Allen J.I. *et al*. Nonpolypoid neoplastic lesions of the colorectal mucosa. *Gastrointest Endosc.*, 2008, 68 (4 Suppl) : S3-47

- S03 -

RISK OF DYSPLASIA AND CANCER IN IBD COLITIS. G. Van Assche, MD, PhD, U of Leuven.

Longstanding IBD colitis, particulary ulcerative colitis, predispose to the development of colorectal cancer (CRC). Despite recent data on a decrease in the absolute cancer risk compared to studies published twenty years ago, all physicians involved in the care of patients with IBD should be aware of this risk. Endoscopic surveillance programs in patients with extensive colitis have been implemented in most countries, but have not changed survival rates for patients. Several factors may explain why surveillance is by no means perfect. First, endoscopic surveillance with random biopsies is notoriously laborious and most endoscopist admit to taking less biopsies than expected. Targeted biopsies with classical endoscopy or at chromoendoscopy are more sensitive and will most likely be the technique of choice in the near future. Second, undetected dysplasia or CRC cancer originating beyond the sequence of low grade to high grade dysplasia, probably account for interval cancers. Thirdly, recent data indicate that CRC occurs also in patients with extensive colitis within 10 years from diagnosis. Chemoprophylaxis with 5-ASA may fill some of the gaps left by endoscopic surveillance, but the evidence to support a role of 5-ASA in chemoprophylaxis is not unequivocal.
Open debates surrounding detection of dysplasia and CRC in patients with IBD are :
– What are the optimal endoscopic screening strategies ?
– Is chromoendoscopy required in all patients to detect flat dysplasia ?
– What is the predictive value of low grade dysplasia and indefinite dysplasia ?
– What is the independent value of mesalamine for chemoprophylaxis ?

- S05 -

APPROPRIATENESS OF COLONOSCOPY IN EUROPE. J.P. Vader. University of Lausanne, Switzerland.

After introducing the challenge of providing quality care in general, the presentation will focus on how appropriate care (as a subset of high quality care) can be defined, evaluated and improved. Results stemming from the EPAGE (European Panel of Appropraiteness of Gastroinestinal Endoscopy) study group will be presented and the process of updating those appropriateness criteria (EPAGE II - 2008) will be described. Examples of the updated criteria and website will be illustrated. Finally, a public health perspective on the potential threat of underuse of colonoscopy will be presented.

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- S07 -

PAEDIATRIC FAMILIAL ADENOMATOUS POLYPOSIS (FAP) COHORT WITH PRACTICAL ASSESSMENT OF GUIDELINES FOR THEIR MANAGEMENT. A. Munck. Hôpital Robert Debré APHP, Paris.

Familial adenomatous polyposis (FAP) is a highly penetrant autosomal-dominant colorectal cancer syndrome with significant morbidity and mortality caused by a germline mutation in the adenomatous polyposis coli (APC) gene, located on chromosome 5q21 (1) ; this rare disease is characterized by the early onset of numerous adenomatous colorectal polyps. Left untreated, there is nearly 100% progression to colorectal cancer (CRC) by the age of 35-40 yr. Associated extraintestinal manifestations include upper gastrointestinal tract polyps, congenital hypertrophy of the retinal pigment epithelium (CHRPE), desmoid tumors, epidermal cysts, osteoma and other extracolonic malignancies. Over 800 mutations have been described and several genotype-phenotype correlations have been observed, though there is heterogeneity in clinical course even between family members with the same mutation.

Guidelines for the management of FAP (2) have advocated that “family members that are found to carry the mutation should be advised to undergo periodic examination of the rectosigmoid from the early teens and of the upper GI tract from age 25-30 to monitor adenoma development” which has successfully decreased the incidence of morbidity and mortality from CRC. In paediatrics, studies have involved only small series or case reports (3-5).

The aims of this study were to collect a large paediatric multicentre cohort with a survey up to 18 yr of age, including clinical presentation, genetic analysis, and outcome and to discuss the statements of the guidelines as the challenges are now to define both optimal screening and therapeutic modalities.

A total of 71 patients issued from 49 different families and followed in 11 paediatric GI units were included in the study. Among children presenting a familial FAP, 95% were diagnosed on the basis of a surveillance programme offered to subjects at risk but already 18% were symptomatic at a median age of 10.3 y, 4 de novo mutation patients were seen at 11.3 y. In this study all patients underwent as part of standard practice for initial screening a colonoscopy. Adenomatous polyps were present in 80%. It is noteworthy to mention that 9 children (13%) had an intact rectosigmoid with colononic adenoma above ; one patient having a high grade dysplasia adenoma.

A colectomy was performed in 44 patients at a median age of 13y. Indications were numerous/innumerable polyps for most cases, high grade dysplasia adenoma in 3 children, one polyp size over 1cm and familial severity expression (n = 4). Ileal pouch-anal anastomosis was performed in 84% and ileo rectal anastomosis for the remaining. The rate of complication was similar but loco regional infections, septicemia and high number of bowel movements occurred only in the former one. The choice between both procedures remains controversial (6). Including colonoscopy and colec-

tomy histology specimens, 6 children had high grade dysplasia adenoma with one cancer in situ at 8.8 y ; among them 4 were clinically asymptomatic.
Our patients developed 63 extra-colonic manifestations. Among them, 7 had gastric adenoma and 24 duodenal adenoma Spigelman stage 1. One infant had a hepatoblastoma.
The pathogenic APC germline mutation was identified in 41 out of 49 families with 29 different mutations. Mutational "hot spots" codons such as 1309 and 1061 were present in 10 families (15 children). Large genomic deletions were found in 5 families (n = 7) and 3 families were phenotype-positive, genotype-negative. In our study, we demonstrated important phenotypic discrepancies in severity of the disease within each subgroup (codon mutations usually associated with attenuated, classical intermediate or classical severe phenotype) in agreement with the literature (7,8).
Given the molecular-clinical correlations, it is appropriate to provide specifically tailored advice to families with early onset mutations ("hot spot mutations"), to start endoscopic investigations at any age in symptomatic patients and in families with severe dysplasia at young age. We wish to emphasize that 14% of our cohort had an intact rectosigmoid with colonic adenoma above. Furthermore, at paediatric ages 8.5% had high grade dysplasia adenoma, half of them being clinically asymptomatic.

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- S08 -

ROLE OF VASOACTIVE SUBSTANCES AND CELLULAR EFFECTORS IN THE PATHOPHYSIOLOGY OF CIRRHOTIC PORTAL HYPERTENSION.

W. Laleman. KULeuven Gasthuisberg.

In the last decade, knowledge regarding mechanisms involved in the pathogenesis of portal hypertension has taken unprecedented levels. However, many aspects still remain to be elucidated. Portal hypertension is primarily caused by an increase in resistance to portal flow and secondly by an increase in splanchnic blood flow. In a later phase, these changes lead to a hyperdynamic circulation with increased cardiac output and decreased systemic vascular resistance and perfusion pressure. Regional alterations in vasoreactivity (vasodilation and vasoconstriction) play a central role in the pathophysiology of portal hypertension by contributing to increased intrahepatic resistance, hyperdynamic circulation, and expansion of the collateral circulation. Among vasoactive substances, which are activated in portal hypertension, nitric oxide (NO) is considered as the most important vasodilator. Endothelin-1 and cyclooxygenase-derived prostaglandins are the main vasoconstrictor factors. The imbalance between the hyperresponsiveness and overproduction of vasoconstrictors and the hyporesponsiveness and impaired production of vasodilators are the main mechanisms responsible of the increased vascular tone in the sinusoidal area of the liver. In addition to an imbalance in vasoactive substances, a major role has been attributed to activated hypercontractile hepatic stellate cells which cause vascular remodelling as an adaptive response to the disturbed balance in vasoactive substances . The present paper aims to elucidate on available knowledge and novel mechanisms gathered over the last years with regard to cirrhotic portal hypertension and the increased intrahepatic vascular resistance in particular.

- S09 -

NEW EVOLUTIONS IN LIVER ADENOMA. P. Bioulac-Sage. Service d'Anatomie Pathologique, Hôpital Pellegrin - CHU Bordeaux- France ; Groupe de Recherche pour l'Etude du Foie / INSERM U889, Université Bordeaux 2.

Hepatocellular adenomas (HCA), solitary or multiple, are rare benign monoclonal tumors occurring mainly in young women under oral contraceptives ; more rarely, they can occur in the context of glycogenosis or androgen intake. HCA bleed rather frequently and transform rarely into hepatocellular carcinoma. Recent identification of genes recurrently mutated in HCA and good genotype/phenotype correlations provided the basis of a new patho-molecular classification in different HCA subgroups which can be identified by immunohistochemical markers[1]. Furthermore, recent progress in imaging permitted to strongly suggest the diagnosis with quite specific MRI patterns for some HCA subtypes[2].
1- *HNF1α mutated HCA*. Bi-allelic-inactivating mutations of the *TCF1* gene inactivating HNF1□□have been identified in 35% to 50% of HCA. HNF1α mutations are of somatic origin in 90% of cases, whereas in 10% one mutation is germline. HNF1α mutated HCA had characteristic pathological features, including mainly a marked steatosis. The target gene FABP1 was found to be downregulated and absence of L-FABP (liver fatty acid binding protein) expression easily diagnosed this subgroup.
2□*β-catenin mutated HCA*. β-catenin mutations leading to activation of the Wnt/ β-catenin pathway represented 15% to 18% of HCA, characterized by an overexpression of the target gene glutamine synthetase and an aberrant nuclear β-catenin staining. These β-catenin activated HCA are at greater risk of malignant transformation and often difficult to differentiate from well differentiated HCC.
3- *Inflammatory HCA* (45-50%) defined by the presence of inflammatory infiltrates, sinusoïdal dilatation and dystrophic arteries corresponded to the entity previously called "telangiectatic FNH". Small in-frame deletions that target the binding site of gp130 for IL-6 have been recently reported in 60% of these inflammatory HCA[3], defined by an overexpression of inflammatory proteins in tumour hepatocytes, such as serum amyloid A and C-reactive protein, both at the mRNA and protein levels. Inflammatory HCA occurred more frequently in patients with high body mass index ; they can be also mutated for β-catenin and therefore are probably at risk of HCC.
4- *Unclassified HCA*. Less than 10% of HCA do not express any of these phenotypic markers.
Taking into account noticeable differences between the HCA subgroups, in terms of clinical and prognostic features, phenotyping may become an important tool for HCA management strategy.

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- S11 -

CYSTIC PANCREATIC LESIONS : RADIOLOGICAL APPROACH AND MANAGEMENT. B. Op de Beeck. UZ Antwerpen.

The radiological and clinical challenges are to determine the benign or malignant nature of the lesion and its potential resectability. The heterogeneity among cystic lesions and overlap in imaging characteristics should cause radiologists to approach the specific characterization of cystic pancreatic masses with a substantial degree of humility. This lecture presents our experience on the characterization of cystic pancreatic lesions using CT and MRI. First of all, true cystic pancreatic neoplasms should be differentiated from pseudocysts. If it is not a pseudocyst, differentiate the tumour by : age, gender, location and morphology. The most useful signs for differentiating cystic pancreatic lesions are : the location of the lesion (parenchymal or intraductal), the size of the cysts, and the chemical nature of the content (mucinous or serous). The most useful sign for predicting malignancy is a solid mural nodule with contrast uptake. MRI is equal to or only slightly superior to thin-section helical CT. MRI is therefore preferable for the follow-up of small cystic pancreatic lesions to limit cumulative radiation dose in relatively young patients for whom conservative management is chosen, or as a problem-solving secondary examination in selected patients. Recommending an appropriate management approach based on imaging findings, in conjunction with clinical information and clinical consultation, may therefore be more important than attempting to assign a specific diagnosis to a cystic pancreatic lesion.

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- S12 -

EARLY DETECTION OF DYSPLASIA AND CANCER IN BARRETT'S ESOPHAGUS. R. Bisschops. KULeuven.

Recently a series of new imaging modalities have been introduced, with potential benefit for early detection of Barrett's associated neoplasia. These include mainly a combination of higher resolution endoscopy (megapixel CCD or HDTV) and spectral modification (NBI, FICE, Epk-i) of the visible light. This development partially coincided with a revival of chromo-endoscopy in Barrett's esophagus. The higest spatial resolution is provided by confocal endomicroscopy, enabling in vivo histology. The use of methylene blue (MB) chromo-endoscopy is controversial. Most studies agree on the fact that the detection of SIM is better using MB. A heterogeneous staining pattern is associated with the presence of dysplasia and the absence of uptake of MB is indicative of the presence of dysplasia. Although MB staining does not add to the detection of dysplasia in comparison to random biopsies in one study, it is useful to relocalize dysplasia. Acetic acid enhanced magnifying endoscopy allows to detect the pit pattern to predict the presence of SIM and high grade dysplasia or cancer (1-2).The use of NBI and FICE in Barrett's esophagus was first met with enthusiasm. Irregular vessels and mucosal pattern are indicative of intra-epithelial neoplasia. Recent data suggest that high definition white light endoscopy already allows to detect suspicious lesions and that NBI does not add to the detection. Hence multimodal imaging with Autofluoroscence as a red flag technique is now evaluated (3). For the detection of Barrett's dysplasia super high resolution endoscopy is probably more important than spectral modification. Current studies have all been performed in expert centers and in a population with a high prevalence of high grade dysplasia (ranging 20-60%) and with prototype imaging systems. These data need to be confirmed in a general screening population in non-expert centers. Currently, it is advisable to use the best available endoscope in terms of resolution, however additional techniques (NBI, FIC, i-scan or AFI) are currently not mandatory. In patients with known dysplasia however, they are useful for tissue characterization and relocalization of dysplasia.

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Further reading : ASGE Technology Status Evaluation Report : Narrow Band Imaging and multiband imaging. *Gastrointest. Endosc*., 2008, 67 (4) : 581-589.

- S13 -

TREATMENT OF BARRETT : HOW AND HOW MUCH ? P. Eisendrath. ULB Erasme.

Treatment of Barrett oesophagus is still an area of controversy in therapeutic endoscopy. Most experts recommend that low-grade intraepithelial neoplasia (low grade dysplasia) and non-dysplastic intestinal metaplasia should not be treated outside prospective clinical trial considering the low risk of carcinoma development (1). In patients with high-grade intraepithelial neoplasia (high grade dysplasia, intramucosal carcinoma), endoscopic mucosal resection (EMR) is positioned as the last step diagnostic procedure. This diagnostic/therapeutic procedure allows optimal histological grading and appropriate treatment decision (2). Total Barrett eradication may represent an adjuvant treatment to EMR in this multifocal/polyclonal disease. If radiofrequency ablation appears to be the most attractive method for total barrett eradication, long term efficacy and safety data should be carefully evaluated.

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BELGIAN PANCREATIC CLUB

Invited lecture

- T01 -

THE DENDRITIC THERAPY WITH ITS POTENTIAL APPLICATIONS IN PANCREATIC CANCER. S. Van Gool. UZ Leuven.

Several adult tumors remain a major challenge for the patients and physicians. Amongst these tumors, pancreatic carcinoma (PC) and high grade glioma (HGG) are tumors with very poor prognosis in spite of optimal oncological treatment. During the last years, novel treatment approaches include targeted therapy, anti-angiogenesis, gene therapy and immunotherapy. Active specific immunotherapy relies on the induction of NK and T cell activity against tumor cells after stimulation with Dendritic Cells (DC) that present tumor antigens in the context of MHC molecules. We established immunotherapy for HGG. Proofs of principle of loading patient-derived DC with patient-derived tumor lysate and of the stimulating capacity of this cellular product DCm-HGG-L to generate an effective and specific antitumoral immune response were demonstrated. Further studies on mice reveal delicate balances between tumor cells, effector T cells, regulatory T cells, memory T cells and macrophages in the tumor microenvironment upon DC vaccination. Finally, a model of care has been established in order to be able to treat patients referred by several institutions in and outside Belgium. In the evaluation of DC vaccinations as a potential treatment for patients with pancreatic cancer, one should evaluate several critical questions. 1/ Is there a spontaneous interaction between pancreatic cancer and the immune system ? 2/ What is the preclinical evidence of DC as functional antigen-presenting cells in the context of immunotherapy against pancreatic cancer ? 3/ What is the preclinical evidence of immunotherapy for pancreatic cancer ? 4/ What is the current status of active specific immunotherapy in clinical practice ? At this time, a review of the literature provide a strong rationale on the potential applicability of autologous DC loaded with autologous tumor lysate for patients with pancreatic cancer. Moreover, we have established a world leading translational research program on the development and improvement of active specific immunotherapy for HGG with DCm-HGG-L. Finally, we are running larger-scale phase I/II clinical trials for children and adults with (relapsed) HGG according to a newly developed model of patient care that include advanced personalised cellular therapy. These three facts strongly invite us to set-up a collaborative effort to implement DCm-PC-L into an early phase I/II clinical trial. Maximal surgical reduction of the tumor load thereby obtaining enough tumor material for the production of the lysate and thereby modulating the immune suppressive intratumoral milieu might be a prerequisite for optimal immune therapeutic efficacy.

- T02 -

SIMILARITIES BETWEEN TOTAL MESORECTAL EXCISION FOR RECTAL CANCER AND TOTAL MESODUODENAL EXCSION DURING PANCREATICODUODENECTOMY FOR ADENOCARCINOMA OF THE PANCREATIC HEAD : THE NEED FOR EFFECTIVE LOCOREGIONAL LYMFADENECTOMY. F. Berrevoet, F. Goudsmedt, L. Libbrecht, X. Rogiers, R. Troisi, M. Peeters, B. de Hemptinne. UZ Gent.

Introduction : The overall 5-year survival rate of pancreatic head carcinoma is less than 10%. Although the benefit from extended lymfadenectomy during pancreaticoduodenectomy has been questioned and R0 resections are difficult to obtain, both local and distant spread through lymf node dissemination around the superior mesenteric artery are the main reason for recurrence. We assessed the effectiveness of both R0 resections and the number of lymf nodes resected comparing a standard operative strategy with a new mesoduodenal excision during pancraeticoduodenectomy.
Methods : For this study 26 concecutive patients who underwent an pancreatoduodenectomy with mesoduodenal excision between march 2007 and oktober 2008 were included. The included indications were resectable tumoral laesion in the common bile duct, the pancreatic head, the processus uncinatus or the ampulla. These results were compared with previous results using the traditional pancreatic head resection.
Results : Anastomotic leakage occurred in three patients. Postoperative ileus was the most frequent early complication both in pylorus preserving as well as in antrectomy patients. Other complications included cholangitis, acute renal failure, cardiac ischemia, and pneumonia. Most complications were treated conservatively, but two patients with cardiac ischemia were in need of a coronary artery bypass.
Considering mesoduodenal excision, a mean of 12.6 ± 4 lymf nodes were resected in the specimen, of which 14.3% had timorous involvement in 57.7% of all investigated patients. After pathological examination R0 margins were obtained in 84.6% of patients.
Conclusions : Pancreatoduodenectomy using a mesoduodenal excision technique facilitates the complete skeletonization of the superior mesenteric artery and consequently increases the number of lymph nodes removed. Whether these data lead to an improved patient survival or to an increased perioperative morbidity needs confirmation in further analyses.

- T03 -

WHEN IG G4 AND CA 19-9 YIELD CONFLICTING RESULTS IN A PATIENT WITH PANCREATIC DISEASE : LESSONS TO BE LEARNED. W. Van Steenbergen, F. Claus, R. Aerts, T. Roskams. KULeuven Gasthuisberg.

In patients presenting with a pancreatic mass, the differential diagnosis between autoimmune pancreatitis and pancreatic cancer can be difficult and mostly relies on serologic and imaging studies. Increased concentrations of serum IgG4 are indicative for autoimmune pancreatitis whereas a marked increase in CA 19-9 points in the direction of pancreatic cancer. The diagnosis of autoimmune pancreatitis can avoid unnecessary surgery and should lead to treatment with steroids.
We present a patient presenting with a pancreatic mass with an increased IgG4 of 8 g/l and with a moderate elevation of CA 19-9 of 242 kU/L ; although radiologic findings with double duct sign and diffusion restriction were suggestive of malignancy, treatment with steroids was started. However, under treatment with steroids, the patient developed itching and dark urine, and a rise in liver tests was measured. Moreover, CA 19-9 rose to 3000 kU/l. Based on these findings, a multidisciplinary proposal for Whipple resection was made. The resection specimen, however, did not show any evidence for cancer. It showed a picture of chronic pancreatitis with a marked plasma cell-containing inflammatory infiltrate characteristic of autoimmune pancreatitis.
Abnormal values of CA 19-9 has been reported in about half of the patients presenting with autoimmune pancreatitis ; in most cases elevated levels are within six times the upper limit of normal (Kamisawa *et al*. A J. Gastroenterol., 2003) and have a tendency to normalize after steroid therapy. In our patient, a dramatic rise in CA 19-9 was seen under steroid therapy, and it was mainly based on this finding that a surgical resection was carried out.
The case illustrates the difficulties that may arise in the differential diagnosis between autoimmune pancreatitis and pancreatic cancer. Diffusion restriction on MRI, double duct sign and upstream dilatation of the pancreatic duct, as well as a marked rise in CA 19-9 even during treatment with steroid, were all in favour of a diagnosis of cancer. The correct diagnosis, however, was autoimmune pancreatitis, as it was suggested by the high level of serum IgG4.

- T04 -

VITAMIN D DEFICIENCY IN PATIENTS WITH CHRONIC PANCREATITIS : A CASE-CONTROL COMPARATIVE STUDY. M. Arvanitakis, B. Gulbis, J. Devière, M. Delhaye. ULB Erasme.

Background : Vitamin D has a key role in the good health of the osteo-muscular system, as well as decreasing the risk of many chronic illnesses, such as cardiovascular, auto-immune, infectious diseases, and even cancer. Healthy subjects living in higher latitudes have an increased risk for vitamin D deficiency. Patients with chronic pancreatitis (CP) are even more exposed, because of exocrine failure and reduction in fat absorption. The aim of this study was to evaluate vitamin D levels in patients with CP compared to a population of patients with other diseases, unrelated to fat malabsorption.
Methods and patients : Based on biochemical laboratory databases, we retrospectively selected patients with CP and controls in whom vitamin D was dosed from January to December 2008. Controls were matched for age, gender and season of testing. Parathyroid hormone (PTH) and vitamin B12 levels were also noted, and ratio of PTH to vitamin D levels was calculated. Clinical data was collected by retrospective review of patients' files.
Results : Sixty-six patients, including 45 males, were included in the study. Mean age was 53 (18-85) and mean body mass index (BMI) was 23.8 (13.7-35). Five patients were under therapy with antivitamin K agents. Thirty three patients had diseases unrelated to fat malabsorption (irritable bowel syndrome, colon polyps etc). The remaining had severe CP with a mean period since onset of symptoms of 84(1-228) months. Exocrine failure was present in 14/33 CP patients and 10 were supplemented with pancreatic enzymes. Patients with CP had a significant lower BMI than controls (22.3 vs 25.2, $p = 0.009$). In the whole series, mean levels of vitamin D were 15.5 (6-50) ng/ml, which was under the normal range of our laboratory (20-60 ng/ml). All patients had normal vitamin B12 levels. Interestingly, there was no significant difference between vitamin D levels or the ratio PTH/Vitamin D among CP patients and controls. Severe vitamin D deficiency (< 10 ng/ml) was present in 26/66 (39.4%) patients. Active alcoholism ($p = 0.031$) and antivitamin K therapy ($p = 0.007$) were factors associated with severe vitamin D deficiency, but not CP. Finally, vitamin D supplementation in CP patients did not seem to be significantly associated with increased vitamin D levels.
Conclusions : Vitamin D levels are low in CP patients, as well as in patients with diseases not related to fat malabsorption. This is probably associated to reduced exposure to sunlight. Additional risk factors are alcoholism and antivitamin K therapy. Adequate vitamin D supplementation is recommended.

- T05 -

PATIENTS SUFFERING FROM CHRONIC PANCREATITIS WITH AN INFLAMMATORY MASS IN THE PANCREATIC HEAD BENEFIT SIGNIFICANTLY FROM A FREY PROCEDURE AT LONGTERM FOLLOW-UP.
F. Berrevoet, F. Goudsmedt, S. Laurent, X. Rogiers, R. Troisi, B. de Hemptinne. UZ Gent.

Introduction : Patients with chronic pancreatitis suffering severe pain pose a therapeutic question. Currently employed therapeutic options in conservative and operative treatment of chronic pancreatitis mainly adress the symptoms and eventually evolving complications of the disease. Chronic pancreatitis with an inflammatory mass in the head of the pancreas has been considered the classic indication for a resective procedure. A possible combination of drainage of the pancreatic duct and resection of the inflammatory mass has been proposed by Frey. The aim of this study was to assess the effectiveness of the Frey procedure in relieving intractable pain in patients with chronic pancreatitis including both short- and longterm quality of life.
Materials and methods : In total 48 patients were operated for chronic pancreatitis of which 15 consecutive patients presenting with an inflammatory mass in the pancreatic head were included in this analysis. Perioperative morbidity and mortality were prospectively recorded. Both endocrine and exocrine function were assessed at 6 months, 1 year and yearly thereafter and specific pain questionnaires were performed at the time of the retrospective analysis.
Results : There was no mortality ; short-term morbidity consisted mainly of wound infection and urinary tract infections without major pancreas related morbidity. During follow-up, 73.3% of the patients remained pain free at time of analysis, while 20.0% (n = 3) needed a second operation to resolve stenosis from the choledocho-jejunal anastomosis. Recurrent episodes of pancreatitis occurred in 2 patients.
Conclusions : The Frey procedure is an effective and safe operation technique to treat uncontrollable pain chronic pancreatitis with the presence of an inflammatory mass in the pancreatic head. In these patients surgery seems the primary choice for treatment compared to repetitive stent placement. Stenosis at the level of the distal common bile duct is the ‘Achilles heel’ of the procedure and should be adressed with specific attention.

- T06 -

THE INTERLEUKIN-33 RECEPTOR ST2 DOWN-REGULATES THE SEVERITY OF ACUTE PANCREATITIS : A TRANSLATIONAL STUDY IN HUMAN AND MICE.
A. Lemmers, C. Moreno, M. Arvanitakis, R. Ouziel, D. Degré, E. Quertinmont, V. Vercruysse, P. Demetter, O. Le Moine, A.N. McKenzie, M. Delhaye, J. Devière, T. Gustot. ULB Erasme.

Background and aim : ST2 is a subunit of the receptor for interleukin-33 (IL-33), a new cytokine from the IL-1 / inflammasome family. Caspase-1 and IL-1² have been shown to enhance pancreas and systemic inflammation in acute pancreatitis (AP). We studied soluble ST2 (sST2) expression in human AP and the function of ST2 in mice experimental AP.
Methods : The plasma from 45 consecutive patients enrolled within 24 hours after the onset of AP were collected in our hospital on days 0,1,2,7 and 30, and were assayed for sST2 by ELISA. ST2 deficient and wild type mice were submitted to two models of AP : Choline-deficient ethionine-supplemented diet (CDE) for 72 hrs or Cerulein (10 hourly injections). The CDE model was also performed on caspase-1 deficient mice. The severity of the disease was evaluated by serum hydrolase measurement, histological assessment and serum cytokines quantification by ELISA.
Results : In our cohort of AP patients, 19% had a necrotizing AP and 27% were severe according to Atlanta’s criteria. Etiologies were biliary, alcohol or other for 60, 20, and 20% respectively. The plasma sST2 level of AP patients was increased from day 0 to day 30 compared to healthy subject (D0 2914 [87-2x10^6] or D30 119 [0-1267] vs HS 56.5 [0-546] pg/ml, n = 16 ; $p < 0.01$ or $p < 0.05$ respectively). From D1 to D7, sST2 level was higher in necrotizing than in edematous AP (Day 1 : 5584 [215-2x10^6] vs 1147 [15-2x10^6] pg/ml respectively, p = 0.02). Day 0 sST2 levels correlated with hospital stay, the presence of severe AP and/or need for ICU (Á = 0.313, p = 0.039 ; Á = 0.358, p = 0.017 ; Á = 0.316, p = 0.037 respectively).
In mice, ST2-/- mice presented significantly higher serum amylase, lipase, and pancreas histologic scoring for edema, inflammation and necrosis than wild type in both models. After 72 hours of CDE diet, ST2-/- serum disclosed more IL-6 than wild type ($p < 0.05$). Furthermore, serum IL-33 increased during CDE diet in wild type mice and was blunted in caspase-1 deficient mice.
Conclusions : The ST2 pathway is activated in human AP and early sST2 level correlated with the severity of the disease. ST2 receptor is a negative regulator of AP severity as shown on 2 rodent models. ST2 ligand IL-33 increased in the sera of mice during CDE diet in a caspase-1 dependent manner, suggesting that inflammasome-dependent IL-33 is released during AP and binds to ST2 to regulate the inflammatory burst. This study opens new perspectives to unravel acute pancreatitis inflammatory burst.

- T07 -

GENETIC AND PHENOTYPIC CHARACTERIZATION OF PATIENTS WITH CHRONIC OR RECURRENT "GENETIC" PANCREATITIS. C. Hamoir, X. Pepermans, J.B. Habyalimana, K. Dahan, J. Gigot, A. Geubel, P.H. Deprez. UCL Saint-Luc.

Background and aims : Chronic or acute recurrent pancreatitis is considered to a complex multigenic disease. «Genetic» pancreatitis (GP) is associated with mutations mainly in the cationic trypsinogen (*PRSS1*), *SPINK1* and *CFTR* genes. Natural history of GP remains poorly documented. The aims of this study were to assess genetic, clinical and morphological characteristics of patients diagnosed with GP.
Methods : Inclusion criteria were the presence of *PRSS1*, *CFTR* and *SPINK* gene mutation in patients with idiopathic recurrent or chronic pancreatitis. Genetic testing was performed in 331 probands referred to our centre from 1999 until 2008. Whole coding region of *PRSS1* was analysed while a specific exon 3 scanning for *SPINK1* and screening for a panel of 36 common *CFTR* causing mutations were done. Further complete *CFTR* sequencing (sequencing and MLPA) was performed in case of positive targeted panels.
Results : Seventeen of 331 pts (5.1%) had a disease causing mutation in *PRSS1*, another 9.8% (15/152) had *SPINK1* substitutions (p.N34S) and 37/331 (11%) had *CFTR* by mutation targeted testing. Of the 37 pts with a *CFTR* mutation 3 carried a *SPINK1* and four a *PRSS1* mutation. Moreover 1 pt was transheterozygote *PRSS1*/*SPINK1*. Median ages at first symptom and diagnosis, were 29 [range : 3-65], 36 [3-66] and 30 [1-84)] y, respectively. Follow-up now extends to a median of 6.8 y. GP was responsible for pancreatic pain in all patients, with a mean of 4.8 [0-15] acute pancreatitis attacks, responsible for 2 [0-15] hospitalisations accounting for 14 [0-60] days. Smoking was observed in 13 pts (21.6%). Severity of chronic pancreatitis was calculated with the M-ANNHEIM score with 17 pts with minor, 26 increased, 8 advanced, 5 marked and 3 exacerbated severities. Calcifications were seen in 44.6%, endocrine insufficiency in 18.9% and exocrine insufficiency in 25.4%. Endoscopic treatment was done in 45% (sphincterotomy in 27, stone extraction in 11, and stenting in 25) and surgery in 5 (8.2%). Interestingly, 7 pts (11.5%) had also a pancreas divisum, 6 associated with *CFTR* mutations and 1 with *PRSS1*. Five pts presented with a cancer (2 adenocarcinoma, 2 malignant IMPT, 1 glucagonoma) during follow-up, all of them with a *CFTR* mutation and smoking habits.
Conclusions : In total, multistep testing of *PRSS1*, *SPINK1* and *CFTR* genes identified genetic variants in only 19% of patients considered clinically as candidate for genetic testing, a rate lower than previously reported. Our cohort is characterized by a high rate of cancer, mainly associated with *CFTR* mutations and smoking, rather than *PRSS1* mutations that should bemore typically associated with an increased risk of cancer.

Invited lecture

- T08 -

NEW ADVANCES IN GENETICS AND PANCREATIC DISEASES. C. Cano. INSERM, Marseille, France.

Pancreas cancer is the forth leading cause of cancer death in western countries, with a death/incidence ratio of 0.99. Nowadays, curative treatment of this deadly cancer is restricted to resection surgery with per-operative radiochemotherapy, which is available for only a few patients (5-10% of diagnosed patients) for whom surgery is not contraindicated. Indeed, around 90% of pancreas cancer patients present an inextirpable and/or metastasized tumour by the time of diagnosis and survival among these patients does not overcome 5% after 5 years. Therefore, a great deal in the treatment of pancreas cancer, and in particular of its most abundant form (95% of cases) sporadic pancreatic ductal adenocarcinoma (PDAC), is the development of reliable screening tests allowing an early detection and adapted treatment of the disease. Since risk factors of PDAC are still matters of debate, a better knowledge of genetic anomalies linked to this disease is essential to the identification of new markers and new therapeutic targets. Classic genetic anomalies characterized in PDAC include mutations inducing either activation of oncogenes and/or inactivation of tumour suppressor genes. After describing the most relevant of these classic genetic anomalies and their kinetics of appearance during PDAC progression, a new class of genomic alterations linked to sporadic pancreatic adenocarcinoma will be discussed. These newly identified genomic alterations named Copy Number Variants (CNVs) are characterized by genomic DNA stretches whose copy number is variable within a genome. We have analyzed whole genome CNVs in peripheral blood of sporadic PDAC patients and healthy individuals using the GeneChip Human Mapping 500K Array Set, and evaluated the data obtained using the PCA algorithm. By these means, several genes thus far unrelated to pancreatic cancer were identified as new markers for the disease. Our results show a series of CNVs-enclosed genes discriminating patients from control individuals that are probably required for sporadic pancreatic cancer development. Knowledge of these genes within CNVs may help understanding the physiopathology of PDAC and perhaps reveal new gene targets for preventive strategies. Moreover, specific CNVs patterns represent new markers for pancreatic adenocarcinoma.
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- T09 -

DIAGNOSIS AND HISTOLOGICAL CONFIRMATION OF DIFFUSE MERKEL CELL CARCINOMA RELAPSE BY ENDOSONOGRAPHY. P. Gast. ULg Sart Tilman.

Case report : Merkel cell carcinoma (MCC) is an uncommon but malignant cutaneous neuroendocrine carcinoma with a high incidence of local recurrence, regional lymph node metastases, and distant metastases. We report the case of a 54 -year- old spanish man referred to oncologic consultation for the diagnostic work up of a three centimeters left inguinal adenopathy, appeared five months ago without any other symptom or cutaneous lesion. The usual work up being normal, the adenopathy was removed, histology found a neuro-endocrine carcinoma, with immunohistochemical profile compatible with merkel cell carcinoma. No primary cutaneous lesion was found, abdominal and thoracic CT scan were normal, TEP scan showed remaining suspicious left inguinal adenopathies. Radical lymphadenectomy, external radiotherapy (54 gy) and systemic chemotherapy (etoposide 100 mg/m^2-cisplatine 30 mg/m^2) were proposed to the patient. Five months after the end of the chemotherapy, follow up examinations were normal. Two months later, follow up showed mesenteric and retroperitoneal adenopathies and an enlarged pancreatic head at TEP and abdominal CT scan. The patient was referred for an echoendoscopy with fine needle aspiration. Several lesions were found and punctured : a two centimeters hypoechoïc lesion of the head of the pancreas, one of the local adenopathies ; and a mediastinal adenopathy (aorticopulmonary window) was also punctured with a second needle. All the samples were positive for a very undifferenciated neuroendocrine carcinoma . Immunohistology was compatible with the diagnosis of cutaneous neuroendocrine carcinoma, leading to the diagnosis of a diffuse relapse of the disease.

- T10 -

SEPTICAEMIC SHOCK IN A PATIENT WITH CHRONIC PANCREATITIS : REPORT OF AN UNUSUAL CASE AND ITS SUCCESSFUL OUTCOME BY ENDOSCOPIC TREATMENT. W. Van Steenbergen. KULeuven Gasthuisberg.

Chronic pancreatitis can lead to local complications such as pseudocyst formation, bile duct stenosis, vascular changes, duodenal stenosis, and also to fistula formation to the peritoneum or to the thorax, resulting in pancreatic ascites or in a pancreaticopleural fistula, respectively.
For the purpose of this meeting, we would like to report a patient with a complex history of chronic pancreatitis complicated by pseudocyst formation, pancreaticopleural fistula and recurrent abces formation at the tail of the pancreas for which a surgical resection of the tail of the pancreas had been performed. Multiple endoscopic procedures had already been carried out. In April 2008, however, after resolution of all previous problems, and during an otherwise asymptomatic period, he suddenly developed a severe septicaemic shock that was not related to any of the abovementioned complications. The complication was due to a very rare complication of chronic pancreatitis that consists of a communication between the pancreatic duct and the portal vein, a so-called ‘pancreatico-portal fistula’. This complication was treated by ERCP with the temporary insertion of a nasopancreatic drainage catheter in an attempt to close the fistula. This endoscopic treatment was followed by a complete resolution of clinical symptoms, and the patient remained free of further complications during further follow-up.
The case will be presented with its clinical and radiological findings illustrating the presence of the pancreatico-portal fistula. A short overview will be given on the meaning of this complication that has only very rarely been reported in the literature. Attendants to the meeting will learn this entity as a potential complication of chronic pancreatitis.

- T11 -

INCIDENTAL FINDING OF A PANCREATIC CYSTIC LESION WITH A VERY HIGH CEA LEVEL IN CYST FLUID. M. Delhaye, M. Arvanitakis, S. Debroux, P. Eisendrath, P. Demetter, C. Matos, J. Closset. ULB Erasme.

Incidental cystic lesions of the pancreas are increasingly being detected with the widespread use of abdominal imaging procedures, such as CT and MRI. However, their accurate pre-operative characterization and differentiation remain sometimes difficult.
We report the case of a 49-year-old man who presented with a renal colic. His past medical history included hyperlipidemia and viral hepatitis. He denied a history of weight loss, alcohol abuse, abdominal trauma or prior pancreatitis. He smoked 10 cigarettes/day.
Laboratory studies were all normal except for a serum CA19-9 at 291 U (Nd 37 U), which decreased at 60 U one month later.
On admission, the CT revealed a large cystic lesion measuring 6 cm in diameter, in the region of the pancreatic head.
A MRI further showed a multilocular cystic lesion ($51 \times 53 \times 55$ mm) extending above and posteriorly to the pancreatic head, hyperintense on T2-weighted pictures and isointense on T1-weighted pictures, suggesting a fluid content rich in proteins. The lesion did not enhance after gadolinium administration. There was no vascular encasement and no pathological lymph nodes.
A PET-scan did not show hypermetabolic activity in the area of the cystic lesion.
EUS displayed an heterogeneous, 68 mm, cystic lesion with regular wall, adjacent to a normal pancreatic parenchyma. Analysis of the fine-needle-aspirated cyst fluid demonstrated abundant necrotic material with no malignant cells and no epithelial cells, but a very high CEA level (29100 ng/ml) and low levels of amylase and lipase (149 U/L and 4 U/L, respectively). The bacteriological analysis showed rare colonies of aspergillus fumigatus, but no mycobacteria. The patient developed slight abdominal discomfort that persisted during 10 days after the puncture but resolved easily with paracetamol and tramadol.
Two months later, when the symptoms had resolved, a second EUS-guided FNA was performed. Again, the cyst fluid analysis revealed the presence of inflammatory cells, a high CEA level (10550ng/ml) and a negative bacteriological culture.
Because of the confirmation of a high CEA level in the cyst fluid, we suspected a mucinous cystic tumour and we proposed a duodenopancreatectomy.

DIGESTIVE AND ABDOMINAL IMAGING

- V02 -

ENDOSCOPIC DEVICES FOR THE EXPLORATION OF THE SMALL BOWEL. T.G. Moreels. UA Antwerp.

The small bowel has gained new attention since the development of the wireless videocapsule in 2000, opening up the last 'black box' of the gastrointestinal tract. Although conventional push-enteroscopy has been available for 3 decades, new enteroscopes have now been developed to explore the entire small bowel endoscopically in order to perform all conventional endoscopic procedures in the small intestine. In 2003 Fujinon introduced the Double-Balloon Enteroscope which proved very useful for both diagnostic and therapeutic interventions in the small bowel. Following the concept of balloon-assisted enteroscopy with an overtube, Olympus introduced the Single-Balloon Enteroscope in 2007. More recent adaptations come from Spirus Medical (EndoEase Discovery SB Overtube) and from Smart Medical Systems (NaviAid Balloon-Guided Enteroscope). The results of multicenter trials are awaited in order to determine the clinical value of these new devices.

- V04 -

WHAT IS THE ADEQUATE PREPARATION FOR THE EXAMINATION OF THE SMALL BOWEL BY VIDEOCAPSULE ? I. Demedts. KULeuven Gasthuisberg.

Videocapsule endoscopy (VCE) has rapidly gained recognition as an excellent tool for examination of the small bowel, recently receiving reimbursement in Belgium for obscure bleeding indications. Standard preparation for VCE is overnight fast, often preceded by liquid diet the day before. However, incomplete small bowel transit and poor visibility due to luminal content and air bubbles may limit its accuracy. In the past, several studies have assessed whether bowel preparation and/or prokinetics can improve visual quality and completion rate and consequently influence diagnostic yield ; with conflicting results. Many of these studies were hampered by poor design (retrospective, not controlled), low numbers and absence of a validated scoring method for bowel cleanliness/mucosal visibility.
Recently, 3 meta-analyses were published (2 full papers, 1 abstract) comparing PEG or NaP versus standard preparation. All agree that PEG/NaP improves visual quality, but has no effect on completion rate. Whether diagnostic yield is improved is less clear : Rokkas *et al*. report improved diagnostic yield, Marmo *et al*. no difference in diagnostic yield, the meta-analysis by Niv does not include data on diagnostic yield. In a prospective randomized study, Postgate *et al*. compared standard preparation to different combinations of magnesiumcitrate, senna and metoclopramide and found no difference in visual quality, completion rate nor in diagnostic yield, but did find reduced patient acceptability for these preparations.
In summary, although PEG and NaP improve visual quality, especially in the distal small bowel segments, this does not seem to lead unequivocally to a higher diagnostic yield ; other bowel preparation regimens appear not to improve standard preparation. In view of recent warnings concerning NaP preparation for large bowel cleansing, advocating its use in small bowel preparation seems not warranted. As for PEGpreparation, the optimal dose and administration protocol is still debated.
Conclusion : Standard preparation of overnight fast, preceded by liquid diet the day before, is adequate preparation for VCE in daily clinical practice.
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