

# ABSTRACTS

## XXIIth Belgian Week of Gastroenterology 2010

### ABSTRACTS

- A01 — A36 Belgian Association for the Study of the Liver (BASL)  
B01 — B18 Research Group “Gastrointestinal Regulatory Mechanisms (OG-FWO)”  
D01 — D71 Joint Meeting of Gastroenterology  
E01 — E03 Belgian Group of Pediatric Gastroenterology, Hepatology and Nutrition  
H01 — H04 Belgian HP Study Group  
I01 — I12 IBD Research Group  
N01 — N10 Research Group of Clinical Nutrition and Metabolism (SBNC and VVKVM)  
P01 — P33 Gastro-intestinal Pathology Club & the Belgian Group for Digestive Oncology and Research Group  
“Digestive and Abdominal Imaging”  
S01 — S07 Symposium of the six societies  
T01 — T11 Research Group “Belgian Pancreatic Club”  
V01 — V07 Belgian Videocapsule Group

## BASL

- A01 -

CHARACTERIZATION OF THE LIVER PROGENITOR CELL NICHE IN LIVER DISEASES ; POTENTIAL INVOLVEMENT OF WNT AND NOTCH SIGNALING. B. Spee (1), G. Carpino (2), B. Schotanus (3), A. Katoonizadeh (1), S. Vanderborght (1), E. Gaudio (4), T. Roskams (1). (1) KU, Leuven, Belgium, (2) University of Rome, Foro Italico, Rome, Italy, (3) Utrecht University, Utrecht, Netherlands, (4) Sapienza University, Rome, Italy.

**Introduction** : Hepatic progenitor cells (HPCs) hold a great potential for therapeutic intervention for currently untreatable liver diseases. However, in human diseases molecular mechanisms involved in proliferation and differentiation of HPCs are poorly understood.

**Aim** : In the present study activated HPCs and its microenvironment (niche) were investigated in acute and chronic human liver disease by gene-expression analysis and immuno-histochemistry/-fluorescence.

**Material and Methods** : Cryopreserved liver tissues were used from patients with parenchymal versus biliary diseases : acute necrotising hepatitis (AH), cirrhosis after hepatitis C infection (HCV), and primary biliary cirrhosis (PBC) in order to study differentiation of HPCs towards hepatocytic versus biliary lineage. Keratin 7 (K7) positive HPCs/reactive ductules were captured by means of Laser Capture Microdissection (LCM) and gene-expression profiles were obtained by using a customized PCR Array. Gene expression results were confirmed by immunohistochemistry and immunofluorescence double staining.

**Results** : In all disease groups, microdissected HPCs expressed progenitor cell markers such as *KRT7*, *KRT19*, *NCAM*, *ABCG2*, *LIF*, *KIT*, *OCT4*, *CD44*, and *TERT*. In AH, HPCs were most activated and showed a high expression of prominin-1 (CD133) and alpha-fetoprotein (AFP), and a strong activation of the Wnt-pathway. In contrast to parenchymal diseases, HPCs in PBC (biliary differentiation) showed a high activation of Notch signalling.

**Conclusion** : A distinct pattern of HPC surface markers was found between acute- and chronic-liver diseases. Similar to what is known from animal experiments, strong evidence has been found signifying the role of Wnt signalling in proliferation of human HPCs whereas Notch signalling is involved in biliary differentiation. These pathways can be targeted in future therapies.

- A02 -

PRESENCE OF LIVER ANGIOGENESIS AND UPREGULATION OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN A MICE MODEL OF NON-ALCOHOLIC STEATOHEPATITIS (NASH). S. Coulon, A. Geerts, B. Brouwers, D. Vlaeminck, L. Libbrecht, C. Van Steenkiste, F. Heindryckx, H. Van Vlierberghe, I. Colle. University Hospital, Ghent, Belgium.

**Introduction** : Non-alcoholic steatohepatitis (NASH) is one of the major consequences of the current obesity epidemic. It is characterized as a potentially progressive disease that may cause fibrosis, cirrhosis, and liver cancer. Angiogenesis or disturbances of the liver vascular architecture has been linked to progression to fibrosis, cirrhosis and liver cancer in chronic liver diseases. The vascular endothelial growth factor (VEGF) is described as one of the most important angiogenic factors and is also upregulated in some chronic inflammatory diseases. The underlying mechanism of progression of steatosis to steatohepatitis is still unclear, probably initiated and regulated by several inflammatory mediators. Until now, the role of angiogenesis and VEGF has never been studied in the pathophysiology of NASH.

**Aim** : To elucidate the role of the angiogenic factor, VEGF, in a methionine-choline deficient (MCD) dietary mice

**Methods** : Female C57Bl6 mice, aged 9 weeks, were fed on a MCD diet or control diet. Liver samples were taken at different time-points (3 days, 1 week, 2 weeks, 4 weeks and 8 weeks) after starting the diet (n = 8/group). Sections were stained with hematoxylin and eosin (H&E) for routine histology or Sirius red for collagen. Hepatic neovascularization was investigated by immunohistochemical stainings for Von Willebrand Factor (VWF). The expression of VEGF was determined quantitatively by ELISA and immunohistochemically.

**Results** : Histological findings of the liver showed already steatosis in the liver after 3 days of MCD feeding. At 4 weeks, steatohepatitis is well developed with visible liver cell injury (ballooning of hepatocytes), and by 8 weeks pericellular and perisinusoidal fibrosis are present. Immunohistochemical expression of VEGF in the liver of MCD fed mice showed a significant increased VEGF expression after 1 week (p = 0,001), 2 weeks (p = 0,029) and 4 weeks (p = 0,028) of MCD diet in comparison with control diet. After 3 days MCD diet, the expression was not yet increased in comparison to the control diet. Quantitative analysis confirmed these histological findings, there was an increase in the VEGF protein concentration between mice given the MCD diet during 1 week (p = 0,001), 2 weeks (p = 0,086) and 4 weeks (p = 0,015) and there control littermates. Expression of Von Willebrand Factor, an endothelial cell marker which is up-regulated by angiogenic factors like VEGF, showed an increased expression after 8 weeks of MCD diet.

**Conclusion** : These data show for the first time that there is an upregulation of the vascular endothelial growth factor (VEGF) in liver tissue during the development of NASH in a murine model. This leads to angiogenesis after 8 weeks of MCD feeding. These data could give rise to new therapeutic options for NASH.

ACTIVATION OF THE LIVER PROGENITOR CELL COMPARTMENT IS NOT SUFFICIENT TO ENSURE LIVER REGENERATION. A.C. Dusabineza, J. Abarca-Quinones, N. Van Hul, I. Leclercq. Université Catholique de Louvain, Brussels, Belgium.

**Introduction :** Liver progenitor cells (LPCs) are thought to represent a facultative source of hepatocytes when replication of mature hepatocytes fails.

**Aim :** The potential of LPCs to insure liver regeneration is examined in a rat model.

**Methods :** Fischer 344 rats were implanted a 2-acetaminofluorene (AAF)-slow release pellet subcutaneously. A 70% PH was performed and an osmotic minipump filled with BrdU implanted 1 week later (AAF/PH group). They were compared to rats subjected to PH without AAF (PH group). Rats were sacrificed at 7, 14 and 21 days post PH. The liver mass recovery rate was calculated. Cell proliferation was evaluated by mitotic count, Ki67 and BrdU immunohistochemistry (IHC) on liver sections, and CK19 and EpCAM IHC were used to identify LPCs.

**Results :** No mortality was observed in the PH group while 20% of AAF/PH rats died between day 12 and day 20 post PH. Liver mass recovery rate 14days post-PH was  $89 \pm 13\%$  in CTL/PH, and  $76 \pm 17\%$  in AAF/PH rats (NS). In PH, the vast majority of hepatocytes were Ki67+ and BrdU+ and numerous mitotic figures were found in hepatocytes. By contrast, in AAF/PH livers, there was a proliferation of CK19+ and EpCAM+ oval cells forming pseudo-ductular structures irradiating from the portal tract. In addition, isolated intermediate hepatocytes-like cells and isolated oval cells expressing, either one or both, CK19 or EpCAM were found in the vicinity of pseudo-biliary structures and more distantly into the parenchyma. Proliferating hepatocytes (BrdU+ and Ki67+) were restricted to the pericentral area, while numerous Ki67+ and BrdU+ cells were found in all the pseudo-ductular structures as well as in isolated oval and intermediate hepatocytes-like cells. There was an interindividual variability in the extent of the oval cell expansion which was inversely correlated to the amount of intermediate hepatocytes-like cells, the amount of proliferating mature hepatocytes in pericentral zone and the rate of liver mass recovery. In deceased rats, expansion of pseudoductular structures was massive but proliferation of mature hepatocytes was not present.

**Conclusion :** In response to PH, liver mass recovery occurred through proliferation of mature hepatocytes. In AAF-treated rats, liver mass recovery relies on both the expansion of oval cells and proliferation of mature pericentral hepatocytes. Our results suggest that, in face of a massive and acute loss of hepatocellular mass, expansion of the progenitor compartment alone is not sufficient to ensure animal survival. The relative contribution of mature hepatocyte proliferation and terminal differentiation of progenitor cells to allow survival remains to be evaluated.

N-GLYCOSYLATION PATTERNS IN HCC-MICE CHRONICALLY INJECTED WITH DEN AND THEIR EVOLUTION AFTER TREATMENT WITH ANTI-PLGF. B. Blomme (1), F. Heindryckx (1), I. Colle (1), J.M. Stassen (2), N. Callewaert (3), H. Van Vlierberghe (1). (1) University Hospital, Gent, Belgium, (2) VIB, Leuven, Belgium, (3) VIB, Gent, Belgium.

**Introduction :** Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. Prognosis for this disease is poor since HCC is mostly diagnosed at an advanced stage. A biomarker that notifies the clinician in a premalignant phase would be beneficial in the management of HCC.

**Aim :** Our aim was to evaluate the use of N-glycosylation patterns in a mouse model of HCC and their evolution after treatment with anti-PIGF, a potential anti-angiogenic agent.

**Methods :** 5-week-old mice received weekly intraperitoneal injections with N-nitrosodiethylamine (DEN). Mice were sacrificed after 4, 16, 20, 25 and 30 weeks. One group was chronically injected with DEN for 25w and subsequently treated with 20mg/kg PIGF antibodies (anti-PIGF) for 5w. Serum was collected at sacrifice and analyzed with DNA-sequencer-assisted-fluorophore-assisted capillary electrophoresis.

**Results :** After 16 weeks of DEN-injections, a mild fibrosis occurs and dysplastic lesions start to appear. The first neoplastic nodules were observed in the liver at 20w that progressed to vascularized exophytic tumors at 25w and 30w. The number of tumors decreased considerably after treatment with anti-PIGF in mice that were injected with DEN for 25w. Already after 16w, the mice displayed multiple alterations of N-glycosylation with a significant increased peak height of peaks 8, 10, 11 and 13 and a significant decreased peak height of peaks 3, 4, 5 and 6. Maximum phenotype at N-glycosylation level was reached after 20w of DEN-treatment. In addition to the alteration at 16w, there was a significant increased peak height of peaks 2, 7 and 12. In mice that underwent a regimen of 25w DEN followed by 5w anti-PIGF, there was a significant increased peak height of peaks 2 and 3 and a significant decreased peak height of peaks 11, 12 and 13 compared to 30w DEN. In other words, more than half of the altered peaks in HCC significantly evolved in the normal direction after treatment with anti-PIGF.

**Conclusion :** N-glycosylation patterns have potential as a diagnostic/prognostic tool in the management of HCC because significant N-glycan alterations can be observed at an early stage of HCC. Anti-PIGF has a beneficial influence on the N-glycosylation patterns in HCC-mice.

GLYCOGEN SYNTHASE KINASE 3 (GSK3) IN IMMUNE CELLS IS A MAJOR PLAYER IN TLR4-MEDIATED CYTOKINE STORM IN PATIENTS WITH ADVANCED CIRRHOSIS. T. Gustot (1), M. Simon-Rudler (2), M. Fasseu (2), S. Gandoura (2), W. Abdel-Razek (2), J. Devière (1), D. Lebrech (2), R. Moreau (2). (1) ULB Erasme, Brussels, Belgium, (2) INSERM Bichat-Beaujon, Paris, France.

**Introduction** : In advanced cirrhosis, the innate immune response to the TLR4 agonist lipopolysaccharide (LPS, a bacterial component) is excessive, characterized by a 'pro-inflammatory cytokine storm' and hypo-production of the anti-inflammatory cytokine IL-10. This is why sepsis-induced multi-organ failure and death are common in patients with cirrhosis. In non-cirrhotic immune cells, the constitutively active GSK3 is thought to favor LPS-elicited pro-inflammatory cytokine (e.g., IL-12p40, TNF- $\alpha$ , IL-1 $\beta$ ) against IL-10 production, by acting on gene induction.

**Aim** : We investigated the role of GSK3 in LPS-induced cytokine production by immune cells from patients with advanced cirrhosis.

**Methods** : Peripheral blood mononuclear cells (PBMCs) were isolated from 19 Child-Pugh C cirrhotic patients. Cells were pre-incubated with or without GSK3 inhibitor (SB216763, 10  $\mu$ M or lithium chloride, 20 mM) for 1 hour and then stimulated with LPS for 20 hours. Cytokine production was assessed by real-time RT-PCR (after 1 hour of LPS exposure to assess gene induction) and ELISA and (after 20 hours to assess protein secretion). Values are medians (min-max).

**Results** : GSK3 inhibition significantly decreased LPS-induced production of IL-12p40 (38% [3-45] and 40% [3-77] of respective controls, for mRNA and protein levels, respectively) and TNF- $\alpha$  (63% [32-89] and 56% [5-114], respectively). In contrast, GSK3 inhibition resulted in significant increase in IL-10 production (569% [512-781] and 229% [174-485], respectively). These findings are consistent with a significant role of GSK3 in both the induction of genes encoding IL-12p40 and TNF- $\alpha$ , and the inhibition of IL-10 gene in LPS-stimulated cells. Interestingly, GSK3 inhibition did not affect LPS-induced pro-IL1 $\beta$  mRNA levels but significantly and markedly decreased IL-1 $\beta$  protein secretion (it remained only 11% [4-15] of the amount secreted in response to LPS in the absence of GSK3 inhibitor). These results suggest that GSK3 mediates LPS-induced IL-1 $\beta$  production at the posttranscriptional level (translation of pro-IL1 $\beta$  mRNA and/or processing by the inflammasome of the pro-IL1 $\beta$  protein into IL-1 $\beta$ ).

**Conclusion** : Immune cell GSK3 plays a major role, via transcriptional and posttranscriptional mechanisms, in TLR4-mediated cytokine storm in patients with advanced cirrhosis. Thus, GSK3 inhibition may be a novel approach in the treatment of severe sepsis in these patients.

THE RAS INHIBITOR FARNESYLTHIOSALICYLIC ACID INHIBITS EGF AND IGF2-INDUCED CELL GROWTH IN HEPATOCARCINOMA CELL LINES. N. Charette (1), C. De Saeger (1), Y. Horsmans (2), I. Leclercq (1), P. Starkel (2). (1) Université Catholique de Louvain, Brussels, Belgium, (2) UCL Saint-Luc, Brussels, Belgium.

**Introduction** : EGF and IGF2 signaling are upregulated in the majority of hepatocarcinoma (HCC), leading to activation of two main intracellular signaling pathways, the ras-raf-MAPK and the PI3K-Akt-mTOR pathways. Farnesylthiosalicylic acid (FTS) inhibits ras by dislodging its activated form from its membrane docking sites and/or directly interferes with the mTOR-raptor complex.

**Aim** : The aim of this study was to evaluate the effect of FTS on EGF- and IGF2-induced cell growth and signaling through ERK and AKT-mTOR in three HCC cell lines (HepG2, Huh7 and Hep3B).

**Methods** : In EGF and IGF2 stimulated cells, cell viability was assessed by a WST-1 assay after three days of culture with dimethylsulfoxide (DMSO, solvent) or increasing concentrations of FTS ranging from 25 to 200  $\mu$ M. Cell proliferation in EGF- or IGF2-stimulated cells was quantified by a BrdU incorporation assay after one day in presence of DMSO or FTS at concentrations ranging from 50 to 150  $\mu$ M. ERK, phospho-ERK, AKT, phospho-AKT, p70 and phospho-p70 (mTOR activation) were determined by immunoblotting.

**Results** : FTS induced a significant dose-dependent decrease in cell viability in the three tested cell lines stimulated by EGF or IGF2. IC50, the concentration at which 50% of cell growth is inhibited compared with DMSO control, was estimated between 50 and 75  $\mu$ M for EGF-stimulated HepG2 and Hep3B, and between 75 and 100  $\mu$ M for EGF-stimulated Huh7 and IGF2-stimulated cells. Moreover, growth factor-induced DNA synthesis was suppressed by FTS as from 100  $\mu$ M. FTS treatment had no impact on ERK phosphorylation. Akt phosphorylation was only decreased in EGF-stimulated HepG2 cells. However, p70 phosphorylation was almost completely abrogated by FTS in all three cell lines, stimulated either by EGF or IGF2.

**Conclusion** : FTS inhibits both EGF- and IGF2-induced cell growth in HCC cell lines in a dose-dependent manner. This effect is principally associated with a strong inhibition of mTOR, independent of Akt. Persistence of ERK phosphorylation in these cells provides a rationale for testing FTS together with an inhibitor of the ras-raf-MAPK pathway (e.g. sorafenib).

ANGIOGENIC CHANGES IN A NEW MOUSE MODEL FOR HEPATOCELLULAR CARCINOMA ASSESSED WITH STATE-OF-THE-ART IMAGING TECHNOLOGY. F. Heindryckx (1), B. Vandeghinste (2), N. Charette (3), D. Slaets (2), L. Libbrecht (1), C. Casteleyn (2), S. Staelens (2), P. Starkel (3), A. Geerts (1), I. Colle (1), H. Van Vlierberghe (1). (1) University Hospital, Ghent, Belgium, (2) Ghent University, Ghent, Belgium, (3) UCL Saint-Luc, Brussels, Belgium.

**Introduction** : An efficient and representative mouse model is the corner stone of a successful experiment. The growing incidence of hepatocellular carcinoma (HCC) in the Western countries has led to an expanding interest of scientific research in this field. Therefore, there is a vast need of experimental models that mimic the natural pathogenesis of HCC in a short time period. Furthermore, the validation of an efficient imaging technique could contribute to the early detection of HCC in patients.

**Aim** : The goal of our study was (1) to develop an efficient mouse model for hepatocellular carcinoma research, (2) to assess time-dependent angiogenic changes and (3) to investigate tumour growth and neo-vascularisation using state-of-the-art imaging techniques.

**Methods** : 5-week-old male mice received weekly intraperitoneal injections with N-nitrosodiethylamine (DEN) (35 mg/kg bodyweight) and samples were taken at several time points. Histology, ELISA and immunohistochemical stainings were used to identify the HCC-lesions and to quantify angiogenic factors VEGF and PlGF. HCC livers (25W) were perfused with Batson's n°17 solution to produce vascular casts (arterial and venous). A state-of-the-art multimodal microPET/CT was used for in vivo detection of HCC-lesions with [18F]-fluoromethylcholine ([18F]FMCH) and for 3D-reconstruction of the vascular casts.

**Results** : After 16W of DEN-injections a mild fibrosis (F1-F2) and dysplastic lesions appear, resulting in a pre-malignant environment. An increase of angiogenic factors VEGF and PlGF takes place, but not explicit enough to induce an increase in endothelial cells, which were upregulated after 20W. After 25W of DEN-injections, the dysplastic lesions have progressed to vascularised exophytic tumours which are macroscopically visible and give rise to a further increase in angiogenic factors, leading to the formation of new blood vessels. HCC-lesions were characterised by an increased uptake of [18F]FMCH, allowing visualisation of these hotspots (> 3mm) with microPET/CT. The vascular casts of HCC-livers clearly revealed the chaotic pattern and hierarchically disorganisation of tumour induced blood vessels. Arteries formed a circumferential mantle around the hepatic tumours, while the central tumour regions showed a lower arterial density.

**Conclusion** : While most DEN-induced models take at least one year to develop tumours, weekly injections with DEN give rise to tumour occurrence after 25W. The well vascularised orthotopic tumours are a representative model for HCC and can serve as an excellent platform for the development of new therapeutic targets. Furthermore, [18F]FMCH PET imaging may be clinically relevant for the visualization of HCC-lesions.

LIVER PROGENITOR CELL EXPANSION IN THE INJURED MOUSE LIVER : DO KUPFFER CELLS PLAY A ROLE ? N. Van Hul, N. Lanthier, J. Abarca-Quinones, M. Petit, Y. Horsmans, I. Leclercq. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : In chronic injury, liver progenitor cells (LPCs) proliferate before undergoing differentiation. Previous work from our lab suggested that extracellular matrix (ECM) deposition and activation of mesenchymal cells during this process condition LPCs expansion and differentiation. Kupffer cells (KC) are active modulators of hepatic fibrosis.

**Aim** : To analyse the role of KC in a murine model of induced LPC proliferation.

**Methods** : To follow the evolution of the LPC/ECM reaction, a choline deficient diet, supplemented in ethionin (CDE) was administrated to C57Bl6 mice for 3 or 7 days. Control mice received normal diet. KC were depleted at day -1 by one intravenous injection of clodronate encapsulated liposomes. (immuno)Histochemistry (IHC) revealed the presence of LPC, KC and ECM and gene expression was evaluated by RT-PCR.

**Results** : After three days of CDE diet, albumin expression strongly decreased confirming the selective hepatocytic damage while collagen already accumulated in the periportal area to values ten times higher than in controls. Expansion of LPC, assessed by an increase of cytokeratin 19 (CK19) positive cells, did not occur until day 7. Massive F4/80 positive KC activation was seen at day 3, with KC predominantly located around the portal tracts. After 7 days, the number of activated KC decreased, but still remained higher than in controls. At the 3-day time point, the clodronate-induced depletion of KC, confirmed by a drastic fall in F4/80 and CD68 both by IHC and RT-PCR, was associated with a significant decrease in expression of pro-fibrotic genes TGF- $\beta$  and collagen I while  $\alpha$ -SMA remained unchanged compared to the control group (RT-PCR). At the 7-day time point, no difference was seen between clodronate-treated and control CDE groups whether in terms of KC, LPCs or deposition of ECM, possibly due to the KC repopulation of the liver by circulating monocytes

**Conclusion** : Our data to date demonstrate that, in the CDE model, beside ECM deposition and activation of matrix producing cells, massive activation of KC is also a significant component of the micro-environmental changes preceding LPCs expansion. Depletion of KC in the early phase is associated with a decreased expression of pro-fibrotic genes such as TGF- $\beta$  and collagen I. Multiple clodronate injections are currently investigated in order to obtain long lasting KC depletion to analyse the influence of KC on LPC reaction.

2-LOG DROP IN VIRAL LOAD AT ONE MONTH : A NEW STOPPING RULE IN RELAPSE AND NON-RESPONDERS HCV PATIENTS RE-TREATED WITH PEGYLATED-IFN AND RIBAVIRIN ? P. Deltenre (1), M. Corouge (2), V. Canva (2), M. El Nady (2), A. Louvet (2), H. Castel (2), F. Wartel (2), S. Dharancy (2), J. Henrion (1), P. Mathurin (2). (1) Hôpital de Jolimont, Haïne-Saint-Paul, Belgium, (2) Centre Hospitalier Régional Universitaire, Lille, France.

**Introduction** : Early identification of previously treated HCV patients with low probability of SVR is mandatory. EPIC and REPEAT trials have recommended undetectable HCVRNA at week 12 as the stopping rule. It is still unsettled whether earlier virological criteria may be useful.

**Aim** : To identify, in relapsers and non-responders re-treated with Peg-IFN/Ribavirin, the earliest and most optimal time at which the decline in viral load (VL) becomes the best predictor of SVR and the best cut-off of VL reduction predictive of SVR.

**Methods** : We used an optimal approach specifically devoted to find the earliest and most accurate virological criteria (both highly sensitive and specific) predictive of SVR. The prediction of SVR was expressed by the area under the ROC (AUROC) curves of reduction in VL (in logs) at different time points during treatment.

**Results** : 184 patients (67% male, 17% genotype 2-3, 43% F3/4) underwent 315 treatment courses. Characteristics (median values) of patients were : age 46 years, duration of infection 22 years, ALT 1.8 ULN, baseline VL 6 logIU/ml. 28.1% reached SVR. Reduction of VL at 1 month was as effective for predicting SVR as reduction of VL at months 2 and 3 (AUROC curves :  $0.723 \pm 0.043$ ,  $0.680 \pm 0.043$ , and  $0.686 \pm 0.039$ , respectively). A 2-logs VL decline at 1 month was a good predictor of SVR (sensitivity 82%, specificity 61%, PPV 45%, NPV 90%), meaning that patients without 2-logs VL decline at 1 month had only 10% probability of SVR. Undetectable HCVRNA at 3 months was also predictive of SVR (sensitivity 95%, specificity 63%, PPV 51%, NPV 97%). Patients with 2-logs drop at 1 month had 89% probability of being HCVRNA negative at 3 months whereas those without had 18% probability of being HCVRNA negative at 3 months. The 2-logs drop criterion at 1 month was as efficient as undetectable HCVRNA at 3 months in term of correctly classified SVR patients : 45% vs. 51% (NS).

**Conclusion** : In previously treated patients, 2-logs VL decline at 1 month predicts SVR. This criterion seems as useful as undetectable HCVRNA at 3 months and may be used early on as a stopping rule in patients with poor tolerance.

MANAGEMENT AND OUTCOME OF BILIARY COMPLICATIONS AFTER LIVER TRANSPLANTATION. S. Van Cleven, H. Peeters, D. De Looze, M. De Vos, A. Geerts, X. Rogiers, R. Troisi, B. De Hemptinne, L. Defreyne, P. Vanlangenhove, I. Colle, H. Van Vlierberghe. University Hospital, Ghent, Belgium.

**Aim** : The aim of this study was to document the prevalence, predictive factors, treatment and survival of patients with anastomotic and nonanastomotic biliary complications after orthotopic liver transplantation in a single center. The results were then reflected to the current literature.

**Methods** : All patient data were collected retrospectively from hospital records between January 2004 and October 2008. In total 197 transplantations were performed for 177 patients. Patients were divided into those with complications (anastomotic and/or nonanastomotic) and those without biliary complications. Both groups were compared for several clinical factors. Statistical analysis was used to demonstrate predictive factors for complications.

**Results** : The overall biliary complication rate was 28.9% (n = 57), including 41 strictures and 8 leakages. Seventy-three per cent of the strictures were anastomotic and 27% nonanastomotic. By means of univariate analysis, risk factors for biliary anastomotic complications were donor age (p = 0.028), donor height (p = 0.041), donor gender (p = 0.039) and HBD dead whole donor (p = 0.017). Risk factors for nonanastomotic complications were donor age (p = 0.012) and hepatic artery thrombosis (p = 0.02). Binary logistic regression showed that donor age was an independent risk factor for biliary anastomotic complications. Donor age and hepatic artery thrombosis were independent risk factors for nonanastomotic complications. Anastomotic strictures were successfully treated with repeated stenting by ERCP (success rate = 86.67%). ERCP for nonanastomotic complications was less effective (success rate = 50%). PTC and the combination with ERCP were 100% successful. One nonanastomotic stricture needed retransplantation.

**Conclusion** : Donor age was an independent risk factor for both anastomotic and non-anastomotic biliary complications. The increasing need for donations has led to the use of 'extended criteria donors', like advanced donor age. This could result in more anastomotic strictures when older donors were used. The experience of the surgeon could also be a predictor for anastomotic strictures, however this issue was beyond the scope of our research. Nonanastomotic complications involving the intrahepatic biliary ducts are mostly related to ischemic injury to the peribiliary vascular plexus. A well known risk factor is a hepatic artery thrombosis after transplantation. ERCP for anastomotic strictures is highly successful. Nonanastomotic strictures were more difficult to treat with ERCP. Nonanastomotic strictures were treated with PTC or ERCP combined with PTC.

HEPATITIS B VIRUS (HBV) INFECTION IN BELGIUM : RESULTS OF THE BELGIAN ASSOCIATION FOR THE STUDY OF THE LIVER (BASL) REGISTRY OF 1421 HBSAG CHRONIC CARRIERS. P. Deltenre (1), W. Laleman (2), M. Van Gossum (3), A. Lenaerts (4), I. Colle (5), M. Adler (6), P. Michielsen (7), C. Assene (8), J. Delwaide (9), H. Orlent (10), H. Reynaert (11), F. D'Heygere (12), P. Langlet (13), G. Robaey (14), C. de Galocsy (15), R. Brenard (16), D. Sprengers (17), M.C. Mairlot (18), C. Preux (19), V. Lefèbvre (20), J. Henrion (1). (1) Hôpital de Jolimont, Haïne-Saint-Paul, Belgium, (2) University Hospital Gasthuisberg, Leuven, Belgium, (3) ULB Saint-Pierre, Brussels, Belgium, (4) CHU, Charleroi, Belgium, (5) UZ, Gent, Belgium, (6) ULB Erasme, Brussels, Belgium, (7) UZ Antwerpen, Edegem, Belgium, (8) Hôpitaux Iris Sud Molière, Brussels, Belgium, (9) Ulg Sart Tilman, Liège, Belgium, (10) AZ Sint-Jan, Brugge, Belgium, (11) UZ, Brussels, Belgium, (12) AZ Groeninge, Kortrijk, Belgium, (13) ULB Brugmann, Brussels, Belgium, (14) ZOL, Genk, Belgium, (15) Hôpitaux Iris Sud Bracops, Brussels, Belgium, (16) Hopital St Joseph, Gilly, Belgium, (17) AZ St-Augustinus, Wilrijk, Belgium, (18) Clinique Saint-Jean, Brussels, Belgium, (19) CHU Tivoli, La Louvière, Belgium, (20) CHR, Namur, Belgium.

**Introduction :** The epidemiology of HBV infection has changed in Western Europe. Few data are available at a national level.

**Aim :** To assess the epidemiologic characteristics of HBV infection in Belgium.

**Methods :** BASL members were asked to report all HBsAg+ patients between March 01, 2008 and February 28, 2009.

**Results :** 1421 patients (mean age 42 years, 67% male) from 26 centres were included. 71% were prevalent cases. 52% were Caucasians and 25% black Africans. Risk factors for HBV infection were : transfusion (14%), intravenous drug use (9%), surgery (6%), sexual behaviour (38%), and familial transmission (33%). 92 patients (12%) were co-infected : 26 with HDV, 28 with HCV, 32 with HIV, 2 with HDV-HCV and 4 with HCV-HIV. Liver biopsy was performed in 641 patients. Fibrosis distribution was F0 = 16%, F1 = 24%, F2 = 24%, F3 = 19% F4 = 17%. Independent predictive factors for F3-4 were age > 40 years (p < 0.001), male sex (p < 0.01), HDV co-infection (p < 0.05), bilirubin > 1.5mg/dl (p = 0.04) and activity score  $\geq 2$  (p = 0.01). According to HBeAg status, viral load (VL) and ALT values, 10 patients were immunotolerants, 622 inactive carriers (group 1), 249 had HBeAg+ hepatitis (group 2) and 413 HBeAg- hepatitis (group 3). 127 patients (9%) could not be classified. Compared to groups 2 and 3, group 1 patients were younger (41 vs 43 years, p = 0.01), less frequently male (59 vs 74%, p < 0.0001), had lower median VL (2 vs 3.5 log IU/ml, p < 0.0001) and less frequently ALT > 2N (0 vs 25%, p < 0.0001). Compared to group 3, group 2 patients were younger (41 vs 44 years, p = 0.001), had a higher median VL (4.58 vs 3.11 log IU/ml, p < 0.0001), more frequently ALT > 2N (31 vs 21%, p < 0.01) and underwent more frequently treatment (81 vs 72%, p < 0.01). Liver biopsy was performed in 467 patients in groups 2 and 3. F3-4 was similar (32 vs 38%, NS).

**Conclusion :** In Belgium, half of HBsAg+ patients are inactive carriers. One third has HBeAg- chronic hepatitis. F3-4 is reported in 35% when a liver biopsy is performed. Patients with HBeAg+ chronic hepatitis are younger and have higher VL and ALT values than HBeAg- patients.

IMPACT OF CURRENT TREATMENT PRACTICE AND DIFFERENT SCENARIOS IMPROVING SCREENING, ACCESS TO TREATMENT AND TREATMENT EFFICACY ON HCV-RELATED MORTALITY IN BELGIUM : A MATHEMATICAL MODELING APPROACH. P. Deltenre (1), C. Moreno (2), P. Mathurin (3), M. Adler (2), A. Louvet (3), H. Castel (3), F. Wartel (3), V. Canva (3), J. Henrion (1), I. Lonjon-Domanec (4), S. Deuffic-Burban (5). (1) Hôpital de Jolimont, Haïne-Saint-Paul, Belgium, (2) ULB Erasme, Brussels, Belgium, (3) Centre Hospitalier Régional Universitaire, Lille, France, (4) Janssen-Cilag, Issy-Les-Moulineaux, France, (5) INSERM U795, Lille, France.

**Introduction :** Viral eradication by drug therapy reduces HCV-related mortality. Screening and accessibility to anti-viral therapy differ across European countries and are lower in Belgium than in neighboring countries. It is unknown if Belgian current treatment practice impacts HCV-related mortality.

**Aim :** To assess the impact of current treatment practice on HCV-related mortality in Belgium and to evaluate the impact of different scenarios improving screening, treatment access and treatment efficacy.

**Methods :** The Markov model of HCV progression was based on published epidemiological data, treatment practice and reports of competitive and hepatocellular carcinoma mortality. It predicted HCV-related mortality until 2025 for all HCV infections occurring in Belgium, taking into account genotype (G), screening and treatment.

**Results :** In 2009, 33% of the patients were HCV-RNA negative (20% cured from treatment). 58% were aware of their HCV status. Repartition by fibrosis stages was : F0-1 = 48%, F2 = 19%, F3 = 13%, F4 = 20% (32% decompensated). Compared to a scenario without treatment, current treatment practice will reduce HCV-related mortality by 11% until 2025 (1,300 deaths, 95%CI : 300-2000) : 9% in G1-4 (800 deaths), 16% in G2-3 (500 deaths). Compared to the impact of current treatment practice, the impact of the treatment on HCV-related mortality until 2025 will increase by : A/ 8%

if 75% of the patients were screened in 2012 (100 additional lives saved, 95%CI : 20-170) ; **B/** 15% if the number of patients treated between 2010 and 2015 increase by 50% (190 additional lives saved, 95%CI : 70-320) ; **C/** 25% if **B/** and **C/** were combined (310 additional lives saved, 95%CI : 100-550). **D/** 6% if a new molecule improving viral eradication by 40% in G1 patients was to become available in 2012 (80 additional lives saved, 95%CI : 20-120). **E/** 15% if screening reaching 75% in 2012 was added to this new molecule (210 additional lives saved, 95%CI : 40-320).

**Conclusion** : Antiviral treatment will reduce HCV-related mortality in Belgium by 11% in 2025. HCV screening should be reinforced to convert any future improvement in viral eradication into additional lives saved. This modeling approach allowing estimation of the treatment impact on mortality may be helpful to find new strategies to reduce HCV-related mortality.

- A13 -

CONSEQUENCES OF LAPAROSCOPY ON LIVER ISCHEMIA DURING PORTAL TRIAD CLAMPING IN A SWINE MODEL ? N. Gilson (1), B. Nsadi (1), E. Pire (1), J.P. Chera-my-Bien (1), J. Pincemail (1), E. Cavalier (1), C. Le Goff (1), J.O. Defraigne (1), M. Meurisse (1), O. Detry (2). (1) University of Liege, Liege, Belgium, (2) Ulg Sart Tilman, Liège, Belgium.

**Introduction** : During laparoscopy, increased intraperitoneal pressure due to CO<sub>2</sub> pneumoperitoneum decreases liver and mesenteric blood flow. During open hepatectomy, portal triad clamping (PTC) induces only partial ischemia of the liver, as some reverse circulation via the hepatic veins plays an important role in the limitation of ischemic liver injury and maintains some liver metabolism.

**Aim** : We hypothesised that during laparoscopic PTC, increased intraperitoneal pressure may limit hepatic vein reverse circulation, inducing a more severe hepatic ischemic injury, and our aim was to test this hypothesis in a swine model.

**Methods** : 15 pigs were randomized in three groups : one hour 15 mmHg CO<sub>2</sub> laparoscopy and 3 hours of observation (scopy group), one hour open PTC (open PTC group) and one hour PTC under laparoscopy (scopy PTC group) under venous splenofemoral extracorporeal circulation, followed by 3 hours of reperfusion. Central venous pressure (CVP), heart rate, mean arterial pressure (mAP), and core body temperature, were continuously followed. Microcirculatory flow and partial oxygen tension (ptiO<sub>2</sub>) in the liver tissue was continuously monitored. Bilirubin, INR, liver enzymes, lactate, IL6, IL10, TNF ± , VitE, VitC, glutathion, lipid peroxyds, were measured at baseline, after 60 min of PTC, and after 1 and 3 hrs of reperfusion. Data are presented as mean ± SEM.

**Results** : There was no difference of mAP or heart rate between the groups. PVC was higher during pneumoperitoneum. PTC induced a reduction of microcirculatory liver flow to 22% and 9.5% of basal values in the open PTC and scopy PTC, respectively (p < 0.01). After 10 min of PTC, ptiO<sub>2</sub> decreased from 43,1 ± 4,89 mmHg to 28,88 ± 4,42 mmHg in the open PTC, and from 36,4 ± 4,39 mmHg to 4,4 ± 1,9 mmHg in the scopy PTC group (p < 0.01). All values returned to normal after unclamping. After 3 hours of reperfusion, the ASAT were significantly increased in the scopy PTC group.

**Conclusion** : This experiment in a swine model demonstrates that the pneumoperitoneum increases ischemic liver injury during PTC by a reduction a the reverse circulation coming from the hepatic veins. During laparoscopic hepatectomy, PTC should be avoided as it produces a liver ischemia comparable to total vascular exclusion.



CLINICOPATHOLOGICAL FEATURES OF HEPATOCELLULAR ADENOMAS CLASSIFIED ACCORDING TO THE BORDEAUX CRITERIA. L. Libbrecht (1), S. Rogge (1), F. Heindryckx (1), C. Verslype (2), W. Pauwels (3), R. Troisi (1), A. Geerts (1), I. Colle (1), H. Van Vlierberghe (1). (1) University Hospital, Gent, Belgium, (2) University Hospital, Leuven, Belgium, (3) St-Lucas, Gent, Belgium.

**Introduction** : A French study group recently proposed a molecular-immunohistochemical subclassification of hepatocellular adenomas (Bioulac-Sage et al. *Hepatology* 50 : 481) of which the clinical relevance needs to be further explored.

**Aim** : We applied this classification to an independent series to investigate the clinical and histopathological features of the different subgroups.

**Methods** : Paraffin blocks from 46 hepatocellular adenomas were retrieved from the archives of the department of Pathology, UZ Gent or were received as consult cases from other hospitals. Sections were immunostained for liver fatty acid binding protein (LFABP), serum amyloid a, beta-catenin, glutamine synthetase and cytokeratin 7 for subclassification and for glypican-3 and reticulin to confirm malignant transformation. Histopathological features were assessed using routine stainings and clinical data were retrieved from the patient files.

**Results** : 42 patients were female and oral contraceptive use was documented in 32 patients. Mean age at diagnosis was 39 years. 8 adenomas were unclassifiable (17%), mainly due to insufficient tissue because of bleeding. We identified 4 beta-catenin-activated adenomas (9%), 9 LFABP-negative adenomas (20%) and 25 inflammatory adenomas (54%) of which 8 were also beta-catenin-activated (18%). Importantly, multiple (micro)adenomas or recurring adenomas were always of the same subtype, with the exception of one case. Maximal adenoma diameter was higher in the 23 patients who had signs of bleeding ( $p = 0.02$ ), but there was no correlation between subtype and bleeding. Well-differentiated hepatocellular carcinoma (HCC) was present in 9 adenomas, which were all beta-catenin-activated ( $p < 0.0001$ ). Malignant transformation showed no correlation with size. The presence of multiple adenomas was not associated with bleeding or malignancy. Multiple (micro)adenomas and recurrence was more frequently seen in the LFABP-negative subtype than in the inflammatory subtype ( $p = 0.03$  and  $p = 0.01$ ). These two subtypes did not differ regarding body mass index (BMI), but the presence of glycogenated hepatocyte nuclei in the surrounding liver was associated with a higher BMI ( $p = 0.04$ ). Activation of beta-catenin in the inflammatory adenomas was correlated with absence of steatosis ( $p = 0.06$ ) and presence of nuclear atypia ( $p < 0.0001$ ), bilirubin ( $p = 0.0002$ ) and rosettes ( $p = 0.04$ ).

**Conclusion** : While bleeding risk was determined by adenoma size, risk of malignant transformation was associated with beta-catenin activation. Multiplicity and recurrence after resection was more frequent in LFABP-negative adenomas than inflammatory adenomas. Several morphological features are helpful to detect beta-catenin activation in inflammatory adenomas.

LIVER TRANSPLANTATION FOR ALCOHOLIC LIVER DISEASE : A RETROSPECTIVE ANALYSIS OF RECIDIVISM, SURVIVAL AND RISK FACTORS PREDISPOSING TO ALCOHOL RELAPSE. E. Deruytter, C. Van Steenkiste, A. Geerts, H. Van Vlierberghe, F. Berrevoet, B. De Hemptinne, X. Rogiers, R. Troisi, I. Colle. University Hospital, Ghent, Belgium.

**Introduction** : Alcoholic liver disease (ALD) is, after virus related causes, the second most frequent indication for liver transplantation.

**Aim** : The aim of this single-center study was to evaluate the alcohol relapse rate and long-term survival after liver transplantation for ALD and to identify risk factors predisposing to alcohol relapse.

**Methods** : In this retrospective analysis, 108 patients transplanted for ALD between 2000 and 2007 in the UZ Ghent were included. "Relapse" was defined as any drinking after transplantation, "harmful drinking" as more than 2 units per day for women and 3 units per day for men after transplantation. All but 3 patients were more than 6 months abstinent before registration on the transplant waiting list. A wide range of demographic and psychosocial variables was obtained from a questionnaire and the medical records. Descriptive statistics were performed on the data : Fisher's exact tests for the categorical variables and independent samples T-tests or Mann-Whitney U tests for the continuous variables. Subsequently, a multivariate analysis (logistic regression) was performed, but no predictive factors could be identified, probably due to underpowering of the study. Survival was studied with Kaplan-Meier analysis.

**Results** : The mean follow-up period was 55 months. Relapse was observed in 29% of the patients, of which 15.9% in harmful drinking and 13.1% in occasional drinking. A significant association was found between relapse (any drinking) and the duration of the pre-transplant sobriety ( $p = 0.021$ ) on the one hand and the presence of a first degree relative with alcohol abuse ( $p = 0.037$ ) on the other hand. Relapse in harmful drinking was found to be significantly associated with the duration of the pre-transplant sobriety ( $p = 0.011$ ), the number of attempts to quit ( $p = 0.018$ ) and the presence

of a first degree relative with alcohol abuse ( $p = 0.017$ ). During the follow-up period 28 patients (25.9%) died. The one-year survival rate was 87% and the five-year survival rate 74.4%, taking the duration of the follow-up period into account. The Kaplan-Meier analysis showed no significant difference in survival between non-drinkers, occasional drinkers and harmful drinkers. The most frequent causes of death were infection (9 patients), neoplasia (8 patients) and cardiovascular disease (6 patients). One patient died due to liver failure caused by harmful drinking.

**Conclusion :** The current system of allocation for ALD with six months abstinence used in this center has good results regarding survival and recidivism comparable with literature. Risk factors associated with alcohol recidivism in harmful drinking are pre-transplant sobriety duration, the number of attempts to quit and the presence of a first degree relative with alcohol abuse, while predicting recidivism remains controversial.

- A16 -

EARLY TRANSPLANTATION IMPROVES SURVIVAL OF NON-RESPONDERS TO CORTICOSTEROIDS IN SEVERE ALCOHOLIC HEPATITIS : A CHALLENGE TO THE 6 MONTH RULE OF ABSTINENCE. C. Moreno (1), V. Lucidi (1), N. Bourgeois (1), J. Duclos-Vallée (2), J. Dumortier (3), V. Leroy (4), T. Gustot (1), N. Boon (1), N. Clumeck (1), P. Deltenre (5), F. Pruvot (6), V. Donckier (1), P. Mathurin (6). (1) ULB Erasme, Brussels, Belgium, (2) Hôpital Paul Brousse, Villejuif, France, (3) Hôpital Edouard Herriot, Lyon, France, (4) CHU, Grenoble, France, (5) Hôpital de Jolimont, Haine-Saint-Paul, Belgium, (6) Centre Hospitalier Régional Universitaire, Lille, France.

**Introduction :** In severe alcoholic hepatitis (SAH), non-responders to steroids (NRS) can be early identified and have a 6-month survival around 30%. As most deaths occur within 2 months, early liver transplantation (eLT) in NRS patients (pts) is attractive but highly controversial as it challenges the 6-month abstinence rule prior to LT.

**Aim :** 1. To determine whether eLT in NRS improves 6-month survival. 2. To evaluate alcohol relapse.

**Methods :** 5 LT centers performed eLT in NRS undergoing their 1<sup>st</sup> episode of liver disease and drastically selected using those criteria : absolute consensus of paramedical and medical staff, no co-morbidities, social integration and supportive family members. NRS were identified using Lille score  $\leq 0.45$  or worsening of liver function by day 7. In a case control study, each transplanted NRS was paired to 1 non-transplanted NRS matched for age, sex, DF and Lille score. Alcohol relapse was evaluated and defined as any alcohol consumption after eLT. All pts were advised to remain abstinent and any alcohol consumption was considered inappropriate.

**Results :** 22 NRS pts were listed for eLT within 15 days following the NRS (13-27 days) : age = 47.4 years (42.4-54 years), creatinine = 71  $\mu\text{mol/l}$  (53-131), MELD = 30 (27.5-34.8), DF = 70.5 (57.4-102), Lille score = 0.88 (0.81-0.97). Before eLT, 9 (53%) pts were treated for infections and 6 (29%) for hepatorenal syndrome. During the waiting time, 2 pts died, 2 improved their liver function and 18 underwent eLT within 9 (5-13) days following listing. After eLT, the 6-month survival in transplanted NRS was  $83.3 \pm 8.7\%$ . The 3 deaths all due to aspergillosis occurred at a median of 29.7 days. In the case-control study, there were no difference between the 18 NRS transplanted and their controls in terms of sex (59% of males), age (47.5 [42.4-54] vs 49.5 [46-53] years,  $p = 0.58$ ), DF (70.5 [57.4-102] vs 75.5 [61-88],  $p = 0.7$ ) and Lille score (0.88 [0.73-0.97] vs 0.83 [0.69-0.96],  $p = 0.64$ ). 6-month survival was higher in the transplanted-NRS than in non-transplanted NRS controls :  $83.3 \pm 8.7\%$  vs  $44.4 \pm 11.7\%$ ,  $p = 0.009$ . In non-transplanted NRS controls, 50% to 90% of deaths occurred within the 1<sup>st</sup> and 2<sup>nd</sup> months, respectively. In term of alcohol relapse, none was observed at 1 year, and only 1 relapsed at 917 days. This relapser mentions alcohol drinking of 1 unit three times a week.

**Conclusion :** After a 1<sup>st</sup> episode of liver decompensation, early LT may be proposed in NRS without any therapeutic option because as most deaths occur within 2 months in the watch-and-wait strategy. Despite early LT challenges the 6-month abstinence rule, the present results support future evaluation in drastically selected NRS.

## Invited Lecture

- A17 -

The BASL-BLIC Spring Meeting Lecture

APOPTOSIS, FROM BENCH TO BEDSIDE

Prof. Marc Bilodeau (Montreal, Canada)

- A18 -

A 7 GENE SET ASSOCIATED WITH CHRONIC HYPOXIA OF UNIVERSAL PROGNOSTIC IMPORTANCE IN HEPATOCELLULAR CARCINOMA. Van Malenstein (1), O. Gevaert (2), L. Libbrecht (3), A. Daemen (2), J. Allemeersch (4), F. Nevens (1), E. Van Cutsem (1), D. Cassiman (1), B. De Moor (2), C. Verslype (1), J. Van Pelt (5). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) ESAT, Leuven, Belgium, (3) UZ, Gent, Belgium, (4) VIB, Leuven, Belgium, (5) KU, Leuven, Belgium.

**Introduction :** Hepatocellular carcinomas (HCCs) are heterogeneous tumors with an unpredictable clinical course. There is a need for more objective prognostic criteria to decide on treatment options. Molecular classification of HCCs might provide better insight in prognosis and patient directed therapy. Since the behavior of solid tumors depends in part on tumor microenvironment, we hypothesized that in HCC certain regions exist with a characteristic gene expression related to chronic hypoxia which will induce aggressive behavior.

**Aim :** To assess the prognostic value of chronic hypoxia induced gene expression.

**Methods :** We determined the gene expression pattern for human HepG2 liver cells under chronic hypoxia by microarray (20% O<sub>2</sub> vs. 2% O<sub>2</sub> during 72 hrs). Significant differentially expressed genes were selected and their clinical value was assessed. In our hypothesis-driven analysis we included four published available independent microarray studies of patients with HCC in one single analysis. Three microarray studies were used as training sets to determine a minimal prognostic gene set associated with poor prognostic indicators and one additional study was used for validation.

**Results :** Using computational methods we identified 7 genes, out of 3592 differentially expressed under chronic hypoxia, that showed correlation with poor prognosis in all training sets (272 patients) and this was validated in a 4<sup>th</sup> dataset (91 patients). The expression of the 7 gene set was liver specific compared to other tissues, as tested in published data and experimentally in other cell lines. In a separate analysis on one of the four training sets, the 7-gene set is associated with poor survival (HR 1.39, p = 0.007) and early recurrence (HR 2.92, p = 0.007). Retrospectively, using a hypoxia score based on this 7-gene set we found that patients with a score > 0.35 had a median survival of 307 days, whereas patients with a score ≤ 0.35 had a median survival of 1602 days (p = 0.005).

**Conclusion :** In our analysis we could include available microarray studies of patients with HCC in one single analysis, irrespective of etiology, diagnostic parameters or molecular technique used. We identified a unique, liver specific 7-gene signature associated with chronic hypoxia that correlates with poor prognosis in HCCs.

THE IMPACT OF ELEVATION OF TOTAL BILIRUBIN LEVEL ON SERUM N-GLYCOSYLATION PATTERNS IN MICE AND MEN. B. Blomme (1), C. Van Steenkiste (1), J. Van Huysse (2), I. Colle (1), N. Callewaert (3), H. Van Vlierberghe (1). (1) UZ, Gent, Belgium, (2) AZ Sint-Jan, Brugge, Belgium, (3) VIB, Gent, Belgium.

**Introduction** : The GlycoFibroTest and GlycoCirrhoTest are non-invasive alternatives for liver biopsy that can be used as a follow-up tool for fibrosis patients and to diagnose cirrhotic patients, respectively. These tests are based on the altered N-glycosylation of total serum protein. Our aim was to investigate if other characteristics of liver patients (e.g. elevated total bilirubin levels) could have an influence on N-glycosylation.

**Aim** : The serum N-glycosylation patterns were determined in 26 fibrosis/cirrhosis patients with an elevation in total bilirubin level ( $> 2$  mg/dl) and in 26 fibrosis/cirrhosis patients with a normal total bilirubin level ( $\leq 1$  mg/dl). The same analysis was performed in 8 HCC-patients with elevated total bilirubin levels and 8 HCC-patients with normal bilirubin levels. Patients were selected from all major liver etiologies. Subsequently, two mouse models of chronic liver disease, common bile duct ligation (CBDL) and chronic injections with CCl<sub>4</sub>, were induced in C57Bl/6 mice. Control mice were sham-operated and chronically injected with a saline solution, respectively. CBDL and control mice were sacrificed after 1, 3, 4, 5 and 6 weeks ( $n = 8$  per time point). CCl<sub>4</sub> mice and control mice were sacrificed after 1, 3, 6, 10 and 16 weeks ( $n = 8$  per time point). In analogy with human serum samples, serum N-glycan analysis was done by DNA sequencer-assisted-fluorophore-assisted capillary electrophoresis.

**Results** : The percentage core-fucosylated glycans was significantly increased in liver patients with elevated total bilirubin levels (70% vs. 52,9% in fibrosis/cirrhosis patients and 70,8% vs. 48,7% in HCC-patients, both  $P < 0,001$ ). This increase of core-fucose was not linked to etiology, only to an increase of total bilirubin level. Furthermore, in a multiple linear regression analysis, only markers for cholestasis significantly correlated with core-fucosylation. These results could be reproduced in CBDL-mice. Interestingly, the increase of core-fucose was already observed at 3 weeks after the induction. CCl<sub>4</sub> mice did not show this increase at any time point studied.

**Conclusion** : When studying fucosylation, a distinction has to be made between an increase of core-fucose which is a marker for hyperbilirubinaemia and an increase of branching fucose which, as suggested in literature, is a marker for HCC.

N-GLYCOSYLATION PATTERNS OF SERUM PROTEINS TO MAKE THE DISTINCTION BETWEEN STEATOSIS OF THE LIVER AND NON-ALCOHOLIC STEATOHEPATITIS IN OBESE PATIENTS SCHEDULED FOR BARIATRIC SURGERY. B. Blomme (1), L. Libbrecht (1), P. Pattyn (1), Y. Van Nieuwenhove (1), D. Van de Putte (1), A. Geerts (1), I. Colle (1), N. Callewaert (2), H. Van Vlierberghe (1). (1) UZ, Gent, Belgium, (2) VIB, Gent, Belgium.

**Introduction** : The prevalence of obesity increases in Western countries. Obese patients are prone to develop steatosis or steatohepatitis of the liver. Steatosis is a rather benign condition, but steatohepatitis can develop into cirrhosis and HCC. The difference between conditions can, at present, only be made by a liver biopsy. Our aim was to evaluate the potential of N-glycosylation of serum protein as a biomarker to distinguish patients with steatosis of the liver and patients with non-alcoholic steatohepatitis (NASH) in obese patients scheduled for bariatric surgery.

**Aim** : Serum was collected from 54 patients (12 male/ 42 female, mean BMI 40 (range 29-65)). A liver biopsy was scored using the Brunt score for NASH. The serum protein N-glycosylation patterns were determined using DNA sequencer-assisted-fluorophore-assisted capillary electrophoresis.

**Results** : Liver biopsy revealed that 7 patients had a histological normal liver, 31 patients had significant steatosis and 16 patients were diagnosed with NASH. Furthermore, the NASH patients displayed no or little fibrosis. Three glycans were significantly altered in the serum of NASH-patients compared to steatosis patients. NGA2F and NA3Fbc are significantly increased ( $P < 0,001$  and  $P = 0,042$ ), while NA2 was significantly decreased ( $P = 0,007$ ) in the serum of NASH-patients. Based on these results, a biomarker was put forward :  $\log ([\text{NGA2F}]/[\text{NA2}])$ . We compared our glycomarker to other non-invasive markers for NASH. A multiple linear regression analysis was performed including parameters which were in monovariate significant different between NASH and steatosis : BMI, waist/hip ratio, HOMA-index, adiponectin, GGT, HDL, triglycerides, uric acid and caspase cleaved cytokeratin-18 (CK-18). In this analysis, our glycomarker demonstrated a significant benefit over all other biomarkers for NASH ( $P = 0,035$ ).

**Conclusion** : N-glycomic alterations in NASH-patients reflect the chronic inflammatory condition present in these patients. In this population of bariatric patients, our biomarker based on the N-glycosylation of serum proteins was superior over the current biomarkers for NASH, including CK-18 and adiponectin.

N-GLYCOSYLATION OF IMMUNOGLOBULIN G AND ITS ROLE IN THE ALTERATION OF TOTAL SERUM PROTEIN N-GLYCOME IN TWO MOUSE MODELS OF CHRONIC LIVER DISEASE. B. Blomme (1), N. Callewaert (2), H. Van Vlierbergh (1). (1) UZ, Gent, Belgium, (2) VIB, Gent, Belgium.

**Introduction :** There are two sources of N-glycosylated serum proteins, the liver which produces the vast majority of these proteins and B-cells which produce the immunoglobulins. In humans, we found that the glycosylation of IgGs plays an important role in the alteration of glycosylation in liver diseases. By using B-cell deficient mice ( $\mu$ MT), the role and the magnitude of the alterations in which the liver is involved in two mouse models of chronic liver disease was determined.

**Aim :** Secondary biliary cirrhosis was induced by common bile duct ligation (CBDL) and a micronodular cirrhosis was induced by subcutaneous (SC) injections with the hepatotoxin CCl<sub>4</sub> in both immunocompetent (WT) and B-cell deficient mice. CBDL mice were sacrificed 6w after induction and CCl<sub>4</sub> mice were sacrificed after 16 weeks. Control mice for CBDL were Sham-operated and the control mice for CCl<sub>4</sub> were injected SC with a saline solution. In addition, serum from WT mice were depleted of IgG using protein A/G and the IgG-fraction was collected. N-glycosylation of serum protein was analyzed with DNA sequencer-assisted-fluorophore-assisted capillary electrophoresis.

**Results :** NGA2F/peak 1 is a IgG-specific (but not exclusive) glycan, while peaks 8 and 11 are not present in the N-glycosylation pattern of IgG indicating that these are exclusive to liver produced protein. Results of the statistical analysis are summarized in Table 1.

Table 1 : The alterations of the CBDL and CCl<sub>4</sub> B-cell deficient mice and wild type mice versus their respective control

Peak	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11
CBDL BKO vs. Sham BKO	↑*	↓***	↓**	↓**	↓***	↑***	↑***	↓**			↑***
CCl <sub>4</sub> BKO vs. saline BKO	↓***	↓*	↓**	↓**		↓**		↑*	↑**	↓*	↑***
CBDL WT vs. Sham WT	↑***			↓***	↓***	↑*	↑***	↓***			↑***
CCl <sub>4</sub> WT vs. saline WT		↓*	↓**	↓***		↓**			↑**	↓***	↑***

BKO : B-cell KO ; WT : wild-type \* $P < 0,05$ , \*\* $P < 0,01$ , \*\*\* $P < 0,001$

**Conclusion :** The alterations in B-cell deficient mice (lacking IgGs) were in general also the alterations seen in total serum minimizing the role of IgG N-glycosylation. We were able to confirm the hypothesis that increase of core-fucosylation in hyperbilirubinaemia is hepatocyte driven because IgGs of CBDL mice do not have an increased abundance of core-fucosylated glycans. Therefore, we can conclude that changes in N-glycosylation in two different mouse models of chronic liver disease are hepatocyte and not IgG driven.

MODERATE AND EXCESSIVE ALCOHOL INTAKE IMPACT TRANSPLANT-FREE SURVIVAL : A LONGITUDINAL STUDY IN HCV CIRRHOTIC PATIENTS. P. Deltenre (1), M. El Nady (2), M. Corouge (2), V. Canva (2), A. Louvet (2), H. Castel (2), F. Wartel (2), S. Dharancy (2), J. Henrion (1), P. Mathurin (2). (1) Hôpital de Jolimont, Haïne-Saint-Paul, Belgium, (2) Centre Hospitalier Régional Universitaire, Lille, France.

**Introduction :** Cross sectional studies indicate that excessive alcohol intake increases the risk of complications in HCV cirrhotic patients. To better understand the impact of alcohol intake, longitudinal studies are interesting.

**Aim :** to analyze risk factors for disease progression in cirrhotic HCV patients using a longitudinal study.

**Methods :** We calculated the probability of transplant-free survival. Patients were first analyzed using the delay from the diagnosis of cirrhosis. In sensitivity analysis to avoid the lead-time bias related to earlier diagnosis of cirrhosis in patients with rapid disease progression, we also analyse survival from contamination. The independent prognostic values were assessed by Cox regression.

**Results :** 265 HCV cirrhotic patients (161 male, median age 49 years, 26% genotype 2/3) were included. 71% underwent treatment. Patients were classified into 3 groups : abstainers (Group 1, n = 71), moderate drinkers (< 40g/day, Group 2, n = 79) and excessive drinkers (≥40g/day, Group 3, n = 115). Characteristics (median values) of groups 1, 2 and 3 were : age (53, 50 and 47 years,  $p = 0.01$ ), alcohol intake (0, 20 and 60 g/day,  $p < 0.001$ ), albumin (37, 38 and

34 g/l,  $p < 0.001$ ), TP (79, 82 and 67%,  $p < 0.001$ ), biliubin (14, 14 and 22 mg/l,  $p < 0.001$ ) and Child-Pugh score (5, 5 and 6,  $p < 0.001$ ). **A/** from cirrhosis diagnosis : 10-year transplant-free survival was significantly lower in group 3 ( $16.7 \pm 4.8\%$ ,  $p < 0.001$ ) than in groups 2 ( $39.7 \pm 8.6\%$ ) and 1 ( $65.1 \pm 8.7\%$ ) with significant differences between groups 3 and 2 ( $p = 0.004$ ) and 2 and 1 ( $p = 0.04$ ). Median age at death was lower in group 3 than in the 2 other groups : 54.8 vs 66.4 years  $p < 0.01$ . 10-year transplant-free survival was higher in sustained responders than in non responders ( $54.6 \pm 10.1\%$  vs  $28.4 \pm 4.5\%$ ,  $p = 0.003$ ) and was 80% in abstainers with SVR. In multivariate analysis, age ( $p < 0.0001$ ), alcohol consumption ( $p < 0.0001$ ), Child-Pugh score ( $p = 0.0001$ ) and SVR ( $p = 0.05$ ) were independent prognostic factors of 10-year transplant-free survival. **B/** from contamination : group 3 had lower 30-year transplant-free survival ( $52.8 \pm 6.3\%$ ) than groups 2 ( $67.4 \pm 7.5\%$ ) and 1 ( $92.3 \pm 4.3\%$ ),  $p = 0.001$ .

**Conclusion** : In cirrhotic HCV patients, moderate and excessive alcohol consumptions reduce survival, even when considering the lead-time bias. Conversely, viral eradication improves survival.

- A23 -

OCCURRENCE AND CLINICOPATHOLOGICAL RELEVANCE OF PROGNOSTIC MARKERS (K19, EPCAM, AND AFP) IN HEPATOCELLULAR CARCINOMA. O. Govaere (1), A. Katoonizadeh (1), B. Spee (1), F. De Luca (2), C. Janssen (1), S. Vander Borgh (1), J. Verheij (3), C. Verslype (4), R. Aerts (4), B. Topal (4), J. Pirenne (4), F. Nevens (4), V. Desmet (1), M. Pinzani (2), T. Roskams (1). (1) KU, Leuven, Belgium, (2) University, Firenze, Italy, (3) Erasmus Medical Center, Rotterdam, Netherlands, (4) University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. The heterogeneous nature of HCCs poses a variable clinical outcome which impairs the prognosis for the patient. Currently several markers linked with overall poor survival are being advocated to stratify HCCs into groups of different prognosis. These markers include keratin (K) 19, Epithelial cell adhesion molecule (EpCAM) and  $\pm$ -fetoprotein (AFP). Although these markers have been used to describe subtypes of HCCs, none of these markers have been simultaneously validated on a large series of HCCs.

**Aim** : It is the aim of this study to assess the incidence of K19, EpCAM, and AFP expression from 411 biopsies and correlate the presence of these markers with clinicopathological parameters.

**Methods** : The diagnosis of HCC was based on WHO criteria. A sequential series of paraffin embedded liver biopsies ( $n = 411$ ) from 242 patients with HCC, treated at the University Hospitals Leuven, were included during this study. The expression of K19, EpCAM and AFP was semi-quantitatively assessed by means of immunohistochemistry. An cut-off value of five percent immunohistochemical positivity was applied to exclude false positives. Statistical analysis was performed to correlate marker expression to clinicopathological parameters including tumour-size, -differentiation, microvascular invasion, and metastasis.

**Results** : Out of the 411 HCCs 12 percent showed K19 positivity ( $n = 49$ ), 17 percent EpCAM positivity ( $n = 68$ ), and 7 percent AFP positivity ( $n = 30$ ). K19 expression was significantly correlated with an increased tumour size ( $p = 0.03$ ), decreased tumour differentiation ( $p = 0.0001$ ), metastasis ( $p = 0.0005$ ), and microvascular invasion ( $p = 0.0001$ ). Although 73 percent of the K19 positive HCCs showed EpCAM expression, EpCAM expression itself was not significantly correlated with the clinicopathological parameters. In contrast, AFP expression was significantly correlated with decreased tumour differentiation ( $p = 0.003$ ) and microvascular invasion ( $p = 0.001$ ).

**Conclusion** : In this series of HCCs the incidence of K19, EpCAM and AFP immunopositivity was 12-, 17-, and 7-percent respectively. In addition, correlation to histopathological parameters indicated that both K19 and AFP can be used to signify a more malignant subtype of HCC. However the incidence of AFP in HCC is much lower which implies that K19 is a more relevant marker. In the future these markers can be decisive in establishing guidelines for patient treatment and follow up.

THE FIBRO7-SCORE : A NOVEL SERUM PROTEOME- AND CLINICAL BIOCHEMISTRY-BASED PREDICTIVE MODEL FOR CHRONIC HEPATITIS C LIVER FIBROSIS. K.J. Cheung (1), K. Tilleman (2), D. Deforce (2), I. Colle (1), H. Van Vlierbergh (1). (1) UZ, Gent, Belgium, (2) Ghent University, Ghent, Belgium.

**Introduction** : Liver biopsy is still the standard in the assessment of liver fibrosis in hepatitis C patients, despite invasiveness and potential sampling error.

**Aim** : Aim of this study was to construct and evaluate an alternative serum-test based on proteome-derived and routine biochemical indicators.

**Methods** : Patients characteristics (age, BMI, gender, genotype and inflammatory activity), clinical biochemistry (11) and proteome-derived markers(4) of an untreated hepatitis C patient cohort (n = 76) were studied in accordance with liver fibrosis. Based on 62 patients, an appropriate model was built for the prediction of minor (F0 - F1), moderate fibrosis (F2- F3) and cirrhosis (F4). Performance of the novel model was compared to aspartate aminotransferase to platelet ratio index (APRI) and Hepascore. Statistical analysis was by univariate analysis, linear discriminant analysis (LDA) and receiver operator characteristic (ROC) curve. P-value < 0.05 was considered statistically significant.

**Results** : Liver fibrosis was associated with patient's age, inflammatory activity, 8 biochemical indicators and all four protein markers. A 7-marker panel (Fibro7-score) based on  $\alpha$ -2-macroglobulin, haptoglobin, hemopexin, galectin-3-binding protein, albumin,  $\gamma$ -glutamyltransferase and white blood cell counts, had the lowest error-rate of 25.8% for fibrosis-classification. The Fibro7-score had areas under the curve of 0.89 and 0.99 for the detection of significant fibrosis ( $\geq$  F2) and cirrhosis (F4), respectively. Diagnostic performance was comparable or even better than the APRI and Hepascore.

**Conclusion** : We successfully implemented proteome-derived markers in combination with routine biochemistry in the construction of a novel predictive model for hepatitis C-related liver fibrosis (Fibro7-score). This novel model can reduce liver biopsies and assist treatment management.

DONATION AFTER CARDIAC DEATH LIVER TRANSPLANTATION : IS DONOR AGE AN ISSUE ? O. Detry, A. Deroover, J.P. Squifflet, M.F. Hans, B. Lambermont, S. Lauwick, M. Meurisse, J. Delwaide, P. Honoré. Ulg Sart Tilman, Liège, Belgium.

**Introduction** : Donation after cardiac death (DCD) liver transplantation has been proposed to increase the number of transplantable liver grafts. As older liver grafts may be more sensitive to ischemia, DCD donors older than 55 years are usually not considered suitable for DCD liver donation. Our local policy is to not refuse DCD liver grafts based on age.

**Aim** : The aim of this study is to determine the results of our DCD liver transplantation programme, and to compare the outcome of patients receiving older DCD livers to the younger ones.

**Methods** : We compared the results of DCD liver transplantations in a retrospective manner in our centre from 2003 to 2009. DCDs were divided into two groups according to age : younger donors (Y-DCD) < 55 years, and older donors (O-DCD) > 55 years. We compared donor and recipient demographics, peak laboratory values during the first postoperative week and results at one year. Results are expressed as mean  $\pm$  SEM. P < 0.05 was considered as significant.

**Results** : 33 DCD liver transplantations (Y-DCD n = 15, mean age : 44  $\pm$  2.2 years, extremes 25-53 ; O-DCD n = 18, mean age : 66  $\pm$  1.5 years, extremes : 56-79) were performed in the study period. No difference other than age in donor characteristics was noted between both groups. Mean age of the recipients was not different. Mean cold ischemia was 305  $\pm$  28 min in the O-DCD group and 257  $\pm$  18 min in the Y-DCD group (NS). Peak AST (UI/mL) and peak bilirubin (mg/dL) were 2,944  $\pm$  1432 and 46.8  $\pm$  9.5 in the Y-DCD group and 2,086  $\pm$  494 and 60  $\pm$  12 in the O-DCD group (NS). There was no PNF. Graft and patient one-year survivals were 100% in the Y-DCD group and 94% O-DCD group (NS).

**Conclusion** : In view of our experience, donor age > 55 years should not be a contraindication to DCD donation. DCD liver transplantation with young or older donors could lead to excellent results, if cold ischemia is limited to 5 hours.

HEPATITIS B-DELTA VIRUS CO-INFECTION IN BELGIUM : JOINT ANALYSIS OF PROSPECTIVE DATA OF THE BASL HDV AND HBV REGISTRIES. E. Ho (1), P. Deltenre (2), M. Nkuize (3), J. Delwaide (4), J. Henrion (2), R. Brenard (5), C. de Galocsy (6), I. Colle (7), F. D'Heygere (8), H. Orlent (9), P. Langlet (10), W. Laleman (11), P. Michielsen (1). (1) Antwerp University Hospital, Antwerp, Belgium, (2) Hôpital de Jolimont, Haine-Saint-Paul, Belgium, (3) ULB Saint-Pierre, Brussels, Belgium, (4) Ulg Sart Tilman, Liège, Belgium, (5) Hôpital Saint-Joseph, Gilly, Belgium, (6) CH Joseph Bracops, Brussels, Belgium, (7) University Hospital, Ghent, Belgium, (8) AZ Groeninge, Kortrijk, Belgium, (9) AZ Sint-Jan, Brugge, Belgium, (10) ULB Brugmann, Brussels, Belgium, (11) UZ, Leuven, Belgium.

**Introduction** : Recent data suggest increasing prevalence of HDV infection in Europe. Epidemiological data on this infection are lacking.

**Aim** : A prospective multicentric questionnaire based registry on HDV infection was performed in Belgium from March 1, 2008 till February 28, 2009. The data were compared to those of a concurrent registry on HBV infection.

**Methods** : Data from 40 patients from 14 centers with HDV-HBV co-infection were compared with 1282 HBV mono-infected patients. Active hepatitis B replication is defined as HBeAg + or HBV DNA > 2000 IU/mL HBeAg being negative. Statistical analysis of prospective data of both the BASL HDV and HBV registries was performed to measure possible differences between HDV - HBV co-infected patients and HBV mono-infected patients. Statistical analysis was performed using Mann-Whitney U test for continuous and Chi-Square test for categorical variables.

**Results** : Baseline characteristics : 32/40 (80.0%) males, 19/40 (47.5%) non-Caucasian, 6/40 (15.0%) had active HBV replication, 27/40 (67.5%) had elevated ALT, with 15/40 (37.5%) ALT > 2N. Metavir fibrosis grades F3 and F4 were present in 20/27 (74.1%) patients who received a liver biopsy.

(format : HBV patients ; HDV-HBV patients ; statistical significance) : HBV viral load IU/ml ( $4.27 \times 10^8$  (SD  $4.46 \times 10^9$ ) ; 6395.69 (SD 17997.04) ;  $p = 0.002$ ), HBV active replication (480/1282 (37.4%) ; 6/40 (15.0%) ;  $p = 0.004$ ), anti-HCV antibody positivity (30/1227 (2.4%) ; 5/40 (13.2%) ;  $p = 0.000$ ), ALT > 2N (140/1237 (11.3%) ; 15/40 (37.5%) ;  $p = 0.000$ ), Fibrosis F3F4 (178/517 (34.4%) ; 20/27 (74.1%) ;  $p = 0.000$ ).

Differing patient totals reflect missing data.

**Conclusion** : 3.0% of HBV patients in Belgium are reported to be co-infected with HDV. These patients present with more advanced liver disease, lower HBV DNA levels, and also were more co-infected with HCV.

PLASMA SOLUBLE ST2 RECEPTOR IS INCREASED IN HUMAN ALCOHOLIC LIVER DISEASE AND CORRELATES WITH THE PROGNOSTIC INDEX IN ALCOHOLIC HEPATITIS. A. Lemmers, C. Moreno, R. Ouziel, D. Degré, E. Trepo, E. Quertinmont, V. Vercruysse, O. Le Moine, J. Devière, T. Gustot. ULB Erasme, Brussels, Belgium.

**Introduction** : Immune mechanisms orchestrating liver immune cell infiltration and fibrosis during alcoholic hepatitis are still poorly understood. IL-33, a recently identified cytokine of IL-1 family, was shown to induce Th2-type cytokines by binding to the heterodimeric ST2/IL-1RacP receptor. Th-2 type cytokines are known to play a crucial role in fibrosis. Moreover, IL-33 was recently shown to be overexpressed by hepatic stellate cells in fibrotic human livers.

**Aim** : The present study investigates IL-33-ST2 pathway activation in human alcoholic liver disease.

**Methods** : Plasma of patients with alcoholic liver disease (ALD) or hepatitis C virus infection (HCV) undergoing transjugular liver biopsies and 12 healthy subjects (HS) were assessed for soluble ST2 (sST2) and IL-33 quantification by ELISA. Quantitative RT-PCR was performed on mRNA obtained from liver extracts. Data are expressed as median[range]. Mann-Whitney U test was performed for group comparisons and Spearman test for correlations studies.

**Results** : Etiologies of liver diseases were alcoholic cirrhosis (n = 57), alcoholic hepatitis (Maddrey Discriminant Function (mDF) < 32 ; n = 40, mDF > 32 ; n = 35), or chronic HCV infection (fibrosis score F0-F2 : n = 18, F3-F4 : n = 19).

Plasma sST2 levels were increased in ALD patients (1035 [0-11100]pg/ml) compared to HS (0 [0-213]pg/ml ;  $p < 0.001$ ) or HCV infected patients (0 [0-1435]pg/ml ;  $p < 0.001$ ). Among ALD patients, those with alcoholic hepatitis (AH) had higher sST2 plasma levels than alcoholic cirrhotic patients without AH (1598[0-11100]pg/ml vs 554[6-7591]pg/ml respectively,  $p < 0.01$ ). No difference was found in sST2 plasma levels between low and high fibrosis grade among HCV patients (F0-F2 0[0-1435]pg/ml vs F3-F4 0[0-961]pg/ml ;  $p = 0.4$ ). Among patients with AH, plasma sST2 level correlated with mDF ( $p = 0.34$ ), ALT( $p = 0.35$ ), CRP( $p = 0.26$ ) and MELD score( $p = 0.44$ ), but not with hepatic venous pressure gradient. IL-33 was not detected in the plasma.

In the liver, ST2 mRNA expression was higher in ALD patients (with or without alcoholic hepatitis) than in HCV patients (1192[0-11286] vs 344[0-3522] ST2 mRNA copies/1e<sup>6</sup>  $\beta$ -Actin mRNA copies ; n = 25 vs 18 ;  $p < 0.01$ ). In ALD patients, liver ST2 mRNA expression correlated with plasma sST2 levels ( $p = 0.41$ ,  $p < 0.05$ ).

**Conclusion** : In alcoholic liver disease, plasma sST2 levels are associated with the activation of liver ST2 pathway. Plasma sST2 level is dramatically increased during AH and correlates with disease prognostic index.



CIRCULATING ANTIBODIES TO COMMENSAL BACILLUS SUBTILIS FLAGELLIN AS A NEW BIOMARKER FOR BACTERIAL TRANSLOCATION-DRIVEN INCREASED MICROBE-HOST INTERACTIONS IN PATIENTS WITH CIRRHOSIS. . Gustot (1), A. Lemmers (1), C. Moreno (1), D. Degré (1), R. Ouziel (1), D. Franchimont (1), D. Lebrech (2), R. Moreau (2), J. Devière (1). (1) ULB Erasme, Brussels, Belgium, (2) INSERM Bichat-Beaujon, Paris, France.

**Introduction :** In cirrhosis, intestinal bacterial translocation is common, increases microbe-host interactions, induces inflammation and contributes to disease progression. To date, there are no accurate biomarkers for immune response to bacterial translocation. *Bacillus subtilis*, common commensal gut bacteria, expresses flagellin, which is recognized by Toll-like receptor 5 and is highly antigenic. Thus, we reasoned that circulating antibodies to *B. subtilis* flagellin may be a new biomarker of response to bacterial translocation in patients with cirrhosis.

**Methods :** Plasma levels of antibodies (IgG and IgA) to *B. subtilis* flagellin were measured by home-made ELISAs (values expressed in optical density (OD) as median [min-max]) in healthy subjects (HS, n = 17), patients with alcoholic cirrhosis (Child-Pugh A n = 12, B n = 13 and C n = 11) without bacterial infection, with infections (n = 11) and patients with HCV-related liver fibrosis, moderate (F1, n = 8) or advanced (F4, n = 8) according to METAVIR classification.

**Results :** IgG and IgA levels against *B. subtilis* were significantly higher in patients with Child-Pugh B or C alcoholic cirrhosis than in HS (for IgG, 1.56 [0.78-2.12] and 1.72 [0.76-2.36] vs. 0.99 [0.52-1.64] and for IgA 1.78 [0.63-2.54] and 1.83 [1.23-2.2] vs. 0.64 [0.44-1.65], respectively). The presence of bacterial infection did not modify levels of IgG and IgA against *B. subtilis*. Patients with compensated HCV-related cirrhosis (F4) had only higher levels of IgG (1.9 [1.27-2.69]). In alcoholic cirrhosis, circulating levels of IgG and IgA against *B. subtilis* were correlated significantly with hepatic vein pressure gradient ( $r_s = 0.39$  and  $r_s = 0.51$ , respectively), albumin ( $r_s = -0.41$  ;  $r_s = -0.38$ ) and MELD score ( $r_s = 0.43$  ;  $r_s = 0.52$ ). The presence of ascites but not hepatic encephalopathy or acute alcoholic hepatitis was associated with higher plasma levels of anti-flagellin IgG and IgA.

**Conclusion :** In patients with alcoholic cirrhosis and without current bacterial infection, anti-flagellin IgG and IgA plasma levels may be a new specific biomarker for host response to bacterial translocation.

VASCULAR HYPOREACTIVITY IN PARTIAL PORTAL VEIN LIGATION-INDUCED PORTAL HYPERTENSION IS MEDIATED BY A SPECIFIC CYCLOOXYGENASE 2-RELATED MECHANISM. S. Francque (1), W. Verlinden (1), I. Brosius (1), B. De Winter (2), A. Herman (2), P. Pelckmans (1), P. Michielsen (1). (1) UZ, Antwerpen, Belgium, (2) Antwerp University, Antwerp, Belgium.

**Introduction :** Splanchnic vasodilation is one of the factors contributing to portal hypertension (PHT). Vascular hyporeactivity to vasoconstrictors has been demonstrated. Different mediators have been implicated in its pathogenesis. Previous experiments of our group in common bile duct ligated and partial portal vein ligated (PPVL) models of PHT could not confirm a role for NO in an organ bath setting. Indomethacin significantly reduced vascular reactivity and significantly more in controls than in PHT, suggesting baseline reduction in vasoconstrictor prostaglandins in case of PHT. Indomethacin has, however, other vascular effects besides COX inhibition, and is an aspecific COX inhibitor.

**Aim :** To study the role of COX inhibition by piroxicam, which has only COX inhibitory features, and, if confirmed, selectively investigate COX1 and COX2 pathways in PPVL-induced PHT.

**Methods :** Vascular reactivity to phenylephrine (an alpha-agonist vasoconstrictor) of abdominal aortic rings of male wistar rats that had undergone PPVL or sham-operation 2 weeks prior to the experiments was studied in organ bath using piroxicam, SC560 (selective COX1 inhibitor) and NS398 (selective COX2 inhibitor). Contractions were expressed as% of KCl-induced precontraction.

**Results :** Maximum contraction ( $C_{max}$ ) was significantly reduced in PPVL ( $139.30 \pm 4.11\%$ ) (N = 10) vs. controls (N = 15) ( $147.70 \pm 6.44\%$ ) ( $p = 0.027$ ) confirming vascular hyporeactivity. Piroxicam significantly reduced vascular reactivity both in controls (from  $147.70 \pm 6.44\%$  to  $81.03 \pm 8.11\%$ ,  $p < 0.001$ ) and in PPVL (from  $139.30 \pm 4.11\%$  to  $96.38 \pm 6.40\%$ ,  $p < 0.001$ ) and the effect was significantly more pronounced in controls than in PPVL (mean decrease  $45.7 \pm 4.3\%$  in controls vs.  $30.8 \pm 4.2\%$  in PPVL,  $p = 0.037$ ). SC650 had no significant effect on vascular reactivity, both in control and in PPVL. NS398 reduced  $C_{max}$  in controls (from  $172.20 \pm 8.04\%$  to  $105.01 \pm 6.21\%$ ,  $p < 0.001$ ) and in PPVL (from  $151.71 \pm 9.24\%$  to  $109.50 \pm 6.99\%$ ,  $p < 0.001$ ) and the effect was significantly more pronounced in controls than in PPVL (mean decrease  $41.10 \pm 4.54\%$  vs.  $27.44 \pm 3.43\%$ ,  $p = 0.033$ ).

**Conclusion :** Previous results on PHT related vascular hyporeactivity are confirmed in the PPVL model. COX inhibition by indomethacin being more pronounced in controls than in PHT suggesting baseline reduction in vasoconstrictor prostaglandins in PPVL is confirmed by piroxicam. The COX related effect is entirely explained by selective COX2 related mechanisms, whereas COX1 does not seem to be involved.

VASCULAR HYPOREACTIVITY IN STEATOSIS-INDUCED PORTAL HYPERTENSION IS MEDIATED BY A SPECIFIC CYCLOOXYGENASE 2-RELATED MECHANISM. S. Francque (1), W. Verlinden (1), I. Brosius (1), B. De Winter (2), A. Herman (2), P. Pelckmans (1), P. Michiels (1). (1) UZ, Antwerpen, Belgium, (2) Antwerp University, Antwerp, Belgium.

**Introduction** : We previously demonstrated that severe steatosis without significant fibrosis induces portal hypertension (PHT). This PHT is associated with vascular hyporeactivity in abdominal aortic rings, which can not be explained by NO inhibition. Indomethacin significantly reduces vascular reactivity and significantly more in controls than in steatosis, suggesting baseline reduction in vasoconstrictor prostaglandins in case of steatosis. Indomethacin has, however, other effects on vascular reactivity besides its COX inhibitory effect, and is an aspecific COX inhibitor.

**Aim** : To study the role of COX inhibition by piroxicam, which has only COX inhibitory features, and, if confirmed, selectively investigate COX1 and COX2 pathways.

**Methods** : Male wistar rats fed the Methionin-choline deficient diet for 4 weeks underwent haemodynamic measurements. Vascular reactivity to phenylephrine (an alpha-agonist vasoconstrictor) was subsequently studied on abdominal aortic rings in organ bath using piroxicam, SC560 (selective COX1 inhibitor) and NS398 (selective COX2 inhibitor). Acetylcholine-induced vasodilatation was used to check endothelial integrity.

**Results** : Portal pressure was significantly higher in rats with steatosis (N = 10) vs. controls (N = 13) ( $9.4 \pm 0.3$  mm Hg (N = 10) vs.  $2.9 \pm 0.6$  mm Hg,  $p = 0.003$ ). Maximum contraction ( $C_{max}$ ) was significantly reduced in steatosis ( $10.37 \pm 1.36$  mN vs.  $18.67 \pm 1.46$  mN in controls,  $p < 0.001$ ) confirming the presence of vascular hyporeactivity. Piroxicam significantly reduced vascular reactivity both in controls (from  $18.67 \pm 1.46$  to  $10.19 \pm 1.04$  mN,  $p < 0.001$ ) and in steatosis (from  $10.37 \pm 1.36$  to  $7.20 \pm 1.11$  mN,  $p < 0.001$ ) and the effect was significantly more pronounced in controls than in steatosis (mean decrease  $44.7 \pm 4.5\%$  in controls vs.  $31.9 \pm 2.9\%$  in steatosis,  $p = 0.038$ ). SC560 had no effect on vascular reactivity, both in control and in rats with steatosis. NS398 reduced  $C_{max}$  in controls (from  $18.69 \pm 1.49$  to  $10.05 \pm 1.20$  mN,  $p < 0.001$ ) and in steatosis (from  $13.82 \pm 1.87$  to  $10.95 \pm 1.46$  mN,  $p < 0.001$ ) and the effect was significantly more pronounced in controls than in steatosis (mean decrease  $46.63 \pm 4.44\%$  vs.  $19.95 \pm 2.64\%$ ,  $p = 0.0003$ ).

**Conclusion** : Previous results on steatosis-associated PHT and related vascular hyporeactivity are confirmed. COX inhibition by indomethacin being more pronounced in controls than in steatosis suggesting baseline reduction in vasoconstrictor prostaglandins in steatosis is confirmed by piroxicam. The COX related effect is entirely explained by selective COX2 related mechanisms, whereas COX1 does not seem to be involved.

THE VASOPRESSIN-2-RECEPTOR ANTAGONIST SATAVAPTAN IMPROVES PORTAL HYPERTENSION IN THE NON-ASCITIC THIOACETAMIDE CIRRHOTIC RAT WITHIN A NARROW SAFE AND EFFECTIVE DOSE-RANGE. W. Laleman (1), J. Trebicka (2), L. Van Landeghem (1), I. Vander Elst (1), T. Sauerbruch (2), D. Cassiman (1), F. Nevens (1). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) University Hospital, Bonn, Germany.

**Introduction** : The highly selective vasopressin-V2-receptor-antagonist satavaptan is effective in the management of ascites and hyponatremia (HypoCAT-trial), but encountered safety concerns upon validation in a larger cohort with different diuretic regimens (SPARE-1-trial). This indicates a narrow safe and effective dose-range in controlling water balance and suggests heterogeneous effects on splanchnic and systemic hemodynamics in cirrhosis.

**Aim** : To elucidate the effect and safety of multiple doses of satavaptan on the portal hypertensive syndrome in non-ascitic cirrhotic rats

**Methods** : A dose-finding study was performed in a rat model of TAA-cirrhosis with free access to drinking water. Satavaptan (0.01-0.05-0.1-1mg/kg, n = 6/group) or vehicle was injected intraperitoneally 6hrs prior to portal (PP) and mean arterial pressure (MAP) measurements. Aquaretic effects, liver and renal function were evaluated. In the most effective and safe dose, hemodynamic assesment was performed by systemic microspheres (n = 17), plasma-volume-calculation (n = 10) and in-situ-liver perfusion (n = 15).

**Results** : Hemodynamic instability and lethal effects appeared at 1mg/kg, while 0.01 and 0.05mg/kg showed no serious adverse effects but on the other hand resulted in very little beneficial effect. The dose of 0.1mg/kg was considered most effective and safe. Further hemodynamic characterization with 0.1mg/kg showed a decrease in PP ( $9.9 \pm 0.8$ mmHg vs  $15 \pm 1.0$ mmHg for TAA alone,  $P < 0.001$ ) without affecting MAP, cardiac output or systemic vascular resistance. The decrease in PP was associated with a decrease in hepatic vascular resistance ( $1.6 \pm 0.3$  vs  $2.9 \pm 0.5$ mmHg\*min\*100g/ml,  $P = 0.02$ ), also confirmed by in-situ-liver perfusion, and a decrease in plasma-volume ( $P = 0.008$ ) and an increase in portal tributary flow ( $P = 0.01$ ), mesenteric shunting ( $P = 0.03$ ) and hepatic artery flow ( $P = 0.04$ ). Satavaptan further increased renal blood flow ( $P = 0.02$ ) which correlated with urine excretion and inversely with urine osmolality. No liver or renal impairment was observed.

**Conclusion** : Satavaptan improves portal pressure without incapacitating systemic hemodynamics by a potential intra-hepatic effect. However, the safe and effective dose for these effects is narrow-ranged, which might explain largely the adverse events encountered in clinical trials. These findings warrant further studies using vaptans for ascites and portal hypertension.

COMBINATION THERAPY WITH  $\beta$ -CAROTENE AND VITAMIN C SIGNIFICANTLY AMELIORATED BIO-CHEMICAL AND HISTOLOGICAL SIGNS IN LIVER DAMAGE CAUSED BY CCL<sub>4</sub> INTOXICATION IN RATS. S. Wamutu (1), S. Francque (2), S. Chatterjee (3), E. Musisi (1), G.W. Muyomba (1), J. Weyler (3), E. Van Marck (2), G.S. Bimenya (1), P. Michielsen (2). (1) Makerere University, Kampala, Uganda, (2) UZ, Antwerpen, Belgium, (3) Antwerp University, Antwerp, Belgium.

**Introduction** : The antioxidant role of  $\beta$ -carotene is still highly contested partly because of its adverse effects. Vitamin C is considered to be a good antioxidant, also believed to protect other antioxidants in vivo. Vitamin C may, therefore, limit  $\beta$ -carotene toxicity, possibly by delaying its conversion to vitamin A.

**Aim** : To establish whether therapeutic doses of  $\beta$ -carotene in combination with vitamin C would improve experimental liver disease without causing toxicity in rats.

**Methods** : Four groups (each n = 9) of male albino rats were studied. Group I (control) and Groups II and III were treated every third-day, respectively, with 0.5 mL/kg saline (IP) and 0.5 mL/kg CCl<sub>4</sub> (IP) + 0.3 mg pentobarbital in drinking water, for ten weeks. Thereafter, CCl<sub>4</sub> and pentobarbital were stopped. Group III received 50 mg/kg/day of vitamins C and  $\beta$ -carotene each/rat (IM) for further 2 weeks, while group II remained untreated. Group IV was given similar doses of saline for 10 weeks and vitamins for further 2 weeks. Liver homogenates and blood samples were, respectively, assayed for malondialdehyde (MDA) and glutathione (GSH) and liver tests. Biochemical results were analysed using Student's t-test and scores (Brunt & Metavir systems) for liver histology by Pearson Chi-Square.

**Results** : Mean body weight ( $227.0 \pm 9.1$ g) was significantly lower and ALT ( $132.5 \pm 8.9$ U/L) and AST ( $316.1 \pm 23.0$ U/L) were higher in group II compared to, respectively,  $281.5 \pm 20.5$ g,  $102.2 \pm 8.6$ U/L and  $231.1 \pm 18.6$ U/L in the control group ( $p < 0.05$  in all). The combination therapy in group III was associated with significantly higher mean body weight ( $265.0 \pm 3.9$ g) and lower ALT ( $110.5 \pm 5.3$ U/L) and AST ( $256.5 \pm 15.1$ U/L) compared to group II ( $p < 0.05$  in all). Also compared to control, hepatic GSH (nmol/mg tissue) was significantly lower and MDA (nmol/mg tissue) higher in group II (respectively,  $66.92 \pm 4.55$  vs.  $47.13 \pm 3.78$  and  $1.43 \pm 0.09$  vs.  $2.76 \pm 0.11$ ,  $p = 0.004$  and  $0.0001$ ). The combination therapy was associated with significantly lower levels of tissue MDA ( $2.27 \pm 0.07$ nmol/mg tissue) and higher GSH ( $57.55 \pm 1.60$  nmol/mg tissue) compared to group II (respectively,  $p < 0.001$  and  $0.05$ ). This therapy was also associated with significantly lower scores for portal inflammation ( $p = 0.019$ ), ballooning ( $p = 0.012$ ), steatosis ( $p = 0.043$ ) and a trend for fibrosis ( $p = 0.053$ ). No side effects by this combination were observed in group IV.

**Conclusion** : The combination therapy with  $\beta$ -carotene and vitamin C significantly improved biochemical and histological signs of liver damage, ameliorated parameters of oxidative stress and stabilized other changes without causing toxicity in an animal model of chemically induced cirrhosis.

DIFFERENTIAL GENE EXPRESSION PROFILE IN PORCINE LIVERS SUBJECTED TO WARM ISCHEMIA. E. Balligand (1), J. Van Pelt (1), Q. Liu (1), H. Van Malenstein (1), D. Monbaliu (2), V. Heedfeld (1), T. Wylin (1), T. Ayoubi (3), W. Van Delm (3), P. Van Hummelen (4), J. Pirenne (2), K. Vekemans (1). (1) University of Leuven, Leuven, Belgium, (2) University Hospital Gasthuisberg, Leuven, Belgium, (3) VIB, Leuven, Belgium, (4) Rutgers University, New Jersey, United States.

**Introduction** : The molecular and pathophysiological mechanisms underlying warm ischemia (WI) injury without reperfusion in the liver are not fully understood.

**Aim** : To elucidate the pathways involved, we investigated the gene expression by microarray in a porcine model of WI.

**Methods** : Porcine livers were randomly subjected to different WI periods (0', 15', 30', 45' WI, 60' WI : n = 3/group except for 60' WI (n = 4)). mRNA of liver samples were analyzed by expression Affymetrix microarray. Porcine genes were translated into human equivalents (Tsai et al.). Changes in gene expression between 0' WI and each WI group were analyzed by Bioconductor. By using a multifactorial equation based on pH and AST measured in a hypothermic machine perfusion system, we classified the severely from the mildly damaged livers. Then we applied a statistical method : Nearest shrunken centroid.

**Results** : 3530 genes were significantly altered between 0' WI and 15' WI. Of them, 1762 genes were up-regulated and 1768 were down-regulated ( $p < 0.05$ ). Between 0' WI and 30' WI 1924 genes were significantly up-regulated and 2217 were down-regulated. For 0' and 45' WI 1552 genes were up-regulated and 1262 genes were down-regulated. For 60' WI two samples did not pass the quality criteria and were therefore not used to make comparisons to the 0' WI, however the two samples that passed the quality criteria were used in the Nearest shrunken centroid method. After pathway mapping, we identified pathways clustering for cell death, cell proliferation and inflammation being up-regulated in WI, which were not altered in 0' WI ( $p < 0.05$ ). Of note, pathways related to extracellular region/space and regulation of cellular process were only observed between 0' WI and 15' WI. The pathways related to lipid metabolism were

down-regulated and increased with WI time. A molecular signature of 193 probesets was identified. A central key regulator is STAT1.

**Conclusion** : Liver WI is accompanied by changes in gene expression affecting several molecular pathways. After classifying all livers in two groups : the severely and the mildly damaged we could identify a molecular signature with STAT1 as central molecule. This study provides new perspectives to alter mechanisms related to WI in liver transplantation.

- A34 -

ALDEHYDE DEHYDROGENASE ACTIVITY : A NEW STRATEGY TO PURIFY LIVER PROGENITOR CELLS.  
L. Dollé, J. Mei, J. Best, C. Empsen, H. Reynaert, A. Geerts, L. van Grunsven. UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

**Introduction** : Several liver dysfunctions are life-threatening conditions for which the most effective treatments rely on orthotopic liver transplantation (OLT). However, drawbacks related to OLT reduce its application on a large-scale. Recently, the role of progenitor cells in liver repair and fibrosis has been described but strategies for their purification have been quite limited making their characterization and use in regenerative medicine difficult. To address this issue, we developed a liver progenitor cell isolation strategy based on ALDH activity, a main feature shared by many progenitor cells.

**Methods** : High levels of ALDH activity have been proposed to be a common feature of progenitor cells (hematopoietic, neural and mesenchymal stem cells). Using a substrate specific for ALDH1A1 activity, we sort out the liver progenitor cells using a Flow Cytometry Activated Cell Sorter (FACS).

**Results** : In normal liver, the enzyme is expressed in some fibroblasts, bile duct cells and canals of Hering, whereas under injured conditions, these cells overexpress ALDH1A1. By flow cytometry, an ALDH+ cell population can be isolated which co-expresses numerous of markers typically found in Liver Progenitor Cells (LPCs) ; for instance, EpCAM, Prominin-1, CK-19, and ABCG2, shown by immunofluorescence, flow cytometry and RT-qPCR analyses. In cell culture, these cells can give rise to hepatocyte-like cells.

**Conclusion** : The isolation of functional LPCs based on ALDH activity offers a new straightforward strategy for their isolation. Since these cells are located in the canals of Hering and are activated during hepatic injuries, we established a new role of ALDH1A1 in LPC activation.

EFFECTS OF LARGE PORE HEMOFILTRATION IN A SWINE MODEL OF FULMINANT HEPATIC FAILURE. O. Detry (1), N. Janssen (1), E. Cavalier (2), P. Delanaye (1), J.P. Cheramy-Bien (2), P. Honoré (1), J.O. Defraigne (2), B. Lambermont (1). (1) Ulg Sart Tilman, Liège, Belgium, (2) University of Liege, Liège, Belgium.

**Introduction** : Systemic inflammatory response might be involved in pathogenesis of brain oedema and intracranial hypertension complicating fulminant hepatic failure (FHF), by inducing an increase in cerebral blood flow and brain water content. We recently demonstrated in endotoxic shock models in the pig, that large-pore membrane hemofiltration (LPHF) with a 80 kDa cutoff may induce a significant IL-6 and IL-10 clearance and an improvement of hemodynamic stability and survival.

**Aim** : In this study, we used the validated ischemic FHF model in the pig, to evaluate the effects of this 80 kDa LPHF on intracranial pressure (ICP) and cerebral blood flow (CBF) and on hemodynamic parameters, in relation with the clearance of proinflammatory cytokines and the blood liver tests.

**Methods** : 15 pigs were randomised in three groups : sham, FHF, and FHF + LPHF. FHF was performed by porto-caval anastomosis and hepatic artery and bile duct ligation. All pigs were monitored over the following 6 hrs. In the FHF + LPHF group, LPHF was instituted for 4 hrs, from Time 2 to 6 hrs. Hemodynamics, CBF and ICP were continuously recorded. Blood samples (ammonia, lactate dehydrogenase (LDH), aspartate and alanine transaminases (AST, ALT), aromatic amino acids, total bilirubin, glucose, lactate, IL-6, IL-10, TNF- $\alpha$ ) were collected before liver devascularisation (T0), and after two (T2) and 6 (T6) hrs.

**Results** : The FHF groups developed blood characteristics of liver failure, without difference between FHF, and FHF + LPHF, two groups that developed intracranial hypertension. Despite a cytokine clearance, there was no significant difference in CBF and ICP between FHF and FHF + LPHF.

**Conclusion** : In this ischemic FHF pig model, LPHF with a 80 kDa cutoff did not improve liver tests, nor CBF or ICP.

THE EVOLUTION OF LAPAROSCOPIC LEFT LATERAL SECTIONECTOMY WITHOUT PRINGLE MANEUVER : THROUGH RESECTION OF BENIGN AND MALIGNANT TUMORS TO LIVING LIVER DONATION. B. Van Den Bossche (1), F. Berrevoet (1), M. Sainz-Barriga (1), B. De Hemptinne (1), R. Troisi (1). (1) University Hospital, Ghent, Belgium.

**Introduction** : Laparoscopic left lateral sectionectomy (LLS) has gained popularity for its safety and reproducibility, being performed in case of benign and malignant tumours. We report herein the evolution of our experience of laparoscopic LLS for different indications including living liver donation.

**Aim** : We report herein the evolution of our experience of laparoscopic LLS for different indications including living liver donation.

**Methods** : We reviewed the medical files of 37 consecutive patients undergoing laparoscopic LLS for benign, primary and metastatic liver diseases and in case of living liver donation in our institution between January 2004 and January 2009. The mean patient age was of  $53 \pm 15$  years and the M/F ratio of 10/27. Resection of malignant tumours was indicated in 19/37 (51%) patients.

**Results** : All patients but three (deceased for metastatic cancer disease) are alive and well after a median follow-up of 20 months (range 8-46). Liver cell adenomas (72%) were the main indication between benign tumours and colorectal liver metastases (84%) were the first indication in malignancy. One case of live liver donation was performed. Sixteen patients (43%) had a previous abdominal surgery whereas 3 (8%) had a LLS combined to bowel resection. The median operation time was of 195 min. (115-300) and the median blood loss was of 50 ml (0-500). No transfusions were required. Mild to severe steatosis was noticed in 7 (19%) patients and aspecific portal inflammation in 11 (30%). A median free-margin of 3 mm (3-27) was achieved in all cancer patients. Overall recurrence rate in colorectal liver metastases was of 7 (44%) but none recurred at the surgical margin. No conversion to laparotomy was recorded and the overall morbidity was of 8.1%. The median hospital stay accounted for 6 days (2-10).

**Conclusion** : Laparoscopic left lateral sectionectomy without portal clamping can be safely performed in patients with benign and malignant liver diseases accounting for minimal blood loss and overall morbidity, free resection margins and a favourable outcome. As ultimate step of learning curve, laparoscopic LLS could be routinely proposed potentially increasing the donor pool in living-related liver transplantation.

## GASTROINTESTINAL REGULATORY MECHANISMS

## Invited lecture

- B01 -

PHARMACOLOGY OF INFLAMMATORY VISCERAL PAIN. P. Holzer. Graz, Austria.

Peripheral pain is mediated by primary afferent neurons. Their sensitivity to painful stimuli undergoes adaptive and maladaptive adaptations. Inflammation is commonly associated with hypersensitivity, hyperalgesia and, in many cases, allodynia. Ideally, these changes should abate once inflammation has resolved but this is not necessarily the case. The phenotypic changes underlying hypersensitivity of afferent neurons persist in chronic inflammation and are also maintained to a certain degree in postinfectious irritable bowel syndrome (1).

Experimental evidence indicates that basically all primary afferent neurons supplying the gut have the ability to sensitize in response to a number of proinflammatory mediators and display enhanced excitability following induction of inflammation. The mechanisms whereby hypersensitivity and hyperexcitability of afferent neurons are initiated and maintained are of prime interest for the development of new treatment modalities (1).

There is a multitude of molecular nociceptors that transduce painful stimuli to propagated action potentials in nociceptive afferent neurons (1). Hypersensitivity of afferent neurons can arise from changes in the expression and/or function of (i) receptors and sensors at the peripheral terminals of afferent neurons that are relevant to stimulus transduction, (ii) ion channels that govern the excitability and conduction properties of afferent neurons, and (iii) transmitters and transmitter receptors that mediate communication between primary afferents and second-order neurons in the spinal cord and brainstem.

Particular therapeutic potential is attributed to targets that are selectively expressed by afferent neurons and whose number and function are altered in chronic visceral pain (1,2,3). The last decade has seen the emergence of a large number of receptors and ion channels that function as molecular nociceptors and whose expression is altered in inflammatory pain. These novel entities include distinct members of the transient receptor potential (TRP) channel family (e.g., TRPV1, TRPV4, TRPA1), acid-sensing ion channels, protease-activated receptors, corticotropin-releasing factor receptors, neurotrophin receptors and tetrodotoxin-resistant sodium channels (1).

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- B02 -

QUANTIFICATION OF BRAIN ACTIVITY EVOKED BY COLONIC DISTENTION IN RATS USING H<sub>2</sub><sup>15</sup>O MICRO PET. M.M. Wouters (1), P. Vermaelen (1), S. Van Wanrooy (1), A. Van Santvoort (1), O. Welting (2), A. Nemethova (1), D. Vanderghinste (1), R.M. Van Den wijngaard (2), K. Van Laere (3), G.E. Boeckstaens (1). (1) University of Leuven, Leuven, Belgium, (2) Academic Medical Center, Amsterdam, Netherlands, (3) University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : Altered brain processing plays an important role in the development of visceral hypersensitivity in irritable bowel syndrome. fMRI has been used to study central mechanisms in animal models, but is prone to artefacts. In addition, animal manipulation and abdominal electrodes may render acquisitions unreliable.

**Aim** : The aim of the study was to utilize H<sub>2</sub><sup>15</sup>O microPET to evaluate brain activity evoked by visceral pain in response to colorectal distention (CRD) in rats.

**Methods** : During anesthesia with alpha-chloralose, Long Evan rats (n = 7) were scanned in a microPET Focus 220. CRD was performed by inflating a colonic balloon (1, 1.5, 2 ml). Reconstructed brain activity images were coregistered and spatially normalized to a standard space based on the Paxinos atlas. Significant clusters of altered activity were identified by voxel based statistical parametric mapping (SPM2,  $P_{height} < 0.005$  uncorrected). The visceromotor response to distention was recorded by superficial neonatal EMG electrodes. EMG signals were analyzed with Spike2 and normalized to the maximum pain response (2ml). Conventional statistical analysis was performed using a paired Student's t-test.

**Results** : CRD resulted in a volume-dependent increase in visceromotor response (1 ml :  $10 \pm 5\%$ , 1.5 ml :  $42 \pm 11\%$  and 2 ml :  $100\%$ ). A group comparison between distention and baseline conditions revealed a volume-dependent activation in clusters at the sensorimotor cortex ( $P_{\text{height}} = 0.001$ ), periaqueductal gray ( $P_{\text{height}} = 0.001$ ) and bilateral cerebellum ( $P_{\text{height}} < 0.001$ ). In addition, correlation analysis with distention volume yielded a significant negative correlation in the prefrontal cortex ( $r = 0.76$ ,  $P_{\text{height}} < 0.001$ ). Maximal colonic distention (2ml) resulted in 16% deactivation of the prefrontal cortex compared to baseline.

**Conclusion** : Anesthetized rats showed a volume-dependent increase in visceral pain which corresponds to activation of the visceral pain neuromatrix, as reported in humans. Volume dependent deactivation of the prefrontal cortex is also in line with clinical studies. These results demonstrate that  $\text{H}_2^{15}\text{O}$  microPET is a useful tool to quantify brain (de-)activation in response to visceral pain.

- B03 -

LONG-LASTING VISCERAL HYPERSENSITIVITY FOLLOWING ACUTE TNBS-COLITIS IN RATS.  
W. Vermeulen, J. De Man, T. Moreels, S. Nullens, P. Pelckmans, B. De Winter. Antwerp University, Antwerp, Belgium.

**Introduction** : Intestinal inflammation can induce long-lasting visceral hypersensitivity. These alterations in sensory function might be related to symptoms of pain and discomfort in patients with IBD and/or IBS.

**Aim** : In this study, the aim was to evaluate the occurrence of visceral hypersensitivity in female Wistar rats 4 weeks after an episode of acute TNBS-induced colitis.

**Methods** : Colitis was induced chemically in female Wistar rats (200-240 g ; n e 7/ group) by intracolonic instillation of 7.5 mg trinitrobenzene sulphonic acid (TNBS) in 40% ethanol. Control rats received a saline instillation. Twenty-eight days later, abdominal EMG registrations of visceromotor responses to colorectal distensions (10-20-30-40-60-80 mmHg, 20 s, 4 min interval) were performed. To confirm the inflammatory response, colonic tissue was examined endoscopically within 3 days after the inflammatory onset. After 28 days, inflammation was re-evaluated endoscopically, macroscopically and microscopically.

**Results** : Colonoscopy showed that intracolonic instillation of TNBS resulted in severe inflammation within 3 days, associated with ulceration, hyperaemia, edema and stenosis. Twenty-eight days after intracolonic instillation of TNBS, visceromotor responses to colorectal distensions were significantly higher in a subpopulation of about one third of the TNBS-treated group compared to the saline-treated group ( $P < 0.05$  ; two-way ANOVA). A population of about two third of the TNBS-treated group did not show hypersensitivity to colorectal distensions. Colonic tissue examination did not reveal severe endoscopic, macroscopic or histopathological signs of inflammation 28 days after TNBS.

**Conclusion** : A single dose of 7.5 mg TNBS dissolved in 40% ethanol resulted in a self-limiting acute colitis. Twenty-eight days after the onset of inflammation, visceral sensitivity to colorectal distension was increased but only in a subgroup of TNBS-treated rats. These results suggest that inflammation alone does not always result in long-lasting visceral hypersensitivity.

INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITION IMPROVES NITRERGIC DYSFUNCTION IN DIABETES-PRONE BIOBREEDING RATS. S. Kindt, T. Vanuytsel, S. Salim Rasool, W. Boesmans, G. De Hertogh, P. Vanden Berghe, J. Tack. University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction :** Loss of intestinal nitrenergic motor control has been reported in models of type 1 diabetes and following transient intestinal inflammation (Zandecki 2008, Demedts 2007). The Biobreeding (BB) rat, a model of spontaneous type 1 diabetes mellitus, consists of a diabetes-resistant (BBDR) and a diabetes-prone (BBDP) strain, the latter characterized by spontaneous intestinal inflammation and development of diabetes in approximately 50%. We demonstrated that loss of jejunal nitric oxide synthase (nNOS) mRNA expression occurs in the BBDP strain, which is related to transient intestinal inflammation (myeloperoxidase activity (MPO), inducible NOS expression) rather than hyperglycemia (Kindt DDW 2009). Studies in the brain and in enteric neuron cultures suggest that iNOS overexpression downregulates nNOS expression through oxidative stress (DeAlba 1999, Zandecki 2006).

**Aim :** Investigate the role of iNOS in nitrenergic dysfunction in BB rats.

**Methods :** Normalized mRNA expression of jejunal nNOS and iNOS and inflammation in the proximal jejunum of BB rats were analyzed by real-time RT-PCR and MPO measurements in diabetes-resistant (BBDR) and diabetes prone rats with or without hyperglycemia (each BBDP-H and BBDP-N) either with or without pretreatment with aminoguanidine (AG) (added to the drinking water at ; 1g/l for BBDP-H and 2g/l for BBDR and BBDP-N during 16 weeks). AG inhibits iNOS and also the production of hyperglycemia-induced advanced glycation end products.

**Results :** In the BBDR rats, AG had no significant effect on MPO and iNOS expression, but significantly increased nNOS expression ( $p < 0.001$ ). AG treatment in BBDP-N rats did not alter MPO, but decreased iNOS expression significantly ( $p < 0.001$ ) and increased nNOS expression ( $p = 0.16$ ), compared to untreated BBDP-N rats. After AG treatment, no significant differences in iNOS and nNOS expression were found between BBDP-N and BBDR rats.

**Conclusion :** AG decreased iNOS mRNA expression in BB rats and seems to operate as a switch between iNOS and nNOS upregulation. iNOS inhibitors have the potential to prevent post-inflammatory nitrenergic dysfunction.

#### Invited lecture

PHARMACOTHERAPY IN SOMATIC PAIN RESEARCH. G. Hans. Antwerp University Hospital, Antwerp, Belgium.

**Introduction :** Visceral pain is the pain we feel when our internal organs are damaged or injured and it is, by far, the most common form of pain. Until recently visceral pain was not always considered to be a major problem by the very specialists that dealt with it. Obstetricians, gynaecologists, cardiologists, gastroenterologists and urologists were mainly concerned with the diagnosis and treatment of the underlying disease, and their approach was to assume that if the disease went away so would the pain. Only recently has (chronic) pain become a subject that can be treated directly and independently of the accompanying disease as physicians realize that this 'symptom' is often the very centre of the problem.

**Aim :** The aim of this presentation is to provide the participants with information regarding the new insights into the pathophysiology and treatment options for acute and chronic visceral pain. An update of the research into this subject will be provided, as well as recent therapeutic strategies.

**Methods :** Visceral pain shows peculiarities that make it very different from pain affecting the somatic organs (the skin, muscles, joints and bones). For instance, not all internal organs are sensitive to pain and some can be damaged quite extensively without the person feeling a thing. Many diseases of the liver, the lungs or the kidneys remain largely painless and the only symptoms felt by the patient are those derived from the abnormal functioning of these organs. On the other hand, relatively minor lesions in viscera such as the stomach, the bladder or the ureters can produce excruciating pain. There is no close relationship between damage and pain like that seen when the lesions affect a somatic organ. The reasons for this strange situation lie with the innervation of the internal organs. Some viscera are innervated by sensory neurons that signal harmful events (nociceptors) but other internal organs lack this form of sensor, so that injuries or lesions to these organs cannot be translated into signals that the brain would perceive as painful. The internal organs with nociceptors are mostly the hollow viscera (the gut, the bladder, the uterus) and it is from these organs that we get most of our visceral pain sensations. The insides of these organs are, in effect, an extension of the external environment so these organs are in contact with potentially harmful agents. They therefore need to be protected by pain mechanisms. Visceral nociceptors are very similar to those that innervate the skin or muscle. They respond not only to intense mechanical stimuli (distension and overstretching) but also to irritant chemicals and specially to the products of



inflammation. Some visceral nociceptors become active only after inflammation of the mucosa of the organs that they innervate. They are particularly important in signalling pain from inflamed and sensitized viscera.

Another interesting peculiarity of visceral pain is the fact that it is often felt in places remote from the location of the affected organ. This is known as 'referred pain' and it is often a very useful tool to diagnose diseases of internal organs. Patterns of referred pain can be detected in diseases of the gut, the bladder or the internal genital organs, where the pain is felt in the abdomen, the pelvic region or the back, with the patient not being able to locate the pain very accurately. The reason for the 'referral' of visceral pain is the lack of a dedicated sensory pathway in the brain for information concerning the internal organs. The sensory neurons from the viscera connect within the brain with sensory pathways that carry information from the skin and muscles, and the brain interprets the signals that originate from internal organs as coming from the overlying skin or muscles. This is known as 'viscero-somatic convergence' and it is thought to be the neural basis for referred visceral pain.

However, recent studies using brain imaging have shown that the areas of the brain activated by painful visceral stimuli are not exactly coincidental with those turned on during somatic pain. Although viscero-somatic convergence may underlie referred pain, there are also other factors involved in the integration of sensory information from internal organs. This brings us to very important pathophysiological mechanisms such as sensitization, wind-up and hyperalgesia.

**Results** : A remarkable aspect of visceral pain is the development of visceral hyperalgesia – an increased sensitivity to visceral stimulation following an injury or inflammation of an internal organ. The increased sensitivity of the viscera after inflammation has two causes : (1) an alteration of the sensory neurons in the viscera so that they now respond more intensely to naturally occurring stimuli ; (2) an enhanced sensitivity of the sensory pathways in the brain that mediate sensations from the viscera.

Both processes are known as 'sensitization' either peripherally (in the viscera) or centrally (in the brain) and are thought to be responsible not only for the pain produced by the inflammatory disease but also for hyperalgesic sensations that can occur in the absence of an identifiable cause, such as pain in conditions like irritable bowel syndrome. This process of sensitization is currently the subject of a great deal of research, to identify its molecular basis and to find ways to restore normal sensitivity to the distorted system. The aim is to reduce hyperalgesic sensations caused by the regular functioning of internal organs without interfering with the normal sensitivity of the viscera or with the digestive, secretory or reproductive functions of the organ.

**Conclusion** : Unfortunately, currently there are very few specific analgesic drugs for visceral pain, and the therapies commonly used are extensions of those used for somatic pain (as well as sometimes for neuropathic pain). Because of the prevalence of visceral pain, there is a great need for therapies aimed specifically at the conditions that cause the pain. This is particularly the case for diseases characterized by visceral hypersensitivity (such as irritable bowel syndrome), in which the therapeutic aim should be to reduce the increased sensations felt from the bowel without damping sensation in general or impairing the ability of the patient to live a normal life.

- B06 -

INTRAGASTRIC PRESSURE DURING INTRAGASTRIC NUTRIENT DRINK INFUSION AS A NOVEL MINIMALLY INVASIVE METHOD TO ESTIMATE MEAL-INDUCED GASTRIC ACCOMMODATION. P. Janssen, J. Tack. KU, Leuven, Belgium.

**Introduction** : The stomach relaxes upon food intake. This reflex relaxation, also referred to as gastric accommodation, provides a reservoir for the ingested meal while keeping the intragastric pressure (IGP) low.

**Aim** : We set out to study meal-induced stomach relaxation by measuring the IGP during nutrient drink infusion.

**Methods** : In fasted healthy volunteers (n = 5-16) a high-resolution manometer was positioned through the nose with the tip in the pylorus. Moreover, an infusion catheter was positioned through the mouth with the tip 10 cm under the lower esophageal sphincter (LES) or in the pylorus. Studies were performed with the volunteers in supine or sitting position. After a stabilisation period a nutrient drink (Nutridrink 1.5 kcal/ml) or saline was infused intragastrically (60 ml/min). The volunteers scored their satiation until maximum, when the infusion was stopped. The mean IGP was studied in the 5 cm below the LES and presented  $\pm$  SEM with paired ANOVA comparison (P < 0.05 was considered significant).

**Results** : During nutrient infusion in the proximal stomach with the volunteers sitting down the IGP decreased initially, reaching a nadir of  $3.1 \pm 0.5$  mmHg after  $3.9 \pm 0.6$  min, followed by a gradual recovery at a rate of  $0.3 \pm 0.1$  mmHg/min. Maximum satiation was reached after  $961 \pm 79$  ml. No significant differences were found in supine or sitting position, or when the nutrients were infused in the distal or proximal stomach. The IGP during intragastric saline infusion was significantly higher as compared to nutrient infusion. Inhibition of nitric oxide (NO) synthase by intravenous infusion of N<sup>G</sup>-monomethyl-L-arginine (8 mg/kg/hour) significantly increased the IGP before and during nutrient infusion while maximum satiation was reached significantly earlier after infusion  $816 \pm 91$  ml nutrient drink.

**Conclusion** : During nutrient infusion the IGP decreased initially but gradually recovered thereafter. This IGP course was dependent on the composition of the drink and was at least partly mediated by NO. We propose that IGP monitoring provides a minimally invasive alternative to the barostat for the assessment of gastric accommodation.

AN INCREASE IN INTRA-GASTRIC PRESSURE IS ASSOCIATED WITH A HIGHER PROXIMAL EXTENT OF GASTROESOPHAGEAL REFLUX IN HEALTHY VOLUNTEERS. K. Blondeau, V. Boecxstaens, R. Farre, V. Mertens, A. Pauwels, G. Boeckxstaens, J. Tack. University of Leuven, Leuven, Belgium.

**Introduction** : Increasing body weight has been associated with an increase in the prevalence of gastroesophageal reflux disease (GERD). An increase of 1 kg/m<sup>2</sup> in BMI is associated with a 10% increase of the intra-gastric pressure. Proximal extent of reflux has been identified as an important determinant of symptom perception in patients with GERD.

**Aim** : The aim of our study was to investigate the role of an increased intra-gastric pressure on occurrence and proximal extent of reflux in healthy volunteers.

**Methods** : 6 healthy volunteers (5 men, age 30 (19-50) yr) were included in the study. All volunteers included had a normal BMI (mean 23.9 range (22.7-24.6) and normal abdominal circumference [mean 81 range (63-98) cm]. After receiving a standard solid refluxogenic meal (1000 Kcal), high resolution (HR) manometry-impedance recordings were performed for 2 consecutive hours. Intra-gastric pressure was experimentally increased using an inflatable cuff which was placed around the abdomen. The cuff was inflated/deflated every 30 minutes, in a random order. Intra-gastric pressure, number of reflux events, bolus clearance time (sec) and proximal extent of reflux (cm above LES) were measured and compared between inflation and deflation periods.

**Results** : Inflation of the cuff resulted in a significant increase of the intra-gastric pressure [average 38% (31-44%),  $p = 0,02$ ]. The number of reflux events during band inflation tended to be higher compared to deflation periods but this did not reach statistical significance [6 (4-10) vs 12 (6-19),  $p = 0.1$ ]. Reflux events had a significantly higher proximal extent [22.1 (18.6-23.8) cm vs. 13(10.8-18.5)cm,  $p < 0.0001$ ] and clearance time [16(11-30.3) vs. 12 (8-16.5) sec,  $p = 0.006$ ] was significantly longer during inflation compared to deflation periods. None of the volunteers experienced reflux symptoms (heartburn and/or regurgitation) during the study.

**Conclusion** : An increase in intragastric pressure in healthy volunteers provokes a significant increase in the bolus clearance time and proximal extent of reflux. These data indicate that increased abdominal pressure may be an important pathophysiological mechanism underlying the increased clinical manifestations of GERD in obesity.

EFFECT OF AZD3355, A NOVEL GABAB AGONIST, ON REFLUX AND LOWER ESOPHAGEAL SPHINCTER FUNCTION IN PATIENTS WITH GERD WITH SYMPTOMS DESPITE PROTON PUMP INHIBITOR TREATMENT. G.E. Boeckxstaens (1), H. Denison (2), M. Ruth (2), J. Adler (2), D.G. Silberg (3), D. Sifrim (4). (1) University of Leuven, Leuven, Belgium. (2) AstraZeneca R&D, Mölndal, Sweden, (3) AstraZeneca LP, Wilmington, United States, (4) Barts and the London School of Medicine and Dentistry, London, United Kingdom.

**Introduction** : A proportion of patients (pts) with gastroesophageal reflux disease (GERD) continue to have reflux symptoms while taking a proton pump inhibitor (PPI).

**Aim** : This study aimed to assess the effects of AZD3355, a novel GABA<sub>B</sub> receptor agonist, on reflux and lower esophageal sphincter (LES) function when used as add-on treatment in pts with GERD symptoms despite PPI therapy.

**Methods** : In this randomized, double-blind, placebo-controlled, cross-over study, pts received AZD3355 65 mg (capsule) or placebo twice on day 1 (morning and evening) and once on day 2 (morning), in addition to existing PPI treatment (doses within the approved label for any GERD indication). After 5-28 days' washout, pts crossed to the opposite treatment arm. Pts finished a standardized meal 1 h after the morning doses. On day 1, ambulatory impedance-pH monitoring was conducted for 24 h after the first dose. On day 2, stationary manometry and impedance-pH monitoring were analysed until 3 h after the meal.

**Results** : In all, 27 pts were randomized and 25 completed the study. On day 1, AZD3355 reduced the mean total number of reflux events over 24 h by ~35% compared with placebo, with the acid component being the most reduced (Table 1). Esophageal acid exposure (mean% time during 24 h at pH < 4) was lower with AZD3355 than placebo (upright : 1.2% [SD :2.0] vs 3.0% [SD :3.9] ; supine : 0.3% [SD :0.8] vs 1.9% [SD :3.2]). There were fewer proximal reflux events over 24 h with AZD3355 than with placebo (mean 8.7 [SD :8.2] vs 15.4 [SD :14.5]). On day 2, AZD3355 reduced the geometric mean number of transient LES relaxations (TLESRs) by 25% (11.6 vs 15.5 ; ratio 0.75 ; 95% CI : 0.60-0.93) and increased the geometric mean LES pressure by 28% (9.1 vs 7.1 mmHg ; ratio 1.28 ; 95% CI : 1.05-1.57) compared with placebo. There were similar numbers of swallows with AZD3355 and placebo (geometric mean 236 vs 244 ; ratio 0.97 ; 95% CI : 0.82-1.14). AZD3355 was well tolerated : the most common adverse events were headache (8/25 pts on AZD3355, 11/27 on placebo) and paraesthesia (transient ; 5/25 pts on AZD3355, 3/27 on placebo).

Table 1. — Arithmetic mean number of reflux events over 24 h on day 1 (n = 21)

	Placebo	AZD3355 65 mg	Mean difference (95% CI)
<b>Total reflux events</b>	62.7	41.0	-22 (-23 to -15)
<b>Acid reflux</b>	29.1	13.3	-16 (-23 to -8.3)
<b>Weakly acidic reflux</b>	31.2	24.7	-6.5 (-12 to -0.53)
<b>Weakly alkaline reflux</b>	2.3	2.9	0.64 (-2.1 to 3.34)

**Conclusion** : AZD3355 had beneficial effects on TLESRs, LES pressure, total number of reflux events, esophageal acid exposure and proximal reflux events when used as add-on treatment in pts with GERD symptoms despite PPI therapy.

- B09 -

BILE ACIDS IN SPUTUM OF PATIENTS WITH CYSTIC FIBROSIS. A. Pauwels (1), A. Decraene (1), K. Blondeau (1), V. Mertens (1), R. Farre (1), L. Dupont (1), D. Sifrim (2). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) Barts and the London School of Medicine and Dentistry, London, United Kingdom.

**Introduction** : Up to 80% of patients with cystic fibrosis (CF) may have increased gastroesophageal reflux. This could be associated with typical symptoms such as heartburn and/or regurgitation. Important in CF patients is that aspiration of duodenogastric contents may further deteriorate already severely inflamed airways.

**Aim** : The aim of our study was to identify bile acids aspiration in CF patients, and its potential impact in airway inflammation.

**Methods** : In 41 CF patients [24 men ; 24 (11-43) years] sputum was obtained after inhalation of hypertonic saline. Sputum was separated from saliva ; supernatant was collected and tested for bile acids (BA) and neutrophil elastase (an enzyme related to lung inflammation in CF). Lung function and BMI were assessed on the day of sputum collection.

**Results** : 23/41 (56%) patients had detectable BA in sputum. The median concentration of BA in positive sputum samples was 2.2 (1.7-2.9)  $\mu\text{mol/L}$ . BA concentrations were similar in patients with genotype DF508 homozygote, DF508 heterozygote and others (2.2 (1.7-3.2), 2.0 (1.6-2.9), 1.6 (1.4-2.3)  $\mu\text{mol/L}$ ) and was unrelated with BMI and age. Patients with BA in sputum had a significantly higher concentrations of neutrophil elastase compared to patients without [1116 (726-1956) vs. 516 (243-1036) pg/ml,  $p = 0.005$ ]. There was a significant correlation between BA concentration and neutrophil elastase concentration in these patients ( $p < 0.0001$ ,  $r = 0.58$ ). Pulmonary function was related to concentration of BA and neutrophil elastase in sputum. In patients with detectable BA, there was a significant negative correlation between BA and FEV1%predicted ( $p = 0.009$ ,  $r = -0.53$ ) and FVC% ( $p = 0.003$ ,  $r = -0.59$ ).

**Conclusion** : BA can be found in the sputum of half of CF patients, confirming aspiration of duodenogastric contents into the lungs. Aspiration of BA was associated with increased airway inflammation. In patients with BA aspiration, the level of BA was associated with lung function impairment.

**Invited lecture**

DEVELOPMENT OF POSTINFLAMMATORY ANIMAL MODELS  
AND VISCERAL HYPERALGESIA  
N. VERGNOLLE. Toulouse, France.

ENDOGENOUS TACHYKININS MODULATE DISTENSION-INDUCED PERISTALSIS IN THE MOUSE COLON MAINLY THROUGH ACTIVATION OF NK1 AND NK2 BUT NOT NK3 RECEPTORS. A. Deiteren, B. De Winter, S. Nullens, P. Pelckmans, J. De Man. Antwerp University, Antwerp, Belgium.

**Introduction** : Tachykinergic NK receptors are involved in various gastrointestinal functions but their role in colonic peristalsis is not well understood.

**Aim** : We investigated the functional role of NK1, NK2 and NK3 receptors on colonic peristaltic activity by studying the effect of the selective NK1, NK2 and NK3 receptor agonists (septide,  $\beta$ -ANKA and senktide respectively) and antagonists (RP 67580, nepadutant and SR 142801 respectively) on distension-induced peristaltic activity in murine colon.

**Methods** : Using a modified Trendelenburg set-up, colonic peristaltic activity was assessed by quantifying the amplitude and interval of distension-induced pressure waves in proximal and distal colon segments.

**Results** : Gradual distension of the colon segments induced rhythmic and aborally migrating contractions which were recorded as repetitive pressure waves. These pressure waves were abolished by TTX (1  $\mu$ M) and inhibited by hexamethonium (100  $\mu$ M) demonstrating their neurogenic origin. Blockade of NK1 receptors (RP 67580, 2  $\mu$ M) did not affect the interval of the pressure waves but reduced the peristaltic pressure amplitude in the distal but not in the proximal colon. Activation of NK1 receptors (septide, 10-100 nM) marginally enhanced the pressure amplitude and interval in the distal colon without affecting peristalsis in the proximal colon. Blockade of NK2 receptors (nepadutant, 1  $\mu$ M) reduced the peristaltic pressure amplitude and interval in the proximal and distal colon. Activation of NK2 receptors ( $\beta$ -ANKA, 10-100 nM) slightly enhanced the amplitude in the proximal and distal colon without affecting the interval. Blockade of NK3 receptors (SR 142801, 0.1-0.3  $\mu$ M) had no effect on peristaltic activity in the proximal and distal colon. However, activation of NK3 receptors (senktide, 1-10 nM) significantly augmented the peristaltic pressure amplitude and interval in the distal colon and slightly enhanced the amplitude in the proximal colon without affecting the interval.

**Conclusion** : NK3 receptors are functionally active in murine colon, especially in the distal part of the colon. Nevertheless, endogenous tachykinins mainly act on NK1 and NK2 receptors to regulate distension-induced colonic peristalsis with different potency in the proximal and distal colon

PRUCALOPRIDE INDUCES TRANSIENT CONTRACTION AND FACILITATES TACHYKININERGIC CONTRACTIONS IN GUINEA PIG PROXIMAL COLON. F. De Vin (1), M. Choi Sze (1), J. De Maeyer (2), E. Ghoois (2), J. Schuurkes (2), R. Lefebvre (1). (1) Heymans Institute of Pharmacology, Gent, Belgium, (2) Movetis NV, Turnhout, Belgium.

**Introduction :** The 5-HT<sub>4</sub>-receptor agonist prucalopride is well known to facilitate stimulated acetylcholine release from cholinergic nerves in stomach and colon. Prucalopride has also been reported to facilitate non-adrenergic non-cholinergic (NANC) contractions in guinea pig proximal colon, but the transmitter(s) involved were not determined. Therefore, we investigated the influence of prucalopride on NANC responses in guinea pig proximal colon.

**Methods :** Full segments (20 mm) of guinea pig proximal colon were mounted longitudinally in isometric conditions under a load of 40 mN in De Jalon solution containing 3.10<sup>-7</sup> M atropine, 10<sup>-6</sup> M phentolamine and 3.10<sup>-7</sup> M propranolol. Electrical field stimulation (EFS) was applied with 10 s trains at 9V (submaximal), 1ms, 1.5 Hz.

**Results :** Prucalopride (10<sup>-6</sup> M) induced a transient contraction per se that was abolished by as well 10<sup>-6</sup> M tetrodotoxin (TTX), as by 3.10<sup>-7</sup> M of the 5-HT<sub>4</sub>-receptor antagonist GR113808, and reduced by 10<sup>-6</sup> M of the NK1-receptor antagonist FK888. EFS induced on-relaxation, followed by off-contraction ; both responses were TTX-sensitive. The on-relaxation was abolished by 3.10<sup>-4</sup> M L-NAME + 5.10<sup>-7</sup> M apamin ; prucalopride did not consistently influence this NANC on-relaxation. Prucalopride facilitated the NANC off-contraction by 60 to 90% and this effect was abolished by 3.10<sup>-7</sup> M GR113808. The off-contraction was not influenced by 10<sup>-6</sup> M of the NK2-receptor antagonist MEN10627 or 3.10<sup>-7</sup> M of the NK3-receptor antagonist SB222200. In the presence of 10<sup>-6</sup> M FK888 the off-contraction was reduced while the facilitating effect of prucalopride was abolished.

**Conclusion :** In guinea pig proximal colon, prucalopride is able to activate and facilitate tachykininergic neurotransmission through 5-HT<sub>4</sub>-receptors.

EXPRESSION AND DISTRIBUTION OF MAS-RELATED GENE RECEPTORS MRGE AND MRGF IN THE NORMAL AND INFLAMED MURINE ILEUM. L. Avula (1), R. Buckinx (1), K. Alpaerts (1), D. Adriaensen (1), L. Van Nassauw (2), J.P. Timmermans (1). (1) Lab of Cell Biology and Histology, Antwerp, Belgium, (2) Lab of Cell Biology and Histology/Lab of Human Anatomy and Embryology, Antwerp, Belgium.

**Introduction :** Mas-related gene (*Mrg*)-receptors constitute a family of G-protein coupled receptors, of which some members are selectively expressed in nociceptive neurons. Moreover, it is hypothesized that *Mrg* receptors mediate IgE-independent activation of mast cells. Previous studies reported on a role of the *MrgE* receptor in selective pain behavioural responses. The deletion of *MrgE* in mice affected the development of neuropathic pain, as well as the expression of *MrgF* and of a few genes linked to nociception.

**Aim :** Due to lack of detailed data concerning the intestinal expression of *Mrg* receptors, we aimed to investigate the expression of *MrgE* and *MrgF* in the murine ileum, unravelling their putative function during intestinal inflammation.

**Methods :** The expression and distribution of *MrgE* and *MrgF* in the ileum of two murine models of intestinal inflammation - acute intestinal schistosomiasis and TNBS-induced ileitis - were compared with healthy wild type controls. *MrgE* and *MrgF* receptors were localized using immunofluorescence on whole-mount preparations and cryosections. Furthermore, the expression of *MrgE* and *MrgF* mRNAs in the ileal wall of non-inflamed and inflamed ileum was detected by RT-PCR, and a quantitative comparison was performed using qPCR.

**Results :** Immunofluorescence localized both *MrgE* and *MrgF* receptors in distinct neuronal subpopulations and nerve fibres in both enteric plexuses of non-inflamed and inflamed ileum. Significant downregulation of *MrgE* and *MrgF* receptors was observed both in the intestinal schistosomiasis and ileitis models. RT-PCR results corroborated the morphological results showing mRNAs of both receptors to be present in the non-inflamed and inflamed murine ileum. qPCR demonstrated no significant ( $p < 0.05$ ) differential expression of *MrgE* and *MrgF* mRNAs in the schistosomiasis model. However, there was a significant downregulation of both *MrgE* ( $p = 0.001$ , 3 fold) and *MrgF* ( $p = 0.001$ , 6 fold) mRNAs in the ileitis model.

**Conclusion :** This is the first report on the expression and distribution of *MrgE* and *MrgF* in the non-inflamed and inflamed murine ileum. In the schistosomiasis model and during ileitis, the neuronal expression levels of both these receptors were significantly reduced, suggesting a functional role of *MrgE* and *MrgF* in enteric neuronal pathways and a possible involvement in the neuro-inflammatory response.

PRESENCE OF FUNCTIONAL CGRP RECEPTORS ON MOUSE BONE MARROW-DERIVED MUCOSAL MAST CELLS. J. W. Rychter (1), L. Van Nassauw (2), J. P. Timmermans (2), A. B. Kroese (1). (1) UMCU & UU, Utrecht, Netherlands, (2) UA, Antwerp, Belgium.

**Introduction and Aim** : Bidirectional interactions between extrinsic primary afferent nerve endings and mast cells are thought to contribute to the maintenance of gastrointestinal mucosal integrity. The infected or inflamed mucosa shows mastocytosis accompanied by increased density of calcitonin gene-related peptide (CGRP)-containing afferent nerve fibers. CGRP can degranulate mast cells, but it is not known if the activation of mucosal mast cells by CGRP is receptor-mediated or occurs through direct action on the G-protein. Therefore, we investigated the mode of action by which CGRP activates mucosal mast cells.

**Methods** : Mouse bone marrow-derived mucosal mast cells (BMMC) were cultured for 9 days in medium supplemented to selectively promote survival and proliferation of the mucosal mast cell phenotype (containing mMCP-1). For recording of intracellular  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ; 1 image/s), BMMC in culture dishes ( $10^5$  cells/100  $\mu$ l) were loaded with Fura-2 AM (5 $\mu$ M; 20 min). In total,  $Ca^{2+}$  recordings were made from 6071 cells in 173 dishes from 20 cultures. For immunohistochemistry, BMMC on adhesion slides (Paul Marienfeld, Lauda-Königshofen, Germany) were fixed in methanol (10 min) and processed for immunohistochemical staining.

**Results** : BMMC responded to a single application of CGRP with a transient rise in  $[Ca^{2+}]_i$ . The proportion of BMMC responding to CGRP increased dose-dependently (range  $10^{-11}$  M to  $10^{-5}$  M) to a maximum of  $18 \pm 10\%$  at  $10^{-5}$  M (mean  $\pm$  SD; secretagogue C4880 (100 $\mu$ g/ml) 100%), with an  $EC_{50}$  of  $10^{-8}$  M (Boltzmann fit;  $R^2$  0.93). The lag time of the CGRP response decreased from  $63 \pm 20$  s at  $10^{-11}$  M to  $17 \pm 19$  s at  $10^{-5}$  M. Pre-incubation with the non-peptide CGRP receptor antagonist BIBN4096BS (Boehringer, Germany;  $10^{-5}$  M; 1h) completely inhibited BMMC activation by CGRP over the entire concentration range (ANOVA  $p < 0.001$ ; 68 dishes), without affecting the BMMC response to substance P ( $10^{-7}$  M; t-test  $p = 0.49$ ; 17 dishes). Pre-incubation of BMMC with  $LaCl_3$  (5 mM; 40 s) to block the influx of extracellular  $Ca^{2+}$  did inhibit their response to C4880 (t-test  $p < 0.0001$ ; 20 dishes), but did not affect the response to CGRP  $10^{-6}$  M (t-test  $p = 0.18$ ; 20 dishes). The presence of the CGRP receptor on BMMC was confirmed by immunoreactivity after incubation with combinations of antibodies against the two components of the CGRP receptor (RAMP1 and CRLR) and against mMCP-1.

**Conclusions** : We conclude that functional CGRP receptors ( $EC_{50}$   $10^{-8}$  M) are present on BMMC and that their activation causes mobilization of  $Ca^{2+}$  from intracellular stores. Receptor-independent activation of BMMC by CGRP was not observed over the concentration range used (up to  $10^{-5}$  M). CGRP receptors thus constitute a component in the bidirectional communication between CGRP-containing afferent nerve endings and mucosal mast cells.

ROLE OF THE GPR39 RECEPTOR, A MEMBER OF THE GHRELIN RECEPTOR FAMILY, IN THE REGULATION OF GLUCOSE HOMEOSTASIS IN A MOUSE MODEL OF DIET-INDUCED OBESITY. P.J. Verhulst, A. Lintermans, S. Janssen, J. Buyse, J. Tack, I. Depoortere. KU, Leuven, Belgium.

**Introduction** : The GPR39 receptor is a member of the family that also includes receptors for ghrelin and motilin. The downregulation of GPR39 mRNA in adipose tissue of obese type 2 diabetic patients suggests that GPR39 may contribute to the pathogenesis of the disease.

**Aim** : Our aim was to study the role of GPR39 in body weight control and glucose homeostasis in a mouse model of type 2 diabetes.

**Methods** : GPR39 knockout (GPR39 $^{-/-}$ ) and wild-type (GPR39 $^{+/+}$ ) mice were fed a high-fat diet (HFD) (60% kcal from fat) or standard diet (SD) during 30 weeks. An oral glucose tolerance test (OGTT; 2g/kg) was performed at week 15 and 30. Changes in plasma insulin and ghrelin levels were measured by radioimmunoassay. Respiratory quotient (RQ) was determined by indirect calorimetry.

**Results** : Expression of GPR39 mRNA in epididymal adipose tissue of fasted GPR39 $^{+/+}$  mice was reduced after 30 weeks on a HFD (SD :  $5.5 \pm 0.5$  vs HFD :  $3.7 \pm 0.5$ ;  $P < 0.05$ ). Body weight, fat percentage and energy intake was increased in the HFD-group but did not differ between both genotypes. A positive correlation ( $r$  : 0.79,  $P < 0.001$ ) was observed between GPR39 expression in adipose tissue and fasted blood glucose levels of GPR39 $^{+/+}$  mice on a HFD but not on a SD. Within the HFD-group, blood glucose levels were lower ( $P < 0.01$ ) in GPR39 $^{-/-}$  than in GPR39 $^{+/+}$  mice between week 22 and 30. Evidence of altered insulin sensitivity was obtained in an OGTT where either a reduction (week 15) or no change (week 30) in blood glucose levels was observed in GPR39 $^{-/-}$  mice compared with GPR39 $^{+/+}$  mice despite significant reductions in plasma insulin levels in GPR39 $^{-/-}$  mice. RQ values were increased in GPR39 $^{-/-}$  mice on a HFD but not on a SD, indicating that GPR39 $^{-/-}$  mice preferentially used carbohydrates as energy source. This is an agreement with the lower blood glucose levels observed in GPR39 $^{-/-}$  mice and the possible alterations in insulin

sensitivity. Plasma octanoylated ghrelin levels were markedly higher ( $P < 0.05$ ) in GPR39<sup>-/-</sup> ( $42.8 \pm 7.6$  pg/ml) than in GPR39<sup>+/+</sup> mice ( $11.8 \pm 1.2$  pg/ml) after 30 weeks on a HFD but not in mice on a SD. Ghrelin levels were inversely proportional to plasma insulin levels (GPR39<sup>-/-</sup> :  $12.1 \pm 1.8$  ; GPR39<sup>+/+</sup> :  $30.7 \pm 6.7$  pg/ml) but were not significantly correlated. Gastric emptying ( $T_{1/2}$ ) was delayed by the HFD but did not differ between both genotypes (GPR39<sup>+/+</sup> :  $126 \pm 6$  min ; GPR39<sup>-/-</sup> :  $125 \pm 7$  min) suggesting that it does not contribute to differences in blood glucose levels between both genotypes.

**Conclusion :** Our results suggest that GPR39 plays a role in the regulation of glucose homeostasis but not in the control of body weight during conditions of increased demand for insulin secretion such as diet-induced obesity.

- B16 -

ROLE OF GHRELIN AND GHRELIN RECEPTORS IN THE EFFECT OF BITTER TASTE RECEPTOR AGONISTS ON FOOD INTAKE AND GASTRIC EMPTYING. S. Janssen, P.J. Verhulst, J. Laermans, J. Tack, I. Depoortere. KU, Leuven, Belgium.

**Introduction :** Ghrelin is an orexigenic hormone with gastroprokinetic properties, mainly produced by the stomach. Ghrelin secretion depends on the nutritional composition of the meal but the factors involved in chemosensation of the ghrelin cell are not known. Transcripts for bitter taste receptors, T2R, and the gustatory G proteins,  $\alpha$ -gustducin and  $\alpha$ -transducin are expressed by the oral cavity and the gastrointestinal mucosa and may be part of a wide chemosensing system of luminal contents.

**Aim :** To investigate whether T2R agonists may affect ghrelin release and consequently food intake and gastric emptying.

**Methods :** The co-localisation of ghrelin with  $\alpha$ -gustducin and  $\alpha$ -transducin was investigated by double immunofluorescence staining of sections of the mouse stomach. Mice were fasted for 16h and gavaged with a bitter taste receptor agonist mixture (denatonium benzoate(10mM), phenylthiocarbamide(10mM), 6-propyl-2-thiouracil (5mM), quinine (1,5mM), D-[-]salicin (5mM)). Blood samples were taken at several time points (0-40 min) after gavage and plasma ghrelin levels were determined by radioimmunoassay. Food intake after administration of bitter taste receptor agonists was monitored and the effect on gastric emptying was determined by the <sup>13</sup>C octanoic breath test.

**Results :** Several ghrelin positive cells were co-localized with  $\alpha$ -gustducin and  $\alpha$ -transducin in enteroendocrine cells of the mouse corpus. Oral gavage of the bitter taste receptor agonist mixture resulted in a time-dependent increase in plasma total ghrelin levels : from  $1295 \pm 105$  pg/ml (0 min) to  $1583 \pm 181$  pg/ml (20 min),  $2196 \pm 93$  pg/ml (30 min) and  $2892 \pm 268$  pg/ml (40 min). Plasma octanoyl ghrelin levels also increased from  $72 \pm 7$  pg/ml to  $231 \pm 49$  pg/ml (40 min). The increase in plasma ghrelin levels after gavage of T2R agonists was accompanied by a significant increase in food intake during the first half hour (saline :  $1.35 \pm 0.09$  ; T2R agonists :  $1.61 \pm 0.07$  g/h). During the following 4 hours food intake was dramatically decreased by 71% in T2R agonists-treated mice compared to 31% in saline-treated mice. Gastric half emptying time ( $T_{1/2}$ ) decreased in parallel after oral gavage of T2R agonists from  $123 \pm 6$  min to  $315 \pm 72$  min. In GHSR<sup>-/-</sup> mice the decrease in  $T_{1/2}$  was even more pronounced (from  $145 \pm 12$  min to  $1177 \pm 379$  min).

**Conclusion :** Oral administration of bitter taste receptor agonists induces an increase in plasma ghrelin levels which results in a temporary increase in food intake. This is followed by pronounced decrease in food intake which may be due to a prominent delay in gastric emptying which also involves activation of ghrelin receptors to counterbalance the effect.

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

**Introduction** : The immotile (primary) cilium is a unique cellular structure (Wheatley DN. Cell Biol Int 2005) which importance has been recently highlighted in signal transduction pathways crucial for embryological development (Eggenchwiler JT. Annu Rev Cell Dev Biol 2007), tissue differentiation (Satir P. Annu Rev Physiol 2007) and diseases (“ciliopathies”) (Fliegauf M. Nat Rev Mol Cell Biol 2007). The presence of a cilium in ICC, known from ultrastructural studies for decennia, has been recently revisited (Junquera C. J Cell Mol Med 2007).

**Aim** : To investigate primary cilium in the mouse GI tract using immunofluorescence (IF).

**Methods** : Double IF procedure for the ICC marker KIT and for the cilium marker ACIII (Bishop GA. J. Comp. Neurol. 2007) was carried out on cryostat sections of PAF fixed E17.5, P14 and adult WT mouse gut.

**Results** : ACIII-ir primary cilium in the gut presented as a single, tiny structure protruding from the perinuclear cytoplasm, approx 0.5 micron diameter by 2 microns length - hence much shorter than in CNS neurons where they can reach up to 10 microns. ACIII-ir cilium were widespread at E17.5, while in the postnatal gut, they were scarce and restricted to ENS neurons and KIT-ir ICC in antrum and jejunum.

**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.

BIOENGINEERING OF CIRCULAR OESOPHAGEAL DEFECTS. V. Coulic (1), V. Maquet (2), P. Delree (3), C. Deprez (1), S. Najar (1), S. Van Daele (1). (1) ULB Brugmann, Brussels, Belgium, (2) KITOZYME, Herstal, Belgium, (3) IRSPG, Gosselies, Belgium

**Introduction** : Up to now plastic surgery of oesophagus circular defects remains a difficult problem . Combined prosthesis composed of living tissues growing on polymer scaffolds have been recently proposed as novel therapeutic solution but still need further investigations. We have first shown that chitosan, as biodegradable and biocompatible natural polymer, can be used for this purpose (BWGE 2007) : chitosan scaffolds do not alter the development of implanted foetal oesophagus and causes only a mild inflammatory reaction in the surrounding tissues.

**Aim** : The aim of the present work was, in a second step, to use chitosan tubes as prosthesis for oesophagus segment repair, either alone or in combination with foetal oesophagus implants.

**Material and methods** : Experiments were carried out on 22 Rats, both males and females ; BW 250-400 g, (with respect of Bioethics rules and local Ethic Committee agreement).

Three experimental series were performed, bridging an oesophagus gap (0.5 – 1.0 cm :) 1°) by a chitosan prosthesis (10 rats) ; 2°) by a foetal oesophagus implant grafted for 1-3 months before in the neck site and grown until reaching a sufficient size (6 rats) ; 3°) by a combination of a chitosan prosthesis and a foetal oesophagus implant, the prosthesis being enwrapped by a flap of grown foetal oesophagus (6 rats).

In the post operation period, clinical observation, ultrasound and optical microscopy investigations were provided.

**Results** : Foetal oesophagus implants grafted in the neck site developed as monstrous cysts up to 2x1 cm with all the morphologic features of adult organ and blood supply from multiple vessels. The use of foetal implant alone to bridge the oesophageal defect (series 2) was difficult because of the alteration of the blood supply during the manipulation and partial necrosis of the replacing segment. On the other hand, the use of chitosan tubes alone (series 1) was more successful, with an animal survival time longer than 2 weeks. A fibrous sheet was formed around the prosthesis but epithelium growth on its inner side was not observed. In all cases, transit problems were present especially for solid food. The combination of chitosan prosthesis with foetal implants either previously grown in vivo (series 3) or cultivated in vitro, using foetal oesophageal cells/tissues cultured on the outer face of the chitosan tube, is ongoing

**Conclusion** : Bioengineering of the oesophagus is one of the promising ways for replacement of oesophagus segments. The main challenges are to optimize the polymer scaffold degradation and the epithelium growth on the inner surface of the “new formed” oesophagus in order to avoid further stricture and to ensure satisfactory motility allowing food transit.



## JOINT MEETING

- D01 -

IMPACT OF TIGHT GLYCEMIC CONTROL IN PATIENTS WITH DECOMPENSATED CHRONIC LIVER DISEASE ADMITTED TO INTENSIVE CARE : RESULTS OF A SINGLE-CENTRE RANDOMIZED CONTROLLED TRIAL. W. Laleman, A. Moonen, C. Verslype, P. Meersseman, F. Nevens, G. Van den Berghe, A. Wilmer. University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction :** Hyperglycemia in critically ill patients (diabetes of injury) has been increasingly recognized as an independent risk factor of adverse outcome. In recent years, tight glycemic control by intensive insulin therapy (IIT) was shown to reduce morbidity and mortality in an unselected group of patients admitted to ICU.

**Aim :** Given the fact that the liver plays a central role in glucose-homeostasis, chronic liver diseases are often associated with impaired glucose tolerance and patients with decompensated chronic liver disease often rapidly develop multi-organ failure, we hypothesized that IIT might favour the outcome of medical critical illness in patients with decompensated chronic liver disease.

**Methods :** This was a pre-planned subgroup analysis of 117 patients with decompensated chronic liver disease out of a large randomized-controlled study (n = 1200, NEJM 2006 ;2 ;354 :449-61) studying the effects of IIT. Patients were randomly allocated to either IIT (glycemia 80-110 mg/dl) or conventional insulin therapy (CIT) requiring insulin above a glycemia of 215 mg/dl. Outcome parameters involved in-hospital-, ICU- and 30-day-mortality, length of hospital and ICU stay, use of intensive care resources (cumTISS-28-score), MELD&SOFA-score, initiation and days of dialysis, mechanical ventilation and vasopressors, bacteremia and adverse events.

**Results :** 117 patients were analysed (CIS-arm n = 49 ; IIT-arm n = 68). Patient demographics showed no differences between groups. Overall, patients were predominantly males (75%), on average 58 years old, with BMI  $25.1 \pm 0.6$ , history of diabetes in 25% of cases, blood glucose on admission of  $157 \pm 5.5$  mg% and SOFA and MELD on admission of  $8.3 \pm 0.5$  and  $15.4 \pm 1.3$ , respectively. IIT reduced blood glucose levels (mean 92.5 mg/dl ; CI 95% : 89.0 to 96.0 vs CIS : 153 mg/dl, CI95% : 147 to 159 ;  $P < 0.001$ ), but did not affect mortality. However, significant differences were observed for IIT with regard to need for dialysis (RR -15.2%,  $P = 0.042$ ) and mechanical ventilation (RR-20.2%,  $P = 0.050$ ). No further differences were observed with regard to cumTISS-28-score, initiation and days of vasopressors and incidence of bacteremia. Hypoglycemia occurred more frequently in the IIT-group (13.2% versus 2%,  $P = 0.001$ ).

**Conclusion :** IIT in patients with decompensated chronic liver disease admitted to ICU is associated with reduced morbidity but not mortality. Further large sample-size multi-centre studies are needed to confirm these findings.

- D02 -

LIVER HISTOLOGY ACCORDING TO THE PRESENCE OF THE METABOLIC SYNDROME IN OVERWEIGHT AND OBESE PATIENTS. A. Verrijken, S. Francque, I. Mertens, G. Hubens, E. Van Marck, P. Michielsen, L. Van Gaal. UZ, Antwerpen, Belgium.

**Introduction :** Paralleling the increasing prevalence of obesity, diabetes, and the metabolic syndrome (MS), non-alcoholic fatty liver disease (NAFLD) has become a common cause of chronic liver disease worldwide. Insulin resistance (IR) is considered a key component of the MS and plays a major role in the pathogenesis of NAFLD.

**Aim :** We wanted to investigate the histologic features of the liver according to the presence of the MS.

**Methods :** MS, as defined by the NCEP-ATP III (2005), was assessed in an overweight and obese population who presented to the obesity clinic between August 2006 and February 2009 without an a priori suspicion of liver disease, and who underwent a liver biopsy because a clinical suspicion of NAFLD was risen. Insulin resistance was estimated using HOMA (Homeostasis Model Assessment).

**Results :** 198 patients were studied. Mean age was  $45 \pm 13$  y ; 66% were female, mean BMI was  $38.3 \pm 6.4$  kg/m<sup>2</sup>. In 71.2% one or more of the liver tests were elevated, 57.1% has the MS according to the NCEP-ATP III criteria (2005). 111 (56.1%) patients did not have fibrosis, 46 (33.3%) had stage 1-2 fibrosis, and 21 (10.6%) had advanced (stage 3-4) fibrosis. A Chi-square test was used to detect a significant association between MS and grade of steatosis ( $p = 0.002$ ). Subjects without inflammation had a significant lower incidence of the MS compared to subjects with signs of inflammation ( $p < 0.001$ ). On the other hand no association was found comparing subjects without fibrosis and subjects with signs of fibrosis. A Kruskal-Wallis test revealed a statistically significant difference in HOMA-IR across the different grades of steatosis ( $p < 0.001$ ) and inflammation ( $p < 0.001$ ), with steatosis grade 3 and inflammation grade 3 presenting the highest scores of HOMA-IR.

**Conclusion :** In an unselected population of overweight patients features of NAFLD are usually mild, 10% however have advanced fibrosis. Besides steatosis inflammation seems to play a major role in both insulin resistance and the MS. 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).

NAFLD LIVER FAT SCORE STRONGLY CORRELATES WITH HISTOLOGICAL SEVERITY OF NAFLD AND NASH IN A LARGE COHORT OF PROSPECTIVELY INCLUDED OVERWEIGHT PATIENTS. S. Francque, A. Verrijken, I. Mertens, G. Hubens, E. Van Marck, P. Michielsens, L. Van Gaal. UZ, Antwerpen, Belgium.

**Introduction** : A non-invasive NAFLD Liver Fat Score (NLFS) calculation has recently been published (Kotronen et al, Gastroenterology 2009). MR spectroscopy was used as golden standard. The score doesn't claim to distinguish between simple steatosis and NASH, and has to date not been related to histology.

**Aim** : To study the relation between the NLFS and liver histology in a large, prospectively included cohort of overweight patients without an a priori suspicion of liver disease, and to test its diagnostic performance in identifying NAFLD and NASH.

**Methods** : Patients presenting to the obesity clinic for a problem of overweight underwent a metabolic and liver assessment. If NAFLD was suspected, a liver biopsy was proposed. Liver biopsy was scored using the NASH CRN scoring system.

**Results** : A series of 194 patients were prospectively included. Mean age was  $45.6 \pm 12.7$  y ; 131 (66.2%) were female. Mean BMI was  $38.3 \pm 6.4$  kg/m<sup>2</sup>. The whole spectrum of NAFLD from normal liver to NASH cirrhosis and with NAS ranging 0-7 and fibrosis 0-4 was present. Mean ALT was  $49.0 \pm 29.7$  U/L. Mean NLFS was  $1.102 \pm 2.284$  (range -2.768 to 11.249). NLFS strongly correlated with the degree of steatosis ( $r = 0.486$ ,  $p < 0.0001$ ), inflammation ( $r = 0.342$ ,  $p < 0.0001$ ), NAS ( $r = 0.461$ ,  $p < 0.0001$ ) and fibrosis ( $r = 0.385$ ,  $p < 0.0001$ ). For the diagnosis of NAFLD, AUROC was 0.724 (95%CI 0.636-0.811). Using the proposed cut-off of -0.640 to predict NAFLD, sensitivity was 82% and PPV 87%, overall accuracy is 75%. In binary logistic regression analysis, ALT is the only independent predictor of NAFLD ( $p = 0.014$ ). AUROC of ALT for the diagnosis of NAFLD is 0.781 (95%CI 0.691-0.870). For the diagnosis of NASH (NAS<sup>35</sup>), AUROC of NLFS was 0.789 (95%CI 0.715-0.863) and of ALT 0.735 (95%CI 0.651-0.818). NLFS is the only independent predictor for the presence of NASH ( $p = 0.009$ ).

**Conclusion** : NLFS, designed using MR spectroscopy as golden standard, correlates well with the degree of steatosis on histology. Its performance in differentiating non-steatosis from NAFLD is, however, less using histology as golden standard, and not better than ALT. NLFS also strongly correlates with inflammation and NAS, and performs better than ALT for identifying NASH. Finally, NLFS correlates with the stage of fibrosis.

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).

LIVER TRANSPLANTATION USING GRAFTS FROM DONORS OLDER THAN SEVENTY AND EIGHTY YEARS OLD. T. Darius, D. Monbaliu, W. Coosemans, R. Aerts, I. Jochmans, J. De Roey, B. Desschans, G. Van Helleputte, D. Claes, W. Laleman, W. Van Steenberghe, C. Verslype, F. Nevens, J. Pirenne. University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : The use of liver grafts from donors older than 70 and 80 yo is an underused option to expand the donor pool and reduce the mortality on the liver transplantation (LTx) waiting list. No large single center data are available.

**Aim** : We reviewed our experience of LTx using septuagenarian and octogenarian donors.

**Methods** : From 03-02-2003 to 18-09-2009, 46 of 377 LTx (12%) were performed using donors > 70 yo. These donors originated from : local 9(20%), national 34(74%), and international hospitals 3(6%). Mean donor age was 77 yo (71-86). 13 (28%) donors were older than 80. Main cause of death was cerebrovascular hemorrhagic/ischemic accidents (77%) and trauma (15%). Mean donor sodium was 144 mmol/L. Liver function tests were normal at time of referral. 43 organs were preserved with University of Wisconsin solution, 3 with HTK.

**Results** : 46 patients (mean lab MELD 16) received a liver graft from a donor > 70 yo. Mean recipient age was 61 (45-75). Indications for LTx were postethyl cirrhosis (22), cryptogenic liver cirrhosis (6), hepatitis C virus (5), and others (13). Mean cold and warm ischemia times were 8h22 (3h34-13h44) and 48' (31'-1h28), respectively. Mean peak aspartate transaminase and alanine transaminase were 1190 IU/L (67-12802) and 1038 IU/L (47-5071), respectively. No hepatic artery thrombosis or primary non function were observed but 2 patients suffered from severe graft dysfunction : 1 was successfully treated with 3 sessions of molecular absorbent recirculating system and 1 spontaneously recovered. Mean hospital stay was 28 days (11-144). The 1 and 5 year patient and liver graft survival were 90,7% and 75,8%, and 88,8% and 73,5%, respectively. 5 (11%) patients developed anastomotic biliary strictures, all endoscopically treated. 1 patient needed retransplantation 57 days post-LTx because of refractory rejection.

**Conclusion** : Short and middle-term survival following LTx with grafts from extremely old donors are excellent. Longer-term follow up is necessary. Brain death due to cerebrovascular ischemia/bleeding is frequent in patients older than 70 yo, but these potential donors are underreported and underused. Larger use of these liver grafts is likely to increase the donor pool and substantially reduce the mortality on the waiting list.

URGENT EARLY ( $\leq 15$  DAYS) ARTERIAL REVASCULARIZATION FOLLOWING HEPATIC ARTERY THROMBOSIS MAY AVOID GRAFT LOSS AND IMPROVE OUTCOME IN ADULT LIVER TRANSPLANTATIONS. A. Scarinci, M. Sainz-Barriga, I. Colle, H. Van Vlierberghe, A. Geerts, B. Van Den Bossche, F. Berrevoet, X. Rogiers, R. Troisi, B. De Hemptinne. University Hospital, Ghent, Belgium

**Introduction** : Hepatic artery thrombosis (HAT) represent a devastating complication of orthotopic liver transplantation (OLT) occurring in 3% to 9% of all transplants and is also one of the main causes of biliary complications (BC) following OLT. Therapy treatments of HAT include : thrombectomy, thrombolysis/angioplasty or liver retransplantation.

**Aim** : Aim of this study was to evaluate outcomes following hepatic artery thrombosis.

**Methods** : We retrospectively analyzed data of 674 adult OLT available from our database between January 1992 and September 2009 (whole-size, LDLT, NHBD and split grafts included). HAT was classified as early (E-HAT) when occurring within the first 30 days after OLT and as late HAT (L-HAT) if diagnosed from 30 days post-OLT. Diagnosis of HAT was suspected clinically and confirmed by Doppler US or angiography. Attempt to revascularization in E-HAT was defined as early (ER) if done prior to 15 days post-OLT and late (LR), if done between 15 and 30 days post-OLT. No attempt to revascularization was done after this period.

**Results** : The median recipient age was of 59 y (range 26-68) and the median follow-up was of 60 months (range 2-181). HAT occurred in 26/674 grafts (3.8%) : E-HAT was recorded in 20/26 (2.9%) and L-HAT in 6/26 (0.9%) cases. HAT occurred in 16(61%) full-size grafts, 6(23%) LDLT and 4(16%) split grafts. ER was done in 16/20(80%) patients leading to 62% graft salvage. However, graft salvage was 81% if the revascularization was performed within the first week post-OLT ( $p = 0.03$  for ER in the 1<sup>st</sup> w and ER in the 2<sup>nd</sup> w). LR was unsuccessful in 100% of cases ( $p = 0.08$  for ER vs. LR). In only 1 patient HAT was discovered incidentally @ 6 m post OLT and remained clinically asymptomatic. The overall incidence of BC in rescued grafts was of 50% without graft loss during FU. Overall mortality following HAT was of 27% (33% following retransplantation). Graft survival was of 79% and 71% ; 50% and 50% @ 1 and 3 y respectively for E-HAT and L-HAT ( $p = ns$ ).

**Conclusion** : HAT in adult liver transplantation lead to a major morbidity and mortality. Urgent revascularization in case of early HAT may decrease graft loss especially when performed within the first week of OLT improving overall outcome.

OUTCOME OF PATIENTS WITH HEPATOCELLULAR CARCINOMA LISTED FOR LIVER TRANSPLANTATION BEFORE AND AFTER THE MELD ALLOCATION SYSTEM. B. Vos, C. Moreno, V. Lucidi, A. Gerkens, A. Buggenhout, N. Bourgeois, V. Donckier, M. Adler. ULB Erasme, Brussels, Belgium.

**Introduction** : Introduction of the Model for End-Stage-liver Disease (MELD) allocation system in the US, with its exceptions for hepatocellular carcinoma (HCC), has increased the likelihood of a patient with HCC to receive a deceased organ liver transplant (LT). What is less clear is the overall and disease-free survival benefit.

**Aim** : The aim of our study was to analyse the effects of this new rule on the outcome of patients listed for LT in our center.

**Methods** : Twenty three patients listed for HCC as a first diagnosis in the pre-MELD era (from 10/1999 to 10/2004) were compared to 24 patients with the same indications in the post-MELD era (from 12/2006 to 12/2008). On an intention to treat basis, the 2 groups were compared for patient characteristics, tumor therapy, rate of transplantation, clinical and p TNM, survival and HCC recurrence.

**Results** : At listing, the 2 groups were identical for age, gender, ABO blood group, underlying liver disease, MELD, Child-Pugh score alpha-foetoprotein level and TNM distribution. By contrast, median tumor number was lower in post-MELD vs. pre-MELD : 1 (range : 1-6) vs. 2 (range : 1-8),  $p = 0.029$ . Specific pre-LT therapy before listing was more frequent in the post-MELD score group : 6/23 (26%) vs. 13/24 (54%),  $p = 0.002$ . There was no significant difference in terms of type of therapy used, neither in the number of patients outside Milan criteria : 5/6 (83%) vs. 5/13 (38%),  $p = 0.076$ . Waiting list outcomes were similar in terms of delisting rate : pre-MELD : 5/23 (21%) vs. 4/24 (17%),  $p = 0.067$  ; death while waiting : 4/23 (17%) vs. 1/24 (4%),  $p = 0.176$  and transplantation rate : 15/23 (65%) vs. 18/24 (75%),  $p = 0.243$ . The median transplantation delay was shorter in the post-MELD group : 4.8 vs. 2.5 months, ( $p = 0.011$ ) but this difference was only significant for the first year (2007) of application of the new allocation system and the median waiting time increased significantly in 2008 : 1.6 up to 3.7 months in 2007 and 2008 respectively,  $p = 0.019$ . No differences were found for post-LT outcomes in terms of recurrences : 2 vs. 4,  $p = 0.547$  ; death at 3 months : 5 vs. 0,  $p = 0.206$  ; death at one year : 7 vs. 3,  $p = 0.450$  and cause of death distribution.

**Conclusion** : Introduction of MELD score for liver allocation has decreased the delay for LT in patients listed for HCC. This was associated with a trend for a reduction of mortality while waiting. The potential benefit on long-term outcome and mostly on post-transplant tumor recurrence should be further evaluated with a longer follow-up.

## Invited lecture

- D07 -

Marc Hautekeete Lecture :  
SEPSIS AND CIRRHOSIS  
J.L. Vincent, Brussels, Belgium.

- D08 -

EVALUATION OF PANCREATIC TUMORS WITH CONTRAST ENHANCED-ENDOSCOPIC ULTRASONOGRAPHY AND EUS-STRAIN RATIO ELASTOGRAPHY. A. Badaoui (1), I. Borbath (2), M. Maffei (2), T. Aouattah (2), C. Gillain (3), T. De Ronde (1), P. Deprez (2). (1) UCL, Mont-Godinne, Belgium, (2) UCL Saint-Luc, Brussels, Belgium, (3) Hopital St Joseph, Gilly, Belgium

**Introduction :** EUS-elastography (EUS-E) is a new imaging tool that might improve the distinction between fibrous, benign and malignant tissue by real-time analysis of stiffness and strain of tissue with determination of colored elastic score and ratio (SRE). The vascular pattern enhanced by contrast agents could also help to differentiate malignant and benign pancreatic lesions.

**Aim :** The aim of the study was to assess the ability of SRE and CE-EUS to distinguish benign and malignant pancreatic lesions.

**Methods :** EUS-E, SRE, CE-EUS, and EUS-FNA were prospectively studied in consecutive patients with pancreatic masses from Jan 2008 to Nov 2009. Qualitative elastography and a strain ratio elastography were carried-out on a Hitachi 8500 platform with an integrated elastography software. SRE was calculated as “focal lesion area strain / surrounding tissue area strain”. Positivity for malignancy were class IV to V for EUS-E and strain ratio cut-off  $> 10$  for SRE. CE-EUS was performed with injection of 2.4 ml Sonovue (Bracco). Gold standards were FNA cytology results, surgery or follow-up.

**Results :** 54 patients have been included with a mean age of the patients was  $60 \pm 16$  years, mean size of pancreatic lesions was  $29 \pm 12$  mm. Results and comparison of methods are shown in table.

*PA = pancreatic adenocarcinoma, NET = neuroendocrine tumour, INF = inflammatory masses*

**Conclusion :** Adenocarcinomas were significantly harder and more hypovascular than NET and inflammatory masses. SRE does not improve sensitivity and specificity of qualitative elastography. Contrast enhanced EUS is mainly helpful to characterize atypical masses such as NET. Both elastography and CE-EUS have high NPV making them important tools in screening high risk patients.

	<b>n</b>	<b>Elastic pattern</b>	<b>SRE</b>	<b>CE-EUS</b>
<b>PA</b>	29	IV-V	23.8 ± 11.8	Hypovascular
<b>NET</b>	7	V	10,1 ± 4,3	Hypervascular
<b>INF</b>	18	II-III -IV	7,1 ± 0,8 (p < 0.05 vs PA)	Hypo or Hyper
<b>Sens</b>		100%	80%	100%
<b>Spec</b>		64%	67%	67%
<b>PPV</b>		87%	89%	78%
<b>NPV</b>		100%	50%	100%

- D09 -

ENDOSCOPIC ULTRASOUND (EUS) GUIDED TRANSLUMINAL DRAINAGE OF PANCREATIC DUCT OBSTRUCTION : LONG TERM OUTCOME. M. Ergun, T. Aouattah, C. Gillain, H. Piessevaux, J.F. Gigot, C. Hubert, P. Deprez. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : EUS guided transluminal drainage (ETLD) has been reported as an alternative to surgery for pancreatic drainage when transpapillary access of the main pancreatic duct is impossible.

**Aim** : The aim of the study was to investigate feasibility and long term clinical outcome of ETLD.

**Methods** : We analyzed our single centre experience with this technique over a 10 year period. We retrospectively reviewed our database and collected clinical data including demographic information, indication for pancreatic drainage, technique and complications about the procedure. Follow-up information was obtained by reviewing charts, telephone contact and scheduled visits.

**Results** : ETLD was attempted in 19 patients (25 interventions) over the study period. Mean age was 62 y (range 36-78), 5 patients (26%) were women. The most common indication for the procedure was post-Whipple symptomatic anastomotic stricture (n = 9). Indications for a Whipple procedure were IPMT (n = 7), ampulloma (n = 1), chronic pancreatitis (n = 1). Complete rupture of MPD (n = 6) and chronic pancreatitis with tight stricture (n = 4) were other indications. Transluminal drainage was performed by transgastric (n = 10) or transbulbar (n = 4) route or with a rendezvous technique (n = 5), using 22 or 19 g FNA needle (n = 10), 0.035 guidewire and a 6.5 Fr cystogastrostome (n = 9). Wirsungography was successfully performed in 24/25 (96%) attempts, although drainage ratio was 21/25 (84%) ETLD, resulting in a success rate of 16/19 (84%) pts. There were 2 procedure related complications : bleeding which was treated endoscopically with hemoclips and perigastric collection which resolved spontaneously. Mean follow up was 51 months (range 3-120 months). Stent dysfunction occurred in 9 (47%) pts : stent exchange was performed in 5 and redo ETLD in 4 pts (migrated stents). Out of 16 pts with successful ETLD, 14 were evaluated for long term pain resolution which was observed in 93% of pts.

**Conclusion** : EUS guided transluminal pancreatic drainage is feasible achieving 84% technical success and 93% clinical long term resolution of pain, with a very low complication rate. It might therefore replace surgery in tertiary centres.

COMPARISON OF SPORADIC GENETICALLY DETERMINED PANCREATITIS WITH AN AGED MATCHED CONTROL GROUP OF PATIENTS WITH IDIOPATHIC PANCREATITIS. C. Hamoir, X. Pepermans, K. Dahan, H. Piessevaux, A. Geubel, J.F. Gigot, P. Deprez. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : Idiopathic pancreatitis is considered to a complex multigenic and multifactorial disease. Genetically determined pancreatitis is associated with mutations in the cationic trypsinogen (PRSS1), SPINK1 and CFTR genes.

**Aim** : The aims of this study was to compare clinical and morphological characteristics of patients diagnosed with genetically determined sporadic pancreatitis (GP) with an aged matched control group.

**Methods** : Inclusion criteria were the presence of PRSS1, CFTR and SPINK gene mutation in patients with sporadic idiopathic recurrent or chronic pancreatitis. Genetic testing was performed in 351 probands referred to our centre from 1999 until 2009. Aged matched patients with idiopathic pancreatitis and negative genetic testing served as controls.

**Results** : 68 patients were compared in both groups (14 PRSS1 mutations, 17 SPINK1, and 43 CFTR). Of the 43 pts with a CFTR mutation 3 carried a SPINK1 and 1 a PRSS1 mutation. One pt was transheterozygote PRSS1/SPINK1. Follow-up now extends to a median of 6.8 y. Four pts (5.8%) presented with a cancer during follow-up, all of them with a CFTR mutation and heavy smoking habits. Comparison with control group is shown in table.

	PRSS1	SPINK	CFTR	GENETIC	CONTROLS	P value
Age onset y	24	31	31	27 [3-65]	38 [1-73]	0.001
N panc bouts	5.1	2	5	4.7 [1-10]	4.1 [1-10]	0.05
Age Calcific.	28y	36y	33y	36y	41y	0.02
Diabetes	16%	30%	17%	16%	7.5%	0.09
Cancer	0	0	4	5.8%	0%	0.05

**Conclusion** : Comparison of patients diagnosed with genetically determined sporadic pancreatitis (GP) with an aged matched control group showed interesting significant differences including but higher cancer risk (linked to CFTR mutations and not PRSS1) in the genetic group. Search for mutations might therefore be of interest in patients, including smokers, younger than 40 years.

HEPATOCELLULAR CARCINOMA AND UNDERLYING LIVER CIRRHOSIS ; INFLUENCE ON TREATMENT OR A PROGNOSTIC VALUE ? C. D. Witjes, R. A. De Man, F.A. Eskens, R. S. Dwarkasing, P. E. Zondervan, C. Verhoef, J. N. Ijzermans. Erasmus Medical Center, Rotterdam.

**Objective** : Hepatocellular carcinoma (HCC) occurs in cirrhotic and non-cirrhotic livers. We investigated whether presence of underlying liver cirrhosis affected treatment and survival of HCC.

**Aim** : To compare patients with HCC having undergone curative treatment in cirrhotic and non-cirrhotic livers.

**Methods** : Retrospective analysis of all diagnosed HCC cases in a single reference center setting. Survival at 1 and 3 years after treatment and recurrence rates were assessed.

**Results** : Between 2000-2008, HCC was diagnosed in 597 patients. Underlying cirrhosis was present in 393 patients (66%) and absent in 204 (34%) patients. Treatment with curative intent was pursued in 239 patients through either partial liver resection, liver transplantation or radiofrequency ablation. The group of patients without cirrhosis contained significantly more females (41% vs 20%) ( $p < 0.001$ ), showed less hepatitis B/C infection (22% vs 51%) ( $p < 0.001$ ) and larger tumour size (median 80 mm (range 3-227) vs median 35 mm (range 3-200)) ( $p < 0.001$ ). Patients without cirrhosis were mainly treated by partial liver resection (85% vs 28%) less by liver transplantation (1% vs 35%) in comparison to patients with cirrhosis. Median follow-up was 48 months (range 1-102). Overall 1 and 3-year survival rates in patients with non-cirrhotic HCC and cirrhotic HCC were 57%, 33% and 55, 32%, respectively. Patients who had undergone potential curative treatment of HCC in non-cirrhotic livers had 1 and 3-year survival rates of 83% and 62%. Patients with potential curative treatment of HCC in cirrhotic livers had 1 and 3-year survival rates of 77% and 57%. HCC with and without cirrhosis had a recurrence rate of 62% and 38% (NS). One year recurrence rate of HCC was 41% in patients with non-cirrhotic livers and 59% in patients with cirrhotic livers (NS).

**Conclusion** : Underlying liver disease is not a prognostic factor for survival after treatment. The presence or absence of underlying cirrhosis did influence treatment of choice, however did not influence recurrence rate or 1 and 3-year survival rates.

IS A MORE AGGRESSIVE SURGICAL MANAGEMENT OF KLATSKIN TUMOUR GRANTED BY BETTER PATIENTS' MORTALITY AND SURVIVAL RATES ? J.F. Gigot, C. Hubert, B. Radu, C. Sempoux, P. Deprez, L. Annet, P. Scalliet, P. Goffette. UCL Saint-Luc, Brussels, Belgium.

**Introduction :** Klatskin tumour is a challenging disease to manage, due to difficult anatomical situation, close relationship with hilar vascular structures, loco-regional invasiveness and associated cholestasis.

**Aim :** To evaluate the potential benefits of a more aggressive multidisciplinary approach to Klatskin tumours.

**Methods :** Longitudinal experience (56 patients) in a university hospital in Belgium in 2 successive periods of treatment (Group 1 : 14 patients, Group 2 : 42 patients) according to a change in treatment philosophy with more aggressive surgery and adjuvant radiation therapy (external beam radiation and endoluminal brachytherapy with Iridium 192). A more aggressive surgery included 1) a non-touch cancer technique, 2) transection at the secondary biliary divisions, 3) routine frozen sections on surgical margins, 4) an extensive retrograde lymphadenectomy, 5) caudate lobectomy in Bismuth type II and III, 6) routine left or right extended hepatectomy, 7) vascular resection and pancreatoduodenectomy, when indicated.

**Results :** The extent of ductal extension according to the Bismuth classification was always underestimated by imaging studies compared to pathology. In Group 2, the resectability rate was 89.5%. The rate of R2, R1 and R0 resection moved from 14%, 43% and 43% in Group 1 to 0%, 21% and 79% in Group 2. The rate of severe complications (Dindo grade 3 or 4), of surgical reoperation and mortality was 29%, 29% and 20% in Group 1 and 33%, 17% and 4.8% in Group 2. The UICC tumour classification was similar in both groups. The actuarial overall and disease-free survival was 0% and 0% in Group 1 and 43.3% and 40.5% in Group 2 ( $p < 0.00001$ ). In Group 2, local resection was associated to significantly poorer survival compared to hepatectomy. Adjuvant radiation therapy decreases survival in R0 resections, improved survival in R1 resections but was associated to 18% of related-complications and 11% of related-death rate.

**Conclusion :** A more aggressive multidisciplinary approach significantly improves long-term survival of patients suffering from Klatskin tumours. However, radiation therapy is associated to a significant rate of severe related complications, justifying restricting its use only to R1 surgical resection at final pathology.

GENE EXPRESSION PROFILING OF CIRCULATING PANCREATIC TUMOR CELLS OBTAINED BY A NEGATIVE DEPLETION STRATEGY. G. Sergeant (1), R. Van Eijdsen (2), T. Roskams (1), V. Van Duppen (1), J. Van Pelt (1), B. Topal (1). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) VIB, Leuven, Belgium.

**Introduction :** Most cancer deaths are caused by metastases, which are the end-results of hematogenous dissemination and growth of tumor cells in distant organs.

**Aim :** To study gene-expression profiles of circulating tumor cells (CTC) in patients with resectable pancreatic ductal adenocarcinoma (PDAC).

**Methods :** In 10 patients with pancreatic cancer, CTC were isolated from 20ml EDTA-treated whole blood using density centrifugation (Oncoquick®), followed by a negative depletion fluorescence activated cell sorting (FACS) procedure combining anti-CD45, anti-CD34, and 7-AAD. Total RNA was isolated from 4 subgroup samples, i.e. CTC, hematological cells (G), original tumor (T), and non-tumoral pancreatic control tissue (P). After RNA quality control, samples of 6 patients were eligible for further analysis. Whole genome (Affymetrix GeneChip HG-U133\_Plus\_2) microarray analysis was performed after double linear amplification of RNA.

**Results :** Using stringent statistical analysis 8,152 genes were finally retained to compare expression profiles of CTC vs. other subgroups, and 1,059 genes found to be differentially expressed. Data were functionally analyzed with 'Ingenuity Pathway Analysis' software and further explored with AmiGO. The pathway with the highest expression ratio in CTC was p38 mitogen-activated protein kinase (p38 MAPK) signaling, known to be involved in cancer cell migration. In the p38 MAPK pathway TGF- $\beta$ 1, cPLA2, and MAX were significantly upregulated. In addition, 9 other genes associated with both p38 MAPK signaling and cell motility were over-expressed in CTC.

**Conclusion :** Gene expression profiles can be obtained from CTC without a priori selection markers. p38 MAPK signaling and cell motility seem to play a pivotal role in CTC promoting metastasis, and may represent novel therapeutic targets.

LONG-TERM RESULTS OF ADULT AND PAEDIATRIC PATIENTS OPERATED FROM CONGENITAL BILE DUCT CYSTS : A MULTI-INSTITUTIONAL STUDY OF 98 PATIENTS. C. Hubert (1), J.Y. Mabrut (2), J. Baulieux (2), C. Ducerf (2), E. De La roche (2), C. Partensky (3), O. Boillot (3), M. Audet (3), R. Dubois (3), J.P. Chappuis (3), C. Gouillat (4), J. Lerut (1), R. Reding (1), J.B. Otte (1), J. de Ville de Goyet (1), P.J. Kestens (1), J.F. Gigot (1). (1) UCL Saint-Luc, Brussels, Belgium, (2) La Croix-Rousse Hospital, Lyon, France, (3) Hôpital Edouard Herriot, Lyon, France, (4) Hôtel Dieu Hospital, Lyon, France.

**Introduction** : Long-term results of adult and paediatric patients operated for congenital bile duct cyst (BDC) are not well known.

**Aim** : To report the results of long-term results of BDC in 93 patients from 4 European institutions, including 54 adults (Group 1) and 39 children aged < 15 years (Group 2).

**Methods** : Retrospective multi-institutional study.

**Results** : According to Todani classification, there were type I BDC in 71%, type II in 4%, type III in 3%, type IV-A in 20% and type IV-B in 1%. A complicated clinical presentation was significantly more frequent in adults (41%) than in children (5%) ( $p = 0.003$ ) and in the 11 patients with prior cyst-enterostomy (82%) ( $p = 0.0001$ ). Synchronous biliary carcinoma was encountered in 5% in the whole series ; 9% in adults and 0% in children. The mortality rate was zero and the complications ( $p = 0.03$ ) and surgical reoperation rates ( $p = 0.003$ ) were significantly greater in adults patients. Eighty-seven patients had a follow-up > 6 months (mean FU : 92 months, range : 6 - 313 months), 84% being alive and disease-free. The 3 patients suffering from synchronous cholangiocarcinoma died within 10 months postoperatively. One of the 2 patients suffering biliary papillomatosis died at 17<sup>th</sup> month, the second being alive 68 months later. According to the Mayo Clinic classification of results (1), excellent and good results were significantly better in Group 2 (91% versus 60% ;  $p = 0.0004$ ), especially for TODANI type 1 BDC (93% versus 63% ;  $p = 0.01$ ). Results were worse in TODANI type IV BDC (67% versus 44%). Metachronous biliary carcinoma was diagnosed in two patients who died 67 and 84 months postoperatively. An additional therapeutic procedure (hepatectomy or percutaneous treatment) was necessary in 6 (30%) patients presenting with type IV-A BDC.

**Conclusion** : Clinical presentation and surgical management of congenital bile duct cysts are more complicated in adults and previous cyst-enterostomy procedures. Complete extra-hepatic cyst excision is the treatment of choice but cannot avoid complications of intrahepatic disease. A long-term follow-up is mandatory to detect late biliary complications or metachronous carcinoma.

1. Gigot et al. J. Hep. Bil. Pancr. Surg. 1996, 3 : 405-411.

MANAGEMENT OF MALIGNANT BILIARY OBSTRUCTION BY ENDOSCOPIC ULTRASONOGRAPHY-GUIDED DRAINAGE. A. Badaoui (1), C. Gillain (2), T. Aouattah (3), I. Borbath (3), T. De Ronde (1), P. Deprez (3). (1) UCL, Mont-Godinne, Belgium, (2) Hopital St Joseph, Gilly, Belgium, (3) UCL Saint-Luc, Brussels, Belgium.

**Introduction** : ERCP with stent placement is currently the standard procedure for the management of malignant biliary obstruction. In case of failure EUS guided drainage may represent an alternative to percutaneous transhepatic biliary drainage catheter and surgery represent alternatives that are associated with high morbidity and mortality rates.

**Methods** : We report 7 patients with malignant biliary obstruction managed by endoscopic ultrasonography-guided biliary drainage (EUS-GBD) in whom ERCP had previously failed. Causes of obstruction were gallbladder cancer (1), colorectal cancer with hilar involvement (1), pancreatic adenocarcinoma (4) and ampulloma (1). The procedure was performed with a linear-array echoendoscope (EG 3830UT, Pentax) under ultrasound, endoscopic and fluoroscopic guidance. The left biliary or the distal common bile duct (CBD) were punctured with a 19-g needle (Cook) under EUS-guidance. Before inserting a 0,035-inch guide wire (Boston Sc) into the biliary duct, cholangiography was performed. If necessary, the tract was enlarged with a 6 Fr cystostome (Endoflex) before insertion of covered metallic stent or plastic stent between the left biliary duct and the gastric wall or between the CBD and the duodenal wall.

**Results** : A rendezvous procedure (RDV) could only be achieved in 1 patient with transgastric and hepatic access and placement of a plastic stent by ERCP. EUS-guided choledocoduodenal stent placement was performed in 2 patients with distal duct obstruction, and 4 patients with hilar or extensive obstruction were managed by EUS-guided left biliary drainage with gastrohepatic stent placement. The mean duration for procedures was 50 minutes (range 45-55). There were no technical differences between both procedures. IV antibiotics were administered during 1-7 days. The post-procedure course was favourable with a rapid normalization of hyperbilirubinemia. One hepaticogastrostomy was complicated with an asymptomatic pneumoperitoneum which rapidly regressed. Chemotherapy was initiated in 5 patients and surgery was performed in 1 patient after EUS-GBD. The mean follow-up was 4 months (range 1-6).

**Conclusion** : EUS-GBD should be considered in patients with hilar and distal malignant obstruction in whom ERCP is unsuccessful. Rendezvous technique is rarely achieved and proper selection for proximal and distal access depends mainly on location of obstruction, easiness of puncture, and echoendoscope stability.



BACTERIAL DNA IS RARELY DETECTED IN THE SERUM AND THE ASCITIC FLUID OF NONINFECTED OUTPATIENTS WITH CIRRHOSIS AND ASCITES TREATED BY PARACENTESIS. T. Serste (1), F. Bert (2), V. Leflon (2), C. Chauveau (2), T. Asselah (2), C. Francoz (2), F. Durand (2), D. Valla (2), R. Moreau (2), M. Nicolas-Chanoine (2). (1) ULB Saint-Pierre, Brussels, Belgium, (2) Hôpital Beaujon, Clichy, France.

**Introduction** : Bacterial DNA (bactDNA) has been found in the serum and/or the ascitic fluid (AF) of 30-40% of in-patients with cirrhosis and nonneutrocytic ascites, and is thought to be related to intestinal bacterial translocation. However, little is known on the presence of bactDNA in body fluids of outpatients.

**Aim** : The aim of the present study was to evaluate the presence of bactDNA in AF and serum in a prospective cohort of cirrhotic outpatients with tense ascites.

**Methods** : 31 consecutive patients who underwent scheduled therapeutic paracentesis in our out-patient clinic (Hôpital Beaujon, France) were enrolled between May and August 2009. Of these patients, 14 had a single paracentesis and 17 patients had consecutive paracenteses (including 8 patients with e 5 paracenteses). Overall, 100 sera and AF specimens were obtained. The presence of bactDNA was investigated by PCR amplification of the 16S ribosomal RNA gene followed by automated nucleotide sequencing of PCR products.

**Results** : Age of patients was  $58 \pm 10$  (mean  $\pm$  SD) years and 80.6% were male. The main cause of cirrhosis was alcohol (41.9%), hepatitis C (25.8%), and cryptogenic in 6.2%. The MELD score was  $17.3 \pm 4.9$  and there were 54.8% Child-Pugh C patients. The AF protein level was  $12.5 \pm 5.3$ g/L. There were 2 episodes of spontaneous bacterial peritonitis. Of the remaining 98 non neutrocytic AF (neutrophil count  $80 \pm 79$  elements/mm<sup>3</sup>), only one was both culture-positive and bactDNA-positive (*Streptococcus mitis*). Of the 98 serum samples, 2 samples from 2 distinct patients were bactDNA-positive for *Lactococcus lactis*. Interestingly, previous and subsequent samples from these patients were bactDNA negative.

**Conclusion** : In contrast to previously reported for inpatients, BactDNA is rarely detected in the serum and the AF of noninfected outpatients with cirrhosis and tense ascites. Most patients in whom samples were repeated remained consistently bactDNA-negative over time.

THE "LIVER FIRST STRATEGY" IN STAGE IV COLORECTAL CANCER DISEASE : THE GHENT UNIVERSITY HOSPITAL EXPERIENCE. S. Laurent (1), K. Geboes (1), O. Varin (1), B. Van Den Bossche (1), A. Scarinci (1), F. Berrevoet (1), X. Rogiers (1), P. Pattyn (1), W. Ceelen (1), Y. Van Nieuwenhove (1), B. De Hemptinne (1), M. Peeters (2), L. Libbrecht (1), R. Troisi (1). (1) UZ, Gent, Belgium, (2) UZ, Antwerpen, Belgium.

**Introduction** : Disease progression occurs frequently in patients with advanced synchronous liver metastases from colorectal cancer (CRC) while treating the primary tumour (PT) precluding curative treatment. High-impact chemotherapy followed by resection of liver metastases before treating the PT has been proposed recently as a new "liver first strategy".

**Aim** : We analyzed retrospectively our experience focusing on resectability rate, tumour recurrence and overall outcome.

**Methods** : Following multidisciplinary conference discussion, 15 patients with a mean age of 57,1 years [range 27-80] diagnosed with non-symptomatic colonic (n = 7) or rectal (n = 8) cancer and synchronous liver metastases were treated with the liver first strategy at the Ghent University Hospital between April 2007 and October 2009.

**Results** : The median follow-up was 6 months (range 1-17). Preoperative chemotherapy (CT) consisted of Folfex or Folfiri +/- bevacizumab. Resectability of liver metastases at diagnosis was scored according to Nordlinger et al. (Ann Oncol. 2009) : 10 (66,6%) patients were considered as not optimally resectable whereas 5 (33,3%) were initially resectable. Disease progression to irresectability was observed in 1 patient (6,6%). Downstaging according to the RECIST criteria was obtained in 12 (80%) patients. R\_0\_ resection was recorded in 13 (86.6%) patients and only one grade II postoperative complication was observed. Pathology reports showed 1 patient with complete response and an average tumoral cell necrosis of  $33\% \pm 20\%$ . Resection of the PT has already been performed in 12 patients while 2 others are waiting. No complications occurred after resection of the PT but disease recurrence after metastasectomy was recorded in 5 (33%) patients. The median time to recurrence was 7 months (range 6-9).

**Conclusion** : "Liver First Strategy" allowed, in our experience, a relatively high resectability rate and R"0" resection in stage IV colorectal cancer. Good initial control and down staging of the liver metastases was achieved with minimal morbidity following surgery of metastases and PT. High-impact CT in this protocol appears to be a promising strategy in patients with advanced synchronous liver metastases from CRC. A prospective multicentric Belgian trial we are leading is ongoing.

PHASE II STUDY OF HELICAL TOMOTHERAPY FOR OLIGOMETASTATIC COLORECTAL CANCER. M. De Ridder, B. Engels, A. Sermeus, H. Everaert, B. Neyns, H. Versmessen, D. Verellen, D. Urbain, G. Storme/ UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

**Introduction** : Long-term survival can be obtained in patients with oligometastatic colorectal cancer (CRC) undergoing resection with curative intent (Nordlinger et al, Lancet 2008).

**Aim** : The aim of this study was to evaluate the efficacy and toxicity of helical tomotherapy in the treatment of oligometastatic CRC patients, who were not amenable for metastasectomy or (further) systemic treatment.

**Methods** : CRC patients with  $\leq 5$  metastases were enrolled. No limitations concerning dimension or localization of the metastases were imposed. Patients were treated on the Tomotherapy Hi•Art II system, combining helical intensity-modulated radiotherapy with daily megavoltage computed tomography positioning. A total dose of 40 Gy was delivered in daily fractions of 4 Gy. Whole-body fluorodeoxyglucose-positron emission computed tomography (PET-CT) was performed at baseline and 3 months after the initiation of radiotherapy (RT), to evaluate the metabolic response rate. Side effects occurring within 3 months after the start of RT were scored using the NCI CTC AE v 3.0 scale.

**Results** : We report the results of the first 23 patients. A total of 54 metastases were treated. Most common sites were the lung, liver, pelvis and mediastinum. One patient (4%) experienced grade 3 vomiting necessitating interruption of the treatment ; 2 patients (9%) grade 2 diarrhea and dysphagia respectively. Twenty patients were evaluated by post treatment PET-CT. Four patients achieved a complete and 7 a partial metabolic response, resulting in an overall metabolic response rate of 55%. Five patients showed disease progression, of which 2 progressed in an irradiated site.

**Conclusion** : Helical tomotherapy is a promising new treatment modality for the treatment of oligometastatic colorectal cancer, with a metabolic response rate of 55%. Ten fractions of 4 Gy result in a good local control with limited toxicity.

ANASTOMOTIC COMPLICATIONS FOLLOWING IVOR LEWIS ESOPHAGECTOMY IN PATIENTS TREATED WITH NEOADJUVANT CHEMORADIATION ARE RELATED TO RADIATION DOSE TO THE GASTRIC FUNDUS. W. Ceelen, C. Vande Walle, T. Boterberg, K. Geboes, S. Laurent, D. Van de Putte, Y. Van Nieuwenhove, O. Varin, P. Pattyn. UZ, Gent, Belgium.

**Introduction** : Neoadjuvant chemoradiation (CRT) is increasingly used in patients with locally advanced esophageal cancer. Some studies suggested that CRT results in increased overall surgical morbidity. No data are available on the influence of CRT on anastomotic complications following Ivor Lewis esophagectomy using a gastric substitute.

**Aim** : To investigate the effect of total radiotherapy dose received by the gastric fundus and proximal esophagus on intrathoracic anastomotic complications following Ivor Lewis esophagectomy (gastric substitute and intrathoracic anastomosis).

**Methods** : Clinical and pathological data were prospectively collected from all patients treated with neoadjuvant chemoradiation (36 Gy in 20 fractions combined with 5-FU and cisplatin) followed by Ivor Lewis esophagectomy. Using radiotherapy (RT) planning CT scans, target volumes were drawn encompassing the proximal esophagus region and the gastric fundus. Within these target volumes, dose-volume histograms were analysed to generate the D50 (total dose to 50% of the target volume). We studied the ability of the D50 to predict anastomotic complications (leakage, ischemia, or stenosis). Dose limits were derived using receiver operating characteristics (ROC) analysis.

**Results** : Fifty four patients were available for analysis. Radiotherapy resulted in either T or N downstaging in 51% ; complete pathological response was achieved in 11%. In hospital mortality was 5.4% and major morbidity occurred in 36% of patients. Anastomotic complications (AC) developed in 7 patients (13%). No significant influence of the D50 on the proximal esophagus was noted on the anastomotic complication rate. The median D50 on the gastric fundus, however, was 33 Gy in patients with AC and 18 Gy in patients without AC ( $P = 0.024$ , Mann Whitney U test). Using ROC analysis the D50 limit on the gastric fundus was defined as 29 Gy.

**Conclusion** : In patients undergoing neoadjuvant CRT followed by Ivor Lewis esophagectomy, the incidence of AC is related to the RT dose on the gastric fundus, but not on the dose received by the proximal esophagus. When planning preoperative RT, efforts should be made to limit the total dose on the gastric fundus.

RESECTION OF LIVER METASTASES AFTER NEO-ADJUVANT TREATMENT WITH BEVACIZUMAB : : THE GHENT UNIVERSITY HOSPITAL EXPERIENCE. K. Geboes (1), B. Van Den Bossche (1), S. Laurent (1), M. Craninx (2), B. Claerhout (3), M. Hanssens (4), B. Berghmans (4), B. Van Overberghe (4), L. Verstraete (4), O. Varin (5), F. Berrevoet (5), X. Rogiers (5), B. De Hemptinne (5), C. Cuvelier (5), H. Monirath (5), L. Libbrecht (5), M. Peeters (6), R. Troisi (5). (1) Ghent University, Ghent, Belgium, (2) AZ Heilige Familie, Reet, Belgium, (3) AZ Alma, Eeklo, Belgium, (4) AZ Sint-Elisabeth, Zottegem, Belgium, (5) Ghent University, Ghent, Belgium, (6) Antwerp University Hospital, Antwerp, Belgium.

**Introduction** : Resection of colorectal liver metastases in selected patients has become standard of care, with reported 5-year survival rates between 20 and 55%. Resectability rate is an important secondary endpoint in studies evaluating efficacy of chemotherapeutic regimens. It is important however, to assess prognostic factors for disease recurrence after resection, because this will enable us to make a better distinction between patients who might benefit from surgery and patients who will not.

**Methods** : We analyzed our experience in patients pretreated with Folfex or Folfiri + bevacizumab retrospectively, focusing on tumour recurrence. 37 patients with a mean age of 58 years [IQ range 52-65] diagnosed with liver-only metastases of colonic (n = 24) or rectal (n = 13) cancer underwent liver resection between September 2007 and October 2009. Resectability of liver metastases was scored at diagnosis according to Nordlinger et al (Ann Oncol. 2009). Pathologic response was reported as the mean of the percentage of viable cancer cells remaining within each tumour according to Blazer et al (J Clin Oncol. 2008).

**Results** : The median follow-up since liver surgery was 14 months (IQ range 5-15). 17 patients had disease recurrence. The median time to recurrence was 6 months (IQR 4-9). 17 (46%) patients were considered as initially not optimally resectable and 20 (54%) as resectable. R0 resection rate was higher in patients that were considered resectable (90 versus 53%), and recurrence rate was lower (30 versus 59%). Only 1 patient (3%) had complete response (no residual cancer cells), as opposed to 6-12% of patients in other series after induction chemotherapy with bevacizumab. 19 (51%) patients had major response (1-49% residual cancer cells), 17 (46%) had minor response (3-50% residual cancer cells). There was no difference in recurrence rate between the two groups. Surprisingly, recurrence rate was higher in patients with metachronous liver disease. The difference was not caused by a lower R0 resection rate or % of major pathological responses.

**Conclusion** : In our experience, pathological response did not predict recurrence after preoperative chemotherapy, whereas the initial presentation as resectable disease and the absence of a positive surgical margin did.

SPECT IMAGING OF APOPTOTIC TUMOR RESPONSE IN A COLORECTAL TUMOR MODEL. C. Vangestel (1), N. Van Damme (1), G. Mees (2), S. Staelens (3), C. Van de Wiele (3), M. Peeters (4). (1) UZ, Gent, Belgium, (2) UMC, Groningen, Netherlands, (3) University of Gent, Gent, Belgium, (4) UZ, Antwerpen, Belgium.

**Introduction** : Molecular imaging of apoptosis is of great importance in oncology to determine therapeutic efficacy and to assess tumor response.

**Aim** : To determine optimal timing for detecting apoptosis in vivo after different apoptosis-inducing treatments, changes in  $^{99m}\text{Tc}-(\text{CO})_3\text{-His Annexin A5}$  ( $^{99m}\text{Tc}\text{-Ann A5}$ ) accumulation in tumors over time were examined and compared to those of caspase-3 activation in a colorectal cancer model.

**Methods** : COLO205-bearing mice were randomly divided into 5 different treatment groups receiving a single dose (ip) of 5-FU, Irinotecan, Oxaliplatin, Bevacizumab or Panitumumab, and control group.  $^{99m}\text{Tc}\text{-Ann A5}$  was injected iv (0.5 mCi) in mice 4, 8, 12, 24 and 48 hours after start of the treatment and also to control mice (n = 3 in each time group). Biodistribution study (n = 6) and microSPECT imaging were subsequently performed for the mice 3.5 hours after injection of the radiotracer. The results were correlated to histological analysis for apoptosis (caspase-3 activation).

**Results** : Biodistribution studies showed rapid clearance of  $^{99m}\text{Tc}\text{-Ann A5}$  from the blood. Radioactivity strongly accumulated in the kidneys (at 8h p.i.  $29,88\% \pm 2,49$  of the injected dose (ID)) and moderate in the liver (at 8h p.i.  $12,81\% \pm 3,06$  of the ID). 5-FU and Irinotecan-treated mice showed two peaks of  $^{99m}\text{Tc}\text{-Ann A5}$  uptake in the tumors. For 5-FU an average increase of 49% ID/volume (v) at 8 hours and a second increase of 27% ID/v at 12 hours posttreatment compared to controls was observed, while for Irinotecan a first peak (8%) occurred 8 hours posttreatment and a second larger peak of 58% of 24 hours posttreatment was observed. A gradual increase of  $^{99m}\text{Tc}\text{-Ann A5}$  uptake in the tumors of oxaliplatin-treated mice was observed with a peak of 70% ID/v at 24 hours posttreatment, compared to controls. Quantitative  $^{99m}\text{Tc}\text{-Ann A5}$  tumor uptake correlated well with the number of apoptotic cells as determined by caspase-3 immunostaining. Bevacizumab-treated mice showed no increased uptake of  $^{99m}\text{Tc}\text{-Ann A5}$  in the tumors. A single peak of  $^{99m}\text{Tc}\text{-Ann A5}$  uptake in Panitumumab-treated mice was observed of 38% ID/v at 24 hours posttreatment. However, both treated groups showed an increase of apoptotic cells determined by caspase-3 immunostaining at 4 hours and 24 hours posttreatment.

**Conclusion** : This study reveals that different therapies induce apoptosis at different rates and extent which may provide clinicians with relevant info on disease activity and therapeutic efficacy.

INDOLEAMINE 2,3-DIOXYGENASE EXPRESSION IN THE TUMOUR INVASION FRONT IS AN INDEPENDENT PROGNOSTIC FACTOR IN PT1-4N1MX STAGED COLORECTAL CANCER. L. Ferdinande (1), A. Mathieu (2), C. Decaestecker (2), T. Van Maerken (1), A.M. Negulescu (2), X. Moles Lopez (2), C. Cuvelier (1), I. Salmon (2), P. Demetter (2). (1) UZ, Gent, Belgium, (2) ULB Erasme, Brussels, Belgium.

**Introduction** : Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme that induces immune tolerance by modulating T cell responses. Carcinomas may actively create an immunosuppressive state via the expression of IDO.  
**Aim** : In this study, we examined a possible contribution of IDO on this phenomenon and investigated whether IDO expression has prognostic value in colorectal cancer.

**Methods** : IDO gene and protein expression was investigated by quantitative PCR and Western blotting in 3 colon cancer cell lines in basal state and after stimulation with IFN-gamma. Semiquantitative immunohistochemistry was used to evaluate IDO expression in tissue microarray materials of 264 pT1-4N0-2Mx staged colorectal cancer resection specimens. Results were correlated with clinical variables and with the presence of CD3+ and CD8+ T lymphocytes as measured quantitatively using the Spot Browser software program.

**Results** : In vitro the expression of the IDO gene and protein was found to be dependent on IFN-gamma stimulation. Using multivariate survival analyses, we identified higher IDO expression in the tumour invasion front as being an independent adverse prognostic factor in pT1-4N1Mx staged colorectal cancer. It was significantly associated with overall survival ( $p < 0.001$ ) and with the development of metachronous metastases ( $p < 0.01$ ). IDO expression was not associated with the presence of CD3+ or CD8+ T lymphocytes.

**Conclusion** : Our results indicate that higher IDO expression in the tumour invasion front is involved in the progression of colorectal cancer and correlates with impaired clinical outcome, suggesting that IDO is an independent and reliable prognostic indicator for colorectal cancer.

ENDORECTAL ULTRASOUND AND MAGNETIC RESONANCE IMAGING IN STAGING OF RECTAL CANCER IN ROUTINE DAILY PRACTICE. E. Cesmeli (1), K. Geboes (1), P.J. De Munck (1), W. Ceelen (1), B. Claerhout (1), J. Van Ongeval (2), A. De Backer (2), P. Smeets (1), P. Pattyn (1), M. Peeters (3), M. De Vos (1). (1) UZ, Gent, Belgium, (2) St-Lucas, Gent, Belgium, (3) UZ, Antwerpen, Belgium.

**Introduction** : A correct pre-operative staging of rectal cancer is important because overall survival and disease-free survival depend on TNM stage and treatment strategy. Magnetic resonance imaging (MRI) and endorectal ultrasound (EUS) both have high accuracy for local staging of rectal cancer. In this study we evaluated the accuracy of both techniques in our institution

**Methods** : In this retrospective study we analysed 102 consecutive patients in whom a pre-operative staging with EUS and MRI for rectal cancer was performed between march 2005 and july 2009. All EUS examinations were performed using a 10 MHz radial scanning instrument (Olympus GF-UM160) by the same experienced gastroenterologist. MRI was performed using a high-spatial-resolution phased-array MR technique at 1.5 Tesla. T- and N-stage were assessed by EUS and MRI according to the UICC TNM classification (6th edition). Imaging data were compared with the final pathology report

**Results** : In total, 102 patients met the inclusion criteria. Only 43 patients received no neo-adjuvant therapy ( $n = 15$ ) or only a short course of radiation therapy ( $5 \times 5$  Gy,  $n = 28$ ). These 43 patients were used for the comparison with pathology. Pathological T-stages were : 6 (14%) with pT1 carcinoma, 12 (27,9%) with pT2, 22 (51,1%) with pT3 and 3 (7%) with pT4. The details of performance of EUS and MRI are depicted in the tables below.

EUS	Sensitivity	Specificity	PPV	NPV	Accuracy
T1	100%	100%	100%	100%	95,3%
T2	50%	93,5%	75%	82,8%	81,4%
T3	86,3%	76,2%	79,2%	84,2%	81,4%
N+	70%	78,8%	50%	89,6%	76,7%

MRI	Sensitivity	Specificity	PPV	NPV	Accuracy
T2	66,6%	1,9%	30,7%	76,4%	48,8%
T3	36,3%	71,4%	57,1%	51,7%	53,4%
N+	60%	7%	40%	85,6%	69,7%

In our series, EUS was more accurate in T- and N staging. MRI is important because of its superiority in assessing circumferential resection margin (CRM). However, in our series, CRM was reported in only 11 patients. The confrontation of both examinations could potentially alter treatment strategy in 46,5% of these patients.

**Conclusion :** This study clearly demonstrates the superiority of EUS for the local staging of rectal cancer. MRI is important to assess the circumferential resection margin, but this is underreported in our series. Combining both examinations is still essential in the therapeutic management of rectal cancer

- D24 -

LAT-1 EXPRESSION AND 4-FMP PET IMAGING IN COLORECTAL CANCER. M. De Ridder, B. Engels, A. Sermeus, H. Everaert, B. Neyns, H. Vermessen, D. Verellen, D. Urbain, G. Storme/ UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

**Introduction :** LAT1, a neutral L-type amino acid transporter, and its transmembrane component CD98 are known to be highly expressed in gliomas. Therefore, LAT1 is recently suggested as a novel molecular target in brain tumors that may affect the outcome of conventional chemo and radiotherapy.

**Aim :** In this study, we examined the expression of LAT1 and CD98 in colon cancer using real time RT-PCR and immunohistochemistry (IHC). Next, we evaluated the feasibility of using [18F]-4-methyl-phenylalanine (4-FMP), a radiolabelled amino acid, as PET tracer.

**Methods :** Tumor and normal mucosa specimens were collected after surgery (n = 22), and total RNA was extracted using an ABI PRISM 6100 nucleic acid prep station and miRNeasy mini kit (Qiagen). RT-PCR was performed in an ABI PRISM 7000 sequence detection system. The relative gene expression of LAT1 and its trans-membrane heavy chain CD98 was normalized to the housekeeping gene GUS, and expressed as  $\Delta Ct$ . IHC staining was performed on paraffin sections using a polymer peroxidase method, and the membrane located LAT1 was quantified by computer-assisted image analysis. PET images were acquired at different time points (5-30-60 min) after administration of 4-FMP. The tracer uptake in the tumor was assessed semiquantitative and expressed as a tumor/intestine ratio (T/I).

**Results :** In preliminary experiments, we have compared the integrity and yield of RNA samples isolated according to the Applied Biosystems and Qiagen methodology. The RNA integrity parameter RIN was found to be high and comparable,  $8.8 \pm 0.6$  and  $8.2 \pm 0.2$  respectively. However, RNA yield was consistently higher using Qiagen kit ( $460 \pm 140 \mu\text{g/ml}$ ) compared with Applied Biosystems ( $49 \pm 26 \mu\text{g/ml}$ ). Therefore, further experiments were performed using Qiagen methodology only. The mean  $\Delta Ct$  values for LAT1 and CD98 for colorectal cancer were  $1.3 \pm 2.3$  and  $1.3 \pm 1.1$  respectively, and were overlapping with those of gliomas ( $0.6 \pm 1.4$  and  $-2.7 \pm 1.2$ ). 9/22 tumors showed higher LAT1 levels than in gliomas, and in 19/22 tumors those levels were higher than in normal mucosa ( $3.9 \pm 1.3$ ). The expression of both LAT1 and CD98 was remarkably heterogeneous (up to 300 and 20-times respectively), and was up to 50-times higher than in normal mucosa. The heterogeneity of the LAT1 gene was confirmed by IHC, which suggested in positive tumors a broad range (14-61%) of membrane staining. Contrasting, normal mucosa showed low (0.3-7%) LAT1 staining. In line colorectal cancer showed an early (5 min) and specific uptake of 4-FMP with T/I ratios ranging from 1,88 – 7,88. T/I ratios at later time points decreased, indicating a rapid washout.

**Conclusion :** This study demonstrates that LAT1 is highly and heterogeneously expressed in human colorectal cancer. This overexpression was confirmed in vivo in patients using a L-type amino acid PET tracer, such as 4-FMP.

PROGNOSTIC SIGNIFICANCE OF N STAGE, NODAL HARVEST, AND LYMPH NODE RATIO IN LOCALLY ADVANCED ESOPHAGEAL CANCER PATIENTS TREATED WITH NEOADJUVANT CHEMORADIATION. W. Ceelen (1), C. Staelens (2), L. Maes (2), V. Ochieng (2), L. Libbrecht (2), T. Boterberg (1), O. Varin (1), D. Van de Putte (1), Y. Van Nieuwenhove (1), P. Pattyn (1). (1) UZ, Gent, Belgium, (2) UZ, gent, Belgium.

**Introduction** : Clinical studies in colorectal cancer and gastric cancer have shown a prognostic impact of nodal harvest and of the lymph node ratio (LNR), defined as the number of positive nodes divided by the total number of examined nodes in node positive patients. It is unclear to what extent neoadjuvant chemoradiation (CRT) in esophageal cancer interferes with nodal staging.

**Aim** : We aimed to define the prognostic significance of the nodal harvest and the LNR in patients with locally advanced esophageal cancer treated with neoadjuvant CRT.

**Methods** : Esophageal cancer patients were staged using endoscopic ultrasound with biopsies and CT scan. Patients staged T3-T4 N+ M0 underwent neoadjuvant CRT (36 Gray in fractions of 1.8 Gray combined with cisplatin and 5-fluorouracil) followed by Ivor Lewis esophagectomy and two field lymphadenectomy after a 6-8 weeks waiting period. Nodal parameters were related to outcome using univariate (Kaplan Meier) and multivariate (Cox proportional hazards regression) analysis. Data are presented as mean  $\pm$  SD.

**Results** : From May 2004 to June 2009, 130 patients were treated (mean age  $59 \pm 10$  yrs ; 86% men). The mean distance from the dental arch to the most proximal extent of the tumour was  $34.6 \pm 4.4$  cm ; 73% were adenocarcinomas. A history of Barrett's metaplasia was present in 26% of patients. A mean of  $13 \pm 9$  nodes were identified in the resection specimen. After a mean follow up of  $28 \pm 33$  months, overall 5 year survival (OS) was 37%. Outcome was significantly better in ypN0 (N = 72) versus ypN1 (N = 58) patients (median survival 34 and 16 months respectively ; P = 0.012). In the subgroup of node negative patients, nodal retrieval was not independently associated with OS (hazard ratio 0.95 – 1.04 ; P = 0.9). In node positive patients, however, the LNR was identified as a prognostically significant variable (hazard ratio 1.3 – 10.4 ; P = 0.017).

**Conclusion** : In patients with locally advanced node positive esophageal cancer treated with neoadjuvant therapy followed by surgery, the LNR represents an independent prognosticator. Use of the LNR could allow to stratify node positive patients as a basis for adjuvant chemotherapy and/or targeted therapy.

RADIOFREQUENCY ABLATION IN BARRETT'S ESOPHAGUS. FIRST BELGIAN SAFETY AND EFFICACY DATA. R. Bisschops (1), T. Lerut (1), H. Willekens (1), R. Pouw (2), G. De Hertogh (1), J. Bergman (2), P. Naftex (1), P. Rutgeerts (1), G. Coremans (1). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) AMC, Amsterdam, Netherlands.

**Introduction** : Recently a combination of endoscopic resection (ER) of visible lesions followed by radiofrequency ablation (RFA) has been reported as a new treatment paradigm for Barrett's esophagus (BE) with high grade intra-epithelial neoplasia (HGIN) or early mucosal cancer (EC). ER and RFA both are effective but only limited data are available about possible adverse events, in particular with regard to a combined treatment.

**Aim** : To evaluate RFA related adverse events and efficacy in the treatment of Barrett's related HGIN or EC in a Belgian study population.

**Methods** : All RFA procedures between 2/2008 and 10/2009 were prospectively monitored for procedural related complications, eradication of dysplasia and intestinal metaplasia (IM). All patients with BE  $\leq 12$  cm with confirmed LGIN, HGIN or EC after ER of all visible lesions pre-RFA (ER  $< 2$ cm length ;  $< 50\%$  circumference ; no invasion  $> T1sm1$  ; no N+ on EUS ; 2 endoscopies post-ER/pre-RFA with 4-quadrant/2cm biopsies to exclude residual EC), were eligible for RFA. Six to 12 weeks after ER, balloon-based circumferential RFA (c-RFA) was performed, followed every 2-3 months by additional RFA until clearance of BE was obtained, with a maximum of 2 c-RFA's and 3 focal RFA's. Two patients with visible lesions or early cancer were treated out of protocol.

**Results** : 54 RFA procedures were performed in 27 patients (20 male) with a mean age of 63 years. 20/27 (74%) of patients underwent a ER for visible lesions prior to RFA. Worst ER histology was : LGIN (n = 3), HGIN (n = 5), EC T1a (n = 11), EC T1bsm1 (n = 1). Worst pre-RFA histology was : intestinal metaplasia (IM) (n = 6), LGIN (n = 8), HGIN (n = 11), indefinite for dysplasia (n = 1), adenocarcinoma (n = 1). By November 2009, 11 patients had finished the entire treatment requiring a median of 2 RFA procedures (range 2-5). 63% of procedures were performed under conscious sedation (midazolam and pethidine). The ablation procedure lasted on average  $19.9 \pm 1$  minutes. The majority of procedure related complications occurred during c-RFA (n = 35) and were all mild : mucosal laceration during sizing or circumferential ablation (n = 6/35), minor bleeding at a biopsy site (n = 1/54). Stenosis occurred in 3/26 patients, 2 after c-RFA, requiring 2-6 dilations. One of these patients had a very poor esophageal healing after c-RFA during 9 months. One additional patient had an anesthesia related pneumonia and one patient was re-admitted for 4 days due to dysphagia and retrosternal pain, one week after c-RFA, with unremarkable findings on endoscopy. After a median

follow-up of 257 days (range 131-588) the response for eradication of dysplasia was 100%. Eradication of IM was obtained in 10/11 (91%) patients.

**Conclusion :** This is the first report on a combined treatment of ER and RFA in a Belgian population. ER of visible lesions followed by RFA of the remaining Barrett is an effective and safe treatment in well selected patients. Eradication of dysplasia is achieved in all patients without any major complications.

- D27 -

**OTILONIUM BROMIDE IMPROVES FREQUENCY OF ABDOMINAL PAIN, SEVERITY OF DISTENTION.**  
J. Tack. University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction :** Otilonium bromide (OB) is a spasmolytic agent mainly acting by blocking L-Type Calcium channels in human intestinal and colonic smooth muscle cells

**Aim :** To confirm the therapeutic efficacy of OB in symptom control in a superiority trial versus placebo in adult patients with Irritable Bowel Syndrome (IBS).

**Patients :** 356 patients ( $46.16 \pm 19$  yr, 71% female) with IBS according to Rome II criteria (25.63% diarrhea, 30.98% constipation, and 43.38% mixed-type) and  $\geq 2$  episodes of abdominal pain/week during two weeks of run-in period were included after exclusion of any organic disease.

**Experimental design :** Double-blind, randomized, parallel group, placebo-controlled phase IV, multinational study. OB (40 mg tid) or placebo was administered for 15 weeks and follow-up was extended 10 weeks after the end of treatment.

**Measurements :** Frequency (4-point categorical scale) and intensity (VAS, verbal rating scale) of abdominal pain, severity of bloating, number of stools, patient/investigator assessment of global treatment efficacy on IBS symptoms, quality of life scores (IBSQoL) and adverse events were weekly assessed from baseline and during treatment phase and the follow-up period.

**Results :** OB (n = 179) and placebo (n = 177) groups were homogeneous in terms of demographics, anthropometric measurements, severity of symptoms and subtypes of IBS. Both OB and placebo strongly reduced the frequency intensity of abdominal pain and IBS symptoms during treatment phase ( $p < 0.0001$ ). Compared to placebo, OB provided significantly greater reduction of weekly frequency of episodes of abdominal pain at the end of treatment period (primary endpoint,  $-0.90 \pm 0.88$  vs  $-0.65 \pm 0.91$ ,  $p = 0.03$ ), reduction of severity of abdominal bloating ( $-1.15 \pm 1.16$  vs  $-0.91 \pm 1.12$ ,  $p = 0.02$ ) and global efficacy by patient assessment ( $1.29 \pm 1.08$  vs  $1.04 \pm 1.14$ ,  $p = 0.04$ ). No difference was found between OB and placebo for intensity of abdominal pain, the stool frequency, the proportion of patient responders, quality of life scores and safety measurements. During post-treatment follow-up OB was superior to placebo in withdrawal rate due to symptom relapse (10.4% vs 27.2%,  $p = 0.009$ ), in global efficacy on IBS symptoms and in time to symptom relapse ( $p < 0.05$ ).

**Conclusion :** This study shows OB is safe, well tolerated and superior to placebo in reduction of frequency of abdominal pain, severity of abdominal bloating and protecting from symptom relapse in patients with IBS. These results further confirm that patients with IBS can improve during and following treatment with OB.

EFFICACY AND TOLERABILITY OF THE NOVEL REFLUX INHIBITOR, AZD3355, AS ADD-ON TREATMENT IN PATIENTS WITH GERD WITH CONTINUED SYMPTOMS DESPITE PROTON PUMP INHIBITOR THERAPY. G. Boeckstaens (1), H. Beaumont (2), J.G. Hatlebakk (3), D.G. Silberg (4), J. Adler (5), H. Denison (5). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) Academic Medical Center, Amsterdam, Netherlands, (3) Haukeland University Hospital, Bergen, Norway, (4) AstraZeneca Pharmaceuticals LP, Wilmington, United States, (5) AstraZeneca R&D, Mölndal, Sweden.

**Introduction** : Some 20-30% of patients (pts) with GERD continue to have reflux symptoms despite proton pump inhibitor (PPI) therapy, outlining the need for new treatment options.

**Aim** : This proof-of-concept study evaluated the efficacy and tolerability of the GABA<sub>B</sub> receptor agonist AZD3355, a novel reflux inhibitor, as add-on treatment in such pts.

**Methods** : A double-blind multicentre phase II study (NCT00394472) was conducted in adult pts (aged 18-70 years) with continued GERD symptoms despite  $\geq 6$  weeks' PPI therapy at approved doses. Pt eligibility, based on symptom recall over the last 7 days, included  $\geq 3$  days with at least mild heartburn and/or regurgitation. Pts were randomised, after an 8-12 day run-in, to 4 weeks of AZD3355 65 mg (capsule) bid or placebo (PL) as add-on to existing PPI therapy. Symptom intensity was recorded twice daily using an eDiary. The primary variable was treatment response (at most one 24-hour period with heartburn or regurgitation of not more than mild intensity during the last 7 days of treatment).

**Results** : A total of 244 pts completed the run-in period and were randomised (66% male ; mean age, 50 years), of whom 232 were analysed for efficacy (AZD3355, n = 114 ; PL, n = 118). At baseline, daily symptoms of heartburn and/or regurgitation were experienced by 76% of pts (n = 181) and such symptoms were moderate-to-severe in 87% (n = 206). The proportion of responders (primary variable) was significantly greater for AZD3355 vs PL (16% vs 8% ; one-sided p = 0.026). The cumulative proportion of responders was also significantly greater for AZD3355 vs PL (log-rank p = 0.0195). Over the 4-week treatment period, the proportion of symptom-free days was higher with AZD3355 vs PL for heartburn (36% vs 21%), regurgitation (37% vs 23%), and either symptom (19% vs 10%). The most common adverse events (AEs) on active treatment were diarrhoea (10.7% [PL, 3.3%]), paraesthesia (transient, 8.2% [4.9%]) and nausea (7.4% [3.3%]). A total of 7 pts (4%) discontinued add-on treatment with AZD3355 due to AEs.

**Conclusion** : This proof-of-concept study shows that add-on treatment with AZD3355 significantly improves GERD symptoms and is well tolerated in pts with GERD with continued symptoms despite PPI therapy. Such findings provide a rationale for further clinical development of this novel reflux inhibitor.

SYMPTOM SEVERITY AND QUALITY OF LIFE IN PATIENTS WITH CHRONIC CONSTIPATION : POOLED RESULTS FROM THREE IDENTICAL RANDOMISED PLACEBO-CONTROLLED TRIALS WITH PRUCALOPRIDE. D. Dubois (1), R. Kerstens (2), L. Vandeplassche (2), J. Tack (3). (1) Patient Value Solutions, Huldenberg, Belgium, (2) Movetis, Turnhout, Belgium, (3) University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : Prucalopride (PRU) is a selective 5-HT<sub>4</sub> agonist, effective for the treatment of chronic constipation (CC) in female patients.

**Aim** : To evaluate the effect of PRU on constipation-related symptoms and disease-specific quality of life (QOL) in patients with CC using combined data from 3 pivotal trials.

**Methods** : Data from 3 identical pivotal phase III trials, with treatment duration of 12 weeks, were combined. Each trial had 3 parallel treatment groups : placebo, PRU 2mg and PRU 4 mg. Constipation-related symptoms were evaluated using the validated Patient Assessment of Constipation SYMptoms (PAC-SYM) questionnaire with 12 items in 3 subscales : abdominal (4 items), stool (5 items), and rectal symptoms (3 items). Constipation-related QOL was assessed using the validated PAC-QOL self-report questionnaire with 28 items in 4 subscales : physical discomfort (4 items), psychosocial discomfort (8 items), worries and concerns (11 items), and satisfaction (5 items). The satisfaction subscale of the PAC-QOL was defined as the main secondary QOL in these trials. Both PAC-SYM and PAC-QOL items were rated on a 5-point Likert scale and were assessed at baseline, week 4 and week 12.

**Results** : At both week 4 and week 12 the proportion of patients with an improvement  $\geq 1$  point from baseline on overall PAC-SYM score was significantly higher for PRU 2 mg compared with placebo : 32.0% and 15.9% at week 4 and 33.2% and 21.5%, respectively, at week 12. Similar effects were observed with PRU 4 mg and on each of the three symptom subscales. The proportion with  $\geq 1$  point improvement from baseline on overall PAC-QOL scores and on the satisfaction subscale was significantly higher for both the PRU 2 and 4 mg compared with placebo at weeks 4 and 12. At week 12, the proportion for overall PAC-QOL was 36.5% on PRU 2 mg compared to 18.6% on placebo. For the satisfaction subscale the proportion at week 12 was 44.0% on PRU 2 mg compared with 22.2% on placebo, representing



an improvement in satisfaction of more than 20%. The clinical meaningfulness of 1-point improvement on both PAC-SYM and PAC-QOL is supported by the fact that most patients with such an improvement also showed clear improvements in other clinically relevant parameters such as the percentage of patients with an average of e3 spontaneous complete bowel movements per week over the 12 week treatment period (primary endpoint).

**Conclusion :** Prucalopride at 2mg significantly alleviates constipation-related symptoms, and improves disease-related QOL and satisfaction with treatment and bowel function in patients with severe chronic constipation.

- D30 -

ESD IN BARRETT'S ESOPHAGUS HIGH GRADE DYSPLASIA AND MUCOSAL CANCER : PROSPECTIVE COMPARISON WITH CAP-EMR. P. Deprez, H. Piessevaux, T. Aouattah, C.P. Yeung, C. Sempoux, A. Jouret-Mourin. UCL Saint-Luc, Brussels, Belgium.

**Introduction :** The role of endoscopic submucosal dissection (ESD) in high grade dysplasia (HGD) and superficial adenocarcinoma in Barrett's esophagus is not established because of the expertise needed to avoid severe complications. Many studies however showed the importance of R0 resection in upper GI superficial cancers, that can only be achieved by en-bloc resection with free deep and lateral margins.

**Aim :** To evaluate efficacy and safety of ESD compared with cap-mucosectomy (EMR-C).

**Methods :** Prospective randomized study, EMR-C being performed with soft oblique 18mm or 12-14 mm straight cap and single use snares whereas ESD was done with Flex-knife, IT-knife, Hook-knife or Dual-knife (Olympus).

**Results :** (expressed as median and range) : 25 pts in each group (68 y, 29-82 ; 45 men) were treated between 09/2006 and 11/2009. Lesions were Paris IIA in 13, IIB in 31, and IIC in 6. Barrett size was C2 (0-11) M5 (1-12). Final histology was HGD in 25, T1m2 in 5, T1m3 in 9 and T1sm in 4. Comparative results are shown in table. ESD duration decrease significantly correlated with increasing expertise. Cost for ESD significant decreased with increasing experience. Complications included 2 perforations during ESD and 1 during EMR-C all treated with CSEMS for 4 weeks, and strictures in 44% and 20% of ESD and EMR-C respectively. They were treated by endoscopic dilatation in all cases (1-16 sessions 20mm CRE balloon) and biodegradable ELLA stent in 1pt. No significant differences in complete remission of intestinal metaplasia and neoplasia could be seen between ESD and EMR-C at last follow-up (15 months, range 1-26).

	EMR-C	ESD	P value
En-bloc resection	None (1-11 specimens)	All but I	0.00001
Surface resected (mm <sup>2</sup> )	1488 (185-3194)	2453 (600-5400)	< 0.01
Procedure time (min)	61 (20-130)	150 (64-334)	< 0.001
Devices cost (€)	264 (60-515)	486 (247-1019)	< 0.001
R0 (free deep and lat margins)	24%	64%	< 0.05
CR for intestinal mataplasia at last F-up	84%	84%	NS
CR for neoplasia at last F-up	100%	100%	NS

**Conclusion :** Both cap-EMR and ESD achieved CR rates higher than 95%. Although ESD provides significantly higher en-bloc specimens and R0 resection than piece-meal cap-EMR, no significant differences could be shown in remission rate (100%) of neoplasia during follow-up. ESD should therefore only be considered in large mucosal adenocarcinoma, that cannot be resected en-bloc with large caps, and in expert centres for further long term validation.

THE EUROPEAN ACHALASIA TRIAL : A RANDOMIZED MULTI-CENTRE TRIAL COMPARING ENDO-SCOPIC PNEUMODILATION AND LAPAROSCOPIC HELLER MYOTOMY AS PRIMARY TREATMENT. G.E. Boeckstaens (1), V. Annese (2), S. Bruley des Varannes (3), S. Chaussade (4), M. Costantini (5), I. Elizalde (6), U. Fumagalli (7), M. Gaudric (4), E. Metman (8), J. Pérez de la Serna (9), W.O. Rohof (10), A.J. Smout (11), J. Tack (12), G. Zaninotto (13), O.R. Busch (10). (1) University of Leuven, Leuven, Belgium, (2) Ospedale CSS-IRCCS, San Giovanni Rotondo, Italy, (3) Human Nutrition Research Center, Nantes, France, (4) INSERM U567 Institut Cochin, Paris, France, (5) University of Padua, Padua, Italy, (6) Hospital Clinic, Barcelona, Spain, (7) Istituto Clinico Humanitas, Milan, Italy, (8) Hôpital Saint Antoine, Paris, France, (9) Hospital Universitario, Madrid, Spain, (10) Academic Medical Center, Amsterdam, Netherlands, (11) UMC, Utrecht, Netherlands, (12) University Hospital Gasthuisberg, Leuven, Belgium, (13) University Padova, Padova, Italy.

**Introduction** : Achalasia is currently treated by pneumatic dilation (PD) or laparoscopic Heller myotomy (LHM) with success rates varying between 70 and 90%. Randomised clinical studies with adequate power comparing the clinical efficacy of PD and LHM are currently lacking.

**Aim** : The aim of the study was to compare the treatment success of these two different therapies in a large multi-centre randomised clinical trial.

**Methods** : From Feb. 2003 to Feb. 2008, 204 newly diagnosed achalasia patients (117M, 83F ; age 19-74 mean 46) from 5 European countries agreed to participate. Subjects underwent physical examination, esophageal manometry, endoscopy and radiographic assessment of esophageal emptying, and filled out several questionnaires (SF-36, GERD-HRQOL, OES24). Symptoms (weight loss, dysphagia, retrosternal pain and regurgitation) were assessed using the Eckardt score (each symptom scored from 0-3). Patients were subsequently randomised to either PD (n = 94) (Rigiflex balloon, 30 and 35 mm) or LHM (+ Dor anti-reflux procedure) (n = 106). Patients were re-evaluated at 1, 3, 6, 12 months after treatment followed by a yearly visit. Treatment was considered unsuccessful if Eckardt score > 3. Retreatment for recurrent symptoms was allowed once during the first two years of follow up in the PD group.

**Results** : Of the 204 randomised patients, 4 patients had pseudo-achalasia and were excluded. 4 perforations occurred after PD compared to 11 peroperatively recognized perforations (1 converted to open) during LHM. Median follow-up (FU) was 38 months (range 0 - 82 months). 13 PD patients required redilation during the first 2 years. Clinical outcome and sphincter function after 1 and 2 years of FU are summarized in Table 1. SF-36, GERD-HRQOL and OES24 scores were similar in both groups. No effect of age or sex could be demonstrated on the success rate. Esophageal acid exposure and the occurrence of esophagitis were similar in both groups. Dropout rates were similar in both groups (8% vs 17%, LHM vs PD after 2y FU).

**Conclusion** : After 2 years of FU, PD and LHM have a comparable success rate of 92-87%. Lower esophageal sphincter pressure is higher in PD after 1 year FU. Based on these data, we conclude that either treatment can be proposed as initial treatment, although further FU is required to evaluate long-term outcome.

Table 1

7	Baseline		1 year FU		2 year FU	
	LHM	PD	LHM	PD	LHM	PD
Patients in FU	106	94	98	81	97	78
Successful treatment (%)			89	93	87	92
Eckardt score	7,4 ± 0,2	7,0 ± 0,2	1,3 ± 0,1	1,4 ± 0,2	,1 ± 0,1	1,3 ± 0,1
LESP (mmHg)	30,5 ± 1,3	33,4 ± 1,7	10,2 ± 0,7	14,1 ± 1,1*	10,4 ± 0,9	11,7 ± 1,0
Barium Swallow (cm of esophageal stasis after 5min)	12,4 ± 0,7	12,3 ± 0,7	3,3 ± 0,5	3,7 ± 0,7	3,4 ± 0,6	4,8 ± 0,7

\* P < 0.01, Student's t-test

REPORT (GLEM-LOK) ON CURRENT BARRETT'S ESOPHAGUS MANAGEMENT IN BELGIUM : COMMENTS AND RECOMMENDATIONS. R. Bisschops (1), P.H. Deprez (2), J.C. Debongnie (3), C. de Galocsy (4), A. Elewaut (5), R. Fiasse (2). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) Cliniques universitaires Saint-Luc, Brussels, Belgium, (3) Clinique St. Pierre, Ottignies, Belgium, (4) Bracops, Anderlecht, Belgium, (5) UZ, Gent, Belgium.

**Introduction** : Surveillance of Barrett's Esophagus (BE) has a beneficial effect on survival of esophageal adenocarcinoma and allows early detection and organ preserving endoluminal therapy.

**Aim** : To assess management and surveillance of BE in Belgium.

**Methods** : A questionnaire on definition, surveillance and treatment of BE, was distributed through the different GLEM-LOKs and returned by 72 respondents.

**Results** : Sixty% of responders use a combination of endoscopic findings (Prague classification) and histology (intestinal metaplasia) to define BE. The majority of BE biopsies are reviewed by one pathologist (90%) who will perform a p53 staining for 22% of the responders. During endoscopy the majority (56%) tries to biopsy visible lesions first before taking biopsies according to the Seattle protocol. Eleven% just take random biopsies and 3% only sample visible lesions. Only 3% of the responders sometimes use fiber endoscopy. Twenty-one% always try to use high definition endoscopy and 15% always use (virtual) chromo-endoscopy. For surveillance of non dysplastic BE, there was a statistically significant difference ( $p < 0.001$ ) in the follow-up interval between short ( $< 3$  cm) and long segment BE : 61% prefer a 12 month interval for long segment BE, in comparison to 32% for short segment BE. In case of low grade (LGIN) or high grade (HGIN) intra-epithelial neoplasia all responders intensify endoscopic surveillance. For LGIN, 44% and for HGIN, 48% adhere to internationally advised repeat endoscopy after 6 and 3 months respectively. In case of LGIN, 63% will increase the dose of PPI prior to follow-up endoscopy. In general, PPI\_s are prescribed to control reflux symptoms (37%), to reduce the BE length (24%) or the risk for cancer (38%). Remarkably, 47% refer patients with LGIN for mucosectomy after confirmation of the diagnosis. In case of HGIN, virtually all responders refer or treat patients, rather than proposing follow-up endoscopies. Half of the responders will refer or treat patients without repeat endoscopy. Endoluminal therapy (mucosectomy) is the first treatment option for HGIN. Only 11% refer patients for primary surgery.

**Conclusion** : Adherence to international surveillance guidelines is rather good among Belgian endoscopists according to this GLEM-LOK survey. The awareness of endoluminal therapy as a first organ saving treatment option for early BE neoplasia is remarkably high. This is reflected in a prompt referral of patients for treatment without awaiting follow-up endoscopy.

BELGIAN GUIDELINES FOR THE MANAGEMENT OF UPPER GI BLEEDING. I. Colle, J. Deflandre, J. Delwaide, E. D'Hondt, P.F. Laterre, O. Lemoine, E. Macken, A. Penalosa, A. Wilmer for the Upper GI bleeding Work group, on behalf of the BASL, BeSEDiM, BSGiE, SIZ, SRBGE and VVGE.

These guidelines will be published in one of the next issues of Acta Gastro-Enterologica Belgica.

EARLY METABOLIC RESPONSE EVALUATION WITH 18-FDG PET-CT IN CT3-4N+ CANCER OF THE OESOPHAGUS AND GE-JUNCTION TREATED WITH INDUCTION CHEMORADIOTHERAPY IS NOT A USEFUL TOOL TO PREDICT HISTOPATHOLOGICAL RESPONSE AND OUTCOME. G. Sergeant, C. Deroose, G. De Hertogh, J. Moons, W. Coosemans, H. Decaluwé, G. Decker, P. De Leyn, P. Nafteux, D. Van Raemdonck, L. Mortelmans, A. Lerut. University Hospital Gasthuisberg, Leuven, Belgium.

**Aim** : To assess the value of 18-FDG-PET-CT as a tool to select patients with advanced cancer of the oesophagus and gastro-oesophageal junction (GEJ) who will benefit from induction chemoradiotherapy (CRT).

**Methods** : In a prospective, blinded study from 2004 - 2008 a total of 55 patients with advanced oesophageal cancer (cT3-4 N0/+) of the oesophagus (n = 42) and GEJ (n = 13), underwent PET-CT scan prior to CRT, 2 weeks after initiation of the first cycle of chemotherapy (blinded interval PET) and after completion of 2 more cycles of chemotherapy with concomitant radiotherapy. Early response (R), late R and complete metabolic R were defined by a decrease in mean standardized uptake value (SUV) of > 35%, > 50% and 100% respectively. The Mandard tumor regression (TRG) scoring system was used to score pathological response. Postoperative deaths (n = 5) were excluded from survival analysis. Cancer-specific survival (CSS) rates were estimated by the Kaplan-Meier method from the start of therapy until cancer-specific death or until last follow-up. For the time-to-recurrence (TTR) analysis postoperative deaths and patients with progressive/non-resectable disease at the time of post-induction re-evaluation or surgery (n = 13) were excluded.

**Results** : Mean decrease in  $\Delta$  mean SUV% of the tumour was  $36.4 \pm 29.3\%$  on interval PET and  $70.3 \pm 27.0\%$  after completion of CRT. No significant difference was found for CSS (p = 0.77) and TTR (p = 0.49) between early R (n = 26/22) and early nonR (n = 24/20) as well as for CSS (p = 0.61) and TTR (p = 0.57) between late R (n = 42/34) and late nonR (n = 8/8). Assessment of changes between early and late response identified 4 separate groups : early R and late R (n = 24), early R and late nR (n = 2), early nR and late R (n = 18), early nR and late nR (n = 6). PPV and NPV for early response in relation to late response were 92% and 25% respectively. A significant difference in CSS (p = 0.03) but not in TTR (p = 0.74) was found in patients (n = 14) who had complete metabolic regression (100%) on  $\Delta$  Mean SUV% at the completion of CRT. Complete tumor regression (TRG1) was demonstrated in 16/46 (34%) resected tumors. PPV and NPV for early R in relation to TRG1 were 44% and 76% respectively. PPV and NPV for late R in relation to TRG1 were 41% and 100% respectively.

**Conclusion** : Early metabolic response evaluation on 18-FDG PET-CT (using a 35% decrease in mean SUV as cut-off) does not seem to be a good predictor of histopathological response (TRG) and outcome in patients with advanced oesophageal cancer treated with induction CRT. Only complete metabolic response (100% decrease in mean SUV) after completion of CRT has a significant prognostic value. Hence, our data do not support the use of early non-response on interval PET to guide decisions on early cessation of induction RCT in advanced oesophageal cancer. Further prospective multicenter studies are necessary to define optimal cut-off values for metabolic response evaluation with PET-CT for oesophageal cancer.

MOLECULAR RECLASSIFICATION OF CROHN'S DISEASE BY CLUSTER ANALYSIS OF GENETIC VARIANTS. I. Cleynen (1), J.M. Mahachie John (2), L. Henckaerts (3), W. Van Moerkercke (1), P. Rutgeerts (1), K. Van Steen (4), S. Vermeire (1). (1) KU, Leuven, Belgium, (2) Ghent University, Ghent, Belgium, (3) UZ, Leuven, Belgium, (4) University of Liege, Liege, Belgium.

**Introduction** : Crohn's Disease (CD) has a heterogeneous presentation with differences in disease location and behavior. The genetic susceptibility to CD is well known. Recent genome-wide association scans (GWAS) and meta-analysis have identified over 30 susceptibility loci. Efforts in linking CD genetics to clinical subphenotypes have not been very successful. Instead, patients may be better grouped at the molecular level.

**Aim** : To investigate if clusters of CD patients can be identified based on genetic markers, hereby treating the clinical subphenotypes as unknown.

**Methods** : 46 SNPs identified from CD GWAS were genotyped in 875 CD patients and 367 healthy controls (HC). Patient genotypes were analyzed by Latent Class Analysis (LCA), using bootstrapping as validation of the models. Random Forest (RF) analysis was used to see if specific clinical subphenotypes could be attributed to the identified clusters.

**Results** : 6 CD clusters were identified. They are defined by specific combinations of *TNFSF15* (rs7869487), *IRGM* (rs13361189), and 6q23.3 (rs946227) genotypes (Figure 1). Cluster E was the most frequent (302/875 CD), but was not disease specific : 126/367 HC were allocated to this cluster according to its specific SNP combination (p = NS). However, clusters A, D and F were highly CD specific : only 1 HC was assigned to cluster A (1/367 HC vs 59/875 CD, p < 0.0001, positive predictive value PPV = 98%), 20 to cluster D (20/367 HC vs 239/875 CD, p < 0.0001, PPV = 92%), and 3 to cluster F (3/367 HC vs 62/875 CD, p < 0.0001, PPV = 95%). The commonly applied clinical subphenotypes (age at diagnosis, disease location, behavior, and surgery) are insufficient to classify patients according to the clusters.

**Conclusion :** We identified highly CD specific clusters, pointing to a non-random clustering of molecular markers in CD patients. The formed CD clusters may contribute to disease pathogenesis. The SNP combinations determining the CD specific clusters could be promising disease predictors in the future. This technique may serve as a first step to reclassify CD patients based on molecular grounds.

- D36 -

TELAPREVIR (TVR) Q8H OR Q12H COMBINED WITH EITHER PEGINTERFERON (PEG-IFN, P) ALFA-2A OR ALFA-2B AND RIBAVIRIN (RBV, R) IN TREATMENT-NAÏVE GENOTYPE 1 HEPATITIS C : FINAL RESULTS OF THE RANDOMIZED, OPEN-LABEL, MULTICENTER PHASE 2 STUDY C208. F. Nevens (1), Y. Horsmans (2), H. Van Vlierberghe (3), J. Delwaide (4), M. Adler (5), P. Marcellin (6), E. De Paepe (7), S. De Meyer (7), M. Beumont-Mauviel (7). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) UCL Saint-Luc, Brussels, Belgium, (3) Ghent University, Ghent, Belgium, (4) Ulg Sart Tilman, Liège, Belgium, (5) ULB Erasme, Brussels, Belgium, (6) Hôpital Beaujon, Clichy, France, (7) Tibotec bvba, Mechelen, Belgium.

**Introduction :** The treatment of chronic Hepatitis C especially for patients infected with genotype 1 still needs improvement.

**Aim :** C208 is an open-label, multicenter Phase 2 exploratory trial evaluating TVR 750mg q8h or 1125mg q12h, with PEG-IFN-alfa-2a (180¼g/wk) or PEG-IFN-alfa-2b (1.5¼g/kg/wk) plus RBV (PR) in treatment-naïve chronic HCV genotype 1(G1) infected patients.

**Methods :** In total, 161 patients were randomized to receive TVR q8h with PEG-IFN-alfa2a/RBV (1000 or 1200mg/day) (A,n = 40) or PEG-IFN-alfa-2b/RBV (800-1200mg/day) (B,n = 42), or TVR q12h with PEG-IFN-alfa-2a/RBV (C,n = 40) or PEG-IFN-alfa-2b/RBV (D,n = 39). After 12 weeks patients received additional PR for 12 or 36 weeks, based on virologic response to treatment. Patients with viral breakthrough ( $\geq$  1-log increase in HCV RNA above nadir) discontinued TVR and were scheduled to complete a total of 48 weeks of PR.

**Results :** Baseline characteristics were comparable between treatment arms. The proportion of patients with undetectable HCV at weeks 4 and 12 were : 80%,93%[A] ; 69%,93%[B] ; 83%,83%[C] ; 67%,85% [D], respectively. SVR rates were comparable among groups (85%[A] ; 81%[B] ; 83%[C] ; 82%[D]) . SVR rates were comparable for the pooled TVR q8h and q12h treatment arms (SVR 83% and 82% respectively), and the pooled PEG-IFN-alfa-2a and PEG-IFN-alfa-2b treatment arms (SVR 84% and 82%, respectively). 18% of patients had to complete 48 weeks of therapy. Breakthroughs during the TVR treatment phase occurred in 1[A], 3[B], 2[C] and 3[D] patients. Total exposure to TVR (AUC24h) was similar across treatment arms. The incidence of AEs (> 25% patients in any group) was comparable between arms. Discontinuations of all drugs due to AEs (mainly rash and anemia) was 8% (n = 13). These events improved upon cessation of treatment.

		Patients				Final histology
LGD	HGD	Ca m1-m3		Ca sm1-sm3		T2
ESD mono	26	11	9	3	3	0
Other	9	0	5	0	1	1

**Conclusion** : A high proportion (> 80%) of chronic HCV G1 naive patients achieved an SVR with all TVR-containing regimens studied, regardless of PEG-IFN type or TVR dosing schedule. Treatment duration was 24 weeks for most patients. These results support further evaluation of a q12h TVR dosing regimen in treatment-naïve chronic HCV G1 patients.

- D37 -

COLORECTAL ESD MAY AVOID SURGERY IN EARLY NEOPLASMS. M. Maffei, H. Piessevaux. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : There are some surgical indications for early colorectal neoplasms in which lymphadenectomy is not necessary. Staging techniques such as EUS are insufficiently reliable to distinguish subtle submucosal invasion in superficial colorectal neoplasms. There is room for a combined diagnostic and potentially therapeutic endoscopic intervention in these superficial lesions, providing optimal histological T staging is obtained. Endoscopic submucosal dissection (ESD) may be used in this setting.

**Aim** : To evaluate colorectal ESD in neoplasms, not amenable to conventional snaring or mucosal resection, which were referred for conventional surgery.

**Methods** : Thirty-five patients were considered for ESD (21 men ; median age 72 years). ESD was performed using single lumen HD Olympus scopes equipped with various caps. Submucosal injection was made with a mixture of saline, epinephrine 1/100.000, methylene bleu and methylcellulose. Peripheral incision was done with a Flexknife or Dualknife. Actual dissection was performed with Hookknife or Dualknife. Endpoints were monobloc resection and complication rates and need for subsequent surgery.

**Results** : Two patients were excluded because of contra-indication for ESD (multiple polyps suggestive of familial adenomatous polyposis and an obviously locally advanced colorectal cancer). The rate of en bloc resection was 26/35 (74%). Median operation time was 127 minutes (range : 33-337). Median specimen size was 38 mm (range : 15-70 mm). There was no significant post-procedure bleeding. Perforation occurred in 12/35 patients (34%). However, all but one was managed conservatively. Nine out of 12 perforations were located in the right colon. After final histological analysis and multidisciplinary consult surgical indication was retained in 11/35 patients. In all 3 patients with monobloc resection where surgery was decided for oncological reasons, no residual tumor was found. After a median follow-up of 22 months, all patients are in complete remission.

		Patients				Final histology
LGD	HGD	Ca m1-m3		Ca sm1-sm3		T2
ESD mono	26	11	9	3	3	0
Other	9	0	5	0	1	1

**Conclusion :** Using colorectal ESD, surgery was avoided in 23/35 patients, for the definitive treatment of superficial colorectal neoplasms. Perforation rate in this series was high, but very rarely needed surgery. It is expected that this rate will decrease with improving experience.

- D38 -

CHEMOTHERAPY INDUCTION FOLLOWED BY PREOPERATIVE CHEMORADIATION VERSUS PREOPERATIVE CHEMORADIATION ALONE IN LOCALLY ADVANCED RECTAL ADENOCARCINOMA : A RANDOMIZED CONTROLLED PHASE II STUDY. R. Maréchal (1), B. Vos (1), M. Polus (2), T. Delaunoy (3), M. Peeters (4), P. Demetter (1), P. Coucke (2), B. Frederick (5), A. Hendlisz (6), A. Demols (1), P. Van Houtte (6), J. Van De stad (1), J.L. Van Laethem (1). (1) Erasme Hospital - ULB Brussels, Brussels, Belgium, (2) Ulg Sart Tilman, Liège, Belgium, (3) Hôpital de Jolimont, Haine-Saint-Paul, Belgium, (4) University Hospital, Ghent, Belgium, (5) RHMS, Baudour, Belgium, (6) Institut Jules Bordet, Brussels, Belgium.

**Introduction :** In locally advanced rectal cancer (LARC), the optimal therapeutic sequence remains an important clinical question.

**Aim :** The aim of the study is to assess the feasibility, efficacy and impact of a short-course oxaliplatin-based chemotherapy induction in conjunction with preoperative radiochemotherapy (RCT) for patients with LARC in a randomized phase II study.

**Methods :** Eligible patients had stage III LARC defined by MRI and EUS. Patients with LARC were randomly assigned to receive either Arm A (A) : concomitant RCT (radiation : 45Gy, 1.8Gy/fraction, 5days/week ; 5-fluorouracil : continuous infusion, 250mg/m<sup>2</sup>/day) or Arm B (B) : two courses of FOLFOX6 schedule (Oxaliplatin, 100mg/m<sup>2</sup> ; 5-fluorouracil, 400mg/m<sup>2</sup> bolus followed by 2000/m<sup>2</sup> continuous infusion) followed by concomitant RCT (idem A). Surgery was planned 6 to 8 weeks after RCT in both arms. Primary endpoint was pathological response and secondary endpoints were toxicity and sphincter preservation rate.

**Results :** Fifty-six patients out of the 110 planned patients were enrolled at 6 sites and 52 assessable for interim analysis were analysed. The two groups are well balanced in terms of age, sex, performance status, clinical TNM stage, tumor location and size. Completion of full sequence was similar in both groups : 25/26 (96%, A) vs. 23/26 (88%, B) patients (p = 0.343) but chemotherapy dose reduction was higher in chemotherapy induction group : 0/26 (0%, A) vs. 4/26 (15%, B) patients (p = 0.040). About surgery, there was no significant difference in terms of type, R0 resection, number of lymph nodes resected or invaded, longitudinal and circumferential margin. Patients with lower third tumor treated with chemotherapy induction tended to show a higher sphincter preservation rate : 6/9 (A) vs. 9/9 (B) patients (p = 0.058). Tumor downstaging occurred in 15/26 (58%, A) vs. 17/26 (65%, B) patients (p = 0.763). Complete pathological response was also similar : 6/26 (23%, A) vs. 3/26 (12%, B) patients (p = 0.331). The tumor regression grades (Dworak)

distribution was similar ( $p = 0.746$ ). Adverse events in any grade were similar : 20/26 (77%) vs. 21/26 (81%) patients ( $p = 0.655$ ) but grade 3, 4 toxic effects was significantly higher in chemotherapy induction group : 2/26 (8%, A) vs. 9/26 (35%, B) patients ( $p = 0.017$ ).

**Conclusion** : Addition of oxaliplatin-based chemotherapy induction to preoperative chemoradiation is feasible for patients with LARC without compromising the preoperative RCT completion although preliminary results do not indicate increased locoregional impact on standard therapy.

- D39 -

MALIGNANT TUMOURS AND PATTERNS OF MORTALITY IN 200 PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS (4 TO 29 YR FOLLOW UP). J. Fevery, L. Henckaerts, S. Vermeire, P. Rutgeerts, F. Nevens, W. Van Steenberghe. University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : The outcome of PSC is greatly improved by liver transplantation, but patients remain at high risk of malignancies and possibly of other complications..

**Aim** : To study the prevalence of malignancies in patients with PSC, and the patterns of mortality.

**Methods** : We studied 200 PSC patients diagnosed before October 2005 ; follow up 10 years (median, range 4 - 29).

**Results** : Till now 57 patients died. Overall, malignancies occurred in 21.5% (43/200) of all patients, and were the cause of death in 27 of the 57 who died (47.4%). Colorectal carcinomas (CRCa) ( $n = 11$ ) and dysplastic colon adenomas ( $n = 7$ ) presented often and were the cause of death in 6. Cholangiocarcinoma (CCa) occurred in 12 patients but led to death in 11. Pancreas CA was seen in 4 and HCC in 3 (all with cirrhosis).

Mortality due to cirrhosis or septic cholangitis was documented in 14 ; it was related to fulminant colitis in 2, to LT in 6 and unrelated to PSC in 8 patients. CCa occurred at a younger age in males. It was diagnosed in 42% simultaneously with or shortly after the diagnosis of PSC, later on, CCa developed in 0.8% per year. CRCa or dysplasia developed 9.8 yrs (median, 1-32 yrs) after the diagnosis of IBD or 6.3 yrs (0-22yrs) after the diagnosis of PSC ; 10 had UC, 5 Crohn's colitis, 1 indeterminate and 1 no IBD. The lesions were localized in rectosigmoid in 10, in the left colon in 2 and in the caeco-ascendens in 3 ; double locations were present in 2 patients : caeco-ascending plus splenic flexure or rectosigmoid respectively. The high incidence of CRCa might result from the special nature of IBD in PSC.

**Conclusion** : Because CCA is often detected at or shortly after the diagnosis of PSC, extensive investigation is required at the time of diagnosis especially in a jaundiced patient, and close follow up during the first two years.

Yearly CA 19.9 and CT or MRI is needed to detect CCa, HCC (in case of cirrhosis) or pancreas CA. Because CRCa occurs often, regular colonoscopy (2 yrs if IBD, 5 yrs if no IBD) is necessary. Since the use of transplantation, malignancies and not hepatobiliary failure are the major cause of death.



ASSESSMENT AND PROGNOSTIC VALUE OF REGRESSION AFTER NEOADJUVANT THERAPY IN RECTAL CANCER : THE ROLE OF TUMOR SHRINKAGE AND FRAGMENTATION. M. Hav, C. Cuvelier, K. Geboes, S. Laurent, N. Van Damme, T. Boterberg, W. Ceelen, P. Pattyn, L. Libbrecht. UZ, Gent, Belgium.

**Introduction** : Patients with locally advanced rectal cancer receive neoadjuvant radio(chemo)therapy, causing a variable decrease in tumor mass. There are different ways of assessing this change, but there are conflicting data on their prognostic significance.

**Aim** : This study evaluates the prognostic relevance of different ways to assess tumor regression extent in the resection specimen.

**Methods** : Seventy-six consecutive patients who received neoadjuvant radio(chemo)therapy (radiotherapy alone : n = 6) between January 2006 and June 2009 were included. Resection specimens were examined according to PROCARE guidelines. We evaluated ypT, ypN, tumor differentiation, and lymphovascular, extramural vascular and perineural invasion. Extramural tumor deposits (ETD) were recorded and lymph node ratio (LNR) was defined as the ratio of metastatic to total examined lymph nodes. The circumferential resection margin (CRM) was measured according to Quirke. Regression was graded according to Dworak, which is based on the amount of tumor in relation to fibrosis. T-downstaging was considered present when ypT < cT. cT, cN and distant metastatic disease-free survival (DFS) were collected from the clinical files.

**Results** : cT, cN, tumor differentiation and perineural, lymphovascular and extramural vascular invasion showed no prognostic relevance. Positive ypN, and especially higher LNR, was associated with shorter DFS (p = 0.05 and 0.0025, respectively). Patients with ypT < 3 or T-downstaging had a longer DFS (p = 0.04 for both), indicating a more favorable prognosis if regression is accompanied by a decreased maximum infiltrative depth, referred to as shrinkage. Increase of CRM was associated with longer DFS (p = 0.0183), indirectly supporting the hypothesis that tumor shrinkage reflects a good therapy response. LNR was lower in patients with T-downstaging (p = 0.002), indicating that shrinkage is accompanied by a decrease of nodal tumor load. Dworak regression grade did not correlate with T-downstaging (p = 0.3623), nor with ypT (p = 0.7020). This implies that tumor mass decrease is sometimes associated with fragmentation rather than shrinkage, i.e. residual small tumor nests are scattered throughout the rectal wall. Dworak regression grade showed no prognostic value (p = 0.4672), suggesting that fragmentation does not reflect a good response to neoadjuvant therapy. ETD was clearly associated with a poorer DFS (p = 0.003).

**Conclusion** : Assessment of tumor shrinkage after neoadjuvant therapy via T-downstaging or CRM helps to predict DFS in rectal cancer patients. Shrinkage of primary tumor is associated with a decreased nodal tumor load. Assessing regression based on the amount of tumor in relation to stroma does not accurately discern fragmentation from shrinkage, which is most likely the reason why Dworak regression grading did not have a prognostic value.

BELGIAN MULTICENTER EXPERIENCE WITH THE BIODEGRADABLE ELLA-STENT IN BENIGN STRICTURES OF THE DIGESTIVE TRACT. UNDER THE AUSPICES OF THE BSGIE. M. Ibrahim (1), A. Vandermeeren (2), V. Van Maele (3), P.H. Deprez (4), I. Ruytjens (5), T. Moreels (6), E. Macken (6), J. Verhofstadt (7), P. Vergauwe (8), D. De Looze (9), O. Le Moine (1). (1) Erasme Hospital - ULB Brussels, Brussels, Belgium, (2) CHIREC-Cavell, Brussels, Belgium, (3) AZ St-Lucas, Gent, Belgium, (4) UCL Saint-Luc, Brussels, Belgium, (5) ZNA Middelheim, Antwerpen, Belgium, (6) UZ, Antwerpen, Belgium, (7) Ziekenhuis, Dendermonde, Belgium, (8) AZ Groeninge, Kortrijk, Belgium, (9) UZ, gent, Belgium.

**Introduction** : Benign strictures of the digestive tract remain a therapeutic challenge for gastroenterologists and surgeons. Whether anastomotic, chemical or induced by radiotherapy, those strictures are often treated by endoscopic balloon dilatation or bougienage but with a transient clinical benefit for the patient and the need to repeat the procedures. Stenting is a new modality which allows dilatation for weeks. However, metal stents are not indicated in benign diseases and plastic stents have a high rate of migration and need a second look for removal. Therefore, there is still room for new developments in this setting.

**Aim** : To collect the Belgian experience with the newly developed ELLA biodegradable stent. This stent has large flares and open mesh to avoid migration and is built with sugar polymers allowing its progressive digestion over around 2 months avoiding the need of a second look for stent removal.

**Methods** : Retrospective analysis of the clinical background and results for patients in whom this stent was placed for a benign disease of the GI tract. Data collection is not yet finished, these are preliminary results.

**Results :** Fifteen patients received a total of 18 ELLA-BD stents. Median age at stenting was 49 [30-72] years. The causes of strictures were post surgery in the upper GI tract in 11. These patients had a median of 10 [2-105] endoscopic procedures (dilatation, stents) before ELLA stenting over a median period of 9 [1.5-115] months. Median follow-up after ELLA stenting was 7 [1-18] months. The placement of the stent was easy in all but 2 cases (including 1 failure). Six patients presented mild to severe pain after stent placement. The median primary patency of the stent (dysphagia relief without any intervention) was 79 [0-360] days. Five patients did not need any reintervention during follow-up but some others developed severe mucosal hyperplasia persisting after stent removal and needing again iterative dilatations. The need for additional procedures after ELLA stenting was 2 [0-7] for the follow-up period.

**Conclusion :** The concept of biodegradable stent is appealing for the treatment of benign strictures of the GI tract. However in its current design, the ELLA stent needs improvements to reduce post-procedural pain and hyperplasia. A better selection of patients who will benefit of this stent on the long term is also mandatory.

- D42a -

OUTCOME OF NON VARICEAL UPPER GASTROINTESTINAL BLEEDING IN BELGIUM. H. Piessevaux, C. Taeter. UCL Saint-Luc, Brussels, Belgium.

**Introduction :** Non-variceal upper gastrointestinal bleeding (NVUGIB) accounts for the majority of emergency hospital admissions for acute GI bleeding and is associated with significant mortality and morbidity.

**Aim :** It is not known how recent therapeutic advances in pharmacologic and endoscopic therapy have influenced outcome in these patients and if Belgian patients are managed similarly to other European countries.

**Methods :** Therefore we analysed the Belgian findings of an observational, retrospective cohort study (ENERGIB ; NCT00797641). This trial was conducted between January-March 2009, with 2661 patients consecutively admitted to hospital for endoscopy for overt NVUGIB in 123 sites in : Belgium, Greece, Italy, Norway, Portugal, Spain and Turkey.

**Results :** The 26 Belgian sites recruited 427 patients (mean age 69 ; 59% male). The Belgian cohort was characterized by a high proportion of patients with one or more comorbidities (76.3 vs. 68.3%) and a high occurrence of NVUGIB while in hospital for another reason (29.7 vs. 14.7%), compared to the whole dataset. While the endoscopic diagnoses and therapies were not different, the Belgian cohort is characterized by a high admission rate to an intensive care unit (17.2 vs. 6.3%) and a low pre-endoscopy PPI use (35.0 vs. 68.9%). Despite the highest mean number of endoscopies being performed (1.74 vs. 1.34/hospital stay), Belgian patients had a poor outcome : 15.8 vs. 12.3% continued bleeding or re-bleeding, 10.1 vs. 7.7% complications and 8.0 vs. 5.2% mortality. In a multivariate analysis, controlling for the known predictors of poor outcome, Belgium performed significantly worse than Greece (Odds ratio : 0.20 (95%CI 0.06-0.66) and Portugal (OR : 0.38 (0.15-0.93) and borderline worse than Spain and Turkey in terms of mortality at 30 days.

**Conclusion :** Belgian patients with NVUGIB have a poor outcome, even controlling for known predictors. Further analysis is warranted to understand these findings and their implications for the standard of care.

HEALTHCARE RESOURCE CONSUMPTION FOR NON-VARICEAL UPPER GASTROINTESTINAL BLEEDING IN BELGIUM. H. Piessevaux, C. Taeter. UCL Saint-Luc, Brussels, Belgium.

**Introduction :** Non-variceal gastrointestinal bleeding (NVUGIB) and its complications impose a substantial burden on health care resources.

**Aim :** To help with the implementation of preventive measures for cost savings, we studied the variation in and the predictors of resource utilization for the management of NVUGIB in Belgium.

**Methods :** Therefore we analysed the Belgian findings of an observational, retrospective cohort study (ENERGIB ; NCT00797641). This trial was conducted between January-March 2009, with 2660 patients consecutively admitted to hospital for endoscopy for overt NVUGIB in 123 sites in : Belgium, Greece, Italy, Norway, Portugal, Spain and Turkey.

**Results :** The 26 Belgian sites recruited 427 patients (mean age 69 ; 59% male). The Belgian cohort was characterized by a high proportion of patients with one or more comorbidities (76.3 vs. 68.3%) and a high occurrence of NVUGIB while in hospital for another reason (29.7 vs. 14.7%). Belgian patients tended to have long hospital stays (8.73 vs. 6.88 days), high admission rates to an intensive care unit (17.2 vs. 6.3%), frequent need for angiographic coiling (1.64% vs. 1.17%) and had a high control endoscopy rate (53.52 vs. 28.64%). In a multivariate model, being treated in Belgium was a significant predictor of undergoing additional endoscopies as compared to all countries (Odd's ratios ranging from 0.09 (0.05-0.16) vs. Turkey to 0.32 (0.17-0.59) vs. Portugal.

**Conclusion :** Belgian patients with NVUGIB consume a large amount of healthcare resources, apparently more than in other European countries. Further analysis is warranted to understand these differences. Cost-evaluations will be necessary to quantify the budgetary impact of these observations.

INTESTINAL MUCOSAL GENE EXPRESSION OF ENDOTHELIAL CELL ADHESION MOLECULES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE BEFORE AND AFTER FIRST INFlixIMAB TREATMENT. I. Arijs (1), R. Quintens (2), K. Lemaire (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) University Hospital Gasthuisberg, Leuven, Belgium, (2) KU, Leuven, Belgium, (3) Ulg Sart Tilman, Liège, Belgium.

**Introduction :** Inflammatory bowel disease (IBD) is characterized by an increased leukocyte recruitment from the circulation into the gut wall. This migration is primarily regulated by adhesion molecules and chemokine receptors on lymphocytes, which allows them to respond to their endothelial ligands.

**Aim :** This study investigated the impact of anti-inflammatory therapy with infliximab on the intestinal mucosal gene expression of endothelial cell adhesion molecules (CAMs) in IBD patients.

**Methods :** The gene expression of 41 endothelial CAMs was investigated in actively inflamed mucosa from 61 IBD patients [24 ulcerative colitis (UC), 19 Crohn's colitis (CDc) and 18 Crohn's ileitis (CDi)] before and 4-6 weeks after their first infliximab infusion and in normal mucosa from 12 control patients (6 colon and 6 ileum), using Affymetrix Human Genome U133 Plus 2.0 Arrays. The patients were classified for response to infliximab based on endoscopic and histologic findings. Data was analyzed using Bioconductor software. Moderated *t*-statistics were used for comparative analyses. A false discovery rate < 5% combined with a > 1.5-fold change was considered significant.

**Results :** No genes were differentially expressed in inflamed colon when comparing UC and CDc before therapy, but large differences were observed with normals. In colonic IBD (IBDc ; UC and CDc taken together) before therapy, the mRNA expression of *CCL2-4*, *CCL11*, *CCL19-20*, *CX3CLI*, *CXCL1-2*, *CXCL5-6*, *CXCL8-11*, *CXCL13*, *ICAM1-2*, *JAM2-3*, *MADCAM1*, *PECAM1*, *SELE*, *SELP*, *THY1* and *VCAM1* was significantly increased, while the mRNA expression of *CD58* and *CD99* was significantly decreased in inflamed colon as compared to control colons. Most of these dysregulations at baseline in colonic IBD was widely restored by infliximab therapy, and only *CCL20*, *CXCL1-2* expression remained significantly increased after therapy in the colon of IBD responders. Compared to CDc at baseline, the mRNA expression of *CCL5* and *TECK* was significantly increased, and the expression of *CCL28* and *CXCL1* was significantly decreased in CDi at baseline. In CDi before therapy, the expression levels of *CCL28*, *CXCL1-2*, *CXCL5-6*, *CXCL8*, *CXCL11*, *MADCAM1*, *PECAM1* and *THY1* were all significantly increased, while *CD58* expression was

significantly decreased in inflamed ileum as compared to control ileums. No genes remained dysregulated after therapy in CDi responders as compared to control ileums.

**Conclusion :** Our data demonstrate that many endothelial CAMs are upregulated in inflamed IBD mucosa. Controlling the inflammation with infliximab restores most of these dysregulations in IBD. These results allow to identify targets for selective anti-migration therapy.

- D44 -

SCREENING COLONOSCOPY : CONVENTIONAL IMAGING VERSUS PANCOLONIC INDIGO CARMINE DYE. S. Mouzyka, A. Vinnytska. Hospital LISSOD, Kiev, Ukraine.

**Introduction :** The adenoma-carcinoma sequence developed is accepted in principle for colorectal cancer. As polypectomy is able to interrupt the transformation of benign to malignant lesions, the early detection and completely removal of these lesions is essential.

**Aim :** The aim of our study was to assess the detection rate of colorectal lesions by comparing chromocolonoscopy with standard white light colonoscopy.

**Methods :** In one institution, 206 asymptomatic patients (F118, M88 ; mean age 51) participated in study of screening video colonoscopy, performed by a single endoscopist. Informed consents were obtained in all cases. Patients were excluded if the bowel preparation was inadequate, if they had an earlier diagnosed colorectal neoplasia or inflammation, or if they were receiving anticoagulant medication. Patients received intravenous propofol prior to intubation of the colonoscope. Complete colonoscopy was performed in 204 (99%) cases. Conventional white light colonoscopy was performed in 114 patients. A further 92 patients were examined using pancolonial 0,2% solution indigo carmine dye. All lesions identified during screening colonoscopy in both groups were removed completely by cold forceps or snare polypectomy. The two groups were similar with regard to age, gender and rate of complete colonoscopy. Mann-Whitney U Tests were used to determine differences between conventional colonoscopy and chromoendoscopy.

**Results :** There was not anything complications after colonoscopy. Totally 172 lesions were detected during colonoscopy in both groups, with flat lesions 119 (70%) and protuberant lesions 53 (30%). Results of colorectal lesions detection are shown in Table, the difference between two groups was significant (p value less than 0.05).

	Lesions/patients	Patients with lesions	Patients with adenoma	Adenoma/patients
<b>Conventional colonoscopy</b>	0,63	30,7%	22%	0,38
<b>Chromoendoscopy</b>	1,08	58,7%	39%	0,65
<b>P</b>	0,0001	0,0001	0,007	0,01

**Conclusion :** The results of our comparative study show that for the detection of colorectal lesions in asymptomatic patients chromocolonoscopy is superior to standard colonoscopy. Pancolonial indigo carmine improves the detection rate of colorectal adenomas.

- D45 -

DIAGNOSTIC (DIS)AGREEMENT BETWEEN CONVENTIONAL MANOMETRY CRITERIA AND THE CHICAGO CLASSIFICATION OF ESOPHAGEAL MOTILITY DISORDERS IN TERTIARY CARE PATIENTS. S. Kindt, N. Rommel, J. Tack. University Hospital Gasthuisberg, Leuven, Belgium.

**Background :** High resolution manometry (HRM) provides a high spatial resolution topographical pressure plot of esophageal body and lower esophageal sphincter (LES) motility, whereas conventional manometry (CM) provides a limited number of single line plots. The Chicago classification has been proposed as a systematic approach to the interpretation of HRM plots (Kahrilas 2008). However, it remains unclear how this new classification relates to the conventional classification of esophageal motility disorders.

**Aim :** We aimed to address the diagnostic outcome of HRM plots and the Chicago classification over CM.

**Methods :** Manometric data from 95 consecutive patients referred to a tertiary care centre were analyzed separately in a blinded fashion by 3 assessors according to the conventional criteria using CM based on virtual sleeve manometry plots (Conv) and HRM plots (HRM) or the Chicago classification (Chicago) using HRM plots. Percentage of subjects with normal peristalsis, peristaltic dysfunction, aperistalsis, hyperperistalsis and achalasia were recorded. Also the presence or absence of normal LES relaxation and hypotensive LES was assessed. The percentage of agreement between all three assessors is reported.

**Results :** Normal esophageal motility was reported in 37 patients with Conv vs. 27 with HRM and 21 with Chicago, with an agreement in 71%. A non-specific motility disorder was diagnosed in 8 patients by Conv and 11 by HRM. According to Conv aperistalsis, mild and severe peristaltic dysfunction, spasm or hyperperistalsis, and achalasia were present in 3, 8, 8, 10 and 21 subjects, as opposed to 6, 9, 13, 11 and 18 according to HRM and 8, 13, 33, 10 and 10 patients according to Chicago (agreement in 88, 77, 70, 90 and 79%). LES function was normal in 49 subjects with Conv, 48 with HRM vs. 57 according to Chicago, with agreement in 51%. Absence of LES relaxation and hypotensive LES were detected in 20 and 17 vs. 18 and 28 vs. 10 and 15 according to Conv, HRM and Chicago resp. (agreement in 73 and 68% resp.).

**Conclusion :** Compared to Conv as well as conventional HRM interpretation, use of the Chicago classification increases the diagnosis of esophageal peristaltic dysfunction at the expense of achalasia diagnoses. The Chicago classification is more likely to consider LES function preserved than expected by conventional criteria. The therapeutic implication of the Chicago classification, especially as to small defects in the peristaltic wave and LES function as well as the differentiation between achalasia and aperistalsis should be addressed.

SUBCLINICAL INTESTINAL DYSBIOSIS IN UNAFFECTED FIRST-DEGREE RELATIVES OF CROHN'S DISEASE PATIENTS. M. Joossens (1), G. Huys (2), V. De Preter (1), P. Rutgeerts (3), P. Vandamme (2), S. Vermeire (1). (1) University of Leuven, Leuven, Belgium, (2) University of Gent, gent, Belgium, (3) University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : Bacteria play an unarguable role in the intestinal inflammation in Crohn's disease (CD). A general dysbiosis of the intestinal microbiota has been described in CD patients with a decrease in fecal numbers of *Faecalibacterium prausnitzii*. Unaffected relatives of CD patients display some CD-like features as increased gut permeability or antimicrobial responses and these are considered as subclinical markers.

**Aim** : We hypothesized that dysbiosis might also be present in a subset of first-degree relatives of CD patients as they have an increased risk for CD.

**Methods** : Fecal samples of 80 unaffected first-degree relatives of CD patients (48 siblings, 31 parents of CD patients and one subject that had both affected siblings and offspring) and 55 healthy controls were subjected to community fingerprinting of the predominant microbiota using denaturing gradient gel electrophoresis (DGGE) analysis. DGGE profiles were processed with BioNumerics software and non-parametric statistical analyses were performed with SPSS v17.0 (Bonferroni corrected p-values are given). Because of the reported lower number of *F. prausnitzii* in CD patients, we also quantified the number of *F. prausnitzii* in this cohort with real-time PCR.

**Results** : When comparing DGGE profiles of siblings of CD patients with parents of CD patients, no differences were found. Therefore, we compared DGGE banding patterns of all first-degree relatives of CD patients with the unaffected controls and found three bands to be disproportioned. The first band belonged to band-class 8.55 and was more present in unaffected relatives ( $p = 0.042$ ), whereas the bands belonging to band-class 11.35 and 16.89 were both less present in unaffected relatives of CD patients as compared to healthy controls ( $p = 0.019$  and  $p = 0.006$  respectively). Band-class 16.89 was purified and could be assigned to *Collinsella aerofaciens*. Interestingly, for all three bands a similar trend as in their unaffected relatives was seen in CD patients (not significant). Concerning the *F. prausnitzii* counts on the other hand, no difference between unaffected relatives of CD patients (11.59 log<sub>10</sub>/g ; IQR : 11.10-11.94 log<sub>10</sub>/g) and healthy controls (11.39 log<sub>10</sub>/g ; IQR : 10.62-11.84 log<sub>10</sub>/g) was observed.

**Conclusion** : Unaffected first-degree relatives of CD patients display an altered composition of their predominant microbiota, but this does not lead to overt disease. Whether this subclinical alteration of the predominant microbiota in first-degree relatives initiates further imbalance and thereby reinforces susceptibility to CD, needs to be studied in prospective follow up studies.

COMPARISON OF COMPLICATION RATES OF PUSH-TYPE AND PULL-TYPE PERCUTANEOUS ENDOSCOPIC GASTROSTOMY (PEG) IN ONCOLOGY PATIENTS. E. Van Dyck, T. Moreels, E. Macken, B. Roth, P. Pelckmans. UZ Antwerpen, Edegem, Belgium.

**Introduction** : Percutaneous endoscopic gastrostomy allows enteral tube feeding and can be performed using the conventional pull-type procedure or the newer push-type procedure. The push-type procedure is advocated in patients with head-neck or upper gastrointestinal (GI) malignancies and allows transnasal endoscopy using an ultra-thin upper GI endoscope during placement.

**Aim** : We retrospectively analysed the safety of both the push-type and the pull-type PEG procedure in oncology patients with head-neck or upper GI malignancies.

**Methods** : From January 2006 until December 2008 we placed 299 PEG-tubes in our Endoscopy Unit. In 56 patients with head-neck or upper GI malignancies, treated with chemo- and radiotherapy, push-type PEG (Fresenius Freka Pexact) was placed in 23 (41%) and pull-type PEG (Fresenius and Nutricia) in 33 (59%) patients. Retrospectively we recorded patients' characteristics and short term (less than 8 weeks) complications and mortality.

**Results** : For the push-type PEG male/female ratio was 18/5 (78% / 22%) with a mean age of  $64 \pm 3$  years. For the pull-type PEG the ratio was 27/6 (82% / 18%) with a mean age of  $65 \pm 2$  years. Short term complications were encountered in 11/23 (48%) push-type PEG patients as compared to only 4/33 (12%) pull-type PEG patients ( $P < 0.05$ ). Accidental removal of the tube occurred in 4/23 (17%) with need for surgical intervention in 1 vs. 0/33 (0%). Wound infection occurred in 3/23 (13%) leading to septic shock and admission to intensive care unit (ICU) in 1 vs. 3/33 (9%). Prolonged bleeding and aspiration pneumonia occurred in 1/23 (4%) each, both treated conservatively vs. 0/33 (0%). Finally, 2/23 GI perforations (9%) resulted from a difficult placement procedure vs. 1/33 (3%), leading to urgent surgical intervention and admission to ICU. Both push-type PEG patients died shortly after during their stay at ICU, resulting in an overall mortality rate of 9% vs. 0% and ICU admission rate of 17% vs. 3% in this selected group of oncology patients ( $P < 0.05$ ).

**Conclusion** : The push-type Freka Pexact PEG procedure for enteral tube feeding leads to significantly higher complication and mortality rates in patients with head-neck or upper GI malignancies treated with chemo- and radiotherapy. Therefore, it is suggested to use the conventional pull-type PEG tube placement in this group of patients, if possible.

EFFICACY AND SAFETY PROFILE OF CERTOLIZUMAB PEGOL : RESULTS FROM THE COMPAS PROGRAMME IN BELGIUM. S. Vermeire (1), K. Vandenbroek (1), M. Noman (1), F. Van De Mierop (2), F. Mana (3), F. Baert (4), E. Louis (5), P. Caenepeel (6), E. Humblet (6), C. Claessens (7), G. D'Haens (8), T. Moreels (9), H. Peeters (10), P. Potvin (11)(12), G. Van Assche (1), P. Rutgeerts (1), D. Franchimont (13). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) Sint Augustinus Ziekenhuis, Antwerpen, Belgium, (3) UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium, (4) H.-Hart Hospital Roeselare-Menen, Roeselare, Belgium, (5) Ulg Sart Tilman, Liège, Belgium, (6) ZOL, Genk, Belgium, (7) AZ Sint-Jozef, Turnhout, Belgium, (8) Imelda GI Clinical Research Centre, Bonheiden, Belgium, (9) UZ, Antwerpen, Belgium, (10) UZ, Gent, Belgium, (11) ZNA Middelheim, Antwerpen, Belgium, (12) St-Jozefkliniek, Bornem, Belgium, (13) Erasme Hospital - ULB Brussels, Brussels, Belgium.

**Introduction :** Certolizumab pegol, a pegylated anti-Fab fragment to TNFalpha, has proven efficacy for the treatment of Crohn's disease (CD) as shown by the large randomized Precise studies. Certolizumab is approved for treatment of CD so far only in the US and Switzerland but a compassionate use programme (COMPAS) is available in Europe.

**Aim :** We hypothesized that the unique structure of Certolizumab pegol, lacking the Fc part of the immunoglobulin (Ig), may lead to less side effects like auto-immunity and skin related paradoxical inflammation. We therefore retrospectively analyzed all CD patients included in the Belgian COMPAS program for efficacy and tolerability of certolizumab pegol.

**Methods :** A total of 41 CD patients (8 male, 33 female) treated with certolizumab pegol since the start of the COMPAS program were reviewed.

**Results :** Infliximab was the first and adalimumab the second anti-TNF in 40/41 patients. Main reasons for discontinuation of the first anti-TNF included primary non-response (7.3%), secondary loss of response (56.1%) or side effects (31.7%). The respective rates for the second anti-TNF were 21%, 45% and 30%. After initiation of certolizumab pegol, 85% of patients reported short term clinical response, including 32.5% of patients with complete disappearance of all symptoms. In 15%, no response was observed despite a minimum of 3 injections. Skin related side effects occurred in 15 patients under Certolizumab pegol (36%) with psoriasiform eczema (n = 8) and xerosis cuti (n = 3) as the main manifestations. Eight of these patients developed their skin reactions de novo under Certolizumab pegol. Of 32 patients (78%) who had antinuclear antibodies (ANA) measured during certolizumab pegol, 11 (34%) had positive ANAs of whom 3 were anti-dsDNA positive. There were no other serious adverse events reported.

**Conclusion :** Treatment with certolizumab pegol as third anti-TNF in CD patients with lack of response or loss of response to infliximab and adalimumab was associated with good short term efficacy. Patients are further followed to assess also the long term efficacy. The presence of antinuclear antibodies as well as skin manifestations does nevertheless also occur under certolizumab pegol and represent therefore a true class effect of anti-TNFs which is unrelated to the presence of the Ig Fc portion.

On behalf of the Belgian IBD research group.

SERUM ADIPONECTIN STRONGLY CORRELATES WITH THE DEGREE OF STEATOSIS AND INFLAMMATION BUT NOT FIBROSIS IN A PROSPECTIVELY INCLUDED COHORT OF NAFLD PATIENTS. S. Francque (1), A. Verrijken (1), I. Mertens (1), M.R. Taskinen (2), G. Hubens (1), E. Van Marck (1), P. Michielsens (1), L. Van Gaal (1). (1) UZ, Antwerpen, Belgium, (2) Helsinki University Central Hospital and Biomedicum, Helsinki, Finland.

**Introduction :** Recent reports suggest a difference in adiponectin and/or adiponectin/leptin ratio between NASH and simple steatosis, and a correlation of adiponectin with fibrosis. Studies are, however, hampered by important bias or retrospectivity and yield conflicting results.

**Aim :** To study the relation between liver histology, adiponectin and leptin in a prospectively included cohort of overweight patients.

**Methods :** Patients presenting to the obesity clinic for a problem of overweight underwent a metabolic and liver assessment. If NAFLD was suspected, a liver biopsy was proposed. Liver biopsy was scored using the NASH CRN scoring system.

**Results :** A series of 62 patients were prospectively included. Mean age was  $46.3 \pm 12.7$  y ; 45 (72.5%) were female. Mean BMI was  $37.1 \pm 6.6$  kg/m<sup>2</sup>. The whole spectrum of NAFLD from normal liver to NASH-cirrhosis (NAS ranging 0-7, fibrosis 0-4) was present. Mean serum adiponectin levels gradually decreased with increasing steatosis ( $12037.88 \pm 1711.43$  ng/mL in grade 0 vs.  $7049.77 \pm 854.17$  ng/mL in grade 3,  $p = 0.017$  ; Kruskal Wallis for all grades  $p = 0.005$ ) and increasing NAS ( $12039.00 \pm 1358.89$  ng/mL in NAS = 0 vs.  $6502.40 \pm 1390.65$  ng/mL in NAS = 7,  $p = 0.025$  ; Kruskal Wallis test  $p = 0.030$ ). Serum adiponectin also decreased with increasing stage of fibrosis, but without reaching statistical significance. Serum adiponectin levels strongly correlated negatively with the degree of steatosis ( $r = -0.379$ ,

p = 0.002) and NAS (r = -0.372, p = 0.002) but not fibrosis. In regression analysis, adiponectin is a stronger independent predictor of the degree of steatosis (p = 0.004) than CT-measured visceral adipose tissue (VAT) (p = 0.050), as is the case for NAS (p = 0.011). It is, however, not a predictor for fibrosis (p = 0.645) in contrast to VAT (p = 0.019). Leptin levels correlate with BMI (p = 0.001) and VAT (p < 0.001), but not with any of the histological parameters. The adiponectin/leptin ratio showed results comparable to adiponectin alone, with even stronger associations with the degree of steatosis (r = -0.422, p < 0.001) and NAS (r = -0.409, p = 0.001) and no correlation with fibrosis stage.

**Conclusion** : Serum adiponectin and adiponectin/leptin ratio closely and negatively correlated to both the degree of steatosis and the severity of steatohepatitis as expressed by NAS in a gradual way. They are not, however, significant markers of fibrosis. Serum leptin alone has no association with NAFLD histology.

- D50 -

MINIMALLY INVASIVE OESOPHAGECTOMY : A VALUABLE ALTERNATIVE TO OPEN OESOPHAGECTOMY FOR THE TREATMENT OF EARLY OESOPHAGEAL AND GASTRO-OESOPHAGEAL JUNCTION CARCINOMA. P. Naftoux, J. Moons, W. Coosemans, H. Decaluwé, G. Decker, P. De Leyn, D. Van Raemdonck, T. Lerut. University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : For almost two decades, minimally invasive esophagectomy (MIO) has been presented as a valuable alternative to open surgery for the treatment of oesophageal and gastro-oesophageal junction (GOJ) carcinoma. But controversy still remains as to its real value in functional and oncological outcome.

**Aim** : This study is a single institution retrospective comparative analysis of outcome after MIO versus open oesophagectomy (OO) for early oesophageal and gastro-oesophageal junction carcinoma.

**Methods** : Data were obtained from our prospective database. Indications for MIO was cT1N0M0. This subset of patients was compared with a group of patients who underwent OO for dcT2 and N0 or suspicion of N1 limited to the peritumoral area. Surgical outcome, survival and quality of life (QoL) were assessed. All the data had been prospectively collected in a database. Post-operative complications were recorded using the Clavien-Dindo classification, QoL was evaluated using the EORTC approved QoL QLQ-C30 and OES 18 questionnaires before the surgery and every 3 months postoperatively.

**Results** : Between Januari 2005 and April 2009 147 patients (82 OO, 57 MIO and 8 MIO converted to OO) fulfilled the abovementioned criteria (121 male, 26 female, mean age 63,6 year). The predominant histology was adenocarcinoma (75,5%), equally distributed between both groups (MIO versus OO). Pre-operative comorbidities were also equivalent between both groups (p = 0,489). Pathologic staging was equivalent (pT : p = 0,78 ; pN : p = 0,26). Blood loss was less (p = 0,02) but duration of operation was longer (p = 0,0003) in the MIO group. Number of resected lymph nodes was lower in the MIO group (p = 0,01). In hospital mortality between both group was comparable (p = 0,64). The postoperative complications were comparable between both groups (p = 0,112), but respiratory complications and readmission to ICU were more frequent in the OO group (respectively p = 0,01 and p = 0,02). On the other hand, gastro-intestinal complications (p = 0.01) in particular gastroparesis (p = 0.04) were more frequent in the MIO group. 5 year cancer specific survival and recurrence free survival were 96% for MIO versus 90% for OO (p = 0,788) and 97,9% for MIO and 84,8% for OO (p = 0,247) respectively. At 3 months, there was a trend in favour of MIO for pain (general) and gastro-intestinal pain (respectively p = 0,08 and p = 0,09). After 1 year, only gastro-intestinal symptoms (mainly gastroparesis) are significantly different in favour of the OO group (p = 0,03)

**Conclusion** : Taking into account the limitations of a retrospective study, it appears that MIO is a valuable alternative to OO for the treatment of early oesophageal and gastro-oesophageal junction carcinoma with especially less post-operative respiratory complications and re-admission to ICU. At 3 months there is a trend for less pain after MIO but this trend faded out at 1 year.

This study underscores the need for large scale preferably multicentric studies to assess the real value of MIO versus OO.



EXTENT OF LIVER RESECTION IN CAROLI'S DISEASE : A MULTI-INSTITUTIONAL RETROSPECTIVE STUDY OF 33 PATIENTS. C. Hubert (1), J. Y. Mabrut (2), C. Partensky (3), E. Oussoultzoglou (4), D. Jaeck (4), J. Baulieux (2), O. Boillot (3), J. Lerut (1), J. B. Otte (1), J. De Ville de goyet (1), M. Audet (3), C. Ducerf (2), J. F. Gigot (1). (1) UCL Saint-Luc, Brussels, Belgium, (2) La Croix-Rousse Hospital, Lyon, France, (3) Hôpital Edouard Herriot, Lyon, France, (4) Hautepierre Hospital, Strasbourg, France.

**Introduction** : Surgical management of Caroli's disease (named also type V congenital bile duct cysts in Todani classification) remains controversial.

**Aim** : To report the short and long-term results of Caroli's disease in Europe.

**Methods** : Retrospective multi-institutional study of 33 patients (32 adults and one child) suffering from Caroli's disease in 4 European university hospitals included suffering from Caroli's disease.

**Results** : There were 26 patients with unilobar [left = 20, right = 6] and 7 patients with bilobar distribution). The mean duration of symptoms was 54 months. Intrahepatic bile duct stones, congenital hepatic fibrosis and cholangiocarcinoma were associated 61%, 30% and 3%, respectively. A previous therapeutic biliary procedure was performed in 73%. All patients, but one suffering from a right unilobar disease with congenital hepatic fibrosis, underwent liver resection according to the extent of the disease within the liver. Out of 7 patients with bilobar disease, 4 with congenital hepatic fibrosis were treated by liver transplantation and the 3 others without congenital hepatic fibrosis underwent extended liver resection. The 20 patients with left unilobar disease all underwent partial hepatectomy while the 5 patients with right unilobar disease underwent liver resection and liver transplantation for associated congenital hepatic fibrosis in 4 and 1 case, respectively. Postoperative mortality was nil. Postoperative morbidity occurred in 48%. Any postoperative additional procedure for residual intrahepatic stones was required in 15%. Five patients (15%) required reoperation for complications. At final pathology, synchronous cholangiocarcinoma was found in 6%. During a mean follow-up of 95 months in 28 patients with a follow-up time exceeding 6 months, 5 patients developed complications due to recurrent intrahepatic stones leading to reoperative procedures in 3 patients. Patients alive, free of symptoms and disease-free were observed in 83% of left unilobar forms of the disease (6/8 after prior left lobectomy and 9/10 after prior left hepatectomy), in 4/4 of right unilobar forms of the disease and in 3/5 of bilobar forms of the disease (3/3 after liver transplantation and 0/2 after hepatic resection). The 2 patients presenting superficial cholangiocarcinoma were alive and disease free at 89 and 143 months.

**Conclusion** : Unilobar forms of Caroli's disease are successfully treated by radical hepatic resection, especially for left unilobar forms of the disease by preferring left formal hepatectomy to left lateral segmentectomy. For bilobar forms of the disease with coexistent congenital hepatic fibrosis, liver transplantation offers good long term results. In the absence of congenital hepatic fibrosis, the limited number and heterogeneous presentation of patients in this series does not permit to compare the results of liver transplantation to large hepatic resections.

IS THE USE OF IV GADOLINIUM MANDATORY IN LIVER MRI FOR CHARACTERIZING FOCAL LIVER LESIONS AS BENIGN OR MALIGNANT ? M. Antic (1), A. Schiettecatte (1), D. Verdries (1), B. Op de Beeck (2), F. Vandenbroucke (1), S. Dymarkowski (3), J. De Mey (1), M. Dujardin (1). (1) UZ, Brussels, Belgium, (2) UZ, Antwerpen, Belgium, (3) KU, Leuven, Belgium.

**Introduction** : Since the discovery of nephrogenic systemic fibrosis (NSF) the question whether we should complete each liver MRI with enhanced images becomes pertinent.

**Aim** : The purpose of this study is to evaluate whether unenhanced T1- and T2-sequences would be sufficient for the characterization of a liver lesion as benign or malignant.

**Methods** : A total of 70 liver lesions (25 cysts, 28 hemangiomas, 1 abscess, 2 focal nodular hyperplasia's, 11 metastases and 3 hepatocellular carcinomas), obtained from 49 patients were evaluated. Routine MRI liver protocol on a 1.5 Tesla system (Siemens, Symphony) included for the unenhanced sequences : T2-Haste (TE = 60, TE = 90), T2-Haste (TE = 60) FS, T1-in/opposed phase, T1-Flash +/- FS. After injection of 20 ml of a standard Gadolinium chelate at 2 cc/sec injection rate, dynamic T1-Flash FS was performed. Three experienced MR-body radiologists characterized these lesions as benign or malignant in a blinded study. Time between the evaluation of the unenhanced and total images (both enhanced and unenhanced) was one week. No patient information was given.

**Results** : In 12.4% of the lesions, the radiologists' opinion was altered when contrast enhanced images were added to the unenhanced series. The expert readers changed their characterization from benign to malignant in 4.3% (N = 3.0) and from malignant to benign in 8.1% (N = 5.7) of the 70 lesions. Such shift in characterization were observed in the first place in the hemangioma group, and in second place in the metastases. Average accuracy in characterization of a lesion as benign or malignant improved from 89.0% to 98.4% when post-contrast images were also available.

**Conclusion :** In our opinion, the proportion of lesions that had altered general characterization as a benign or malignant lesion on MRI after contrast imaging can not be neglected. In a renally impaired patient, the substantial improvement in focal liver lesion characterization accuracy from Gadolinium administration should be weighed against the small risk of inducing NSF.

- D53 -

COMBINED LIVER AND KIDNEY TRANSPLANTATION. T. Darius, D. Monbaliu, R. Aerts, T. Roskams, E. Lerut, D. Cassiman, C. Verslype, W. Laleman, Y. Vanrenterghem, F. Nevens, J. Pirenne. University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction :** Renal failure in a Liver Transplant (LTx) candidate is a risk factor of morbidity and mortality after isolated LTx. Alternatively, liver disease in a Kidney Transplant (KTx) candidate is a risk factor of morbidity and mortality after isolated KTx. Therefore, Combined Liver and Kidney Transplantation (CLKTx) for combined kidney and liver disease theoretically represents the best option. But in an era of organ shortage, the use of 2 organs in a single recipient can only be justified if the results are satisfactory. International registries indicate higher peritransplant mortality in CLKTx *versus* isolated Tx recipients.

**Aim :** In this study we reviewed our experience with CLKTx.

**Methods :** Between January 1997 and November 2009, 44 patients (6,7% of all LTx) underwent CLKTx. Mean age was 51 years (4-69). Indications for LTx were : hepatorenal polycystosis (n = 18), postethyl cirrhosis (n = 9), hepatocellular cancer (4), and others (13). Indication for KTx was advanced irreversible renal disease : 28 were dialysis-dependent (hemodialysis > 3 months) and 16 had a creatinine clearance between 8 and 39 mL/min.

**Results :** Follow-up is : 2-148 months. 1 and 5 year patient, liver and kidney graft survival are 93,2% and 93,2%, and 90,8% and 90,8%, and 97,4% and 93,4%, respectively. Primary non function of the liver and kidney grafts were observed in 0% and 2,3%(n = 1), respectively. Delayed graft function of the kidney (= transient need for dialysis post Tx) was observed in 9,1%(n = 4). Acute cellular rejection of the liver and kidney graft were seen in 6,8%(n = 3) and 15,9%(n = 7), respectively ; all were successfully treated with steroids. 2,3% (n = 1) developed simultaneous rejection of both grafts. Kidney function remained stable in all recipients except 6,8%(n = 3) who developed terminal renal failure requiring renal replacement therapy because of presumed rejection(n = 1), polyoma virus infection(n = 1)and chronic allograft nephropathy(n = 1),7 days, 42 and 78 months, respectively post-Tx.

**Conclusion :** In patients suffering from irreversible diseases of liver and kidney, simultaneous replacement of both organs is the preferred option and is justified in view of the poor natural history without Tx, the poor results of isolated Tx (liver or kidney), and the excellent survival and the low rate of rejection seen after combined transplantation.

LIVER TRANSPLANTATION FOR ADULT POLYCYSTIC LIVER DISEASE. T. Darius (1), A. Patris (2), D. Monbaliu (1), E. Bonaccorsi (2), R. Aerts (1), W. Coosemans (1), T. Roskams (1), Z. Hassoun (2), O. Ciccarelli (2), Y. Pirson (2), Y. Vanrenterghem (1), F. Nevens (1), J. Lerut (2), J. Pirenne (1). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) UCL Saint-Luc, Brussels, Belgium.

**Introduction** : Liver transplantation (LTx) for adult polycystic liver disease (PLD) has been controversial because : 1) liver function remains stable until late in the disease, 2) other surgical options are available, 3) LTx is *not immediately* life saving but is associated with morbidity/mortality. However, non transplant surgical options usually offer transient/partial palliation and morbidity/mortality of LTx is dramatically decreased. Therefore, LTx for PLD needs to be reevaluated.

**Aim** : We review the experience of 2 LTx centers with isolated LTx and combined liver kidney transplantation (CLKTx) in patients with PLD.

**Methods** : Between 1995 and 2008, 56 patients from 2 LTx centers (KUL/UCL) underwent LTx for PLD. The main indication for LTx was massive handicapping hepatomegaly secondary to small and diffuse PLD. 7 patients with isolated PLD received LTx alone. Of 49 patients with combined PLD and ADPKD, 31 underwent isolated LTx and 18 CLKTx. Among the 18 CLKTx patients, 11 were dialysis-dependent at the time of LTx whereas kidney transplantation (KTx) was performed preemptively in 7 (creatinine clearance (CrCl) : 15,4-38,3 mL/min).

**Results** : All surviving patients became symptom-free after LTx and went back to a normal life style. The 1 and 5 year patient and liver graft survival are 96,3% and 93,6%, and 96,2% and 90,0%, respectively. Of the 31 patients who underwent isolated LTx for combined PLD and ADPKD, 29% (n = 9)(mean pre-LTx CrCl 75,8 mL/min, range 47,6-109,7 mL/min) developed terminal renal failure 0 to 114 months post-LTx. The mean pre-LTx CrCl in the 71% patients who display a stable kidney function after LTx was 77,8 mL/min (range 46,5-152,9 mL/min).

**Conclusion** : This series demonstrates that LTx and CLKTx for PLD provide complete symptom relief and excellent survival. Terminal kidney failure after isolated LTx is due to evolving renal polycystosis and calcineurin inhibitor toxicity. In patients with preserved or mildly affected renal function receiving isolated LTx, strategies to spare the nephron mass are essential. In patients with overt evolving renal impairment pre-transplant, CLKTx is the preferred option, anticipating the need for a later KTx. LTx should be the first line treatment in small diffuse handicapping PLD.

A MUTICENTER INTERNAL AUDIT ABOUT FOLLOWING GUIDELINES ON OSTEOPOROSIS PREVENTION AND DETECTION IN GI DISEASE. D. Baert. Maria Middelaers Ziekenhuis, Gent, Belgium.

**Introduction** : It is well known that several disorders of the GI tract are associated with a significant higher risk for osteoporosis. When we focus on the prevention and detection of osteoporosis in daily practice, it is our perception that gastroenterologists only look sporadically for osteoporosis in their patients with CIBD, celiac disease, postgastrectomy, ... We performed this multicenter audit in order to quantify this problem.

**Aim** : The aim of this study is to audit the frequency in which gastroenterologists are looking for osteoporosis in daily practice, and to audit the available guidelines on the subject for uniformity and implementation feasibility.

**Methods** : In 4 non-university GI departments (Maria Middelaers Ghent, AZ St Lucas Ghent, AZ Jan Palfijn Ghent, St Vincentius Deinze), the total number of bone densitometries (BMD's) for GI disease are retrospectively recorded for 2006-2007-2008. Furthermore, guidelines on the subject according to the American Gastroenterological Association, the British Society of Gastroenterology, the European Crohn's and Colitis Organisation, and the European guidance for management of osteoporosis in postmenopausal women, are reviewed.

**Results** : During the 3 years study period, only 6 BMD's were done in Middelaers (6 doctors), none in Deinze (2 doctors), 3 in Palfijn (3 doctors) and 9 in Lucas (6 doctors). The indications for BMD were CIBD (6), celiac disease (3), pancreatic insufficiency (3), postgastrectomy (2), alcohol abuse (2) and auto-immune hepatitis (2). On average, these gastroenterologists only ask one BMD per year in their patient population. Although the number of BMD's is an indicator with limited value, since the total number of true indications for BMD is unknown, it may indicate the rather limited awareness of gastroenterologists for the osteoporosis problem in GI disease.

Secondly, comparing the available guidelines, they seem sometimes contradictory and generally rather difficult to implement. We distillate 2 simplified measures to be used in clinical practice :

- vit D + calcium : to be supplemented in postmenopausal women, in men > 55 y of age and in patients on corticosteroids.
- BMD : to be performed in persistent inflammation or disease with : weight loss > 10% or BMI < 20 or age > 70 y of age.

**Conclusion** : In the GI departments who participated in this study, there is only a limited use of bone densitometries in daily practice. On average, only one BMD per year per gastroenterologist is performed.

According to the guidelines however, a routine BMD in patients with celiac disease, CIBD, postgastrectomy,... is not advocated. A BMD is recommended in selected patients with higher risk, according to disease activity, weight loss, BMI and age.

In general, in order to better implement guidelines, clinicians should be able to simplify them and integrate them into the patient files and correspondence with the general practitioner, serving as reminders for future follow up.

LIVER TRANSPLANTATION FOR HEPATOCELLULAR FAILURE AFTER BILIOPANCREATIC DIVERSION (SCOPINARO) OR JEJUNO-ILEAL BY-PASS FOR WEIGHT REDUCTION : A BELGIAN SURVEY. A. Geerts (1), T. Darius (2), T. Sablon (1), R. Aerts (2), T. Chapelle (3), G. Roeyen (3), L. Libbrecht (1), T. Roskams (2), F. Nevens (2), S. Francque (3), J. Pirenne (2), R. Troisi (1). (1) Ghent University, Ghent, Belgium, (2) University Hospital Gasthuisberg, Leuven, Belgium, (3) Antwerp University, Antwerp, Belgium.

**Introduction** : The prevalence of obesity has increased dramatically over the last decades and occurrence of non-alcoholic steatohepatitis (NASH), a co-morbidity related to obesity, is increasingly observed. Therapeutic options for morbid obesity include bariatric surgery. Cases of liver failure after this type of malabsorptive weight reduction surgery are rarely reported in the current literature. Fatal liver failure has been recorded after jejunum-ileal bypass but is controversial in bilio-pancreatic diversion (Scopinaro operation).

**Aim** : Aim of this study was to perform a survey to the frequency of performed liver transplantations (OLT) for liver failure after bariatric surgery in Belgian transplant centers.

**Methods** : An enquiry was sent to the six Belgian liver transplant centers to investigate the occurrence of sub acute and chronic liver failure in this setting requiring OLT.

**Results** : After weight-reduction surgery, 10 patients in Ghent, Leuven and Antwerp were listed for OLT due to severe hepatocellular failure. Nine of them underwent a Scopinaro operation and a 1 jejunum-ileal bypass. The median time for developing liver failure after Scopinaro was 5 years. The patient with jejunum-ileal bypass developed a chronic liver failure after 25 years. Seven patients were transplanted, two died awaiting a graft and one is still on the list. Following OLT, 1 patient developed a rapid re-appearance of a cirrhotic liver with liver failure @ 10 months requiring retransplantation (probably a combination of bacterial overgrowth for late dismantling of Scopinaro and weight regain). Two recipients died shortly after OLT because of MOF and, in another one, a de novo cancer was fatal @ 6-y follow-up. The remaining recipients are doing well.

**Conclusion** : Bariatric surgery has been advocated as a safe and excellent treatment for severe obesity but this survey shows that the Scopinaro operation carries a potential risk of liver failure. However, even if there are only 10 cases, we are unaware of the real figures of Scopinaro-induced liver failure. This may be extremely low and the survival advantage (due to reduced obesity) can largely offset this small risk of liver failure. We advise to strictly follow liver function in these patients, eventually, a timely dismantling of the Scopinaro to reverse liver failure or to prevent re-occurrence of NASH after OLT.

LAPAROSCOPIC CHOLECYSTECTOMY IN ACUTE CHOLECYSTITIS : EVIDENCE TO SUPPORT A PROPOSAL FOR AN EARLY INTERVAL SURGERY. K. Boterbergh, F. Berrevoet, I. Adriaenssens, H. Peeters, E. De Wolf, B. Van Den Bossche, X. Rogiers, B. De Hemptinne, R. Troisi, A. De Croo. University Hospital, Ghent, Belgium.

**Introduction** : Early laparoscopic cholecystectomy has been advocated for the management of acute cholecystitis, but the superiority of this approach over delayed-interval operation is not always recognized.

**Aim** : To determine whether there is an increase in morbidity, hospital stay or conversion rate, when comparing acute to delayed surgical treatment for acute cholecystitis.

**Methods** : In this retrospective study 725 patients were evaluated for laparoscopic cholecystectomy over a 6 year period. All patients treated for an episode of acute cholecystitis were analyzed, comparing early (onset less than 5 days) versus delayed (antibiotic treatment and surgery 6 weeks later) surgical intervention. We evaluated the safety and feasibility of an early cholecystectomy for uncomplicated acute cholecystitis. Both grading of cholecystitis, duration of symptoms, reasons for delayed operation, conversions to open procedures and morbidity were evaluated for all patients.

**Results** : In total 147 patients were diagnosed with acute cholecystitis from 01/01/2004 till 20/11/2009. In 72 patients the treatment was early laparoscopic cholecystectomy of which 7 (1%) were converted to an open procedure due to difficult anatomy (n = 2), severe inflammation (n = 4) or others (n = 1). For 36 patients delayed cholecystectomy was the preferred treatment option because symptoms were present for a longer period of time (n = 17) or for other unspecified reasons (n = 19). In this group the conversion rate was 17% (n = 6) and not significantly lower. Two patients had to be operated within the 6 weeks interval (5.5%) with insufficient response to conservative treatment. When considering hospital stay, there was no difference when patients were operated within 3 days or 5 days respectively. Morbidity mainly consisted of pneumonia, wound infection and minor biliary leakage, but was not different between the groups. In total hospital stay was 6.6 days in case of early operation versus 5.4 days in case of delayed surgery (p < 0.05).

**Conclusion** : Early laparoscopic cholecystectomy resulted in a reduced length of hospital stay without an increase in morbidity or conversion rates compared to delayed surgical treatment after antibiotic treatment. This should influence our management in case of urgent admissions for acute cholecystitis.

WILSON'S DISEASE : LONG-TERM FOLLOW-UP OF A COHORT OF TWENTY-FOUR PATIENTS TREATED WITH D-PENICILLAMINE. K. Lowette, K. Desmet, P. Witters, W. Laleman, C. Verslype, F. Nevens, J. Fevery, D. Cassiman. University Hospital Gasthuisberg, Leuven, Belgium.

**Aim** : Detailed data on long-term effectiveness of various drug therapies in Wilson's Disease (WD) are lacking. Therefore, we retrospectively reviewed our patient cohort treated with D-penicillamine.

**Methods** : This study reports on the clinical presentation, the diagnostic evaluation and the disease course in twenty-four WD patients treated long-term (15 +/- 12 years, between 1969 and 2009) with D-penicillamine.

**Results** : The overall survival in our cohort was 91,6%. 22/24 patients had liver disease at presentation, 17/24 patients (71%) had cirrhosis, 11 of whom had complications of cirrhosis. 6/11 of these patients showed hepatological improvement (5/6) or stabilisation (1/6) ; 3/11 were transplanted ; 1/11 died ; 1/11 discontinued follow-up. In the 6/17 cirrhotic patients without complications, improvement (4/6) or stabilisation (2/6) occurred. Of all other patients (7/24), 5/7 showed improvement (3/5) or stabilisation (2/5) ; hepatological deterioration occurred only in one due to poor therapy compliance and 1/7 discontinued follow-up. Neuropsychiatric symptoms were present in 13/24 at presentation and resolved in 1/13, decreased in 7/13, stabilized in 4/13 and worsened in 1/13 (due to poor compliance). In general, we observed a favourable hepatological and neurological evolution with D-penicillamine.

**Conclusion** : Despite the presence of liver disease or neuropsychiatric symptoms at baseline in all but one of the patients, we report beneficial results on liver and neurological disease after very long-term treatment with D-penicillamine, thereby adding to its reputation as "first-line" therapy in WD.

WEIGHT REDUCTION BY MEANS OF AN INTRAGASTRIC BALLOON IN DAILY ROUTINE PRACTICE : RESULTS AT REMOVAL AND IN LONG-TERM. E. Cesmeli (1), H. Troubleyn (2), M. Daems (3), C. Tuytens (1), D. Vansteenkiste (1), T. Onghena (1). (1) AZ St-Lucas, Gent, Belgium, (2) KU, Leuven, Belgium, (3) St-Lucas, Gent, Belgium.

**Introduction** : Intra-gastric balloons are not commonly used as a therapy for obesity due to concerns about the possible side effects and doubts about effectiveness in long-term. Recent publications suggest a sustained effect on weight loss. In this study we present the results in a non-academic community practice.

**Methods** : All patients who had received an intra-gastric balloon (BIB) therapy in our hospital and had retained the balloon for at least 3 months were retrospectively evaluated. Successful therapy was defined as a weight loss  $\geq 10\%$  of weight at baseline. To assess the long-term effects on weight reduction we contacted the patients in whom the balloon was removed for longer than 2 years.

**Results** : 181 patients were treated with an intra-gastric balloon between January 2002 and September 2009. All balloons were placed and afterwards removed by a gastroenterologist under general anesthesia in day hospital. There were no major complications. 10 balloons were removed during the first month due to intolerance, 2 were spontaneously released and 3 balloons were removed elsewhere. The remaining 166 patients fulfilled the inclusion criteria and were used for the analysis. Mean BMI (body mass index) at baseline was 34,7 (range : 27-52) and mean weight 92 kg (range : 66-140). The balloons were removed after a mean of 7 months (range : 3-23). The mean BMI at removal was 30,1 (range : 19,6-48,5). Mean weight loss was : 11,4 kg (range : 0-33). 102 individuals (61,4%) had a  $\geq 10\%$  baseline weight loss by time of the balloon removal. 50 patients could be contacted for the long-term evaluation. Mean time after removal was 5,5 years (range : 2-7). 11 patients (22%) had received bariatric surgery. For the remaining 39 patients the results for mean BMI are depicted in the table below.

BMI at baseline	BMI at balloon removal	BMI after a mean of 5,5 years
33,1 (range : 27,9-45)	28,9 (range : 22,5-37)	30,0 ( 24-37)

16 of these 39 patients(41%) had retained the weight loss of  $\geq 10\%$ .

**Conclusion** : Intra-gastric balloon therapy was associated with a successful weight loss at removal in 61,4%. There were no major complications. In long-term there seems to be a sustained effect in a subgroup of patients. Prospective studies are needed to better define the patients who will benefit from this therapy.

THE ROLE OF SINGLE-BALLOON ENDOSCOPY IN DIAGNOSTICS AND MINIMALLY INVASIVE TREATMENT OF PATIENTS WITH A CLINICAL SUSPICION OF OBSCURE BLEEDING. E. Ivanova, E. Fedorov, M. Timofeev, O. Yudin, P. Tchernaikovich. Moscow University Hospital #31, Moscow, Russia.

**Introduction** : Preoperative diagnostics of intestinal bleeding have always been a difficult problem because much of the small intestine was inaccessible for endoscopic devices.

**Aim** : To evaluate new method of single-balloon enteroscopy (SBE) %SIF-Q180Y (Olympus, Japan) for diagnostic and therapeutic enteroscopy in patients with suspected intestinal bleeding.

From V.2003 to VI.2009 a total of 74 (54 planned, 20 urgent) enteroscopies were performed in 58 patients (m-30, f-28 ; mean age  $59,4 \pm 9,5$  yrs., range 16-89) with suspected intestinal bleeding. VCE was performed in 34 pts. and SBE (from II.2007) - in 40 pts. There were 30 transoral examinations (including 5 therapeutic), 10 colonoscopies and from the both directions in 8 pts. Obvious bleeding had 41 patients and another 17 occult. However, in 18 of the 41 patients with clinically obvious bleeding, abnormalities which were discovered during the esophagogastroduodenoscopy and colonoscopy disagreed with the clinical picture and severity level of bleeding. In the other patients (n = 40), there were no abnormalities of the upper and lower digestive tracts and they were classified as fully obscure bleedings when we made the decision to perform enteroscopy.

**Results** : Total time for transoral SBE ranged from 35 to 130 min. (mean  $69,1 \pm 13,5$  min.) and for transanal from 70 to 120 min. (mean  $93,8 \pm 12,2$  min.). The mean depth of oral SBE insertion was  $314,7 \pm 49,1$  cm and anal -  $193,0 \pm 43,4$  cm.

The source of the intestinal bleeding was confirmed in 33 (56,9%) (m-18, f-15, range 20-89 yrs, mean age  $56,8 \pm 11,9$  yrs.) of the 58 pts. ; intestinal erosions and ulcers were located in 12 (36,4%), vascular pathology in 11 (33,3%) (8 angiodysplasias and 3 phlebectasias), tumors in 7 (21,2%) (3 leiomyomas, 2 lymphomas, 1 adenocarcinoma, 1 Peutz-Jeghers syndrome), Meckel's diverticulum in 2 (6,1%) and lymphatic cysts in 1 (3,0%). No side effects nor complications during SBE were observed. 22 patients received subsequent medical treatment, 5 were endoscopically treated (APC in ulcer jejunum bleeding in 1, clipping of angiodysplasias and phlebectasias in 2, removal of a large epithelial tumor in 1 patient with Peutz-Jeghers syndrome, polypectomy in 1) and surgical treatment was performed in 6 pts.

**Conclusion** : This newfound endoscopic technique helps to solve problems with diagnosing obscure bleeding and to determine the best course of treatment for this category of patients.

SINGLE-BALLOON ENTEROSCOPY IN PATIENTS WITH SMALL BOWEL ABNORMALITIES. E. Fedorov, E. Ivanova, M. Timofeev, O. Yudin. Moscow University Hospital #31, Moscow, Russia.

**Introduction** : Balloon assisted enteroscopy provides stable access into difficult parts of the small intestine.

**Aim** : To evaluate medical and technical aspects of performing single-balloon enteroscopy (SBE) in patients with small bowel abnormalities.

From 14.02.2007 to 01.06.2009 SBE have been successfully done in 78 pts. (m-40, f-38, mean age  $56,1 \pm 10,2$  yrs., range 21-89). There were 116 procedures have been undertaken with XSIF-Q180Y (Olympus, Japan) ; 108 (102 planned, 6 urgent) of them have been performed successfully. There were 80 examinations from the oral route (including 13 therapeutic) and 28 from the anal route.

**Results** : Total time for transoral SBE ranged from 20 to 140 min. (mean  $73,8 \pm 22,1$  min.) and for the transanal from 50 to 120 min. (mean  $79,3 \pm 14,3$  min.). In oral SBE the mean depth of insertion was  $357,0 \pm 17,2$  cm (range 100-500 cm), in anal \_ it was  $192,0 \pm 31,5$ cm (range 30-250 cm). SBE is allowed us to reveal the following abnormalities in 79,4% of pts. (n = 78) : enteritis in 15 (19,2%) incl. 6 with Crohn's disease and 1 with radiation enteritis with stricture (balloon dilation using W-Cook balloon was performed) ; erosions and ulcers in 10 (12,8%) ; tumors in 4 (5,1%) incl. 1 leiomyoma, 2 lymphomas, 1 adenocarcinoma ; benign polyps in 7 (9,0%) ; vessel pathology in 8 (10,3%) (in 2 clipping using EasyClip, Olympus) ; inheritable pathology in 4 (5,1%) incl. 2 with Peutz-Jeghers syndrome (polypectomy of the large epithelial tumors was performed) ; celiac disease in 3 (3,8%) ; lymphangiectasias in 7 (9,0%) ; acute adhesive small bowel obstruction in 4 (5,1%) (decompression tube was set in 2 of them). In all 6 pts. after surgical operations with B-II, Balfur and Roux en Y reconstruction, in spite of long afferent loop, we were able to reach bile ducts, but performed the therapeutic ERCP in only 3 of them due to lack of full range of long instruments. No side effects nor complications during diagnostic SBE were observed. There was 1 case of small bowel perforation after balloon dilation of radiation stricture on the next day. The patient was operated and discharged from the hospital after 10 days later in good condition.

**Conclusion** : SBE guaranties high quality diagnostics and topmost the possibility for getting biopsy and to treat small bowel diseases as well as pancreatobiliary diseases in abnormal anatomical conditions.

ACUTE APPENDICITIS AFTER COLONOSCOPY : AN UNDERVALUATED COMPLICATION ?  
E. Vanderstraeten, D. Baert, E. Monsaert, K. Rasquin, P. Burvenich. AZ Maria Middelaes, Ghent, Belgium.

**Introduction** : Five endoscopists perform about 1600 colonoscopies in our department every year. Earlier data have shown that quality criteria are met. Acute appendicitis is thought to be a rare complication of colonoscopy that has been reported in English-language literature in only fifteen cases. The etiology of this complication has not yet been elucidated. It was estimated that the incidence of appendicitis following colonoscopy is about 0,038%.

**Methods** : Between June and September 2009 three patients (two females, one male, 54yo, 46yo and 44yo) underwent laparoscopic appendectomy following colonoscopy. Only biopsies were taken during these colonoscopies. The indications were red blood loss in the two female patients and surveillance for Crohns disease in the male patient. One of the female patients was diagnosed with a non-obstructive sigmoidal cancer. The main symptom at admission was pain (24 to 48 h after colonoscopy) in all three patients and diagnosis of an appendicitis was made with CT-scan and US.

**Results** : Laparoscopic appendectomy was performed and revealed an acute appendicitis (one with perforation) in the three patients. The postoperative course was uneventful. The oldest patient underwent a laparoscopic sigmoidectomy three weeks later.

**Conclusion** : Diagnostic colonoscopy is a relative low-risk procedure. Appendicitis is a rare but important complication of colonoscopy. Occurring hours to days after the procedure, clinicians should consider postcolonoscopy appendicitis in a patient with postprocedural abdominal pain and patients should be warned about this complication. We add three more patients to the 15 reported cases in literature, therefore it seems prudent to question the real frequency of this complication. Since one of the proposed mechanism consists of reactivation of subclinical disease of the appendix, perhaps CT-scan has to be the preferred examination in patients with right lower-quadrant pain.

CAUSES OF NON-RESECTION OF POLYPS DURING COLONOSCOPY IN HOSPITALIZED PATIENTS.  
M. A. Denis, C. Oxford, K. Azzouzi, O. Dewit, H. Piessevaux, P. Hoang, A. Uytterhoeven, P. Deprez. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : Hospitalized patients (inpatients) are known to be at higher risk of cancelled colonoscopy or failed polypectomy.

**Aim** : The aim of this study was to analyse the causes of failed polypectomy (FP), in order to improve care for patients and avoid a second procedure.

**Methods** : From June 1<sup>st</sup> 2008 to May 31<sup>st</sup> 2009, 2562 colonoscopies were performed in our GI department, of which 484 (19%) for inpatients. Among these, 153 patients (31.6%) required a polypectomy that was cancelled in 28 of them (FP group) : 18% of polypectomies and 5.7% of the inpatients. Data concerning FP patients, including bowel cleansing modalities and procedure findings were retrospectively analysed from nurse charts and medical reports.

**Results** : Reasons for non-resection were insufficient cleansing in 10 patients (36%), coagulation disorders (n = 5), current bleeding (n = 5), neoplasia and need for surgical resection (n = 4), anticoagulant or antiplatelet treatment (n = 4), high number of polyps (n = 4) or technical failure (n = 2), some FP patients having more than one cause of non-resection. Nine out of the 28 FP patients required a second colonoscopy for polypectomy during the same hospitalization or soon after, whilst the other patients were either operated or referred for follow-up at 6 -12 months.

Bowel preparation consisted in a PEG regimen (3 to 7 liters given the night before the colonoscopy) except for one patient who received oral sodium phosphate solution. No differences in type of bowel preparation and tolerance were observed in FP group, except that only 50% of them followed a residue-free diet for 3 days before colonoscopy. Coagulation disorder was thrombocytopenia due to cirrhosis in 80% of the FP patients. Out of the 28 FP patients, 9/13 (69%) treated with antiplatelet or anticoagulant drugs continued treatment. The reason given by the patients was that they had not been required to stop their treatment. In 4 out of those 9 cases, this was the only reason not to perform polypectomy. Concerning the number of polyps, 11/28 patients (39%) had only 1 polyp seen during the colonoscopy, 7 had less than 5 polyps, 7 between 5 and 10 polyps, and 3 patients more than 10 polyps. Size of polyps was lower than 5 mm in 12 patients, 5 had a polyp between 5 and 15 mm, and 11 a polyp higher than 15 mm.

**Conclusion** : The main causes of failed or cancelled polypectomy for inpatients is inadequate bowel preparation, frequently associated with no respect of diet restriction, and lack of patients' information about antiplatelet or anticoagulant treatment management before hospitalization. These results suggest the need for a better inpatients' information and an important role for the nursing during hospital stay.

PROSPECTIVE COMPARISON OF FULL THICKNESS RESECTION OF SUBMUCOSAL TUMOURS WITH AND WITHOUT A NEW GRASPING DEVICE TO HELP TRIANGULATION : THE ENDOLIFTER. P. Deprez, J. Navez, B. Navez, J.F. Gigot, E. Legrand, P. Gianello, C.P. Yeung. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : Endoscopic resection of deep submucosal tumors, leiomyomas, GISTs, or even mucosal lesions with adherence to the muscularis propria cannot be easily or safely performed with EMR or ES techniques and are therefore potential indications for NOTES. Full thickness resection of the digestive wall needs better endoscopic tools.

**Aim** : The aim of our study was to evaluate a new triangulation device that can be adjusted to the tip of a conventional endoscope.

**Methods** : Prospective comparative animal survival study with and without the Endolifter (Olympus) to achieve full thickness resection of a 3 cm artificial submucosal tumor. The endoscope used was a single accessory channel with waterjet facility (GIF-1T160). The pseudotumor was created by injecting 3-5 ml of HPMC and was subsequently marked with a 2 mm margin with Hook knife. Gastric wall was incised after percutaneous CO2 insufflation in the peritoneum, with a Hook knife (Olympus) and circumferential dissection (plus haemostasis) including all gastric layers was done with an IT knife 2. Closure was performed with Brace-bars. The animals were sacrificed at 14 days to check for healing.

**Results** : Full thickness resection was successfully achieved in all 6 pigs. Median resection time from incision to extraction of specimen was 32 min (range 30-52 min) with Endolifter and 62 min (58-73) without Endolifter ( $p = 0.03$ ). Median closure time from application of first Brace-bar until last one was 31 min (22-47) and 30 min (15-39) with and without Endolifter, respectively (NS). Six to 11 Brace-bars were placed for complete closure of the large defect. One closure was incomplete and resulted in pig's death at 12hours post closure. On necropsy, most brace bars were unattached and deep ulcers were observed in all pigs.

**Conclusion** : Full thickness resection of large gastric tumors can be achieved in a reasonable time with conventional endoscopes and might be faster and safer by use of grasping devices such as the Endolifter.

DIFFERENTIAL MUCOSAL EXPRESSION OF TH17 RELATED GENES IN THE SMALL AND LARGE BOWEL OF IBD PATIENTS. S. Bogaert, D. Laukens, H. Peeters, M. Lode, K. Olievier, J. Vandesompele, D. Elewaut, M. De Vos. Ghent University, Ghent, Belgium

**Introduction** : The association of inflammatory bowel disease (IBD) with IL12B, JAK2, STAT3 and CCR6, genes involved in the Th17 pathway, point at the importance of Th17 cells in IBD.

**Aim** : Expression levels of Th17 related genes were studied in colonic and ileal biopsies of healthy controls, Ulcerative colitis (UC) and Crohn's disease (CD) patients.

**Methods** : Colonic and ileal mucosal samples of healthy controls, UC and CD patients were obtained during colonoscopy. Quantitative PCR was performed to analyze the mRNA expression levels of pro-inflammatory cytokines (IL8, IL1A, IL1B, TNF, IL6), Th17 effector cytokines and chemokines (IL17, IL21, IL22, IL26, CCL20) and genes involved in Th17 cell differentiation and amplification (IL23A, TGFB1, STAT3, IL23R, CCR6, JAK2).

**Results** : The expression levels of pro-inflammatory cytokines were increased in the inflamed mucosa of IBD patients and basal in patients in remission. Expression levels of IL8, IL1A and TNF were higher in active UC as compared to CD. In the inflamed colon of IBD patients expression levels of TGFB1, IL23A, STAT3, CCR6, JAK2 (UC only) and the Th17 derived chemokine CCL20 were significantly increased, whereas in the inflamed ileum of CD patients only JAK2 was markedly elevated. Concerning Th17 effector cytokines, expression levels were only detectable in 15-31% of colonic and 31-50% of ileal samples of healthy controls. Although significantly more inflamed samples expressed the Th17 effector cytokines, mRNA levels of IL17 were increased in the colon and only IL22 was increased in the ileum. IL26 was only increased in the inflamed colon of UC patients. Expression levels of IL17 were higher in the inflamed colon of UC patients compared to CD patients.

**Conclusion** : Our data illustrate that the expression of pro-inflammatory cytokines can be used as a objective marker of inflammation. Furthermore, the increased expression of most Th17 related genes in the inflamed colon of IBD patients in contrast to the basal expression in the inflamed ileum of CD patients implies a different immune regulation in the colon and ileum, suggesting different therapeutic approaches for colonic and ileal disease.



DEFINING ENDOSCOPIC RESPONSE IN CROHN'S DISEASE. M. Ferrante, M. Noman, S. Vermeire, G. Van Assche, P. Rutgeerts. KU, Leuven, Belgium.

**Introduction** : Endoscopic healing has become a major goal of treatment in patients with Crohn's disease (CD). In contrast to clinical response rates, endoscopic response rates to placebo therapy have not been described yet. Currently, there is no definition of endoscopic response to be used in clinical trials.

**Aim** : We planned to evaluate the evolution of endoscopic lesions in the ileocolon of CD patients who received placebo therapy.

**Methods** : The study population consisted of 24 patients with moderate to severe CD (CDAI 220-450). All these patients were randomized to placebo therapy for a period of 6 weeks as part of two double-blind placebo-controlled trials investigating novel biological agents. At baseline, all patients had active ileocolonic ulcers. If using corticosteroids or immunomodulatory agents, the dose had to be stable before inclusion for at least 2 weeks for corticosteroids and 8 weeks for azathioprine, 6-mercaptopurine and methotrexate. Throughout the trial, dosing of concomitant medication remained stable. Both at baseline and 6 weeks after start of therapy, ileocolonoscopies were performed and recorded by experienced gastroenterologists at different study sites. The central reader, who was blinded for study number, treatment and recording sequence, calculated both the Crohn's disease endoscopic index of severity (CDEIS) and simple endoscopic score for Crohn's disease (SES-CD).

**Results** : At baseline, the median (interquartile range) CDEIS and SES-CD were 13.23 (10.74 - 18.86) and 16.50 (14.00 - 19.50) points, respectively. There was a close correlation between both scores (Spearman's  $r = 0.810$ ,  $p < 0.001$ ). After 6 weeks of placebo therapy, none of the patients had complete mucosal healing. The CDEIS dropped to 10.18 (5.94 - 14.41) with a median delta of -1.42 (-4.55 - 0.50) points or -8.88 (-43.75 -7.75) percent (Wilcoxon  $p = 0.076$ ). The SES-CD dropped to 13.00 (7.25 - 16.00) with a median delta of -2.00 (-5.75 - 1.00) points or -14.29 (-44.00 -6.90) percent ( $p = 0.012$ ). In two patients both endoscopic activity scores improved dramatically with at least 50% and at least 5 points. In another four patients either the CDEIS or the SES-CD improved with at least 50% and at least 5 points.

**Conclusion** : Although in most patients with moderate to severe CD endoscopic lesions remained stable or worsened under placebo therapy, endoscopic activity scores improved markedly in 6 patients. Based on the quartiles of the distribution of this dataset, we propose that endoscopic improvement of CD ileocolitis should be defined as a decrease of any of the 2 endoscopic activity scores (CDEIS or SES-CD) with at least 50% and at least 5 points. Complete mucosal healing stays the most robust endpoint.

ADALIMUMAB IMPROVES WORK PRODUCTIVITY FOR PATIENTS WITH MODERATELY TO SEVERELY ACTIVE CROHN'S DISEASE : SUBGROUP ANALYSIS OF BELGIAN PATIENTS IN THE CARE TRIAL.

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**Introduction** : Adalimumab is approved for the treatment of severe Crohn's disease (CD) in Europe and has been shown to induce and maintain remission for both anti-TNF-naïve and infliximab-experienced patients (pts) with CD. It's necessary to investigate the impact of adalimumab on work productivity for overall Europe pts as well as the subgroup of Belgian pts and estimate the cost savings associated with the improvement for Belgian pts.

**Methods** : CARE was a multicentre, open-label, European, Phase IIIb trial that included pts naïve to biologic therapy and pts who failed infliximab. Pts with moderate to severe CD (Harvey Bradshaw Index scores  $\geq 7$ ) received induction therapy of 160-mg/80-mg adalimumab at Weeks 0/2, followed by adalimumab 40-mg every-other-week maintenance therapy until Week 20 (pts with flares/nonresponse could receive 40 mg weekly at/after Week 12). Changes from baseline to Weeks 2, 4, 8, 12, and 20 (observed values) on the Work Productivity and Activity Impairment Questionnaire (WPAI), including absenteeism, presenteeism, total work productivity impairment (TWPI) and total activity impairment (TAI) were analyzed by using paired Student *t*-tests. The percentages of pts who reached the minimum clinically important difference (MCID ; absolute change of at least 7%) for TWPI and TAI components were summarised using nonresponder imputation at Weeks 4, 8, 12, and 20. Work-related cost savings were estimated over 1 year using TWPI improvements at Week 20.

**Results** : For pts naïve to anti-TNF therapy and those who had failed infliximab therapy, WPAI improvements were observed as early as Week 2 and were maintained throughout the study for both overall study pts and for the Belgian subset of pts. For the 71 Belgians among 907 study pts included in the TAI analysis, 63% Belgian and 62% overall pts met the MCID at Week 20. For the 32 Belgian pts who were employed ( $n = 442$  for all pts), 47% (48% for all pts) met

the MCID for TWPI at Week 20. Improvement in TWPI after 1 year of treatment was 24% (62 days of full work productivity), which translates to an estimated per-patient indirect cost savings of €7884.

**Conclusion :** The results from Belgian subset were consistent with overall pts in CARE. Adalimumab therapy significantly improved work productivity and reduced daily activity impairment for pts with moderate to severe CD. Reductions in CD-related work loss and productivity impairment could result in significant cost savings.

- D68 -

ADALIMUMAB FOR INDUCTION AND MAINTENANCE OF REMISSION IN ANTI-TNF-NAÏVE AND ANTI-TNF-EXPOSED CROHN'S DISEASE PATIENTS IN THE CARE STUDY : RESULTS FROM BELGIUM. E. Louis (1), F. Baert (2), O. Dewit (3), G. Van Assche (4), G. D'Haens (5), T. Moreels (6), A. Robinson (7), M. Kron (8), P. Pollack (7). (1) University of Liege, Liege, Belgium, (2) Heilig Hartziekenhuis, Roeselare, Belgium, (3) Université Catholique de Louvain, Brussels, Belgium, (4) University Hospital Gasthuisberg, Leuven, Belgium, (5) Imelda GI Clinical Research Centre, Bonheiden, Belgium, (6) Antwerp University Hospital, Antwerp, Belgium, (7) Abbott, Abbott Park, IL, United States, (8) Abbott GmbH&Co.KG, Ludwigshafen, Germany.

**Introduction :** Adalimumab, a fully human, anti-tumour necrosis factor (anti-TNF) monoclonal antibody, is approved for the treatment of Crohn's disease, and has been shown to induce and maintain remission in patients both naïve to or previously exposed to anti-TNF therapy. The Crohn's patients Treated with Adalimumab : Results of a Safety and Efficacy Study (CARE) evaluated efficacy and safety of adalimumab in patients whose treatment approximated usual clinical practice.

**Aim :** In this analysis, we evaluated the induction and maintenance of clinical remission in patients enrolled in CARE at sites located in Belgium. CARE was a multicenter, open-label, Phase IIIb trial conducted in Europe, which enrolled adult patients with Harvey-Bradshaw Index (HBI) scores > 7, including patients naïve to anti-TNF therapy and patients who had failed prior infliximab treatment. Adalimumab induction therapy (160mg/80mg at Weeks 0/2) was followed by maintenance therapy (40mg every other week) through at least Week 20 (patients with flares/nonresponse could receive 40 mg weekly at or after Week 12). Remission (HBI < 5) rates at Weeks 4 and 20 were analyzed overall and by prior exposure to infliximab, using non-responder imputation for subjects with missing values.

**Results :** 72 of the 945 CARE patients were enrolled in Belgium. Of this cohort, 52 patients (72%) were female, 46 (64%) were < 40 years old, 45 (63%) had previously received infliximab, and 43 (60%) were receiving concomitant corticosteroids and/or immunosuppressants at baseline. Remission rates at Week 4 were 51% (37/72) overall, 70% (19/27) in anti-TNF naïve patients, and 40% (18/45) in patients previously treated with infliximab. Remission rates at Week 20 were 44% (32/72) overall, 56% (15/27) in anti-TNF naïve, and 38% (17/45) in infliximab-exposed patients. 22 patients (31%) experienced treatment-emergent serious adverse events ; the most common were infections (9/72, 12.5%) and Crohn's disease (5/72, 6.9%). There were no cases of tuberculosis or lymphoma and no deaths.

**Conclusion :** In patients enrolled in the CARE study at Belgian sites, the majority of whom had previously failed infliximab therapy, the efficacy of adalimumab therapy was similar at Weeks 4 and 20 to that in the entire CARE population and in the adalimumab pivotal clinical trials. Adalimumab was well-tolerated, with overall safety profile consistent with prior reports.

IMPAIRED BUTYRATE OXIDATION IN ULCERATIVE COLITIS IS DUE TO DECREASED BUTYRATE UPTAKE AND A DEFECT IN THE OXIDATION PATHWAY. V. De Preter, G. Vandermeulen, P. Rutgeerts, K. Verbeke. University of Leuven, Leuven, Belgium.

**Introduction :** Butyrate, a colonic metabolite of carbohydrates, is considered as the major energy source for the colonic mucosa. Several *in vitro* and *in vivo* studies have reported an impaired butyrate metabolism in ulcerative colitis (UC) due to a defect in the butyrate oxidation pathway and/or transport.

**Aim :** The aim of the present study was to investigate whether the butyrate oxidation deficiency in UC is a consequence of a reduced butyrate uptake.

**Methods :** Colonic mucosal biopsies were collected during endoscopy of 25 UC patients and of 12 control patients with normal colonoscopy. The activity of UC was assessed using the endoscopic Mayo Score. Butyrate uptake and oxidation was measured by incubating biopsies with <sup>14</sup>C-labeled Na-butyrate and measuring the released <sup>14</sup>CO<sub>2</sub> by b-liquid scintillation counting. Results were corrected for protein content.

**Results :** Both butyrate uptake and oxidation were significantly decreased in UC compared to controls (p < 0.001). Subsequent division of the UC patients into disease activity subgroups using endoscopic criteria (Table 1), showed a decreased butyrate uptake and oxidation in the different subgroups as compared to controls and/or quiescent disease (Mayo 0-1). Butyrate oxidation remained significantly reduced in moderate (Mayo 2) and active (Mayo 3) disease after correction for butyrate uptake suggesting that butyrate oxidation itself is also affected.

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

	Butyrate oxidation	Butyrate uptake	Ratio oxidation/uptake
Controls (n = 12)	21.16 ± 14.04	4.58 ± 2.24	5.37 ± 4.21
UC (n = 25)	5.75 ± 5.39 <sup>a</sup>	2.56 ± 1.04 <sup>a</sup>	2.29 ± 2.24 <sup>a</sup>
ayo 0-1 (n = 11)	10.12 ± 5.53	3.15 ± 0.99	3.17 ± 1.52
Mayo 2 (n = 3)	1.69 ± 1.12 <sup>ab</sup>	2.15 ± 0.27 <sup>a</sup>	0.76 ± 0.42 <sup>a</sup>
Mayo 3 (n = 11)	2.49 ± 1.07 <sup>ab</sup>	2.18 ± 1.03 <sup>a</sup>	0.99 ± 0.38 <sup>ab</sup>

Significantly different from control colon (a) and quiescent UC (b) : p < 0.05

**Conclusion :** Our results indicate that the butyrate oxidation deficiency in intestinal inflammation is not only a consequence of a reduced butyrate transport, but also of a defect in the butyrate oxidation pathway.

IBD AND MONTH OF BIRTH. J.M. Ghilain, M. Vanderhofstadt, M. Schapira, O. Descamps. Hôpital de Jolimont, Haïne-Saint-Paul, Belgium

**Introduction :** Genetic susceptibility and environmental factors (smoking, viral or bacterial infections, appendectomy...) are implicated in the genesis of inflammatory bowel disease (IBD). Some authors postulate that perinatal factors can interfere with the development of IBD like it was shown in some patients' studies with asthma, multiple sclerosis or diabetes mellitus. Four studies have already studied the influence of the month of birth and the occurrence of IBD. The results are controversial : two showed no relation between date of birth and development of IBD while two studies were in favour of such a relation.

**Aim :** The aim of our study is to evaluate in our patients with IBD a possible link between date of birth and later development of IBD.

**Methods :** We included all patients affected with IBD that were seen in our hospital during the last five years. We compared the months of birth of IBD patients with that of a control group constituted by the total of patients registered in our computer database and born in an interval of 5 days around IBD patient's birth date (58000 patients). We have done an x<sup>2</sup> test to compare both population.

**Results :** 507 patients were included, 195 affected by Ulcerative colitis (UC) and 311 with crohn's disease (CD). There isn't any significative difference between men and women. We observed a significant difference for patients born in August-September-October who have less probability to be affected of UC and conversely for patient born in November and December who have an increased risk to develop UC compared with other months of the year (p = 0.0112). The results are less significant concerning CD : we observed a significative peak in August-September and October compared with the rest of the year (p < 0.001) but the result isn't significative for November and December (like it was for UC (p = 0.12)).

**Conclusion :** This study confirmed a significative relation between month of birth and occurrence of ulcerative colitis and Crohn disease. It is the first to show an association between UC and date of birth. People born between August and

October have a reduced risk to develop UC but with an increased risk for CD. Inversely to be born in the end of the year (November and December) increases later development of UC but does not have any impact on future development of CD. This result may indicate that prenatal and perinatal event like respiratory or gastro-intestinal infection seems to modulate the development of inflammatory disease. Moreover, like another Belgian study has already showed, the month of August seems to be associated with a risk for CD.

- D71 -

PLASMA OCTANOYLATED GHRELIN LEVELS IN YOUNG CHILDREN WITH GASTROESOPHAGEAL REFLUX DISEASE (GERD) AND FOOD REFUSAL. E. Dierckx (1), S. Annemans (1), S. Staelens (1), E. De Wever (1), I. Depoortere (2), G. Veereman-Wauters (1). (1) Queen Paola Children's Hospital, Antwerpen, Belgium, (2) University of Leuven, Leuven, Belgium.

**Introduction :** Ghrelin is an orexigenic peptide, mainly produced by the X/A like cells in the oxyntic mucosa of the stomach. Plasma ghrelin levels increase before the meal to regulate the frequency of the meals. Ghrelin o-acetyltransferase octanoylates ghrelin to its active form which binds to the ghrelin receptor to stimulate food intake.

**Aim :** The aim of this study was to determine total (octanoylated and non-octanoylated) and octanoylated ghrelin levels in young children with GERD and food refusal compared to normal controls.

**Methods :** A single centre, prospective observational study was performed in 9 patients aged between 0 and 5 years with GERD and food refusal (energy intake less than 80%). The median growth percentile is p10 in this patient group. The control group consisted of 12 healthy children under 5 years of age with a normal growth and appetite. Their median growth percentile was p50.

Blood samples were taken after an overnight fast. Total and octanoylated plasma ghrelin levels were measured with radioimmunoassay. Statistical analysis was performed using the unpaired t-test. IRB approval was obtained for all subjects.

**Results :** Patients with GERD and food refusal have total plasma ghrelin levels of 957 +/- 174 pg/ml. This was significantly ( $P = 0.004$ ) lower than in the control group where ghrelin levels of 592 +/- 37 pg/ml were obtained. Octanoylated plasma ghrelin levels in the patient group were also elevated (98 +/- 27 pg/ml) in comparison with the control group (41 +/- 5 pg/ml) ( $P = 0.014$ ).

**Conclusion :** Ghrelin is significantly higher in young children with GERD and food refusal. This can be explained as a result of their lower body weight. However, these children do not respond to this endogenous hunger stimulus possibly because of the pain due to pyrosis.

**BeSPGHAN**

**Invited lecture**

- E03 -

ENDOSCOPY FOR GERD IN CHILDREN  
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## BHPSG GROUP

- H01 -

THE DIAGNOSIS OF H. PYLORI RESISTANCE BY OTHER METHODS THAN CULTURE. V.Y. Miendje Deyi (1), Z. Bentatou (1), Y. Maaroufi (2), M. Scaillon (3), S. Cadranel (3), A. Burette (4), M. Reynders (2). (1) ULB Brugmann, Brussels, Belgium, (2) ULB Saint-Pierre, Brussels, Belgium, (3) ULB HUDERF, Brussels, Belgium, (4) CHIREC - Basilique, Brussels, Belgium.

**Introduction :** Antimicrobial resistance in *Helicobacter pylori* (Hp) infection is an important factor of treatment failure ; therefore, a rapid susceptibility testing is needed to improve the management of this infection. Culture allows tests for antibiotic susceptibility. However the result is available within 2 weeks and its accuracy depends on the conditions of transportation and processing of the specimens.

Hp resistance is essentially due to point mutations which can be detected with molecular tests. Genotypic methods included a single method without gene amplification (FISH) and several methods with amplification among which real-time PCR and PCR-RFLP are mainly used, particularly for clarithromycin (CLA)-resistance.

**Aim :** To evaluate a new molecular test GenoType® HelicoDR (based on PCR + hybridization) which allows within 6 hours a molecular detection of Hp and characterisation of mechanisms of resistance for clarithromycin (CLA) and fluoroquinolones (FQs)

**Methods :** From January to November 2009, each gastric biopsy submitted to routine culture was ground in sterile water and an aliquot was frozen at -70°C for further genotypic evaluation.

The main evaluation involved 65 randomly selected gastric biopsy specimens containing Hp (positive culture, one specimen per patient). The second group included 58 specimens from which the analysis was requested by gastroenterologists based on several criteria : discordance between antrum and corpus results (n = 20 biopsies, 7 patients with discordant susceptibility results and 3 with discordant culture results -pos/neg-), negative culture in contrast to other diagnostic methods (n = 13), unavailable phenotypic result (dead strains ; n = 5), unexplained treatment failure with negative culture (n = 10).

On the other hand, the robustness of the molecular method was evaluated using 15 biopsies previously used for Rapid Urease Testing (positive RUT) and stored at room temperature during 10 days.

The molecular results were compared to the phenotypic method (culture + MIC-determination for CLA and FQs). In case of discrepancy in susceptibility testing, a control was processed by the 2 methods by using a single Hp isolate.

### **Results :**

1. Among the 64 biopsies, 37 (58%) genotyping results were concordant with MIC determination, 22 (34%) showed a mix of genotypes, and a true discrepancy was observed in FQs resistance in 5 (8%) specimens (1 resistance only with PCR and 4 only with MIC). Compared to MIC, the sensitivity in detecting resistance was 100% and 95.3% for CLA and FQs respectively.

2. Molecular analysis revealed that the phenotypic differences between antrum and corpus results were mostly related to mixed Hp populations (6 out of 7 patients) or lack of sensitivity of the culture (PCR detected Hp in 3 biopsies for which culture remained negative). All the 13 culture-negative biopsies revealed a positive genotyping result in correlation with other diagnostic methods and consequently susceptibility results became available. The 5 biopsies with unavailable phenotypic result yielded valuable susceptibility results with PCR. For the subgroup of selected biopsies based on eradication failure, 9 (90%) showed a positive result compared to none with culture.

These results suggest a higher sensitivity of the molecular method and the possibility to examine non- viable strains as supplementary advantage.

3. Finally, the 15 samples from positive RUT revealed the presence of Hp with mixed population in 5 (33%) of them.

**Conclusion :** GenoType HelicoDR is a promising molecular test for diagnosis and detection of CLA and FQs resistance in Hp directly from gastric biopsy specimens. Owing to the restricted use of culture (rigorous analysis conditions, time consuming&) we have shown that this new method could be helpful particularly after documented treatment failure. It has an advantage of being robust, rapid and can reveal a mixture of several genotypes reflecting a co-infection or selection for resistant mutants.

Further molecular analyses are needed to assess the specificity of the method and to investigate the point mutations in FQs resistance-determining regions in the 4 discrepancy Hp isolates which did not reveal any mutation with this molecular method.

## Invited lecture

- H02 -

THE CHALLENGE OF RESCUE THERAPY OF HELICOBACTER PYLORI INFECTION. A. Burette. CHIREC - Basilique & E. Cavell, Brussels.

**Introduction** : Triple therapy regimens containing clarithromycin (PPI + clarithromycin + amoxicillin or metronidazole for 7-14 days) are the best validated, most recommended and widely used first line treatments for *Helicobacter pylori* infection (cf. European, USA, Canadian, Japanese, WGO & other guidelines) and remain still the standard therapy with amoxicillin, clarithromycin and metronidazole as “key” antimicrobial agents.

However the efficacy of these currently recommended PPI-based triple therapies appears to be declining this last decade. Indeed the most recent data addressing the efficacy of these regimens reports eradication failures in 15-30% of the cases. The two most important causes of treatment failure include primary or acquired antimicrobial resistance and poor patient adherence with the treatment regimen. Clinically, patient non-compliance with the eradication therapy not only decreases the efficacy of therapy but also increases the likelihood of developing antimicrobial resistance. Patient adherence is influenced by a variety of factors including the duration of therapy, complexity of the treatment regimen and the frequency and severity of treatment associated side effects. Taking the time to talk to the patient and to explain the present viewpoints on *H pylori* and related diseases, giving the rationale for antimicrobial therapy as well as explaining carefully the regimen and informing the patient of the most common side effects and suggesting not to stop therapy without prior medical advice can help to improve compliance. Other factors which may have some impact on treatment response include the patient's country of origin, the indication for *H pylori* treatment (e.g. peptic ulcer vs. dyspepsia), and tobacco use.

All epidemiological studies confirm that the prevalence of *H pylori* infection is decreasing in the western populations so that the proportion of *H pylori* positive patients issued from the immigrated population is increasing. And the lower adherence with the treatment regimen reported in these population may result from a lack of comprehension, so the need to take time to carefully explain the therapy particularly to this kind of patient and to his/her family and verify if the message was well understood.

One must also be aware that patients who fail with their first treatment most probably include a higher percentage of individuals who are unreliable tablet takers, others who have resistant organisms and also a ‘constitutional’ group, where failure will be inevitable.

The background rate of clarithromycin resistance is critically important as its presence negatively impacts the efficacy of the first-line clarithromycin-based regimens. Indeed primary clarithromycin resistance adversely affects the success rate of clarithromycin-based triple therapies by 50-70%. The same considerations are also probably true for quinolones-based triple therapies. Unlike clarithromycin resistance, metronidazole resistance adversely affects the success rate of metronidazole-based triple therapies by 25-30% and can, to some extent, be overcome by lengthening the duration of therapy and/or using higher doses of metronidazole or adding a PPI to bismuth triple therapy.

Emergence of resistance after triple therapies failures is also of concern : after exposure to clarithromycin, emergence of resistant strain has been observed in  $\approx 25\%$  (0-50%) although after exposure to metronidazole, emergence of resistant strain is observed in  $\approx 50\%$  when the infecting strain is clarithromycin-sensitive and  $\geq 50-100\%$  when the infecting strain is clarithromycin-resistant. Emergence of resistant strain is also very common after exposure to fluoro-quinolones. Patients who are not cured with  $\geq 2$  consecutive treatments including clarithromycin and metronidazole will have at least single, and usually double, resistance.

In Belgium, primary resistance to antimicrobials in 2008 was  $\pm 30\%$  (20-45%) for imidazoles,  $\pm 15\%$  (10-20%) for macrolides,  $\pm 25\%$  (20-30%) for fluoro-quinolones but rare for amoxicillin ( $< 1\%$ ) and tetracycline (2%) and 5-15 strains are multi-resistant. We must be aware of these data as resistance evaluation is not widely available.

So we must also be prepared to face treatment failures. Therefore, we have to design a treatment strategy that did not focus on the results of primary therapy alone, but also on the final (overall) eradication rate.

The choice of a ‘rescue’ treatment depends on which treatment is used initially. In Belgium bismuth salts are no more available and the very successful bismuth-based quadruple therapy cannot be used anymore in the second line. On the other hand, furazolidone-based therapies are not available and rifabutin-based therapies not accessible (only available for TBC resistant strain). Therefore, management of first-line eradication failures is becoming challenging.

**General considerations** for second line therapies include : never use clarithromycin or levofloxacin if already included in the first line therapy ; consider prolongation of therapy up to 10 or 14 days (but controversial results of meta-analysis : Europe = ; USA +) ; antimicrobial susceptibility testing and tailored therapy if possible (recommended when resistance rates in the population are  $> 20\%$ ) ; consider regimen combining PPI + amoxicillin + an antibiotic not included in the previous regimen ; if using metronidazole : 500 mg tid instead of bid ; if using amoxicillin : soluble better than tablets & *never forget that post treatment testing is mandatory to confirm cure!*

Several ‘rescue’ therapies have been recommended as alternatives to the second-line bismuth-based quadruple therapy : the quinolones-based triple therapies, the sequential therapy, the concomitant therapy and high dose dual therapy (PPI + amoxicillin).

If a first-line clarithromycin-based regimen was used, a **second-line quinolone (levofloxacin)-based regimen** (including PPI + amoxicillin + levofloxacin) may be used with the advantage of efficacy, simplicity and safety, although the

drawback is the increasing prevalence of quinolone resistant strains in our country. This high level of fluoro-quinolones resistance suggests that levofloxacin based \_rescue\_ therapy may not be very effective in eradicating *Hp* except if the strain is known to be sensitive to quinolones.

**Sequential therapy** is a new concept in eradication therapy : with this form of therapy, antibiotics are administered in a sequence rather than all together. The sequential regimen combines 10-day PPI treatment with amoxicillin for the first 5 days and clarithromycin + metronidazole for the following 5 days. A recent meta-analysis of 10 Italian trials found an eradication rate of 93.4% with sequential therapy as compared to an eradication rate of 76.9% with PPI-triple therapy (Jafri et al, Ann Int Med, 2008). In a recent double-blind randomised comparison of sequential therapy with conventional triple therapy Vaira et al. (Ann Int Med, 2007) found that 10-day sequential therapy had a significantly higher eradication rate (91%) compared with 10-day triple therapy (78%). Of particular interest was the success of sequential therapy in patients with clarithromycin-resistant strains. The eradication rate in patients with clarithromycin-resistant strains of *H pylori* was 89% with sequential therapy and 29% with standard triple therapy. Given this potential benefit in patients with clarithromycin-resistant *H. pylori* infection, one wonders about the use of sequential therapy in patients with persistent infection. At present, however, there is no data available on the use of sequential therapy as salvage regimen. Despite these promising results, the apparent complexity of the regimen and the evidence that its efficacy in Asia is more disappointing tempers enthusiasm for its wide acceptance and further validation of this novel therapy outside Italy is expected before it can be recommended routinely as second-line or even first-line therapy.

Administering all the key antibiotics together (PPI + amoxicillin + clarithromycin + metronidazole) is the basis for **Non-bismuth quadruple therapy** (= **Concomitant** or **Concurrent therapy**) that was originally developed a decade ago to try to decrease the duration of the therapy. With reported eradication rate of > 90% in an ITT basis this therapy seems as performant as the novel sequential therapy (Essa et al., Helicobacter, 2009) and may be an interesting, less complex alternative. In an open-label comparison with sequential therapy from Taiwan, efficacy of concomitant therapy was similar to the one of sequential therapy (Wu et al, GE, 2008). However, as there are no recent data from Western populations with the current rates of resistance, there is a need for well-controlled studies. Further the available studies offer little data on antimicrobial resistance and its effect, if any, on the efficacy of sequential or concomitant therapy.

**High dose dual therapy** (PPI + amoxicillin for 14 days) is another therapeutic option that proved successful. Indeed, if early studies (1993-97) reported eradication rate up to 80% (PP analysis), the best results being achieved with omeprazole 40 mg bid + amoxicillin 1 g bid, subsequently this dual combination achieved disappointing results in many countries and was therefore regarded as a poorly effective regimen when compared with the cure rates reached with the triple therapies when clarithromycin resistance was rare. However this therapeutic approach regains in interest these last years with the declining eradication rates observed with the triple therapies. More recent studies, mostly from Japan, have well demonstrated that differences in the CYP2C19 genotype influence the eradication rate of PPI-based dual or triple therapies. The good results observed in patients with CYP2C19 poor-metabolizer genotypes suggest that if sufficient PPI is provided to achieve the same acid-suppressive effect in rapid metabolizers as it has in poor metabolizers, dual therapy should be successful. Clinical trials in Japan suggested that approximately rabeprazole 20 mg bid or omeprazole 80 mg bid with amoxicillin e2g/d for 14 days is sufficient.

Currently, a standard third-line therapy is lacking, and European guidelines recommend culture in these patients to select a third-line treatment according to microbial sensitivity to antibiotics. However, cultures are often carried out only in research centres, and the use of this procedure as 'routine practice' in patients who failed several treatments seems not to be feasible. Therefore, the growing interest in the evaluation of new genotypic methods used to detect antibiotic resistance in *H. pylori* without the need to culture the organism (like the GenoType® HelicoDR).

Several **non antimicrobial co-therapies** including normal foods or food components, such as cranberry juice, ginger, oregano and broccoli sprouts, food additives such as lactoferrin, pronase and N-acetyl cysteine to reduce the gastric mucus layer and various probiotics have been proposed as a useful adjunct. The purpose of their use was either to reduce the side effects of eradication therapy or to improve the efficacy of the therapy or both. Combination of probiotics with triple therapy may decrease side effects of the eradication regimen but has no consistently reported effect on eradication rates. The relevance of these issues have to be clarified in the future, as therapy for *H. pylori* infection is becoming more and more frequently prescribed.

The evaluation of second or third 'rescue' regimens for problematic cases seems to be worthwhile. In any case however, the selection of the most appropriate antimicrobial drugs following treatment failure is best approached by drug-susceptibility testing. In designing a treatment strategy we should not focus on the results of primary therapy alone : an adequate strategy for treating *H. pylori* infection should consider using several therapies which, if consecutively prescribed, come as close to the 100% cure rate as possible.



NON CONVENTIONAL THERAPIES FOR HELICOBACTER PYLORI. R. Ntounda, M. Nkuize, V. Muls, J. Mulkay, R. Saidi, Y. Nang Atto, M. Van Gossum, T. Serste, M. Buset. CHU Saint-Pierre, 1000 brussels, Belgium.

**Introduction :** The rate of eradication of *Helicobacter pylori* (Hp) is at its lowest levels because of antibiotic resistance and poor compliance [1]. Many agents like antioxidant, N-acetyl cysteine (NAC), sulphoraphane, phenolic agents, probiotics and others were tested. They inhibit Hp growth or have an additive effect in curing Hp infection. Vaccinations are also still in development.

**Results :**

**NAC :** NAC does not have any know activity against Hp, but it could dissolve the mucus biofilm making Hp more vulnerable to the antibiotic attack [2]. Pretreatment with NAC also significantly reduced the Hp load, but did not prevent Hp colonisation [3]. Furthermore, NAC decreased sensitivity and specificity of the Hp stool antigen test [4].

**Antioxidant :** Hp eradication has been shown to attenuate oxidative stress in human gastric mucosa. Adding vitamin C can reduce the dosage of clarithromycin, while preserving the same eradication efficacy [5]. Vitamin C or vitamin E supplementation leads to some short-term protective effects on Hp induced gastritis. However, these effects seem to subside over time when the infection persists [6,7].

**Other unconventional therapeutic agents :** Probiotics, sulphorane and drinking alcohol have shown to somewhat inhibit Hp in various trials. However none of them were really proven effective in a clinical setting on a long term basis [8,9].

**Vaccination :** Vaccination for Hp represents a novel and very important weapon against both infection and gastric cancer. The intramuscular vaccine formulation consisting of VacA, CagA and Hp-NAP (neutrophil activating protein of Hp) plus aluminium hydroxide adjuvant seems a very promising candidate vaccine for the prevention of Hp infection [10].

**Conclusion :** Rates of eradication for Hp with the standard therapies are failing dramatically due to a combination of antibiotic resistance and poor compliance with therapy. Although several unconventional therapeutic agents were shown to be effective in vitro, the results were rather inconsistent and even contradictory in vivo. The most interesting treatment seems to be the mucolytic/antioxidant agents. More controlled studies with larger number of patients are needed to define their place in existing therapeutic strategies. A new intramuscular vaccine is promising but clinical studies are expected.

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PROTON PUMP INHIBITORS : USE OR 'ABUSE' ? V. Mattens, D. Urbain, F. Mana. UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

**Introduction** : Since the introduction of PPIs in 1988, their use has increased tremendously, evolving worldwide in one of the most commonly used medications, particularly for the treatment of gastroesophageal reflux disease (GERD). The tolerability of PPIs in both short- and long-term use is remarkably good. We reviewed recent literature illustrating potential risks associated with inappropriate (long-term) use of PPIs.

**Results** : PPIs create profound acid suppression and secondary hypergastrinemia. Although hyperplasia of enterochromaffin-like cells, development of fundic gland polyps and hyperplastic polyps were observed, it didn't seem to provoke neoplastic changes. However, chronic intake of PPIs in the presence of *Helicobacter pylori* increases the development of an atrophic gastritis, a precursor of gastric cancer.

In susceptible patients, PPIs can increase the risk of bacterial overgrowth and enteric infections. Although data on *Clostridium difficile*-associated diarrhoea are controversial, most hospital-based studies show a statistically significant association with PPI use. The question remains if this association is causal. Information on an association of PPI therapy with spontaneous bacterial peritonitis in patients with advanced cirrhosis is scarce, but suggests a higher risk due to bacterial translocation.

Recent studies have suggested that PPI use may increase the risk for community- acquired pneumonia (CAP) but some arguments counter a causal association. Discordant meta-analyses were published on the association of PPI use with nosocomial pneumonias in mechanically ventilated patients. Properly conducted well-controlled trials are lacking.

Acid suppression by PPI might decrease Calcium absorption, leading to osteoporotic fractures especially in patients with other risk factors for hip fracture. This association has a low magnitude ( $OR < 2$ ) and is dose- and/or duration-dependent.

Recently the debate about medical interactions was reopened due to some retrospective studies, showing an increased risk of in-stent thrombosis in patients on a combination of clopidogrel and PPI, especially omeprazole. However, 3 recent controlled randomised trials did not confirm the negative effect of the combination PPI/clopidogrel.

**Conclusion** : Appropriate use is advised as key point for any medication but especially for a medication used for frequent complaints like GERD. Guidelines for PPI use exist and should be better known by non gastroenterologists to obtain effective, not reflexive treatment.

## IBD RESEARCH GROUP

### Invited lecture

- I01 -

ENDOPLASMIC RETICULUM STRESS AND AUTOPHAGY INFLAME THE GUT. A. Kaser. Queensland Institute of Medical Research, Brisbane, Australia.

Recent insights into intestinal epithelial cell (IEC) biology imply a prominent role of IECs in regulating the intestinal immune system at the interface to the intestinal microbiota. Moreover, intestinal inflammation in inflammatory bowel disease (IBD) may originate from IECs, as highlighted by inflammation that occurs secondary to unresolved endoplasmic reticulum (ER) stress. ER stress-related mechanisms might not only be primary originators of IBD as in the case of *XBPI* polymorphisms, but due to a manifold of intrinsic and extrinsic mediators interfering with these pathways, might also represent an important perpetuator of inflammation. A particularly relevant biologic mechanism intersecting with the ER stress response is autophagy, which has been discovered through genome-wide association studies to play a profound role in Crohn's disease. The recent discovery of impaired autophagy induction by CD-associated *NOD2* variants and *NOD2*'s interaction with *ATG16L1* suggests that the function of several key genetic risk factors of IBD mechanistically converge on these evolutionary conserved "metabolic" pathways, and may highlight novel treatment options.

- I02 -

HAPTOGLOBIN POLYMORPHISMS ARE ASSOCIATED WITH ULCERATIVE COLITIS AND CROHN'S DISEASE. L. Márquez, I. Cleynen, K. Machiels, S. Organe, M. Ferrante, L. Henkaerts, V. Ballet, P. Rutgeerts, S. Vermeire. University Hospital of Gasthuisberg, Leuven, Belgium.

**Introduction :** Haptoglobin (Hp) is an acute phase hemoglobin-binding protein that has antioxidant and immunomodulatory properties. There are two common alleles at the Hp locus on Chromosome 16q22 : Hp 1 and Hp 2 and functional differences between Hp1 and Hp2 protein products have been described, with Hp 1 having a superior anti-inflammatory effect compared to Hp 2. Genotype 2-2 has been shown to be overrepresented in immune diseases as Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE).

**Aim :** Based on the functional differences described for Hp1 and Hp2 and its immunomodulatory role, we studied the Hp locus in Ulcerative Colitis (UC) and Crohn's disease (CD).

**Methods :** We genotyped these alleles in an UC cohort (n = 755), an exploratory cohort of CD patients (CD1, n = 429), a second independent CD cohort (CD2 = 632), and in 452 healthy controls. Hp genotypes were determined using touch-down polymerase chain reaction (PCR). Distribution of alleles was in Hardy-Weinberg equilibrium. Chi-square test was performed to evaluate the differences between groups (SPSS 16.0). P values less than 0.05 were considered statistically significant.

**Results :** The Hp2 allele frequency was significantly higher in UC patients (p = 0.006) and in CD1 (p < 0.0001) and CD2 (p = 0.016), all compared to the same control group (table 1).

Also the distribution of the genotypes was significantly different between UC and controls (p = 0.0046), and between CD1 (p < 0.00003) and CD2 (p < 0.018) and controls, mainly through an overrepresentation of Hp 2-2 in both UC and CD patients.

Furthermore, the differences between CD combined group (1+2) and controls were also significant regarding allele frequency (p < 0.0004) and genotypes distribution (p < 0.0001).

We found no significant association between haptoglobin genotypes and smoking status at diagnosis, familial disease, disease extension, type of medical treatment, need of surgery, dysplasia or extraintestinal manifestations in UC and CD patients.

Table 1. — Allele frequency and genotypes in controls, UC and CD

	Allele 1(Hp1)	Allele 2(Hp2)	Genotype 1-1	Genotype 2-1	Genotype 2-2
<b>CONTROLS</b>	43%	57%	16%	53%	31%
<b>UC</b>	37%	63%	14%	46%	40%
<b>CD (1)</b>	33%	67%	13%	39%	48%
<b>CD (2)</b>	37%	63%	16%	45%	39%
<b>CD (1+2)</b>	36%	64%	15%	43%	43%

**Conclusion** : Polymorphisms in the Hp gene play a role in susceptibility to IBD. The higher frequency of the functional allele 2 in IBD patients suggests a decrease in the Hp anti-inflammatory capacity. Hp 2-2, as in other immune diseases like RA and SLE, is more frequent in CD and UC, and may provoke an imbalance in Th1/Th2 response.

OXYGEN SENSOR INHIBITION ATTENUATES TNF-ALPHA INDUCED INTESTINAL EPITHELIAL DAMAGE BY HYPOXIA INDUCIBLE FACTOR (HIF) 1-DEPENDENT REPRESSION OF FAS-ASSOCIATED DEATH DOMAIN PROTEIN (FADD). P. Hindryckx (1), M. De Vos (1), P. Jacques (1), L. Ferdinande (1), H. Peeters (1), K. Olievier (1), B. Brinkman (2), P. Vandenabeele (2), D. Laukens (1). (1) UZ, Gent, Belgium, (2) VIB, Gent, Belgium.

**Introduction** : Oxygen sensor inhibitors stabilize Hypoxia-Inducible Factor (HIF), which has barrier protective activity in the gut. As the inflammatory cytokine Tumor Necrosis Factor alpha (TNF- $\alpha$ ) contributes to inflammatory bowel disease (IBD) in part by compromising intestinal epithelial barrier integrity, we hypothesized that oxygen sensor-inhibition may have beneficial effects in TNF- $\alpha$ -induced intestinal pathology.

**Aim** : To investigate the effect of oxygen sensor inhibition in TNF- $\alpha$ -induced chronic gut inflammation.

**Methods** : Littermate C57BL/6 TNF $\Delta$  ARE/+ mice, which spontaneously develop chronic terminal ileitis, were treated with DMOG or vehicle, their bodyweights were monitored and intestinal epithelial permeability was assessed. The effect of DMOG pretreatment on TNF- $\alpha$ -induced intestinal epithelial apoptosis was evaluated both *in vitro* and *In vivo*.

**Results** : TNF $\Delta$  ARE/+ mice treated with DMOG showed increased weight gain compared to littermate controls, as well as significant restoration of intestinal epithelial barrier function and clear attenuation of chronic terminal ileal inflammation.

Preincubation with DMOG protected intestinal epithelial cells (IEC's) against TNF- $\alpha$ -induced apoptosis both *in vitro* and *in vivo*, whereas suppression of HIF-1 $\alpha$  augmented the apoptotic response. Subsequent transcriptional studies indicated HIF-1 $\alpha$ -dependent repression of intestinal epithelial Fas-Associated Death Domain protein (FADD) as a substantial underlying mechanism. This led to the identification of a previously unappreciated functional binding site of HIF-1 $\alpha$  (hypoxia-responsive element) in the FADD promoter.

**Conclusion** : We identified a novel innate mechanism to protect IEC's during (inflammatory) hypoxia by direct modulation of death receptor signalling. HIF-1 $\alpha$ -dependent repression of FADD by the PHD inhibitor DMOG protects the intestinal epithelium against TNF- $\alpha$ -induced apoptosis, rapidly restores barrier function and dampens chronic intestinal inflammation. As such, PHD inhibition could represent a promising alternative treatment strategy for IBD.

SMALL ANIMAL PET-CT AS A NON-INVASIVE METHOD TO EVALUATE TERMINAL ILEAL INFLAMMATION IN A MURINE MODEL OF CROHN'S DISEASE. P. Hindryckx, S. Staelens, S. Deleye, H. Peeters, D. Laukens, M. De Vos. UZ, Gent, Belgium.

**Introduction :** TNF $\alpha$  ARE/+ mice spontaneously develop a chronic terminal ileitis which worsens with increasing age and is strikingly similar to human Crohn's disease (CD). As such, they represent an unique model to study promising new therapeutics for intestinal CD. However, animal studies are hampered by several problems such as inter-animal variability and timing or sampling errors. Small animal molecular imaging is an expanding and promising technology that may largely overcome the aforementioned problems.

**Aim :** To investigate the possible use of small animal Positron Emission Tomography (PET) - Computed Tomography (CT) in TNF $\alpha$  ARE/+ mice.

**Methods :** TNF $\alpha$  ARE/+ mice of several ages and their corresponding healthy control mice were fasted overnight, after which they received an IV injection with 1mCi 2-Deoxy-2-[<sup>18</sup>F]Fluoro-d-Glucose and an oral gavage with 200 $\mu$ l of gastrografin. After one hour, a PET and CT scan of the animals was acquired with the Flex Triumph PET/CT (Gamma Medica Ideas, Northridge, LA, USA). First a CT scan was acquired in 2x2 binning mode, with a 50  $\mu$ m spot size, at 70kVp and 175  $\mu$ A in 2048 projections using a magnification of 1.3 followed by a 30 min PET scan in 2 bed positions. Image fusion proceeded flawlessly since both imaging modalities are mounted on the same gantry. The resulting images were analyzed by using VIVID (Gamma Medica Ideas, Northridge, LA, USA) based on Amira and compared with histological inflammation grade.

**Results :** The use of gastrografin as an oral contrast agent allowed easy identification of the murine terminal ileum.  $\mu$ -PET-CT nicely detected terminal ileal inflammation in the TNF $\alpha$  ARE/+ mice, with an age-dependent increase in PET-activity and a good correlation with histological inflammation. Virtually no intestinal PET-activity was observed in other parts of the gut in TNF $\alpha$  ARE/+ mice or at the terminal ileum in healthy control mice of any age, indicating high specificity of FDG as a PET-tracer for intestinal inflammation.

**Conclusion :** Small animal PET-CT is a feasible non-invasive method to evaluate chronic terminal ileal inflammation in TNF $\alpha$  mice and may possibly offer a new opportunity to rapidly screen therapeutic compounds for anti-inflammatory activity in Crohn's disease.

EVOLUTION AND PREDICTIVE FACTORS OF RELAPSE IN UC PATIENTS TREATED WITH 5-ASA AFTER A FIRST COURSE OF SYSTEMIC STEROIDS. C. Bello, E. Louis, J. Belaiche, C. Reenaers. Ulg Sart Tilman, Liège, Belgium.

**Introduction :** Although immunosuppressive drugs are increasingly used in IBD, 5-ASA remains a good option for the induction and the maintenance of remission without steroids in mild to moderate ulcerative colitis (UC). Efficacy of 5ASA as a maintenance treatment after a flare treated with corticosteroids has not been specifically studied.

**Aim :** The primary endpoint of our work was to study the evolution of a cohort of UC patients treated with mesalazine after a first flare requiring oral systemic corticosteroids. The secondary endpoint was to identify predictive factors of relapse and of colectomy in these patients.

**Material and method :** We studied retrospectively a cohort of 143 UC patients, who never received immunosuppressive drugs, suffering from a first moderate to severe flare requiring oral steroids. Among patients responding to steroids, we studied the group treated by mesalazine after the flare. Sex, age at diagnosis, disease duration and location as well as smoking habit were studied as predictive factors of relapse and colectomy.

**Results :** Eighty-five% of UC patients responded to the steroid therapy and 50% (n = 52) achieved a complete clinical remission without steroid. In this group, 67% (n = 35) received oral mesalazine at a dose of 3 to 4 grams per day. Seventy-five patients treated by mesalazine finally relapsed. The median time to relapse was 29 months (range :0,33-156). Fourteen% required a colectomy within a median of 11 months (range : 1-24). The relapse rate and colectomy rate over one year were 26% and 11%, respectively. Proportion of females, E3 location, smoker and disease duration were 33%, 38%, 22%, 36 months in one-year relapsers and 52%, 50%, 8%, 17 months in non relapsers respectively. Globally no clinical or demographic factor.

**Conclusion :** Maintenance efficacy of mesalazine over one year after a flare treated with systemic steroids is reasonably high and such strategy seems thus appropriate. Nevertheless, the relapse rate over longer follow up becomes much higher and an immunosuppressive treatment should certainly be discussed in case of relapse. No predictive factor of relapse could be found.

### Invited lecture

- I06 -

IMPAIRED AUTOPHAGY IN CROHN'S DISEASE : AN OPENED GATE TO INVASIVE BACTERIA.  
P. Lapaquette, A. Darfeuille-Michaud. Université d'Auvergne, Clermont-Ferrand, France.

**Introduction** : Ileal lesions in Crohn's disease (CD) patients are colonized by pathogenic adherent-invasive Escherichia coli (AIEC) due to abnormal expression of the CEACAM6 receptor. These bacteria are able to invade and to replicate within intestinal epithelial cells and macrophages. Recent genome-wide association studies have highlighted a highly significant and replicated association between CD and variants in two separate autophagy genes, ATG16L1 and IRGM.

**Aim** : As defects in autophagy could predispose patients to CD by promoting prolonged survival and/or replication of intracellular microorganisms within host cells, we analyzed whether the cellular autophagy mechanism can control bacterial survival and/or replication of CD-associated E. coli strains.

**Results** : A subpopulation of the intracellular AIEC LF82 bacteria is located within LC3 positive autophagosomes. Thus, functional autophagy can limit intracellular AIEC replication. In IRGM and ATG16L1 deficient cells intracellular AIEC LF82 bacteria have enhanced replication. Autophagy deficiency does not interfere with the ability of intracellular bacteria to survive and/or replicate for any other E. coli strains tested, including non pathogenic, environmental, commensal, or pathogenic strains involved in gastroenteritis. Together our findings stress the central role of autophagy in specifically restraining only the pathogenic Adherent-Invasive E. coli strains associated with ileal CD.

**Conclusion** : This indicates that AIEC infection in patients with polymorphisms in autophagy genes may have a significant impact on the outcome of intestinal inflammation. From these observations it could be suggested to investigate the presence of mucosa-associated AIEC bacteria, the ileal expression of CEACAM6 and ATG16L1 or IRGM polymorphism in CD patients in order to define CD patients at high risk of developing structuring and fistulizing CD.

### Invited lecture

- I07 -

CROHN'S DISEASE : TH17 AND/OR TH1 DISEASE ?

S. Brand. Munich, Germany.

FATIGUE IN ACTIVE AND QUIESCENT INFLAMMATORY BOWEL DISEASE : A STUDY INVESTIGATING ITS IMPACT ON QUALITY OF LIFE AND POSSIBLE UNDERLYING MECHANISMS. H. Peeters, C. De Roo, A. Malfait, D. Laukens, M. De Vos. UZ, Gent, Belgium.

**Introduction** : IBD patients often mention fatigue as a prominent symptom in both active and quiescent disease. The underlying mechanisms however are not well understood. Studies in chronic fatigue syndrome and malignancies point at specific micronutrient deficiencies (zinc and carnitine) as possible causes for fatigue.

**Aim** : To objectify and quantify fatigue in patients with active and quiescent IBD, to study its impact on quality of life and to determine possible underlying mechanisms of fatigue in IBD.

**Methods** : We included 97 consecutive IBD patients (75 CD, 25 UC) and 50 healthy controls. Fatigue was measured using the multidimensional CIS questionnaire (Checklist Individual Strength), SFQ (Shortened Fatigue Questionnaire) and a VAS (Visual Analog Scale) for fatigue. Quality of life was measured using the IBDQ questionnaire. Two thirds of patients (n = 65) were in clinical remission (CDAI or UCAId150). Blood was drawn for extensive biochemical and haematological testing (including inflammatory and nutritional parameters, serum levels of IL1, IL6, IL8 and TNF ± [ELISA], free and acylcarnitine [HPLC] and zinc [Atomic Absorption Spectrometry]).

**Results** : There were significant correlations between all fatigue measurement scales (CIS-SFQ-VAS  $p < 0.001$ ). Patients with active IBD had significantly higher fatigue scores compared to patients in remission ( $p < 0.001$ ). The latter also had significantly higher scores compared to controls ( $p < 0.001$ ). A significant inverse correlation was found between fatigue and quality of life scores (IBDQ-SFQ  $\Delta = -0.74$   $p < 0.001$ ), however only prominent in remission ( $\Delta = -0.74$   $p < 0.001$ ) and not in active disease ( $\Delta = -0.26$   $p = 0.21$ ). There were no associations between fatigue and underlying biochemical imbalances (including cytokines, carnitine and zinc). We found a positive correlation between fatigue and anti-TNF treatment ( $p = 0.008$ ). Whereas in active IBD this correlation was not seen ( $p = 0.94$ ), anti-TNF therapy was an independent predictor of fatigue in quiescent IBD ( $p = 0.02$ ).

**Conclusion** : Fatigue can be quantified in IBD and is a frequent symptom with major impact on quality of life, in particular in patients in remission. Whereas no biochemical ground could be found for fatigue in IBD (including parameters of nutritional status or inflammation and serum levels of zinc and carnitine), we could identify anti-TNF treatment as an independent predictor for fatigue in the subgroup of patients with quiescent IBD.

IMPACT OF AN EXTENSIVE THIOPURINE METHYLTRANSFERASE (TPMT) GENOTYPING IN INFLAMMATORY BOWEL DISEASE PATIENTS WHO EXPERIENCED MYELOSUPPRESSION DURING ANTI-PURINE THERAPY. O. Dewit (1), T. Moreels (2), F. Baert (3), H. Peeters (4), C. Reenaers (5), M. De Vos (4), P. Van Hootegem (6), V. Muls (7), G. Veereman (8), F. Mana (9), J. Holvoet (10), S. Naegels (10), M. Van Outryve (2), Y. Horsmans (1), J.L. Gala (1). (1) UCL Saint-Luc, Brussels, Belgium, (2) UZ, Antwerpen, Belgium, (3) H.-Hart Hospital Roeselare-Menen, Roeselare, Belgium, (4) Ghent University, Ghent, Belgium, (5) University of Liege, Liege, Belgium, (6) AZ Sint-Lucas, Brugge, Belgium, (7) ULB Saint-Pierre, Brussels, Belgium, (8) Queen Paola Children's Hospital, Antwerpen, Belgium, (9) UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium, (10) ZNA Middelheim, Antwerpen, Belgium.

**Introduction** : Azathioprine (AZA) is an immunosuppressive drug that is widely used in IBD. Among its side effects, myelosuppression (MS) is of major concern. Patients with TPMT deficiency have a high risk of MS when exposed to standard dose of AZA. In a previous study \*, only 27% of Crohn's Disease Caucasian patients with MS had a mutant allele of the TPMT gene associated with enzyme deficiency.

**Aim** : Since this study, a number of new TPMT mutations have been described and our aim was to investigate their clinical relevance. Secondary aim was to evaluate, in TPMT deficient and normal pts, the delay, the severity, the complications, the hospitalization and outcome of MS.

**Methods** : Inclusion criteria were leukopenia (white blood cell count  $< 3000/\text{mm}^3$ ), and /or thrombocytopenia (platelets  $< 100000/\text{mm}^3$ ) while taking AZA therapy. Patients (n = 61, CD : 48, UC :13) were included from 11 Belgian centers. The median treatment dose was 2 mg/kg (0.7-2.5). Data were collected about the onset of treatment, the onset of MS, concomitant medications at that time, complications and hospitalization. For each patient, 30 milliliters venous blood was drawn at distance of MS event, in EDTA-treated tubes and sent to a central laboratory for full TPMT cDNA sequence analysis (exon 4 to 10), specifically designed to avoid the co-amplification of the TPMT pseudo-gene.

**Results** : Forty-six out of 61 were homozygous for the wild-type TPMT genotype (High methylator, HM), 11/61 were heterozygous for at least 1 non functional mutation (intermediate methylator, IM) and 4 were homozygous for non functional mutation (low methylator, LM). In the spectrum of 22 known TPMT mutations, only TPMT 2\*, 3B\* and 3C\* mutated alleles were identified. According to the expected methylator status, median delay between AZA initiation and

MS was 2 months (5 weeks-5 months) in the LM pts, 2,75 months (4 weeks-6 years) in IM pts ; and 6 months (11 days-7 years) in HM. Occurrence of infections was the most frequent complication and was found in TPMT deficient pts 6/15 (40%) as well as normal TPMT pts 15/46 (33%). No death was reported. Hospitalization was necessary in 7/15 pts (47%) with deficient TPMT genotype and in 12/46 (26%) of the patients with normal TPMT genotype. The median duration of hospitalization was 15 days (range 2-42) and similar in both groups.

**Conclusion :** In this series of Caucasian IBD patients, only 1/4 (25%) of MS during AZA was associated with TPMT deficient genotype. However, a shorter median time to MS onset and a proportionally higher rate of hospitalization was recorded in TPMT deficient patients.

\* Colombel et al, Gastroenterology 2000 ; 118 :1025-1030.

- 110 -

HIGH INFLIXIMAB TROUGH LEVELS ARE ASSOCIATED WITH MUCOSAL HEALING IN CROHN'S DISEASE. W. Van Moerkercke (1), C. Ackaert (2), G. Compennolle (2), M. Jürgens (1), I. Cleynen (1), G. Van Assche (1), P. Rutgeerts (1), A. Gils (2), S. Vermeire (1). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) University of Leuven, Leuven, Belgium.

**Introduction :** The introduction of anti-TNF biological agents in the last decade is seen as the largest evolution in the management of IBD. Infliximab (IFX) is a very efficacious treatment and has shown to induce rapid and profound healing. Mucosal healing is associated with deep clinical remission, reduction of hospitalisations and surgical rates and is an important treatment goal.

Nevertheless, primary and secondary failure occurs. Secondary loss of response is at least in part explained by immunogenicity leading to lower trough levels of the drug.

**Aim :** Little is known about the importance of trough levels in tailoring the therapy to the individual patient. We wanted to study whether IFX trough levels (TR) are important to achieve mucosal healing and if they are related to the degree of healing.

**Methods :** We studied serial serum samples in 210 patients with Crohn's disease, all in whom clinical, biochemical and endoscopic data before and after start of IFX therapy were present. Serum was available at time  $t_0$  (before the start of IFX),  $t_1$  (2 to 6 weeks after the first infusion) and  $t_2$  (at the time of the endoscopy). IFX trough levels were determined by an in-house developed ELISA method, in which 1 :150 and 1 :300 diluted serum samples were applied to TNF alpha coated plates. On each plate, an internal standard curve was introduced and a polyclonal HRP-conjugated goat anti-human antibody was used for detection. Endoscopic healing was defined as complete (disappearance of all lesions), partial (clear endoscopic improvement but still ulceration present) or no healing. Data were analysed using non-parametric Kruskal Wallis and Mann Witney test.

**Results :** Complete healing was observed in 39% of patients, partial in 22% and no healing in 39% of patients. Patients who showed (partial or complete) healing had significantly higher IFX trough levels (median 5.00  $\mu\text{g/mL}$  ;  $P_{25}$  0.49 –  $P_{75}$  9.85) compared to patients without healing (median 0.95  $\mu\text{g/mL}$  ;  $P_{25}$  0.35 –  $P_{75}$  6.56 respectively ;  $p = 0.006$ ).

Furthermore, a dose-dependent effect was observed with the highest trough levels detected in patients with complete healing (median 5.77  $\mu\text{g/mL}$  ;  $P_{25}$  1.05 –  $P_{75}$  10.72) followed by partial healing (median 3.89  $\mu\text{g/mL}$  ;  $P_{25}$  0.35 –  $P_{75}$  8.28) and absence of healing (0.95  $\mu\text{g/mL}$  ;  $P_{25}$  0.35 –  $P_{75}$  6.56 ;  $p = 0.013$ ) (see figure).



**Conclusion :** Infliximab trough levels are related with the degree of mucosal healing. In this study, we showed that patients with complete healing under IFX have significantly higher trough levels than patients without healing. Measurement of infliximab trough levels is therefore useful in optimizing therapy since they may allow dose adjustment in patients with low drug levels. For patients with low TR and no healing, we suggest to increase dose and/or decrease the interval. In patients with high TR and no healing switching the therapy is mandatory. Obviously, in patients with high TR and healing we can continue the treatment.

- I11 -

LACK OF CRP RESPONSE IN PATIENTS WITH ACTIVE CROHN'S DISEASE IS CHARACTERIZED BY LOW IL-6 CONCENTRATIONS BUT NOT WITH GENETIC VARIANTS IN THE CRP GENE. M. Jürgens (1), I. Cleyne (1), S. Organe (1), F. Schnitzler (2), V. Ballet (1), M. Noman (1), G. Van Assche (1), P. Rutgeerts (1), S. Vermeire (1). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) University Hospital Grosshadern, Munich, Germany.

**Introduction :** Crohn's disease (CD), a chronic inflammation affecting the entire gastrointestinal tract, is characterized by recurrent flares of inflammatory activity. C-Reactive protein (CRP) has shown to be an accurate marker of inflammation in these patients. Nevertheless, 20-25% of CD patients, will not mount CRP response in case of active inflammation. The reasons for this are unknown.

**Aim :** We investigated if mutations in the gene encoding CRP account for the lack of CRP response. Second, as IL-6 is the strongest trigger for CRP production by hepatocytes, we studied IL-6 levels in patients with and without CRP response.

**Methods :** From a single centre cohort of 718 CD patients under infliximab therapy with serial CRP levels available (mean number of CRP values per patient = 12.3), 34 patients with endoscopic active inflammation and normal CRP at all time points were identified. These patients were compared to 46 age and gender matched CD patients with increased CRP (> 3mg/ml) at all time points. Serum IL-6 was measured using a high sensitivity ELISA kit (abcam®). Eight gene-tagging SNPs (rs16842559, rs3093070, rs3093080, rs1205, rs1130864, rs1800947, rs1417938, rs2211321) were genotyped in all patients.

**Results :** The median IL-6-concentration was significantly lower in patients with absent CRP response (0.65 pg/ml ; IQR 0.09-1.57) as compared to those with CRP increase (7.07 pg/ml ; IQR 3.27-15.08 ; p < 0.001). (see figure). None of the mutations in the CRP gene were associated with CRP levels. Also in patients with increased CRP mutations did not affect changes in CRP after resolution of the inflammation.

**Conclusion :** Serum-concentrations of IL-6 were significantly lower in patients with absent CRP response. Mutations in the CRP gene on the other hand were not associated with CRP expression. Whether the serum concentrations of IL-6 also reflect similar differences at the mucosal level needs further study.

**Invited lecture**

- I12 -

TNFA<sup>ARE</sup> MICE : A MODEL TO STUDY EIMs IN CD.

D. Elewaut, Gent, Belgium.

## NUTRITION GROUP

- N02 -

NUTRITION DAY 2009 : RESULTS FROM A CROSS-SECTIONAL NUTRITIONAL RISK SCREENING ON NINETEEN HOSPITAL WARDS. K. Boeykens, AZ Nikolaas, Sint-Niklaas, Belgium.

**Introduction :** The Nutrition Day in Europe is a yearly well-known European cross-sectional audit to improve knowledge and awareness of malnutrition in hospitals. On this day we performed a hospital wide screening with an instrument based on the Nutritional Risk Screening 2002 (NRS 2002).

**Aim :** The aim was to collect individual nutritional parameters to assess the risk for malnutrition in hospitalized patients. Nineteen hospital wards were freely asked to complete a registration form. Actual body weight and length, weight loss in the previous 3 months, recent food intake and disease severity had to be recorded. A statistical package (SPSS, version 16) was used for data analysis.

**Results :** Study population included 347 patients (68% of the occupied beds) . Forty-four per cent (n = 153) was indicated as nutritionally at risk (NRS e 3). Risk for malnutrition significantly ( $P < 0,01$ ) increased with age e 70 (59,4%). Unintentional weight loss of e 10% was recorded in 16,8% and one in four patients (24,4%) had eaten less than 50% of normal intake in the past week. A BMI of  $< 18,5$  was recorded in 7,8% of patients. High risk wards for undernutrition were oncology, pneumology, geriatrics and abdominal surgery.

**Conclusion :** The high prevalence of the risk for undernutrition accords with data published in the literature. With a simple and validated nutrition screening tool it is possible to identify patients at risk for further nutritional evaluation. Efforts have to be made for broader sensibilisation and implementation of nutritional care pathways (starting with routine screening) in hospitals.

- N03 -

UTILITY AND LIMITATIONS OF NUTRITIONAL SCREENING IN A UNIVERSITY HOSPITAL. E. De Waele, S. Mattens, K. Delanghe, B. De Waele, G. Delvaux. UZ, Brussels, Belgium.

**Introduction :** Malnutrition lengthens hospital stay and increases readmissions. Systematic nutritional screening of patients on admission to hospital is advocated by many organizations but frequently omitted for various reasons including presumed inefficiency.

**Aim :** The aim of the present study was to investigate the feasibility, the advantages and the limitations of at random screening in our hospital

**Methods :** Patients were recruited from 4 departments (general surgery, oncology, geriatrics and internal medicine). Within 24 hours they were screened using the NRS-2002 scoring system and accordingly the nutritional status was categorized as “normal” or “at risk”. All patients were assessed by the same investigator (S.M.) over a 19-month period. Data were analyzed using PASW Statistics 17.0 for Windows.

**Results :** A total of 1729 patients were entered into the study. The male/female ratio was 777/952 and the age (mean  $\pm$  SE) was  $65.4 \pm 0.4$  years (range 16 – 99). Of the 1729 patients, 852 (49.3%) were classified as being “at risk” for malnutrition by NRS-2002. In the age groups of  $< 75$  years and  $\geq 75$  years, the prevalence of “at risk” was 44.4% and 56.5%, respectively. Patients discharged to a nursing home were more frequently (65.9%) “at risk” than those who returned home (46.2%).

**Conclusion :** This study confirmed the high prevalence of malnutrition risk in hospitalized patients and should alert health-care providers of all levels. Early detection by systematic nutritional screening of all admitted patients is very beneficial but represents a huge workload. Linking admission lists to medical diagnoses with a known risk of malnutrition may render the screening process more selective and efficient. Nutritional follow-up of older patients, especially those discharged to nurseries, deserves special attention.

THE NEED FOR NUTRITIONAL SUPPORT IN PREOPERATIVE OUTPATIENTS. B. Geurden, A. Van Piggelen, N. Delmotte, P. Van Aken, J. Weyler, D. Ysebaert. UZ, Antwerpen, Belgium.

**Introduction :** Malnutrition in general surgery patients is a significant problem. Inadequate peri-operative oral intake for more than 10 days is associated with higher mortality and morbidity. Following the ESPEN guidelines on enteral nutrition, nutritional support should be immediately initiated in patients without obvious undernutrition if it is anticipated that the patient will be unable to eat for more than 7 days peri-operatively or in patients who cannot maintain oral intake above 60% of recommended intake for more than 10 days.

**Aim :** In the present study, we aimed to determine the need for early preoperative intervention and nutritional support in candidate surgical patients in an outpatient setting.

**Methods :** The Nutrition Risk Screening-2002 (NRS-2002) scores of 529 consecutive patients were recorded in the preoperative outpatient setting of a university hospital. Patients who had a NRS-2002 score  $\geq 3$  were diagnosed as malnourished.

**Results :** The results demonstrated that nutritional status was normal 84% (N = 444) whereas 16% of the patients (N = 89) were malnourished. 74% of the malnourished patient had unintentional weight loss in the previous three months and their mean weight loss was 11%. Patients with malignant disorders were more likely to be malnourished. Loss of appetite and reduced dietary intake in the last week was a complaint for 11% of the total population in this study. 61% of them had 50% reduced intake and 39% used < 25% of a normal daily intake. Multivariate analysis showed that dietary intake less than 75% of normal resulted in OR 9,5 (5,39-16,66) to have a NRS-2002 score  $\geq 3$ . Mean time before surgery was 15,4 days.

**Conclusion :** According to the NRS-2002, 16% of the preoperative outpatients are malnourished about two weeks before surgery. Additional nutritional counseling is necessary in these patients, resulting in additional oral intake or supplementation of artificial nutrition.

SCREENING AND PREDICTING MALNUTRITION IN LUNG CANCER PATIENTS : VALIDATION OF NEW TOOLS. N. Barthelemy (1), A.F. Donneau (2), S. Haterte (2), S. Streeel (2), A. Albert (2), M. Guillaume (2). (1) Ulg Sart Tilman, Liège, Belgium, (2) University of Liège, Liège, Belgium.

**Introduction :** The assessment and management of nutritional problems are vital to support patients undergoing radiotherapy. Poor nutritional status may occur as a result of pre-existing problems, the age, the cancer itself, or the side effects of treatment. Weight loss, anorexia and cachexia affect many patients with cancer, particularly those with metastatic disease and cancer of the lung, head and neck and gastrointestinal tractus. The altered nutritional status affects patients and their families physically, psychologically and socially. Malnutrition impairs also the outcome of the disease with increased morbidity, mortality, length of hospital stay and healthcare costs.

**Aim :** This prospective study aimed at developing tools (1) to detect malnutrition before radiotherapy and (2) to assess the risk of malnutrition during radiation treatment.

**Methods :** For the study, 47 lung cancer patients treated with curative intent were recruited and evaluated 3 times : before radiotherapy, 2 weeks and 4 months after completion of the treatment. The evaluation was performed using 59 questions. The first part of the questionnaire investigated social support, recent weight loss, the current disease and their relation to nutritional needs, metabolic stress, physical evaluation, the treatment and the patient's functional capacity. The second part of the questionnaire concerned the patient's age, his/her symptoms, functional capacity and smoking habits. Malnutrition was defined using Thoresen's criteria. The validity of the new screening tool was based on the comparison of anthropometric, biological and nutritional variables between patients classified as being at risk of malnutrition or not.

**Results :** Using multivariate stepwise regression, body mass index (BMI) and weight loss over the last 6 months were identified as criteria for malnutrition. The score of malnutrition was computed according to the following equation :  $S = 5.88 - 0.2 \text{ BMI} + 0.05 \text{ WL}$  where WL is the patient's weight loss over the last 6 months expressed in percent of the initial weight and S is the malnutrition score with a threshold value of 1.8.

Low BMI, age (older than 70 years) and the presence of oedema were identified as risk factors for malnutrition during radiotherapy. The risk for malnutrition during radiotherapy is given by the equation :  $R = 3.67 + 0.98 \text{ A} - 0.12 \text{ BMI} + 1.2 \text{ OE}$  where A is the age > 70 years, OE is the presence of oedema and R the risk with a threshold value of 1.2.

**Conclusion :** Two simple tools were determined with the capacities (1) to detect malnutrition in lung cancer patients scheduled for radiotherapy and (2) to assess the risk for these patients to develop malnutrition during radiation treatment. Further studies are needed to validate these tools in larger samples and in other cancer patients populations.

- N06 -

REGISTRATION OF NUTRITIONAL PARAMETERS IN PAPER BASED PATIENT RECORDS AT A UNIVERSITY HOSPITAL. B. Geurden, C. Wouters, N. Delmotte, P. Van Aken, D. Ysebaert. UZ, Antwerpen, Belgium.

**Introduction :** Despite awareness, malnutrition continues to contribute to significant morbidity during and after hospitalization and evidence suggests persistent undertreatment of patients.

**Aim :** In the present study, we focused on the quality of the registration of nutritional parameters in paper based patient records at a university hospital. We investigated our records before introducing a training session for nurses and nursing aids on the screening of malnutrition.

**Methods :** We created a checklist and a system to score the registration of nutrition related data in patient records and used it in patient records of 15 wards. We scored patient records in respectively the two first weeks of June 2008, December 2008 and March 2009 because at the same time the National registration of the revised Belgian Nursing Minimum Dataset was running, which stimulates attention for completing patient records. We included records of patients, hospitalized for at least three days during one of the three periods of registration. At the same time, nurses and nurse aids filled out a short questionnaire about knowledge and attitudes in relation to nutritional parameters.

**Results :** According the criteria, we included 1661 patient records. A maximum score was never observed. Although 70% of the nurses reported consequent registration of every nutritional parameter, only 28% of the patient records scored above the mean.

516 questionnaires were returned (80% respons). 3 out of 10 multiple choice questions on basic knowledge about nutritional parameters were answered incorrect by 39% of the repondents on BMI and malnutrition, 37% on kCal content of food, and 26% on best choice of food in malnutrition. There were no significant differences in knowledge between surgical or medical wards. Nurses scored significantly higher than nurse aids.

**Conclusion :** In sum, no patient record was filled in completely and only 28% had a score above the mean. In contrast, 70% of the nurses reported consequent registration of nutritional parameters.

Basic knowledge by nurses and nurse aids about the relationship between BMI and malnutrition, kCal content of food, and best choice of food in case of malnutrition is poor. This study demonstrates that a change in attitudes in relation to the registration of nutritional parameters in patient records is necessary. Therefore, we recommend the necessity of upgrading nurses knowledge about nutritional parameters. Evidence-based nutritional care is impossible without proper registration of nutritional parameters.

IS EARLY ENTERAL NUTRITION DANGEROUS IN ACUTE NON SURGICAL COLONIC DIVERTICULITIS ?  
G. Van Ooteghem, H. Rouvière, J.F. Gallez, A. El Nawar, A. Nakad. CH Wapi Notre Dame, Tournai, Belgium.

**Introduction :** Acute Colonic Diverticulitis (ACD) is usually treated by parenteral way keeping the bowel at rest, without any clearly defined guidelines about nutrition and administration route. Non-surgical ACD are not a contraindication to enteral nutrition. We study the safety of early feeding by oral liquids with low-fiber in ACD patients who were hospitalized, without any indication for an emergency surgery.

**Methods and patients :** From February 2008 to March 2009, 43 patients (mean age : 60-year-old +/- 10 years) were admitted for ACD. The diagnosis had first been made on clinical and laboratory criteria and confirmed by (CT). Surgical and medical assessments were performed at admission. Immunodepressed patients, pneumoperitoneum, obstruction or septicemia were excluded. Abscess larger than 4cm of diameter was percutaneously drained. Initial treatment was given with Glucose 5% perfusion, intravenous antibiotics and Hydric diet during 24 to 48 hours. In the third day of admission, antibiotherapy was switched to oral administration for 5 up to 10 days depending on the progression. In the same time the patient received oral liquid feeding with low-fiber (intake of calorie/nitrogen : 20-30 Kcal/kg = 6 drinkable bottles of 125ml/day). Laboratory test were checked every two days. Normal but low-fiber diet was introduced 24h hours before discharge. CT and colonoscopy were performed after one month.

**Results :** Thirty three cases of ACD were uncomplicated (6 of them were recurrent) and 10 were complicated with covered perforation and/or abscess. The 33 uncomplicated-cases had good recovery. Mean hospitalization time was 6 +/- 4 days. Five cases had an elective surgery, one because of cancer, and the other four for a recurrent episode of the disease. Mean hospitalization time for the 10 complicated cases was 12 days. 8 of them had good recovery with an assessment after 7 months or more from the initial episode and 2 cases progressed to colonic stenosis during their hospitalization, requiring a sigmoidectomy with a one-time anastomosis, but had good recovery. None of the 43 patients had their disease worsened with the oral liquid nutrition. Mean daily cost was 30 euros.

**Conclusion :** Early enteral nutrition in ACD is not dangerous, it can reduce the mean hospitalization time and the cost of the treatment. Moreover if colonic surgery is necessary during this oral liquid diet, resection and anastomosis in a one-time surgery remains possible. Further studies are necessary to confirm our hypothesis.

A DIETARY INTERVENTION WITH ARABINOXYLAN OLIGOSACCHARIDES REDUCES COLONIC PROTEIN FERMENTATION IN HEALTHY SUBJECTS : RESULTS FROM FAECAL METABOLITE FINGERPRINT ANALYSIS. H. Hamer, V. De Preter, L. Cloetens, G. Vandermeulen, C. Courtin, W. Broekaert, J. Delcour, P. Rutgeerts, K. Verbeke. University of Leuven, Leuven, Belgium.

**Introduction :** The addition of prebiotics to the diet may improve host health by modulation of the colonic metabolism.

**Aim :** To evaluate the effect of a new candidate prebiotic, arabinoxylan oligosaccharides (AXOS), on the colonic metabolism by characterization of the metabolite profile in faeces.

**Methods :** AXOS was administered for 3 weeks at 10 g/day to 20 healthy volunteers (30% male ; age 23(20-45) ; BMI 21(18-26)). Faecal samples were collected before and after the intervention with AXOS. To determine a broad range of fermentation metabolites, metabolic fingerprinting was applied using a purge-and-trap system coupled on-line to a gas chromatograph-time-of-flight-mass spectrometer (GC-TOF-MS). Cluster analysis, based on the relative indices of all identified volatile organic compounds (VOCs) versus 2-ethylbutyric acid as an internal standard, was used to compare the metabolite profiles.

**Results :** A total of 179 different VOCs were identified in the faecal samples, 24 VOCs were present in more than 70% of the samples. The compounds acetaldehyde, butanoic acid, dimethyl disulfide, dimethyl trisulfide and limonene were present in all samples. Cluster analysis of the VOC fingerprints allowed to discriminate baseline samples from the samples after the intervention with AXOS, suggesting that the impact of the intervention exceeded the inter-individual variability. This discrimination was mainly due to protein fermentation metabolites such as p-cresol, branched-chain fatty acids (4-methyl pentanoic acid, 2-methyl propanoic acid and 2- and 3- methyl butanoic acid) and sulphur containing compounds (hydrogen sulphide, carbon disulfide, dimethyl trisulfide and dimethyl tetrasulfide). Faecal short-chain fatty acids did not contribute to discrimination between both groups, although this does not preclude an increased SCFA production in the proximal colon.

**Conclusion :** Dietary intervention with AXOS in healthy subjects has a considerable impact on the colonic metabolism mainly by decreasing proteolytic activity.

AUGMENTER LES VARIÉTÉS DES COMPLÉMENTS MÉDICAUX ORAUX PROPOSÉS (CMO) AUX RÉSIDENTS DE MAISONS DE REPOS ET MAISONS DE REPOS ET DE SOINS INFLUENCE-T-IL L'ADHÉSION A LA PRISE DE CMO, AINSI QUE L'ÉTAT FONCTIONNEL ET LA QUALITÉ DE VIE ? D. Brees. Centre Hospitalier des Ardennes, Libramont, Belgium.

**Introduction :** La dénutrition a une prévalence élevée en milieu hospitalier ainsi que dans les institutions gériatriques (1). Les coûts humains et financiers induits par celle-ci nous amènent à nous poser la question de la stratégie optimale à adopter pour les résidents dépistés à risque de dénutrition ou dénutris.

Lorsque les apports alimentaires sont insuffisants pour couvrir les besoins chez des patients dont le tube digestif est fonctionnel, l'utilisation de compléments médicaux oraux (CMO) est indiquée (2).

**Aim :** La compliance, souvent mauvaise à la prise de CMO, nous a amené à orienter l'objectif de ce travail. Celui-ci consiste en une étude observationnelle dont le but est d'analyser si l'augmentation des variétés de CMO influencerait l'adhésion des résidents à la thérapie, ainsi que leur état fonctionnel et leur qualité de vie.

**Methods :** L'étude s'est déroulée au sein de trois maisons de repos (MR) et une maison de repos et de soins (MRS). La population étudiée au sein des institutions regroupe les résidents à risque de dénutrition ou dénutris (score MNA < 23,5) (3).

Les participants devaient être capables de comprendre les procédures, pourvus d'un esprit leur permettant de donner leur consentement éclairé et capables de communiquer leur avis.

Pour cibler cette population, nous avons exclu les résidents déments, diabétiques, allergiques ou intolérants à un des composants présents dans les produits proposés, sous chimiothérapie, sous alimentation par sonde, dont le pronostic vital est en jeu, qui n'aiment vraiment pas les CMO proposés.

Une analyse comparative de l'adhésion à la prise de CMO (*gamme Fortimel Nutriticia*) a été réalisée entre deux groupes, grâce à l'évaluation de la consommation quotidienne de deux CMO choisis par les résidents durant 30 jours :

*Groupe 1 :* variété de 4 goûts de CMO ayant une même consistance avec une base lactée et sucrée.

*Groupe 2 :* variété de 9 CMO sucrés et salés, chauds et froids, liquides ou plus crémeux.

**Results :** Après un mois de supplémentation, les résultats montrent une bonne adhésion à la prise de CMO, avec en moyenne 72,2% des CMO consommés et sans différence significative entre les deux groupes étudiés

Parallèlement, nous avons observé une amélioration de la qualité de vie ( $p = 0,040$ ), de la force de préhension de la main ( $p = 0,010$ ) et de la capacité motrice des résidents ayant mené l'étude jusqu'à son terme.

	Avant <i>f</i> Arrêt	Arrêt <i>f</i> + 2 semaines
Qualité de vie (/15)	+ 0,5	- 0,46
Echelle de Qualité de vie (%)	+ 6	- 1
Handgrip (libras)	+ 2,4	- 0,5
Timed Up and Go (sec)	- 4,6	+ 2,5

(Significatif – Non significatif)

Les résidents présentent après 4 semaines de complémentation une augmentation significative du poids ( $p = 0,004$ ) soit une moyenne de 1,5kg de prise de poids.

**Conclusion :** La prise de compléments médicaux oraux permet d'améliorer le statut nutritionnel, fonctionnel et la qualité de vie des résidents. Ces effets positifs ont été répertoriés dans les deux groupes sans différence entre les groupes. Un ensemble de facteurs, autres que la prise des CMO proposés, peuvent influencer l'adhésion à la thérapie comme la collaboration du personnel soignant, la stimulation et l'état d'esprit des résidents.

Les diététiciennes ont un rôle clé à remplir afin d'expliquer et de convaincre le résident du bénéfice qu'apportent les CMO sur l'état nutritionnel et fonctionnel du patient.

## PATHOLOGY, RADIOLOGY AND BGDO

- P01 -

PROSPECTIVE RANDOMIZED STUDY COMPARING HEPATIC INTRA-ARTERIAL INJECTION OF YTTRIUM-90 RESIN-MICROSPHERES (HAI-Y90) WITH PROTRACTED IV 5FU (5FU CI) VERSUS 5FU CI ALONE FOR PATIENTS WITH LIVER-LIMITED METASTATIC COLORECTAL CANCER (LMCRC) REFRACTORY TO STANDARD CHEMOTHERAPY (CT). A. Hendlisz (1), M. Van den Eynde (2), M. Peeters (3), L. Defreyne (3), G. Maleux (4), J. Vannoote (5), P. Delatte (1), M. Paesmans (1), J.L. Van Laethem (6), P. Flamen (1). (1) Institut Jules Bordet, Brussels, Belgium, (2) UCL Saint-Luc, Brussels, Belgium, (3) UZ, Gent, Belgium, (4) UZ, Leuven, Belgium, (5) KU, Leuven, Belgium, (6) ULB Erasme, Brussels, Belgium.

**Introduction :** Chemo-refractory colorectal cancer metastatic to the liver only (LMCRC) has a poor prognosis. We hypothesized a significant improvement of the patient's outcome after HAI-Y90 radioembolization of hepatic metastases given along with 5FU CI over 5FU CI alone.

**Aim :** Primary endpoint was time to liver progression (TTLP). Secondary endpoints were time to progression (TTP), overall survival (OS) and safety.

**Methods :** This prospective, multicentric, randomized trial compared arm A : 5FU CI (300 mg/m<sup>2</sup> D1-14 q3weeks) with arm B : HAI-Y90 and 5FU CI (225 mg/m<sup>2</sup> D1-14 followed by 300 mg/m<sup>2</sup> D1-14 q3weeks) until disease progression. Eligibility criteria were : chemo-refractory (5FU, oxaliplatin, irinotecan) LMCRC, PSd2, normal direct bilirubin, and no lung shunting. Cross-over (HAI-Y90 monotherapy) was permitted in arm A after disease progression. Analysis was by intention to treat. To detect an increase in median TTLP from 6 to 18 weeks, 35 local progressions were needed (alpha 5%, power 90%). Distribution of time to events variables was modelled through Cox regression (likelihood ratio tests).

**Results :** Trial randomized 46 patients (pts) of whom 44 were eligible for analysis (23 in arm A and 21 in arm B). Pts' characteristics in the 2 arms were well balanced. Local progression was documented in 41 pts. Median length of follow-up was 108 weeks. Median TTLP were respectively 9 and 24 weeks in arm A and B (Hazard Ratio 0.38 (95% CI :0.20-0.72, p = 0.003)). Median TTP were respectively 9 and 20 weeks in arm A and B (Hazard Ratio 0.51 (95% CI : 0.28-0.94, p = 0.03)) and Overall Survival were 32 and 43 weeks in arms A and B (Hazard ratio 0.92 (95% CI : 0.47-1.78, p = 0.80)). Treatment was well tolerated with few side effects reported, essentially grade 3 asthenia (5 pts ; 22%) in arm A. Most pts (25/44) received further treatment after local progression, including 10 pts with cross-over to HAI-Y90 in arm A, which may explain apparent lack of difference in overall survival.

**Conclusion :** HAI-Y90 with CI 5FU significantly improves TTLP and TTP over CI 5FU alone and is a valid salvage therapeutic option for chemo-refractory LMCRC.

- P02 -

EUS PREDICTS LOCAL RESECTION FOR RECTAL CANCER. E. Cesmeli (1), K. Geboes (1), P.J. De Munck (1), B. Claerhout (1), W. Ceelen (1), P. Pattyn (1), D. De Looze (1), M. Peeters (2), M. De Vos (1). (1) UZ, Gent, Belgium, (2) UZ, Antwerpen, Belgium.

**Introduction :** Pre-malignant (T0) and early T1 rectal tumours are treated by local excision techniques. In this study we tried to assess the accuracy of endoscopic ultrasound (EUS) in selecting patients with rectal neoplasia suitable for local resection

**Methods :** Patients with rectal tumours deemed suitable for local resection by the gastroenterologist were staged using endoscopic ultrasound (EUS). With a radial 10 MHz instrument (Olympus GF-UM160) the depth of invasion in the rectal wall was evaluated. The pre-operative stage predicted by EUS (uT stage) was compared to the definitive histopathology (pT stage)

**Results :** In 65 consecutive patients 66 rectal lesions were evaluated between march 2005 and july 2009. 4 Patients were excluded from the analysis because they had received neo-adjuvant chemo-radiation therapy (2), incomplete staging (1) or the absence of definitive histopathology (1). In 53 patients histopathology confirmed early rectal disease : pT0 disease in 43 patients and pT1 disease in 10 patients. 9 lesions(in 8 patients) showed a higher T-stage : pT2 (5) and pT3 (4). Endoscopic ultrasound predicted early rectal disease correctly in 49 of the 62 lesions. 4 patients were overstaged by EUS : 1 patient with a very large villous adenoma and 3 patients with a recent endoscopic manipulation. The table below depicts the results for early disease in more detail.

EUS	Sensitivity	Specificity	PPV	NPV	Accuracy
pT 0/1	92,4%	100%	100%	69,2%	93,5%



39 of the 53 patients with early rectal disease were treated with local resection : 8 endoscopically and 31 by the surgeon with transanal resection or TEM(transanal endoscopic microsurgery). 14 out of 53 patients were treated with radical resection(LAR and APR). In 5 patients this was due to overstaging with EUS(3) and MRI(2). In 8 patients the rectal neoplasia clinically looked suspicious or technically not feasible for a local resection. In 1 patient radical resection was performed after transanal approach because pathology showed submucosal invasion.

**Conclusion :** Endoscopic ultrasound is very accurate in differentiating T0/1 rectal tumours from more invasive carcinomas. Unnecessary radical excision can be avoided if the selection of these patients is guided by EUS.

- P03 -

LAPAROSCOPIC RESECTION OF COLORECTAL LIVER METASTASES : A SINGLE CENTER CASE-CONTROL MATCHED-PAIRS ANALYSIS. B. Van Den Bossche (1), A. Scarinci (1), F. Berrevoet (1), X. Rogiers (1), L. Libbrecht (2), B. De Hemptinne (1), R. Troisi (1). (1) University Hospital, Ghent, Belgium, (2) Ghent University, Ghent, Belgium.

**Introduction :** The laparoscopic surgical approach has gained wide acceptance for the treatment of several liver pathologies, however, little data are available on the treatment of colorectal liver metastases (CRLM) using this technique.

**Aim :** The aim of this study is to evaluate overall results of liver surgery for CRLM by comparing the open approach (OS) with the laparoscopic (LS) in a case-control matched pairs single center analysis.

**Methods :** Between January 2005 and September 2009 a total 533 liver resections were performed in our institution. During this period laparoscopic liver resection of CRLM was increasingly performed in 40 cases. Data from the LS group were compared with those obtained from a control group undergoing open surgery for a similar indication in a matched-pair analysis. Pairs were matched as closely as possible for age and gender, type of resection, location of the tumoral lesions and surgery date. Endpoints were : overall morbidity, R0 resection rates, recurrence rates and hospital stay.

**Results :** After a median follow-up of 9.95+10.1 month in the LS and 16.9+16.4 month ( $p = 0.02$ ), the mean age in the LS group was of  $64 \pm 11.5$  y and in the open liver group  $62 \pm 9.5$  ( $p = 0.87$ ). Conversion to laparotomy was required in 3(7.5%) out of 40 laparoscopic cases due to uncontrolled bleeding. In both groups 8(20%) major and 32(80%) minor hepatectomies were performed. The overall morbidity in the LS group was 10% (4/40) as in the OS group this was 11/40 (27.5%) ( $p = 0.08$ ). Similar R0 resection rates were found between LS and OS groups : 35/40(87.5%) cases vs. 32/40 (80%) cases respectively ( $p = 0.54$ ). The recurrence rate was of 15% (6/40) in the LS group and 40% (16/40) in the OS group respectively ( $p = 0.02$ ). The three-year disease-free survival was 35.8% and 46.8% respectively for LS and OS ( $p = 0.64$ ). Laparoscopic liver surgery was finally characterized by a shorter hospital stay :  $7.7 \pm 2.7$  days vs.  $9.5 \pm 4.5$  days,  $p = 0.01$ .

**Conclusion :** Despite the flaws of such an analysis and the potential selection biases, according to this experience, laparoscopic resection of colorectal liver metastases can be considered as a good even preferable alternative when possible to open surgery. Short and mid-term outcomes are similar, being the overall morbidity and hospital stay significantly less in the laparoscopic approach. Further data are required before any conclusion can be definitely be drawn.

### Invited lecture

- P04 -

RADIONUCLIDE THERAPY FOR PRIMARY LIVER TUMOURS. B. Lambert. UZ, Gent, Belgium.

Hepatocellular Carcinomas (HCC) receive their blood supply primarily from the hepatic artery, whereas normal liver parenchyma is fed primarily via the portal vein. Intra-arterial administration of radio-active Lipiodol or microspheres will mainly target the tumour, and relatively spare the normal tissue.

Iodine -131 is a gamma and beta emitter with a physical half life of 8 days. Lipiodol is a contrast medium suitable for intrahepatic use. Following injection in the hepatic artery Lipiodol is typically retained in the HCC foci. Lipiodol labelled by therapeutic activities (2,2 GBq) of Iodine-131 is commercially available (Lipiodol®). It is most often used in a palliative setting for HCC without extrahepatic metastasis. It can also be used as an adjuvant therapy following resection. In general contra-indications consist of decompensated liver cirrhosis (Child-Pugh score > 7), pregnancy/breastfeeding, limited performance status and pulmonary symptoms following previous treatment. Due to the gamma emissions of I131 the patient needs to stay in the radionuclide therapy ward for 7 days. Until 2 weeks following discharge the patient is asked to stick to guidelines aiming at minimizing the radiation burden for relatives. At present 3 randomized trials using I-131 Lipiodol have been conducted. Raoul and coworkers compared chemo-embolisation with I-131 Lipiodol and concluded that survival rates were similar but that radionuclide therapy had a more favourable toxicity profile. In a smaller RCT by the same group it was shown that I-131 Lipiodol is feasible in patients suffering portal vein thrombosis. In a 3rd RCT adjuvant I131-Lipiodol therapy 6 weeks following hepatectomy was studied and was shown to reduce recurrent disease. However the latter indication is still under debate.

More recently non-biodegradable 90-Yttrium loaded microspheres were developed. Yttrium-90 is a pure beta-emitter with a physical half life of 64h. Two kinds of microspheres are commercially available, glass microspheres (TheraSphere®) and resin microspheres (SIR-Spheres®). In contrast to I-131 Lipiodol, a more laborious patient preparation is needed. Treatment needs to be simulated by injecting Tc99m-MAA (albumin particles) intra-arterially. If scintigraphy does not depict excessive lung shunt or intra-abdominal extra-hepatic tracer deposition, treatment can be scheduled about ten days later. Indications consist of palliative treatment of intermediated stage HCC (B), as well as patients with limited portal vein thrombosis. Patients with clinical signs of liver decompensation (eg ascites) or with limited performance status are not eligible. In practice a cut off of 2mg/dL is used for bilirubin, or 3 mg/dL in case of radiosegmentectomy. No RCTs are available, but retrospective analysis suggests it is a valuable tool in downstaging patients towards resection or Milan criteria. Due to the fact that Yttrium-90 is a pure beta-emitter, the hospital stay can be reduced to less than 1 day and no additional measures have to be taken to protect the relatives or partner.

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### Invited lecture

- P05 -

SIRT : NUCLEAR MEDICINE CONSIDERATIONS AND PITFALLS.

P. Flamen. ULB Bordet.

**Invited lecture**

- P06 -

**ANGIOGRAPHIC CONSIDERATIONS IN PATIENTS UNDERGOING  
LIVER-DIRECTED THERAPIES.**

G. Maleux. UZ Leuven.

- P07 -

A RANDOMIZED PHASE II STUDY OF TRANSARTERIAL CHEMOEMBOLIZATION WITH DOXORUBICIN ELUTING SUPERABSORBENT POLYMER MICROSPHERES IN PATIENTS WITH HEPATOCELLULAR CARCINOMA. H. Van Malenstein (1), G. Maleux (1), S. Heye (1), V. Vandecaveye (1), W. Laleman (1), J. Van Pelt (2), F. Nevens (1), C. Verslype (1). (1) University Hospital Gasthuisberg, Leuven, Belgium, (2) KU, Leuven, Belgium.

**Introduction :** Transcatheter Arterial Chemo-Embolization (TACE) is a standard treatment option in selected patients with hepatocellular carcinoma (HCC). The use of drug-eluting beads might provide a potential higher local effect of the antimitotic drug and less systemic side-effects.

**Aim :** Evaluate the safety data of the first prospective randomized phase II study with doxorubicin eluting superabsorbent polymer (SAP) microspheres (Hepasphere, Biosphere Medical).

**Methods :** We included prospectively 30 patients with different BCLC stages HCC (A = 3, B = 19, C = 8) and randomly assigned them to receive lipiodol and doxorubicin (n = 14) (classical TACE, control group) or doxorubicin eluting SAP microspheres (n = 16). The doxorubicin plasma level was assessed at different time points up to three hours after infusion to assess the peak concentration (C<sub>max</sub>) and the Area under the Curve (AUC). Biochemical analysis was performed the first two days and one month after the procedure. Side-effects were reported using the Common Toxicity Criteria. The tumor response was assessed six weeks after the procedure with magnetic resonance imaging (MRI).

**Results :** There was a significant lower peak concentration of doxorubicin and smaller AUC in patients receiving SAP microspheres (mean C<sub>max</sub> 495 ± 293.9 ng/mL, mean AUC 69.7 ± 26.9 ng/mL min) compared to the controls (mean C<sub>max</sub>, 1928 ± 560.8 ng/mL, mean AUC 165 ± 32.3 ng/mL min, both p < 0.001). Biochemical analysis showed better preserved liver function and there were four grade 3 and no grade 4 adverse events in the SAP microsphere group, whereas the control patients developed twelve grade 3 and eight grade 4 adverse events. The tumor response was comparable after six weeks.

**Conclusion :** TACE with Doxorubicin eluting SAP microspheres leads to low plasma levels of the antimitotic drug and therefore minimizes toxicity.

INTRADUCTAL PAPILLARY MUCINOUS TUMOR OF THE BILE DUCTS. K. Van den Bergh, D. Vanbeckevoort, D. Bielen, M. Thijs, K. Op de Beeck, R. Vanslebrouck, F. Claus, T. Roland. University Hospital Gasthuisberg, Leuven, Belgium.

**Clinical data** : A 58-year-old woman presented with jaundice, fever and abdominal pain since one day. In the past a cholecystectomy and a lung transplantation (february 2009) were performed. Laboratory work-up revealed abnormal liverfunction tests : the elevation of the serum enzymes reflected cholestasis.

**Radiology** : Ultrasound showed diffuse dilatation of the biliary tree. A disproportional aneurysmal dilatation of bile ducts was present containing a fungating mass in the right liver lobe and sludge and microlithiasis in the choledochal duct. During ERCP a widely open ampulla of Vater and a bile duct filled with thick mucin was observed. The cholangiogram showed extremely dilated intrahepatic and extrahepatic bile ducts with amorphous filling defects in the choledochal duct. CT-scan and MRI confirmed dilatation of the biliary tree and aneurysmal dilatation of intrahepatic ducts in the right liver lobe and showed vascularised papillary masses in the intrahepatic ducts of the right liver lobe. Diagnosis of a biliary IPMT was proposed.

**Pathology** : The patient underwent a right hemihepatectomy. Under macroscopic examination, the cut section revealed a dilated bile duct containing a multicystic mucinous mass. Histologic examination showed a well differentiated mucinous adenocarcinoma.

**Conclusion** : Intraductal papillary mucinous tumors of the bile ducts secrete a large amount of mucin, which results in intermittent obstruction of the segmental or lobar bile ducts or the entire biliary tree, depending on the location of the tumor and the amount of mucin. When generalized biliary dilatation is evident on images that also show disproportionately more severe dilatation in one part of the biliary tree and excessive mucin in the bile ducts, a diagnosis of intraductal papillary mucinous tumor of the bile ducts should be considered.

CORRELATION BETWEEN MAGNETIC RESONANCE IMAGING AND PATHOLOGICAL FINDINGS AFTER NEOADJUVANT CHEMORADIATION IN RECTAL CANCER. M. Mertens, C. Sempoux, M. Habay, F. Zech, A. Kartheuser, P. Scaillet, Y. Humblet, M. Van den Eynde, E. Danse, A. Jouret-Mourin. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : In rectal cancer, recent publications suggest that performing a local excision only or even a strict observation without surgery should be efficient in well-responders after neoadjuvant chemoradiation. However the way to evaluate tumoral stage and response is not defined.

**Aim** : To assess the MRI accuracy in predicting pathological features such as T and N stage, CRM and tumoral regression after neoadjuvant chemoradiation for rectal cancer.

**Methods** : We performed a retrospective analysis of 24 patients with an MRI examination done before and after a standardized long-course of neoadjuvant radiochemotherapy. The T and N stages of MRI's were compared to the pathological findings in the surgical specimens and the CRM evaluation by imaging, to the CRM measurement on the slides. The tumor regression grade assessed by MRI (0 = no regression, 1 = intermediate regression, 2 = complete regression) was compared to the pathological grading based on the Dworak classification, grouping the Dworak grades as follow : 0 = grade 0 or 1 (no response) ; 1 = grade 2 or 3 (mild to moderate response) ; 2 = grade 4 (complete response).

**Results** : The T stage predicted by MRI after chemoradiation was significantly correlated with the pathological T stage ( $r = 0.404$  ;  $p = 0.054$ ) while the correlation was poor for the N stage ( $r = 0.317$  ;  $p = 0.20$ ). The CRM estimation by MRI was significantly correlated with the pathological measurement ( $r = 0.480$  ;  $p = 0.011$ ) whereas for the regression grade, MRI did not reflect the pathological findings ( $r = 0.432$  ;  $p = 0.16$ ) in this small retrospective series.

**Conclusion** : MRI staging after chemoradiation is reliable for T stage and CRM prediction being therefore very useful for the surgeon. By contrast, N stage and tumoral regression estimations are poorly correlated with the pathological results which suggests to be cautious when considering patients as well-responders by MRI only. These results have to be confirmed by a larger and prospective study.

DYSPLASIA IN FUNDIC GLAND POLYPS : WHAT TO DO ? A. Capoen, G. De Hertogh, X. Sagaert, E. Van Cutsem, K. Geboes. UZ, Leuven, Belgium.

**Introduction :** The prevalence of gastric polyps in adults may be as high as 6%. The large majority of these, with a percentage close to 70% are “fundic gland polyps” (FGPs). FGPs are observed as sporadic lesions, often in middle aged women ; they have been associated with the use of proton pump inhibitors, which might explain the rise in incidence and they are also common in patients with familial adenomatous polyposis (FAP) (occurring in 20-84% of patients). FGPs are traditionally seen as a non-neoplastic lesion. However, dysplasia and gastric carcinoma arising from FGPs have been reported, essentially in FAP patients (the prevalence is approximately 44%). Overall, the incidence of gastric cancer in FAP is however low (0.6-4.2%). Recently it has been demonstrated that FAP patients carry germline mutations for the APC gene, indicating that the FGPs need to be considered as potentially neoplastic lesions. These APC mutations are infrequent in sporadic FGPs. In larger studies there seems to be no correlation between gastric cancer and sporadic FGPs.

**Aim :** We reviewed retrospectively a series of 10 patients diagnosed with dysplasia in FGPs in our department.

**Results :** High grade dysplasia was found in 2 patients. Upon review of the medical history, we discovered that 7 patients had previously been diagnosed with FAP (approximately 200 FAP patients in follow up), 1 patient has no previous records in our department. Two patients have no associated FAP.

**Conclusion :** Surgery should be considered in FAP patients with FGP showing dysplasia and implies extensive and careful analysis of the surgical specimen.

REVISION OF A SERIES OF BELGIAN HEPATOCELLULAR ADENOMA ACCORDING TO THE BORDEAUX EXPERIENCE. D. Hoton (1), A. Jouret-Mourin (1), S. Dardenne (1), C. Hubert (1), J. F. Gigot (1), J. Rahier (1), G. Cubel (1), S. Godecharles (1), C. Balabaud (2), P. Bioulac-Sage (2), C. Sempoux (1). (1) UCL Saint-Luc, Brussels, Belgium, (2) University Hospital Pellegrin, Bordeaux, France.

**Introduction :** Light has recently been shed on the genotype/phenotype correlation in hepatocellular adenomas (HCA) by the Bordeaux team, identifying four different subtypes : HNF1a-inactivated HCA, inflammatory HCA, b-catenin activated HCA and unclassified HCA.

**Aim :** To review our surgical series of HCA according to the Bordeaux experience.

**Methods :** We analyzed the clinical data and subclassified our series of HCA using the Bordeaux criteria and the immunohistochemical detections of liver fatty acid binding protein (L-FABP), C-reactive protein (CRP), serum amyloid A (SAA), glutamine synthetase (GS) and b-catenin.

**Results :** From 1992 to 2009, 31 cases of HCA were surgically resected in our institution. All concerned women, aged 16 to 52. None was diabetic. Ten were HNF1a-inactivated HCA (32%) showing steatosis and loss of L-FABP expression and 19 were inflammatory HCA (61%) with inflammatory infiltration, ductular proliferation, sinusoidal dilatation and expression of both CRP and SAA. Two were unclassified (6.5%). Among the 31 HCA, 4 had an abnormal expression of GS, two of them with a nuclear staining for b-catenin suggestive of b-catenin mutation. Bleeding as first manifestation was more frequent in the HNF1a-inactivated HCA group than in the inflammatory HCA group (50% vs 35%). The frequency of high BMI (38% vs 41%) and that of oral contraceptive use (63% vs 59%) was equivalent. Multiple lesions were more frequent in the HNF1a-inactivated HCA group (50% vs 35%). Follow-up (8 to 196 months) was available in 26 cases. Twenty-two women are alive without recurrence and 3 are alive with residual disease, one of them being suspected to have a b-catenin activated HCA. One case recurs.

**Conclusion :** The Bordeaux experience is reproducible in other series of surgically treated HCA and has an important clinical impact.

**Invited lecture**

- P12 -

**RADIONUCLIDE THERAPY FOR NEUROENDOCRINE TUMORS :  
INDICATIONS AND PITFALLS.**

C. DeRoose. UZ Leuven.

**Invited lecture**

- P13 -

**EMPHASIS ON THE KEY ROLE OF THE PATHOLOGIST IN KRAS MUTATION TESTING IN THE SELECTION OF PATIENTS FOR EGFR-TARGETED THERAPY IN COLORECTAL CANCER.** A. Hoorens (1), A. Jouret-Mourin (2), C. Sempoux (2), G. De Hertogh (3), P. Demetter (4). (1) UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium, (2) Université Catholique de Louvain, Brussels, Belgium, (3) University Hospital Gasthuisberg, Leuven, Belgium, (4) Erasme Hospital - ULB Brussels, Brussels, Belgium

Retrospective analyses of several clinical trials have shown that patients with metastatic colorectal cancer and mutation in codon 12 or 13 of exon 2 of the KRAS gene are unlikely to benefit from treatment with the anti-EGFR antibodies cetuximab or panitumumab, and testing for KRAS mutation in this setting is recommended. Pathologists have a crucial role in coordinating the testing for KRAS mutations, whether or not testing is performed in their own laboratory, as mutation analysis is performed on paraffin embedded tissue, often archival tissue, selected by the pathologists. The type of fixative used is a very important issue, as some fixatives do not allow molecular testing. Only properly fixed tissue can be used. Pathologists must select the most appropriate tumoral tissue block for KRAS mutation analysis and hence, must be aware of which KRAS mutation detection methodology is utilized in their reference laboratory. They have to know the sensitivity of the method used, as it is essential that they select a tissue block for KRAS mutation analysis that contains enough percentage of viable tumour cells, as false negative results will occur when the sample is contaminated with high levels of nontumour elements. Pathologists not only have to recognize the area of invasive carcinoma and distinguish it from non-invasive neoplastic components, but they also have to estimate the percentage of necrotic debris and nontumoral compounds such as supporting stroma and infiltrating inflammatory cells. For tests that require a high percentage of tumour cells, macrodissection before extraction of nucleic acids is often indicated. The primary pathologists in addition are responsible for preparation of the pathology report for the tissue block on which the KRAS mutation analysis was performed. They should include the result into the original or a complementary pathology report and transmit the results to the requesting clinician. Finally, pathologists should participate in a multidisciplinary oncologic consult to achieve correct interpretation of the results e.g. in case of potential false negative results.

KRAS MUTATION STATUS IN ENDOSCOPIC AND CORRESPONDING SURGICAL BIOPSIES OF PRIMARY SPORADIC COLORECTAL CARCINOMA. B. Lemmens (1), X. Sagaert (2), H. Prenen (3), K. Geboes (2), S. Tejpar (3), G. De Hertogh (2). (1) KU, Leuven, Belgium, (2) UZ, Leuven, Belgium, (3) University Hospital Gasthuisberg, Leuven, Belgium.

**Introduction** : Metastasized colorectal cancer (CRC) can be treated with monoclonal anti-Epidermal Growth Factor Receptor (EGFR) antibodies. Guidelines require prior testing of tumor tissue for the presence of KRAS mutation, since this precludes effective therapy. Surgical biopsies of the primary tumor are most commonly submitted for testing. However, in about 20% of cases only endoscopic biopsies are available.

**Aim** : Up till now, the results of testing on endoscopic and corresponding surgical biopsies have not been systematically compared.

**Methods** : We selected endoscopic biopsies (EBs) and corresponding surgical biopsies (SBs) of 56 CRC patients. One HE stained slide was evaluated for presence, extent, type and grade of carcinoma or precursor lesions. Four slides were used for DNA-extraction and testing for the common KRAS mutations (G12D, G12V, G13D, G12C, G12A, G12S and G12R) by qRT-PCR. Mutation test results were compared between EBs and SBs and expressed as concordance or discordance.

**Results** : KRAS mutations were detected in 18/56 EBs (32%) and 20/56 SBs (36%). The presence of mutations was significantly associated with low tumor grade ( $P = 0.018$ ) but not with tumor type. There were 7/56 discordant sample pairs (13%). The distribution of results was as follows : 5 (EB wild type, SB mutated), 1 (EB mutated, SB wild type) and 1 (EB mutated, SB different mutation). For the first 5 cases, 2/5 EBs contained adenomatous tissue only ; 1/5 showed a single carcinomatous gland ; 1/5 showed a sufficient amount of cancerous tissue, which was however coagulated ; and 1/5 was discordant for no clear reason. This last case and the 2 other types of discordance may be explained by the presence of different tumor cell clones, which may or may not be sampled during endoscopy.

**Conclusion** : KRAS mutation analysis on an endoscopic tumor biopsy is technically feasible but a wild type result may not always be predictive for successful anti-EGFR therapy. The presence of a sufficient amount of undamaged carcinomatous cells is an absolute requirement. Even then, CRC heterogeneity may hinder a correct response prediction. Surgical biopsies remain the preferred test material.

K-RAS MUTATION DETECTION BY PYROSEQUENCING IN COLORECTAL CANCER. V. Slachmuylder, A. Jouret-Mourin, M. Heusterspreute, C. Sempoux, J.L. Gala. UCL Saint-Luc, Brussels, Belgium.

**Introduction** : KRAS mutations, found in 30-40% of colorectal cancer (CRC) mainly at codon 12 and 13, are associated with poor or absent response to tyrosine kinase inhibitors.

**Aim** : The aim was to validate a rapid, sensitive and high throughput assay for KRAS mutations in CRC patient samples.

**Methods** : To detect a minority of KRAS mutants (MUT) within a wild-type (WT) background, a DNA sequencing assay using pyrosequencing (PSEQ) was used, ie, nucleotide extension sequencing with an allele quantification capability. DNA previously extracted from pancreatic juice in patients with a suspicion of pancreatic tumor was assessed by conventional PCR-RFLP for the presence of codon 12 mutation and reassessed by PSEQ ( $n = 20$ ). The PSEQ limit of detection (LOD) was determined by mixing various amount of cells from a mutated cell line with a fixed amount of wild-type cells and tested in triplicate after total DNA extraction. PSEQ was then tested on a series of formalin-fixed paraffin-embedded tissue blocks prospectively collected from CRC ( $n = 456$ ) : manual dissection of tumor sections on glass slides was followed by automated DNA extraction (EZ1 BioRobot workstation, Qiagen). Sequence analysis ( $n = 40$ ) was performed in parallel. A blind double cross check ( $n = 10$  CLC-DNA per center) was carried out with the Centrum of Human Genetics, UZ, KULeuven that uses a allele specific Q-PCR KRAS assay.

**Results** : Identical results were obtained when comparing PSEQ and RFLP-PCR, as well as sequencing and PSEQ data. The PSEQ-LOD was 8%. KRAS mutation double cross check in both centers also gave concordant results. KRAS mutation rate in the series of CRC was 38%. Ten different mutations have been identified : G12D (33%), G12V (25%), G13D (22%), G12C (9%), G12S (4%), G12A (3%), G13C (1,2%) and G12R, G12F, and a double mutation G12D/G12V (

**Conclusion** : Using PSEQ, KRAS mutation rate and the mutations distribution are in line with data from the literature, as is the 8% LOD (5% LOD reported previously with mixed DNA, not with whole cells). The current PSEQ assay is simple, robust, sensitive, reproducible, high throughput and rapid (turn around time from slides received in CTMA to final results : 5 days, with a week end included). The assay readily identifies each KRAS mutation in codons 12 and 13.

RAPID AND SENSITIVE K-RAS MUTATION DETECTION BY HIGH RESOLUTION MELTING ANALYSIS AND IDENTIFICATION BY PYROSEQUENCING. P. Vannuffel, X. Deghorain, J.L. Dargent. Institut de Pathologie et de Génétique, Gosselies, Belgium

**Introduction** : K-ras exons 12 and 13 mutations in CRC primaries and related metastatic sites may predict resistance to anti-epithelial growth factor receptor (EGFR) inhibitors. Activating mutations in the K-ras gene are found in 35-40% of colorectal tumors. Many diagnostic tools have been developed for K-ras mutation analysis, both laboratory-based and commercially available assays, with variable sensitivity, technical requirements, labor time and costs.

**Aim** : Here, a High Resolution Melting (HRM) assay was developed and optimized for K-ras mutation detection from formalin-fixed paraffin-embedded tissue samples.

**Methods** : HRM analysis were performed on DNA isolated from a panel of 815 samples derived from paraffin sections. Detected somatic mutations were further identified by direct Pyrosequencing of the amplified PCR products.

**Results** : K-ras mutation was detected and identified in 330 (40.5%) samples. This percentage as well as the respective percentage of the different mutations are equivalent to frequencies reported in the literature using ultrasensitive techniques. Moreover, we observed a high concordance of K-ras status analyzed in primary tumors and related metastatic sites.

	Type of mutation	No of mutated samples	Ratio of mutation (%)
G12D	Gly12Asp	129	39,1
G12V	Gly12Val	87	26,4
G13D	Gly12Asp	60	18,2
G12C	Gly12Cys	26	7,9
G12A	Gly12Ala	20	6,1
G12R	Gly12Arg	8	2,1

**Conclusion** : Our results demonstrate that HRM analysis actually represents a rapid cost effective and robust assay for the detection of K-ras exon 2 mutations prior to Pyrosequencing.

KRAS MUTATION DETECTION USING HIGH RESOLUTION MELTING ANALYSIS AND ITS PROGNOSTIC VALUE IN PARAFFIN-EMBEDDED FORMALIN FIXED COLORECTAL CANCER TISSUES. V. Deschoolmeester (1), C. Boeckx (1), M. Baay (1), W. Wuyts (1), E. Van Marck (1), P. Vermeulen (2), P. Pauwels (1), F. Lardon (1), J.B. Vermorken (1), M. Peeters (1). (1) UZ, Antwerpen, Belgium, (2) Sint Augustinus Ziekenhuis, Antwerpen, Belgium.

**Introduction** : With the advent of personalized medicine, there is a compelling clinical need for rapid and accurate molecular characterization of cancers. High resolution melting analysis (HRMA) provides a valid approach to efficiently detect DNA mutations.

**Aim** : In this study, HRMA was validated and implemented to detect *KRAS* hot spot mutations (codon 12/13) in paraffin-embedded formalin fixed colorectal cancer (CRC) samples. Direct sequencing was used to confirm and characterize HRMA results. In addition, the prognostic potential of *KRAS* in CRC was evaluated.

**Methods** : Initially, the HRMA sensitivity was established using a cell line dilution model, enabling us to detect 3% mutant DNA in a background of wildtype DNA. Next, HRMA was validated on 7 cell lines with or without a known *KRAS* mutation. All mutations were readily detectable using HRMA.

**Results** : HRMA revealed abnormal melting patterns in 57/164 (34.8%) CRC samples with G12D substitution the most prevalent type. However, only 50/164 (30.5%) samples could be confirmed by sequencing. The presence of *KRAS* mutation was associated with advanced stage, proximal location and microsatellite stability (MSS). Kaplan-Meier survival analysis revealed a significantly shorter overall and disease free survival for CRC patients harboring a *KRAS* mutation, especially for the G12C substitution. In Cox regression, *KRAS* mutation was still found to be a negative predictor of survival, however, significance was lost.

**Conclusion** : In conclusion, HRMA was found to be a fast, efficient and reproducible screening method for *KRAS* mutation detection by which degraded DNA from formalin-fixed, paraffin-embedded tissues can be tested. Additionally, accurate detection of *KRAS* mutations in CRC shows potential as a prognostic marker and poor outcome could possibly be linked to specific mutations. These results might have an impact on patient selection for adjuvant treatment.



KRAS MUTATION STATUS AND COLORECTAL CANCER PHENOTYPE : IS THERE A CORRELATION ? G. De Hertogh, X. Sagaert, K. Geboes, A. Tertychnyy. UZ, Leuven, Belgium.

**Introduction :** Anti-Epidermal Growth Factor Receptor (EGFR) therapy with monoclonal antibodies in metastasized colorectal cancer (CRC) is only effective when tumor tissue shows no KRAS mutations. In contrast to BRAF mutations, the relation between KRAS mutation status and pathological features of CRC has not been explored in depth.

**Aim :** To identify a possible correlation between CRC KRAS mutation status and tumor grade or other pathological parameters.

**Methods :** We investigated randomly selected surgical biopsies of 154 CRC patients. All tumors were ordinary adenocarcinomas. One HE stained slide was used for cancer grading following the classical 2-tiered or 4-tiered scheme and also according to the Nottingham Breast Cancer Grading System (NGS). We also evaluated other parameters such as quality of the tumor margin, presence of budding, desmoplasia, lymphocytic infiltrate, and vascular or perineural invasion. Serial slides were used for DNA-extraction and testing for the common KRAS mutations (G12D, G12V, G13D, G12C, G12A, G12S and G12R). The presence or absence of mutations was correlated with the microscopic features of the tumors.

**Results :** KRAS mutations were detected in 69/154 tumors (45%). The presence of a mutation was significantly associated with low "classical" tumor grade (47% vs only 11% for high-grade cancers,  $P = 0.0425$ ) and low or intermediate combined NGS grade (91% vs 9%, respectively,  $P = 0.0395$ ). The relation with NGS grade could be attributed exclusively to tumor growth pattern, with tumors showing more than 10% tubule formation having a 1 in 2 chance of being mutated and those with score 3 only a 1 in 5 chance ( $P = 0.0036$ ). We further observed a tendency for tumor budding and desmoplasia to be associated with KRAS mutation ( $P$ -values 0.0719 and 0.0912, respectively). No relation was observed with the other investigated parameters.

**Conclusion :** While the biological meaning of these findings is unclear, they may assist pathologists in the selection process of patients eligible for anti-EGFR therapy. A poorly differentiated tumor or one showing less than 10% gland formation has a high a priori chance of being wild type for KRAS.

K-RAS MUTATION IN COLORECTAL CANCER : AN ONE YEAR EXPERIENCE. H. El Housni, P. Demetter, B. Dessars, P. Heimann. ULB Erasme, Brussels, Belgium.

**Introduction :** KRAS mutational analysis is now considered as a major molecular tool for predicting response to anti-EGFR therapy for metastasized colorectal carcinoma, and has become standard practice to identify patients that will benefit or not from this therapy.

**Aim :** We report on our own experience on KRAS mutational testing based upon close collaboration between the departments of pathology and Human Genetics.

**Methods :** At first, the GI pathologist is responsible for selecting the most appropriate tissue block containing sufficient amount of tumor cells needed for molecular analysis. Appropriate isolation of tumor cells and DNA extraction are also performed in the department of pathology. The KRAS mutation detection is then performed in the molecular lab where the methodology used is an allelic discrimination method based on technology previously published (1,2), and which allows to detect the 7 most frequent mutations (in codon 12 and 13 of the KRAS gene) accounting for more than 95% of the activating KRAS mutations. Briefly, seven independent PCR are performed using a pair of primers designed to amplify both wild type and mutant alleles, and discrimination is obtained by using hydrolysis probes specific to each 7 mutated and wild type alleles. The quantitative sensitivity offered by this method has been assessed on colorectal carcinoma cell lines and varies from  $\pm 3$  to 10-20% according to the mutation tested.

**Results :** In one year, a total of 324 samples have been tested on routine, among which 41% (133/324) displayed one of the 7 hot-spot mutations. The p.G12D mutation was the most frequent encountered in our series (36%).

These results are in perfect concordance with the ones published by De Roock (1) et al as well as by another study (3) aiming at comparing the quantitative sensitivity of several methodological approaches for KRAS mutation detection.

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**Conclusion** : These good results emphasize the need for a perfect collaboration between both pathologists and molecular biologists. The good sensitivity of KRAS mutation analysis depends on the choice by the pathologist of the most appropriate tissue block to be tested, on a DNA extraction of quality and on the choice by the molecular biologist of the most reliable and sensitive KRAS mutation test.

**FAPA Association invited lecture**

DIAGNOSTIC ALGORITHM OF LYNCH SYNDROME.  
H. Vasen. Leiden, The Netherlands.

**GI PATHOLOGY CLUB MEETING – 06 MARCH 2010**  
**The President's Day**

- P26 -

GASTRIC CANCER AND MIMICS OF GASTRIC CANCER. F. Carneiro. IPATIMUP, Porto, Portugal.

Carcinomas of the stomach are a very heterogeneous group of lesions in terms of architectural diversity, pattern of growth, cell differentiation and histogenesis. This is reflected in part by the diversity of the various histopathological classifications on record. Although the most commonly used classifications are those of WHO and Lauren, several other classification systems have been proposed, including those presented by Ming, Mulligan, Goseki and Carneiro (1-5). Mimickers of gastric carcinoma encompass intraepithelial neoplasia/dysplasia, reactive and artifactual changes as well as peculiar aspects displayed in lymphomas, mesenchymal tumours and metastases.

Herein we will focus on some lesions that constitute a diagnostic challenge in the differential diagnosis with gastric carcinoma, such as : gastric dysplasia-like epithelial atypia associated with chemoradiotherapy (which may be misinterpreted as carcinoma) (6-8), atypical histiocytic infiltration (simulating diffuse carcinoma) (9), artifactual signet ring cells (sometimes seen in biopsies of gastric lymphoma) (10), and peculiar artifacts due to procedural trauma (mimicking diffuse carcinoma) (11). Further, one should be aware of “extremely” well-differentiated adenocarcinoma that may mimic complete-type intestinal metaplasia in the stomach (12) and mimics of signet ring cells *in situ*, including telescoped normal glands (13). A final mention is due to metastatic cancer, such as breast cancer (the presence of metastatic lobular breast cancer may resemble advanced gastric cancer with features of linitis plastica) (14,15) and mesenchymal tumours such as keratin-positive gastrointestinal stromal tumours of the stomach mimicking gastric carcinoma (16).

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SIGNIFICANCE OF I.E. LYMPHOCYTES IN THE SMALL BOWEL. V. Vincenzo Villanacci. University, Bologna, Italy.

Increased lymphocyte infiltration in architecturally normal intestinal mucosa was noted for the first time in the 70s in biopsies of patients with dermatitis herpetiformis [1, 2]. The significance of this infiltration was later studied by Marsh, who showed that single doses of gluten in patients with celiac disease (CD) treated was obtained an increase in intraepithelial lymphocytes (IEL) proportional to the administered dose of gluten, and he also demonstrated that this increase occurred well before the onset of the atrophy of intestinal villi [3]. Through this experiment, Marsh then realized that the various lesions described in patients with CD represent a dynamic continuous spectrum that evolves from the injury as mild (the only increase in IEL) to severe (complete villous atrophy and crypts hypertrophy) and resolves in the opposite direction with the introduction of gluten-free diet. It is important, however, to note that Marsh himself states explicitly that none of these lesions is specific for MC, as each may be due to various causes. In particular, the only increase in IEL (Marsh 1 lesion) was also described in infectious and parasitic diseases (giardiasis, cryptosporidiosis, infectious enteritis and enteropathy with AIDS) in tropical sprue, in graft versus host disease, food intolerances in transitional (at milk protein, egg, soy, chicken, fish) [3]. Furthermore, a recent study of duodenal biopsies showed that intraepithelial lymphocytosis showed that the condition is frequently found in patients with autoimmune diseases and in patients taking NSAIDs, and this study suggested that only 10% of intraepithelial lymphocytosis is associated to CD[4] In terms of anatomical and pathological finding an increased number of IEL in an architecturally normal duodenal biopsy should therefore raise the suspicion of a potential CD. This is a particular form of CD characterized by the presence of “stigmata” of celiac disease, whith antiendomysio antibody positivity in a patient who, while taking gluten, has an architecturally normal intestinal biopsy [5, 6,7,8,]. The finding of a lesion Marsh 1 must therefore be marked, so that they can be carried further serological investigations to clarify whether the patient is suffering from MC potential. The message for the pathologist is a correct morphological evaluation of the duodenal mucosa for the clinician. Other considerations on IEL concerning the complications of CD in particular Refractory Sprue : this condition reproduces the same clinical picture as collagenous sprue but can be identified by immunohistochemical staining, demonstrating that T lymphocytes, which in normal conditions express CD3 and CD8, in this case present only the expression of CD3 and not of CD8 (9).

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PATHOLOGIC PREDICTORS OF MICROSATELLITE INSTABILITY IN COLORECTAL CANCER. J. Greenson. University of Michigan Medical Center, ANN ARBOR, United States.

**Introduction :** Molecular genetic studies of colorectal carcinoma (CRC) have found that 10 to 15% of CRCs have high-level microsatellite instability. Microsatellite instability most often occurs as a result of sporadic methylation of hMLH1, however, germline mutations in DNA mismatch repair genes also predispose to MSI-H CRCs in patients with Lynch Syndrome. MSI-H colorectal cancers have been shown to have a better overall prognosis compared to microsatellite stable cancers (MSS). There is also evidence that MSI-H CRCs are less responsive to 5 fluorouracil based chemotherapy regimens than MSS tumors, although the evidence is conflicting. Because of these clinical differences and the profound importance in recognizing Lynch Syndrome, there has been increasing pressure on pathologists to identify microsatellite unstable colorectal cancers. While this can be accomplished by testing all CRCs for microsatellite instability and/or mismatch repair protein expression, this is a costly approach. Many MSI-H tumors have characteristic histologic features which some centers have used as a primary screen to select cases that warrant further testing for MSI.

**Aim :** The aim of our study was to develop a statistical model that would predict the likelihood of microsatellite instability based on the histologic features of a tumor.

**Methods :** 1649 consecutive CRCs from northern Israel were examined for a wide range of histologic changes including ; numbers of tumor infiltrating lymphocytes (TIL cells) per high-power field, presence of a Crohn's-like reaction, histologic heterogeneity, presence of a circumscribed growth pattern, lack of dirty necrosis, presence of mucinous differentiation, grade of differentiation, and tumor location. Microsatellite instability was determined by microdissecting normal and tumor tissue from unstained, recut slides of paraffin-embedded tumors. Five NIH consensus microsatellite markers were used, and only tumors with complete scoring on all 5 markers were included in the study. A tumor was considered to be MSI-H if two or more markers showed instability.

**Result :** 198 of 1649 tumors were MSI-H (12%). Univariate analysis showed that TIL > 2/HPF, well or poor differentiation, a lack of dirty necrosis, mucinous or focal mucinous differentiation, histologic heterogeneity, signet ring or focal signet ring differentiation, right sided location, expansile growth pattern, Crohn'slike reaction, age less than 50, low stage (I and II vs III and IV) and female sex were statistically significant predictors of microsatellite instability. Multivariate analysis found that TIL cells per high-power field had the strongest association with unstable tumors. The lack of dirty necrosis, the presence of a Crohn\_s-like reaction, right-sided location, any mucinous differentiation (mucinous or focally mucinous) and well or poor differentiation were independent predictors of MSI-H (Table 3). Age less than 50 was the only clinical factor that was an independent predictor of MSI-H. Logistic regression analysis came up with a formula that allows one to predict the likelihood of MSI based on these histologic features. The following website shows how this works : <http://sitemaker.umich.edu/gruber.lab/files/msi-pre.htm>. Using this histological model, one finds that greater than 50% of all CRCs have less than a 5% chance of being MSI-H.

**Conclusion :** This study reinforces that one can predict the likelihood of microsatellite instability based on the histologic features of a given tumor. Finding increased TIL cells is the single best marker of MSI-H, but the combination of increased TIL cells, a Crohn's like reaction, well or poor differentiation, mucinous differentiation, lack of dirty necrosis, right-sided location and age less than 50 provides a robust model for predicting MSI. At our institution we use this model to determine whether to send the tumor for MSI testing. Any tumor with more than a 5% likelihood is sent for testing (as are any tumors in patients under 50).

## SIX SOCIETIES SYMPOSIUM

Over- and undernutrition

- S05 -

SHOULD WE STILL USE DIETS IN GASTROENTEROLOGY ? E.M.H. Mathus-Vliegen, MD, PhD, gastroenterologist, professor in Clinical Nutrition, Academic Medical Centre, University of Amsterdam, AZ Amsterdam Amsterdam, The Netherlands (e-mail e.mathus-vliegen@amc.uva.nl).

A diet is the type and range of food that people regularly eat. Diet as an adjunctive word means drinks or foods which have been specially produced so that they do not contain many calories (diet margarine, diet coke). Dieta in the Greek language is defined as instructions and rules for life style and behaviour with guidelines for food and fluids in harmony with the common consent. Food prescriptions were an essential part of the holistic approach of mankind. In our days, a diet is a feed with specific requirements, prescribed to an individual for medical reasons.

To be on a diet is a reason for lamentation for some subjects, others take pleasure in it as attention is paid to them and their complaints are taken seriously. To be on a diet has also become a cult, and every cult has its magic : the placebo effect should therefore not be underestimated. The invincible trust in a diet may result in severe deficiencies, especially when people continue to adhere to a temporary intended advice. To meddle with their firm conviction and to come forward with scientific proofs to the contrary may also not be applauded. On the one hand there is a blind faith in the wholesome effects of the diet, on the other there is hypocrisy when diets are difficult to comply with. Davies et al. (1975) made an assessment in four hospitals of the food eaten by 40 patients on special diets restricted in energy, carbohydrate, fat, protein, and sodium. They clearly demonstrated that the assumption that the *diet prescribed is the diet consumed* is false.

Reminiscent of the statement by Dunkerley, already in 1976 - no specialty is so over-laden with unproved diets as is gastroenterology - the question remains : should we still use diets in gastroenterology? The answer is negative for those diets that proved to be senseless. The gastric and duodenal "bland" ulcer diet being "thermally bland, chemically bland, mechanically bland and psychological bland" did not hasten ulcer healing and pain relief and may therefore be addressed as "senseless bland". Normal food is recommended.

The bowel rest in intestinal and colonic disorders by total parenteral nutrition provided nutrition to the patient as a whole but starved the small and large bowel and deprived them from nutrients essential for replication and healing. Also, the prescription of a bland, soft and low residue diet did not make sense. However, in diverticular disease we have to be modest and unpretentious as to the attainments with high fibre diets. Historical and epidemiological data and a biologically plausible mechanism support the use of fibre-enriched diets, but 6 randomised placebo-controlled studies are inconsistent in their conclusions, being in favour of the use of fibre in 2 and recommending against its use in 4. The international recommendation as to the use of dietary fibre (3.4 g per MJ/d) is based on the dose relationship between fibre intake and stool weight and volume and its preventive effect on cardiovascular disease. In inflammatory bowel disease, emphasis is put on an adequate protein and energy intake and nutrition support has an important role to play, not so much as primary treatment but more as an adjunct and supportive measure. Here, more attention is being paid to the specific composition of the food with respect to n-3 fatty acids, glutamine, short-chain fatty acids and butyrate, and probiotics, although as yet, no clear recommendation as to its use can be given. In the irritable bowel syndrome (IBS), it is unlikely that food is responsible for all the symptoms. An interpretation of the evidence reveals a limited role for exclusion diets, a move away from high-fibre diets towards manipulation of fibre fractions of the diet, an evaluation of the effects of caffeine on gut function and the necessity for individual dietary assessment to identify dietary issues pertinent to the patient's symptoms (such as oligosaccharide intolerance). Probiotics are still an unsettled issue in IBS.

In liver disease, the fat-restricted and protein-restricted diets are out of date. The knowledge of the pathophysiology of liver disease and its role in the intermediary metabolism and the recognition of the disastrous course in the presence of malnutrition has resulted in a recommendation of adequate energy and protein intakes, in which several strategies may be of help such as a nibbling eating pattern or an extra late-evening meal and dairy or vegetable proteins in case of meat protein intolerance. The role of branched-chain aminoacids (BCAAs) is limited. Nutritional guidelines do not include BCAAs in their recommendation, although they may be advised as nutritional supplements.

The answer is affirmative for those diets in gastroenterology that result in the relief of symptoms in lactase deficiency and in constipation. In celiac disease the symptomatic relief and restoration of the villous atrophy to normal by a gluten restricted diet are an established phenomenon.

Controversy as to dietary advices exists mainly in gastro-oesophageal reflux disease and in pancreatitis. The purpose of treatment in GORD is to reduce the abnormal reflux of gastric contents and to minimise the tissue damage by the refluxate. Lifestyle modifications are aimed to reduce the reflux. Despite the deficiency of studies in GORD patients, the validity of some of the life-style modifications, such as selective food and beverage avoidance and weight loss, remains consistent with our current understanding of the pathogenesis of GORD, and are founded on well-studied physiologic determinants of gastro-oesophageal reflux in volunteers. It should yet be realized that there is almost no published evidence of the efficacy of dietary measures in GORD patients.

In pancreatitis, the outcomes of pancreatic rest and nutritional support, the timing and route of enteral nutrition and the timing and nutrient composition of oral support are still heavily debated, as are the modifications in composition of enteral feeds and the addition of probiotics. As yet, the axiom of pancreatic rest is outmoded and in mild acute pancreatitis the patient usually consumes normal food within 5-7 days. In more severe disease enteral nutrition is indicated, and when not tolerated, either the deep intrajejunal route should be tried and if impossible, parenteral nutrition should be given. Specific supplements added to enteral nutrition such as arginine, glutamine, and n-3 fatty acids may be associated with a positive impact on outcome, but apart from glutamine, studies are too few to make recommendations. With regard to probiotics, the probiotic mixture that was used in the study by Besselink et al. (2008), is not an option. To summarize the answer to the question : “Should we still use diets in gastroenterology?” the answer is affirmative, but diets should only be used when a sound pathophysiologic mechanism underlies the dietary prescription and randomised clinical trials have proven its value. But also, the diet should conform to healthy eating advices in order to not induce other secondary diseases.

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- S07 -

THE ROLE OF NUTRITIONAL SUPPORT FOR CANCER CACHEXIA. J-M. Argilés. Hospital Clinic, Barcelona, Spain.

The anorexia-cachexia syndrome, characterized by a marked weight loss, anorexia, asthenia and anemia is invariably associated with the presence and growth of the tumour and leads to a malnutrition status due to the induction of anorexia or decreased food intake. In addition, the competition for nutrients between the tumour and the host leads to an accelerated catabolic state, which promotes severe metabolic disturbances in the host, including hypermetabolism, which leads to an increased energetic inefficiency. Although the search for the cachectic factor(s) started a long time ago, and although many scientific and economic efforts have been devoted to its discovery, we are still a long way from knowing the whole truth. The main aim of the presentation is to review the different molecular mechanisms and catabolic mediators – both humoral and tumoural – involved in cancer cachexia since they may represent targets for promising clinical investigations in the future. In addition, the role of nutrition, nutraceuticals and some promising drugs for the treatment of the cachectic patient, will be discussed.

ANOREXIA NERVOSA. M. Vandewoude. RZ Tienen, Tienen, Belgium

**1. Diagnostic criteria :**

- a) BMI < 17 ;
- b) intense fear of gaining weight ;
- c) unhappy with his/her own body shape ;
- d) amenorrhea.

**2. Prevalence :**

- 1-2% among teenage girls and university students ;
- being 10 times more frequent among girls than boys.

**3. Prognosis :**

- complete recovery (50%) ;
- partial recovery (25%) ;
- developing chronic eating disorder (20%) ;
- death (5%).

**4. Mortality causes :**

- a) consequences of extreme malnutrition ;
- b) sudden death (including refeeding syndrome) ;
- c) suicide.

**5. Differential diagnosis :**

- hyperthyroidism ;
- Addison's disease ;
- chronic infectious disease (including HIV) ;
- malabsorption disorder (e.g. celiac disease) ;
- IBD ;
- carcinoma ;
- depression.

**6. Symptoms :** affecting almost all organ systems ; be aware of :

- osteoporosis (high risk long-term complication) ;
- refeeding syndrome (congestive cardiomyopathy) (high risk short-term complication .
- electrolyte disturbances (K, P, Mg) ;
- GI manifestations : gastroparesis, abdominal bloating, abdominal pain, constipation.

**7. Need for hospitalisation :**

- a) BMI < 13 ;
- b) heart rate < 40bpm ;
- c) hypoglycemia ;
- d) electrolyte disturbances ;
- e) suicide risk

**8. Some topics in treatment :**

- unstable patient : check hypoglycemia, check cardiac rhythm, be aware of Wernicke encephalopathy (treatment : IV fluids, correction of electrolyte disturbances and hypoglycemia, thiamine 100mg IV) .
- edema : bed rest, reassurance, avoid diuretics (if necessary : low dose spironolactone) ;
- gastroparesis : reassurance, prokinetics, avoid cisapride (QT-prolongation !) ;
- obstipation : document, assure fluid intake, bulk agents and fibre ;
- refeeding syndrome : be aware of P and Mg deficiencies .
- osteoporosis : no alternative for refeeding ; vit D and Ca supplements ; no place for hormone replacement ; biphosphonates questioned.



## BELGIAN PANCREATIC CLUB

### Invited lecture

- T01 -

PATHOPHYSIOLOGY OF INFLAMMATORY PANCREATIC DISEASES AND ITS CLINICAL IMPLICATIONS.  
J.L. Frossard. Hôpitaux Universitaires, Genève, Switzerland.

Recent advances have improved our understanding of the pathophysiology of AP. Indeed, the emerging concept for the past 10 years is that AP includes the premature activation and release of pancreatic enzymes in the interstitium, the autodigestion of the pancreas by these enzymes and the multiple organ dysfunction following the release of several mediators in the systemic circulation. Indeed, AP occurs when intracellular protective mechanisms designed to prevent trypsinogen activation or reduce trypsin activity do not function fully. These protective mechanisms include the synthesis of trypsin as the inactive enzyme trypsinogen, enzyme compartmentalization and packaging, synthesis of specific trypsin inhibitors such as serine protease inhibitor Kazal type 1 (SPINK<sub>1</sub>) and low intracellular calcium concentrations. During AP, following the activation of trypsinogen into active trypsin within acinar cells, numerous enzymes such as elastase and phospholipase A<sub>2</sub> as well as complement and kinin systems are activated. Additionally, inflammation is initiated with local production of mediators such as IL-1, IL-6 and IL-8 from neutrophils, macrophages and lymphocytes. TNF- $\alpha$  is also released by local macrophages within pancreatic tissue and its production correlates with the severity of the experimental disease. Interestingly, anti-inflammatory cytokines, such as IL-10 decrease the severity of experimental pancreatitis. Over the last few years, significant evidence has been accumulated that this multisystem pathological condition is caused by the synthesis and release of pro-inflammatory cytokines and chemokines. These pro-inflammatory mediators are thought to be produced within the gland. They can amplify localized pancreatic injury and then translate local inflammation into systemic disease with the involvement of distant organ. In addition to cytokine and chemokine synthesis and release, activation of endothelial cells permits the transendothelial migration of leukocytes that release other harmful enzymes. Decreased O<sub>2</sub> delivery to the organ and generation of O<sub>2</sub>-derived free radicals also contribute to the injury. Thus, regardless of the initial trigger of the disease, the severity of pancreatic damage is related to the injury of acinar cells and to the activation of inflammatory and endothelial cells. Local complications (acinar cell necrosis, pseudocyst formation and abscess) and injury in remote organs (lungs) follow the release of numerous mediators by these cells. Finally, recent studies have also shown a key role played by substance P, a neuropeptide, and hydrogen sulphide (H<sub>2</sub>S), a gas, in the pathogenesis of AP and systemic organ damage.

- T02 -

IMPACT OF MAGNETIC RESONANCE CHOLANGIOPANCREATOGRAPHY IN THE WORK-UP OF PANCREATIC DISEASES. J. Rigaux (1), M. Arvanitakis (1), M. Bali (1), M. Costa Pereira (2), J. Devière (1), M. Delhaye (1), C. Matos (1). (1) Erasme Hospital - ULB Brussels, Brussels, Belgium, (2) Centro Hospitalar de Lisboa Central, Lisboa, Portugal

**Introduction** : MRCP shows comparable diagnostic accuracy as ERCP and tends to replace this procedure for the diagnosis of pancreatic and biliary diseases, as a safe non-invasive method. However, the value of MRCP information and its impact on physician thinking and/or behaviour has not been clearly demonstrated.

**Aim** : To evaluate the ability of MRCP to alter the diagnosis and to prevent diagnostic and/or therapeutic invasive procedures in a series of patients investigated for suspected pancreatic diseases.

**Methods** : Fifty-two selected patients (M :F 31 :21, mean age 58.9 years) who underwent MRCP in our tertiary center for suspected pancreatic disease were included as clinical cases. Thirty-one patients presented as definitive diagnosis a pre-malignant or a malignant pancreatic disease and 21 patients presented a benign condition. All cases were retrospectively assessed by 2 physicians with a special interest in pancreatic disease (a junior and a senior), uninformed of the final diagnosis, first, by using clinical history, physical examination, laboratory results and results of previous imaging procedures and secondly, with the addition of the MRCP results. For each step, each physician suggested the most probable diagnosis rated with a confidence level (0-100%) and proposed further diagnostic and/or therapeutic procedures. The second assessment was performed using a different sequence of the cases and without knowledge of previous assessment results. Diagnostic changes, confidence level changes, reduction rates of invasive procedures and overall accuracy of MRCP were evaluated by a third independent physician.

**Results** : For the junior and the senior physician, the diagnosis was modified by adding the MRCP results in 59.6% and 55.7% of the cases respectively. The overall accuracy of MRCP was 93.2%. The diagnosis was correct in 34.6% and 44.2% of the cases before the MRCP results for the junior and the senior physician respectively and in 92.3% of the cases after the MRCP results for both physicians ( $p < 0.0001$ ), with the improvement in the diagnostic accuracy provided by the MRCP information being statistically significant even for the senior pancreatologist. The gain in confidence added by the MRCP results was statistically significant for both physicians ( $p < 0.0001$ ), as well as the reduction in the rate of invasive procedures (EUS, ERCP, and/or surgery), prevented in more than 50% of the cases.

**Conclusion :** In patients selected for suspected pancreatic diseases, admitted in our tertiary center and investigated by physicians with a special interest in pancreatic disease, MRCP significantly improves the rate of correct diagnosis and reduces the rate of invasive procedures.

- T03 -

WHEN IGG4 AND CA 19-9 YIELD CONFLICTING RESULTS IN A PATIENT WITH PANCREATIC DISEASE : LESSONS TO BE LEARNED. W. Van Steenberghe, F. Claus, R. Aerts, T. Roskams. University Hospital of Gasthuisberg, Leuven, Belgium.

**Introduction :** 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.

**Aim :** We present a patient 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.

**Results :** 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 half of the patients presenting with autoimmune pancreatitis ; in most cases levels are within six times the upper limit of normal and have a tendency to normalize after steroid therapy. In our patients, a dramatic rise in CA 19-9 was seen under steroids.

**Conclusion :** 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 CA19-9 even during treatment with steroids, 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.

INTRAPANCREATIC METASTASES : A SURGICAL INDICATION ? G. Roeyen, T. Chapelle, W. Van Riel, K. De Greef, B. Op de Beeck, J. Somville, M. Huizin, D. Ysebaert, University Hospital Antwerp, Belgium.

In this presentation two case reports of intrapancreatic metastatic disease will be discussed. The literature on this subject will also briefly be reviewed.

The first patient is a 59 year old woman, who underwent a right nephrectomy 15 years ago because of renal cell carcinoma. Follow up never showed recurrent disease and the patient was considered to be cured. She is also recently treated for a breast carcinoma (T2N0). She underwent surgery and has hormone therapy. In her further oncologic follow up, she developed several pancreatic metastases and a second renal cell carcinoma in her left kidney. After diagnostic work-up, the patient underwent a surgical exploration because all known tumor masses could be resected by a pancreaticoduodenectomy combined with a left nephrectomy. As a result of this invasive therapy, hemodialysis would become necessary. During the surgical exploration however, there was evidence on peroperative frozen section for liver metastases of the renal cell carcinoma as well. These liver metastases however were not known on preoperative imaging. Therefore, surgical therapy had to be aborted, the patient was only treated by chemotherapy.

The second patient is a 66 year old man. He underwent an amputation of his right thumb because of an invasive melanoma Clark V. Four years later, in his oncologic follow up, a 6 cm prominent mass was discovered in the pancreatic corpus. Preop imaging characteristics were not conclusive about the nature of this mass. Extrapancreatic disease had been ruled out. The patient underwent a pancreaticoduodenectomy (including resection of the body of the pancreas). Pathologic findings confirmed that this mass was a metastasis of the malignant melanoma. In the abdomen, no other evident metastases were present. Resection margins are free of disease. Adjuvant therapy was not initiated.

VASCULAR RECONSTRUCTION DURING PANCREATODUODENECTOMY FOR DUCTAL ADENOCARCINOMA OF THE PANCREAS IMPROVES RESECTABILITY BUT DOES NOT ACHIEVE PATIENTS CURE. M. Ouaiissi, C. Hubert, A. Dili, D. Glineur, P. Astarci, R. Verhelst, C. Sempoux, P. Deprez, I. Borbath, L. Annet, Y. Humblet, M. Van den Eynde, P. Scalliet, P. Goffette, A. Loundou, J.F. Gigot. UCL Saint-Luc, Brussels, Belgium.

**Introduction :** Combined vascular and pancreatic resection improves long-term survival of patients suffering from ductal adenocarcinoma of the pancreatic head.

**Aim :** To compare the results of surgical resection in patients with pancreatic cancer with or without concomitant vascular resection.

**Methods :** Retrospective study of 149 consecutive patients having undergone pancreato- duodenectomy without vascular resection (Group A : 82 patients), with isolated venous resection (Group B : 67 patients) or with arterial and/or venous resection (Group C : 8 patients). Late 10-year disease-free survival was considered as the indicator of patients\_ disease cure.

**Results :** The duration of surgery and blood losses were significantly more important in groups B and C compared to Group A, but postoperative morbidity and mortality rates were similar. R0 resection was significantly more frequent in Group A (86.6%) compared to Group B (57.6%) and C (50%) ( $p = 0.0002$ ), but tumours were more advanced in these groups, as demonstrated by a lower Karnofsky index, a higher Ca 19-9 plasmatic level, a greater tumour size, a more advanced stage in the AJCC classification and more tumour location in the uncinate process of the pancreas. Ten-year overall and disease-free survival was significantly better in Group A (19% and 20%) compared to Group B (2.8% and 0%) and Group C (0% and 0%). Multivariate analysis proved vascular resection and metastatic nodal status as being independent predictive factors of poor disease-free survival.

**Conclusion :** Vascular resection combined to pancreatoduodenectomy for pancreatic cancer increases local resectability without increasing mortality and morbidity rates but is not associated to improved cure rate. Neoadjuvant therapy might be considered in those patients.

## Invited lecture

- T06 -

SCREENING INDIVIDUALS FOR PANCREATIC CANCER : FOR WHOM ? HOW ? WHEN ? AND HOW FREQUENTLY ? P. Deprez. UCL Saint-Luc, Brussels, Belgium.

Pancreatic cancer (PC) screening should only be proposed in patients at high risk for cancer. The following groups might benefit from screening :

1-Familial pancreatic cancer :

- a.  $\geq 2$  first-degree relatives with PC
- b.  $\geq 3$  relatives of any degree with PC
- c.  $\geq 2$  relatives of any degree with PC, one of whom was aged 50 years or younger at the time of diagnosis ;

2-Hereditary syndromes with a cumulative lifetime risk of PC  $> 10\%$

- carriers of mutations in CDKN2A, PRSS1 and STK11 genes
- patients with a clinical diagnosis of Peutz-Jeghers syndrome but without a known gene mutation

3-Hereditary syndromes with an unknown cumulative lifetime risk, or  $< 10\%$

- carriers of a germline mutation in BRCA2, BRCA1, MLH1, MSH2, APC or p53 in families with PC in 1 FDR or SDR.

Surveillance should be offered from age 50 or 10 years younger than the age at which the youngest family member developed pancreatic cancer, whatever comes first, except in PJS where it should start at the age of 40y. Earlier start might be considered in smokers. Frequency should be every 1-2 years if normal baseline. Algorithms of surveillance and John Hopkins outcomes of screening and treatment will be discussed during the lecture.

In those patients, the Dutch register showed that surveillance is possible, participation rate is high and that the success is dependent on cooperative multi-disciplinary team approach. A learning experience will be necessary to understand the clinically relevant pathologies that will be searched for and detected. There will be a learning curve in dealing with mass lesions, with precursors lesions such as single or multiple IPMNs and PanINs. I suggest that a similar registry should be proposed by the Belgian Pancreatic Club to physicians dealing with patients at high risk for cancer.

- T07 -

ECTOPIC PANCREATITIS IN THE ANTRUM, MIMICKING A SUBMUCOSAL GASTRIC TUMOR. E. Vanhoutte, D. Vanbeckevoort, D. Bielen, K. Op de Beeck, R. Vanslebrouck. University Hospital Gasthuisberg, Leuven, Belgium.

**Purpose** : To discuss the clinical, endoscopic, radiologic and histological manifestations of a gastric ectopic pancreas rest, complicated by an acute pancreatitis and to highlight the difficulties of making an accurate preoperative diagnosis.

**Discussion** : Ectopic pancreas is rare and may manifest as a submucosal mass in any segment of the gastrointestinal tract, although there is a striking preponderance for the antrum and the adjacent duodenum and proximal jejunum. It generally does not cause any symptoms and is discovered incidentally. However diseases of normal pancreas may also occur in ectopic pancreas tissue such as pancreatitis, pseudocyst, insulinoma and pancreatic cancer. The most common symptoms attributed to ectopic pancreas are abdominal pain, gastrointestinal bleeding and obstruction. We report two cases of ectopic pancreas in the prepyloric antrum, complicated by an acute pancreatitis. Both patients presented with subacute abdominal pain. Upper gastrointestinal endoscopy revealed a soft submucosal mass in the distal antrum, without central umbilication. Endoscopic biopsy of the mass was inconclusive. A CT scan confirmed the submucosal mass in the antrum with tubular, cystic or necrotic center. They were both operated for suspected gastric malignancy, type GIST or leiomyoma. Intra-operative examination of frozen sections demonstrated ectopic pancreas tissue centred in the submucosa, with acute inflammation of a focal dilated and ruptured pancreatic duct. Based on the histological findings, the diagnosis of ectopic pancreatitis was made and therefore only local excision of the ectopic pancreas was performed.

**Conclusion** : Ectopic pancreas can be extremely difficult to distinguish from malignancy and therefore, should be included in the differential diagnosis of a submucosal mass in the upper gastro-intestinal tract.

COINCIDENTAL FINDING OF PRIMARY AND METASTATIC TUMOURS IN A WHIPPLE RESECTION SPECIMEN. L. Verset, A. Mathieu, M. Arvanitaki, J. Closset, P. Loi, M.A. Bali, M. Delhay, P. Demetter. Erasme Hospital - ULB Brussels, Brussels, Belgium.

**Introduction :** A 76-years-old woman was admitted to our hospital because of jaundice and loss of weight. At the admission, the bilirubin level was 5 mg/dl ; the alkaline phosphatase level was 223 UI/l. The CEA was slightly increased (4.7 ng/ml) while CA19.9 was normal.

**Aim :** The abdominal MRI showed a solid lesion, 2.5 cm in diameter, regarding to the pancreatic head as well as a dilation of the main pancreatic duct and a distal stenosis of the main bile duct. An echoendoscopy and an ERCP were performed, and a malignant ampulloma was suspected. Biopsies and smears were, however, negative. A PET-scan revealed a hypermetabolic lesion in the inferior right pulmonary lobe, which was confirmed by CT-scan. Transbronchial biopsy and cytological examination did not show any neoplastic cells.

**Methods :** The histological examination of the Whipple resection specimen showed a well differentiated cholangiocarcinoma of the main bile duct (staged pT2N0) as well as a high-grade neuroendocrine tumor that also invaded a para-pancreatic lymph node. The neuroendocrine tumour was positive for TTF-1 immunohistochemistry, suggesting a pulmonary origin.

**Results :** Primary high-grade endocrine tumours of the pancreas are rare ; they constitute less than 5% of all pancreatic endocrine neoplasms. Metastasis from the lung (or other sites) should always be excluded before accepting a specific case as a primary pancreatic tumour. TTF-1 immunohistochemistry is useful in this setting since this marker is often expressed by neuroendocrine tumours from pulmonary origin whereas tumours from pancreatic origin are virtually never positive.<sup>1</sup>

**Conclusion :** Given the literature data, the neuroendocrine tumour described in this case was considered as a primary pulmonary tumour, even in the absence of histological confirmation by means of pulmonary biopsies.

1. Lin X., Saad R.S., Luckasevic T.M., Silverman J.F., Liu Y. Diagnostic value of CDX-2 and TTF-1 expressions in separating metastatic neuroendocrine neoplasms of unknown origin. *Appl. Immunohistochem. Mol. Morphol.*, 2007, 15 : 407-414.

SEPTICAEMIC SHOCK IN A PATIENT WITH CHRONIC PANCREATITIS : REPORT OF AN UNUSUAL CASE AND ITS SUCCESSFUL OUTCOME BY ENDOSCOPIC TREATMENT. W. Van Steenberghe. University Hospital of Gasthuisberg, Leuven, Belgium.

**Introduction :** 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.

**Aim :** 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 abscess 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, 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.

**Results :** 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. The endotherapy was followed by a complete resolution of clinical symptoms, and the patient remained free of further complications during further follow-up.

**Conclusion :** The case will be presented with its clinical and radiological findings illustrating the presence of the pancreaticoportal 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.

HEMOSUCCUS PANCREATICUS CAUSED BY RUPTURE OF A SPLENIC ARTERY ANEURYSM COMPLICATING CHRONIC PANCREATITIS : AN UNCOMMON CAUSE OF GASTROINTESTINAL BLEEDING. N. Hiltrop, J. Hoste, G. Lambrecht, A. Janssen, M. Cool, G. Deboever. AZ Damiaan, Oostende, Belgium.

Hemosuccus pancreaticus is an uncommon cause of gastrointestinal bleeding, most frequently associated with chronic pancreatitis. Erosion of a peripancreatic artery by a pseudocyst can cause a pseudoaneurysm, and rupture occurs in up to 10% of the cases. Bleeding from a pseudocyst wall or rupture of an atherosclerotic or traumatic aneurysm is rare. Angiography, contrast-enhanced computer tomography and endoscopic findings can be diagnostic in the majority. In the absence of pancreas related indications for surgery, angiographic embolization can be therapeutic. A case of a 50-year old women with intermittent gastrointestinal bleeding and anemia is presented. Contrast-enhanced computer tomography showed a splenic artery aneurysm secondary to chronic alcoholic pancreatitis. A distal pancreatectomy and splenectomy was performed.

INCIDENTAL FINDING OF A PANCREATIC CYSTIC LESION DURING THE POSTOPERATIVE FOLLOW-UP OF A RIGHT NEPHRECTOMY FOR RENAL CELL CARCINOMA. M. Delhaye, M. Arvanitakis, M.A. Bali, C. Matos, P. Demetter, J. Devière, J. Closset. Erasme Hospital - ULB Brussels, Brussels, Belgium.

Incidental cystic lesions of the pancreas are being identified with increasing frequency with advances in imaging techniques.

We report the case of a 53 year-old woman who presented with non-specific abdominal pain for many years. Her previous history revealed saphenectomy and hysterectomy. She did not smoke or drink alcohol. She was taken furosemide as only medication.

In 4/2007, she underwent a right nephrectomy for renal cell carcinoma and a cholecystectomy for gallbladder stones. During her postoperative follow-up a focal cystic dilatation of the main pancreatic duct in the body of the pancreas was incidentally discovered.

Five months later, she developed acute abdominal pain increasing after meals, without fever. She had lost 10 kg in 1 year.

Laboratory data showed increase of serum amylase (at  $2.6 \times N$ ) and lipase (at  $3.7 \times N$ ). Liver tests were normal. Tumoral markers CEA was normal, and CA 19.9 slightly increased at 43 U/L ( $N < 37$ ).

Imaging procedures included CT scan, MRI, PET-CT scan and ERCP with pancreatoscopy.

All procedures displayed cystic dilatation of the main pancreatic duct involving the head and the body of the pancreas. During endoscopy pus and blood flew out of the main papilla which was largely opened allowing introduction of the pancreatoscope.

A duodenopancreatectomy was performed.

## SMALL BOWEL GROUP

- V04 -

ATYPICAL AND RARE INDICATIONS OF SMALL BOWEL CAPSULE.. Urbain. UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

Capsule endoscopy for the small bowel induced a revolution in the endoscopic examination of the small bowel. Obscure bleeding remains the most important indication for this technique, but some other small bowel diseases can also be explored.

Acute or chronic gastrointestinal hemorrhage at the level of the small bowel is infrequent and should account for about 5% of gastrointestinal hemorrhage. Therefore, this device has to be used exclusively after performing routine upper and lower endoscopy. As for classical enteroscopy, some lesions encountered by capsule examination are within the field of classical upper- and lower endoscopy (up to 20%). Overt bleeding remains up to now the most accurate indication of capsule endoscopy, especially shortly after the bleeding episode.

Crohn's disease is another field of application of the capsule endoscopy. As a matter of fact, capsule is not reimbursed in our country for this indication. The device is particularly efficient in established Crohn's disease, when recurrence of the disease is suspected. In these patients, retention of the capsule can occur at the level of stenosis. In celiac disease, villous atrophy can reliably be identified by capsule inclusively in parts of the duodenum not accessible for routine endoscopy. Capsule endoscopy is also used for the detection of polyps out of reach of conventional endoscopy like in the Familial Adenomatous Polyposis. Less useful indications of the capsule are abdominal pain of obscure origin (with a very low diagnostic yield), or the measurement of small bowel time transit. Data concerning the detection of small bowel tumors by capsule endoscopy are more and more available, with an additional diagnostic yield varying between 2% and 9% as compared with other techniques. In some tumors like intestinal lymphoma or melanoma, capsule endoscopy can be used for evaluating the real extend of the metastatic disease. In this indication, it seems to be superior to other techniques inclusive the Pet-scan.

- V05 -

CAPSULE ENDOSCOPY FOR OBSCURE GASTROINTESTINAL BLEEDING IN LIÈGE : RESULTS AND LONG-TERM OUTCOME OF THE PATIENTS. S. Bessemans, E. Louis, J. Belaiche, C. Reenaers. Ulg Sart Tilman, Liège, Belgium.

**Introduction :** Despite standard endoscopy evolution, 5 to 10% of patients have unexplained obscure gastrointestinal bleeding (OGB). Capsule endoscopy (CE) is now recommended as the third line of exploration after normal gastroscopy and colonoscopy with high level of sensibility and specificity. However, its impact on patient's outcome and management has been poorly studied.

**Aim :** The primary and the secondary endpoints of this work were respectively to describe the population and the results of CE performed for OGB in Liège and to evaluate their long-term outcome.

**Methods :** We retrospectively studied 98 patients who underwent CE for OGB from 2003 to June 2009. CE findings were devised in three groups : negative, positive with lesions probably not involved in OGB, positive with lesions potentially involved in OGB. Therapeutic interventions were medical, endoscopic or surgical. Symptoms were considered resolved when the patient had cessation of symptoms for at least 6 months post-CE.

**Results :** CE was negative in 31% of patients, positive with lesions probably not involved in OGB in 16,5%, positive with lesions potentially involved in OGB in 52,5%. Anaemia resolution occurred in 55.2%, 50% and 64,6% respectively. The difference was not statistically significant. In the latter group, 64,6% of patients underwent endoscopy that consisted in upper, combined upper and low push enteroscopy or gastroscopy. Ninety-three% of lesions described in CE were accessible for coagulation or biopsies. Seventy-seven% of patients in the third group had anaemia resolution due to management modification (new endoscopy, drug modification or both) and only 22,6% of anaemia resolution were spontaneous which was statistically lower than in the 2 other groups ( $p = 0,0038$  and  $0,0098$ ). Male gender and transfusions were predictive for positive CE whereas other demographic or medical characteristics were not. Haemoglobin levels, and previous transfusions were associated with persistence of anaemia whereas acenocoumarol intake was predictive of absence of anaemia resolution in the group with significant lesions in CE.

**Conclusion :** We observed about 50% of anaemia resolution in all groups but in case of significant lesions in CE, rates of spontaneous resolution of anaemia were low. Modifications of patient's management in this group induced significant increase of anaemia resolution. Acenocoumarol should be interrupted if possible because of pejorative effect of outcome in this group. Moreover, nearly 100% of the lesions involved in OGB were accessible and treatable by push enteroscopy suggesting a reasonably high efficacy and such strategy seems thus appropriate.

CURRENT EXPERIENCE OF SINGLE-BALLOON ENTEROSCOPY IN BELGIUM. T. Moreels (1), M. Aerts (2), P. Christiaens (3), P. Deprez (4), E. Macken (1), V. Muls (5), S. Van Avermaet (6), C. Van Kemseke (7), D. Urbain (2). (1) UZ Antwerpen, Edegem, Belgium, (2) UZ, Brussels, Belgium, (3) Imeldaziekenhuis, Bonheiden, Belgium, (4) UCL Saint-Luc, Brussels, Belgium, (5) CHU Saint-Pierre, Brussels, Belgium, (6) Henri Serruys Ziekenhuis, Oostende, Belgium, (7) Ulg Sart Tilman, Liège, Belgium.

In 2000 wireless videocapsule endoscopy has opened the last “black box” of the gastrointestinal tract, enabling complete endoscopic visualisation of the small bowel. In 2001 the concept of balloon-assisted enteroscopy was initiated with the Fujinon Double-Balloon Enteroscope. The Olympus SIF-Q180 Single-Balloon Enteroscope (SBE) is commercially available since 2007 and it was introduced in Belgian hospitals since 2008. It is a 200 cm long flexible enteroscope with a latex-free balloon-loaded overtube which can be in- and deflated. SBE allows complete visualisation of the small bowel by combining the antegrade and retrograde approach. The 2.8 mm working channel allows all conventional endoscopic interventions in the small bowel. To date, 7 endoscopy centres perform single-balloon enteroscopy, both in Flanders and in the Walloon Region, both in university and peripheral hospital settings. The present review focuses on the current experience of SBE in these Belgian centres. All SBE procedures performed until December 2009 were retrospectively analysed. Procedural characteristics, indications, findings and complications are recorded. Results will be presented at the meeting of the Small Bowel Group during the Belgian Week of Gastroenterology 2010.

THE IMPACT OF CHANGING REIMBURSEMENT POLICY ON THE DIAGNOSTIC YIELD OF CAPSULE ENDOSCOPY IN BELGIUM. S. De Rouck, P. Hindryckx, M. De Vos, D. De Looze. UZ, Gent, Belgium.

**Introduction and aim** : Since the first of July 2008, capsule endoscopy (CE) is partially reimbursed in Belgium for patients with obscure gastrointestinal bleeding (OGIB). A correlation between severity of bleeding (transfusion need, haemoglobin level) and the diagnostic yield of CE has previously been found. We questioned whether the new reimbursement policy influenced the referral pattern and the diagnostic yield of CE for OGIB in clinical practice.

**Methods** : We retrospectively collected data from patients who underwent a CE for OGIB in the University Hospital of Ghent between July 2002 and June 2009. Following data were analysed : number of CE's, indication, number of transfusion-dependent patients, haemoglobin level and relevance of the CE findings.

**Results** : The introduction of CE reimbursement for OGIB was associated with an abrupt increase in the number of patients referred for CE to our center. Simultaneously, the number of CE's with relevant findings dropped dramatically from more than half to less than one third of all examinations for OGIB. Patients referred after the introduction of reimbursement less frequently had transfusion need and had a significantly higher haemoglobin value as compared to patients that were referred before reimbursement.

**Conclusion** : The number of patients referred for CE has risen since the reimbursement of CE. However, there is a trend towards referral of less severe bleeders, with less transfusion need and a higher haemoglobin level. This has a detrimental impact on the diagnostic yield of CE, which inevitably results in a lower cost-benefit ratio.