38th European Pancreatic Club (EPC) Meeting

Abstracts
June 7–10, 2006, Tampere, Finland

Guest Editor
Juhani Sand, Tampere

Contents

Oral Presentations
Abstracts T1–T44

Poster Presentations

Thursday, June 8, 2006
Experimental
Abstracts P1–P24
Clinical
Abstracts P25–P70
Basic
Abstracts P71–P79

Friday, June 9, 2006
Experimental
Abstracts P80–P100
Clinical
Abstracts P101–P146
Basic
Abstracts P147–P156

Author Index

402

Basel · Freiburg · Paris · London · New York · Bangalore · Bangkok · Singapore · Tokyo · Sydney
Cases of Late Clinical Presentation

Pankrin: A New Parameter for the Diagnosis of Acute Pancreatitis in Cases of Late Clinical Presentation

PG. Lankisch¹, B. Weber-Dany¹, C. Doobe¹, T. Finger¹, R. Maisonneuve², A.B. Louvenfels³, V. Keim⁴

¹Department of Internal Medicine, Municipal Clinic of Lueneburg, Germany; ²European Institute of Oncology, Milan, Italy; ³New York Medical College, Valhalla, NY, USA; ⁴Medical Clinic and Policlinic II, University Clinic of Leipzig, Germany

Amylase and lipase levels decrease very rapidly after the onset of acute pancreatitis, and consequently the disease may be overlooked in patients with abdominal pain due to this condition who come in late to the hospital. A recently developed serum assay of pancreatic enzymes (pankrin – ELISA) should be able to overcome this disadvantage of the traditional enzyme tests as it also measures enzymes with long half-lives.

Patients and Methods: Amylase, lipase (Roche Diagnostics GmbH, Mannheim, Germany) and pankrin ELISAs (Bioserv Diagnostics, Rostock, Germany) were performed on admission and on day 2, 3 and 8 in 27 prospectively enrolled patients with a first attack of acute pancreatitis. The enzyme levels were then considered in the context of the following parameters: pain intensity, signs of peritonitis, APACHE II score on admission, Ranson and Imrie score after 48 h, and the result of the computed tomography examination scored for Balthazar.

Results: There was no correlation between the three enzymes and any parameters of the severity of acute pancreatitis. The velocity with which enzyme levels returned to normal was different for each enzyme: about half of the patients already had normal amylase levels after 3 days and almost all after 8 days. In contrast, the return to normal was almost parallel for lipase and pankrin with levels still elevated on days 3 and 8 for three quarters and half of the patients, respectively. On day 8, both lipase and pankrin levels were still detectable in 23 patients. Two out of nine patients with normal lipase had an elevated pankrin level and four out of eleven patients with a normal pankrin had an elevated lipase level.

Conclusion: The combination of pankrin and lipase estimation may be used to diagnose acute pancreatitis in patients with abdominal pain who present several days after the onset of pain.

Intraductal Papillary Mucinous Tumors (IPMTs) of the Pancreas: Contribution of 18FDG-PET to Surgical Decision Making

C. Sperti¹, C. Pasquali¹, S. Bissoli², F. Chierichetti², G. Liessi², S. Pedrazzoli³

¹Department of Medical and Surgical Sciences, Clinica Chirurgica IV, University of Padua, Padova, ²PET Center-Nuclear Medicine, ³Department of Radiology, Castelfranco Veneto Hospital, Treviso, Italy

Introduction: Pancreatic IPMTs are increasingly recognized. Even with current imaging modalities, discrimination between benign and malignant tumour is still unreliable. This study was designed to evaluate the role of 18-Fluorodeoxyglucose positron emission tomography (18FDG-PET) in decision making of IPMTs.

Materials and Methods: From January 1998 to June 2005, 50 patients with suspected IPMTs were prospectively investigated with 18FDG-PET in addition to conventional imaging techniques (helical-CT and/or magnetic resonance cholangiopancreatography). 18FDG-PET was analyzed visually and semiquantitatively using the standard uptake value (SUV). Positivity was assumed when a focal uptake occurred with a SUV of at least 2.5. The validation of diagnosis was made by surgical operation (n = 34), biopsy (n = 4), or follow-up (n = 12). Median follow-up time was 24 months (range 8–84 months).

Results: There were 25 males and 25 females, mean age of 64.3 years (range 37–80). Seventeen patients were asymptomatic. Thirty-two patients underwent pancreatic resection, 2 palliative surgery, and 16 did not undergo surgery. An adenoma was diagnosed in 11 patients, a borderline tumour in 8, a carcinoma in situ in 4, and an invasive cancer in 15. Histology was missing in 12 patients. 18FDG-PET was negative in 11/11 adenomas and 7/8 borderline IPMTs, while positive in 3/4 carcinomas in situ and in 14/14 invasive cancers. Positive 18FDG-PET findings influenced surgical decision making in 9 patients (18%): suggesting surgical resection in 6 patients (4 asymptomatic) without signs of malignancy on conventional imaging, allowing resection of unsuspected colon cancer in 1 patient, and avoiding laparotomy in 2 patient with distant metastases. Furthermore negative 18FDG-PET findings allowed more limited resection in 6 patients and follow-up strategy in 12.

Conclusions: 18FDG-PET is very accurate in distinguishing benign from malignant IPMTs, including non invasive carcinomas. 18FDG-PET is an alternative, non invasive, method useful to select patients for surgical treatment or observation.
**Introduction:** As resulting from many studies, VOCs (volatile organic compounds) are present in breath air. They result from oxidative stress caused also by breast cancer and lung cancer and they allow to discriminate between people affected and non-affected.

**Methods:** In the period between September 2004 and January 2005 we enrolled prospectively patients to breath through a breath sampler in a vial. We needed just one breath per patient (200ml). Kruskal-Wallis and Mann-Whitney-Wilcoxon test were applied and a p < 0.01 was considered significative.

**Results:** We enrolled 125 patients (104m/21f), 26 with pancreatic neoplasms and 99 controls with no cancer history. We considered patients suffering from pancreatic neoplasms and controls. Table 1 resulted 19 (mean age 62.7 ± 6.9) and controls resulted 92 (age 62.3 ± 9.2).

Table 1.

<table>
<thead>
<tr>
<th>VOC</th>
<th>Area</th>
<th>Standard error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>M29 formyl</td>
<td>0.897</td>
<td>0.0298</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aceticacid</td>
<td>0.798</td>
<td>0.0418</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M19</td>
<td>0.812</td>
<td>0.0410</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ethylene</td>
<td>0.823</td>
<td>0.0440</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CH3NH2</td>
<td>0.797</td>
<td>0.0416</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M32</td>
<td>0.849</td>
<td>0.0355</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M60</td>
<td>0.768</td>
<td>0.0429</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M66</td>
<td>0.817</td>
<td>0.0430</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M73</td>
<td>0.699</td>
<td>0.0520</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M74</td>
<td>0.721</td>
<td>0.0563</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M100 heptane</td>
<td>0.760</td>
<td>0.0541</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M105</td>
<td>0.727</td>
<td>0.0472</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M110</td>
<td>0.796</td>
<td>0.0425</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M114</td>
<td>0.805</td>
<td>0.0402</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M115</td>
<td>0.783</td>
<td>0.0424</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

15 VOCs showed significative difference (p < 0.01) between patients suffering from pancreatic neoplasms and controls. Table 1 represents the value of the ROC curve area for each of the 15 VOC.

**Conclusions:** The breath air analysis is a safe, non-invasive, economic and reliable method that allows to discriminate between people with and without pancreatic neoplasms.
relief and reduced opioid use. A RCT of thoracoscopic splanchnicectomy (TS) (Buscher 2002) has shown good pain relief in patients with chronic pancreatitis. No RCT compares opioids, TS and CPB in pancreatic cancer. The NaTTS trial is designed to address this question.

**Methods**: Patients with pain and unresectable upper abdominal malignancy were randomized to medical management (MM; opioids ± adjuvant analgesia), MM + CPB or MM + TS. Pain was assessed by daily diaries using the Brief Pain Inventory (BPI), and by a 4-point scale at each assessment visit. A planned interim analysis (without disclosure of treatment allocation) occurred after entry of 50 patients (45 (90%) pancreatic cancer). Primary endpoints were the number of pain-free days in the first 2 months and the proportion at 2 months reporting good pain relief (GPR: BPI ‘worst’ pain none or mild; reduction of mean BPI score >50% from baseline; or a decrease of >1 point on the 4-point scale.

**Results**: Death occurred before 2 months in 13 (29%) of 45 evaluable patients. Of 32 survivors, 19 (59%) completed diaries for more than 28 days, and 23 (72%) for >15 days. Half the patients achieved GPR at 2 months. Almost all adverse events were disease-related; two patients reported transient hypotension or diarrhoea after CPB.

**Conclusions**: Daily pain diaries are too onerous to provide adequate data in pancreatic cancer; pain questionnaires at assessment visits enable valid analysis of pain relief as primary endpoint. These results confirm the practicality of the first RCT evaluating all three principal options for pain relief in upper abdominal malignancy. NaTTS is open to international participation.

---

**T6**

**Localization and Functional Characterization of PAR-2 Receptor in Guinea Pig Pancreatic Duct Cells**

I. Ignáth1, B. Özsvari1, V. Venglovicz2, Z. Rakonczay Jr1, T. Takács1, J. Lonovics1, K. Borka2, Z. Schaff2, A. Tóth2, A. Varro1, M. Sahin-Tóth4, P. Hegyi1

1First Department of Medicine, University of Szeged, Szeged, 2Second Department of Pathology, Semmelweis University, Budapest, 3Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary; 4Department of Molecular and Cell Biology, Goldman School of Dental Medicine, Boston University, Boston, Mass., USA

**Introduction**: The pathophysiology of acute pancreatitis is associated with pancreatic hypersecretion in the early stages of the disease. Under these conditions, premature activation of trypsinogen has been described in acinar cells, which can activate protease-activated receptor-2 (PAR-2) and trigger cellular responses. However, no data is available on the localization and activation of PAR-2 in pancreatic duct cells. The aim of this study was to investigate the expression of PAR-2 and the effects of trypsin and PAR-2-activating peptide (PAR2-AP) on intracellular Ca2+ levels of guinea pig pancreatic duct cells.

**Methods**: Intracellular Ca2+ concentration was measured on isolated intra/interlobular microperfused pancreatic ducts. Immunohistochemical localization of PAR-2 was performed by immunoperoxidase staining of guinea pig pancreatic tissue.

**Results**: Trypsin and PAR2-AP, even at low concentrations, activated Ca2+ signaling from the basolateral membrane and trypsin from the luminal membrane of the duct cells. Trypsin inhibitor and the Ca2+ chelator BAPTA-AM totally blocked the effect of trypsin on intracellular Ca2+, while a Ca2+-free external solution did not prevent the effect. PAR-2 is expressed on the luminal membrane of intralobular ducts.

**Conclusion**: Our results suggests that PAR-2 may be the target by which pancreatic duct cells are activated. The role of the receptors and their activation in pancreatic epithelia during the inflammation of the pancreatic tissue needs further investigation.

This work was supported by OTKA, MTA, OM.

---

**T7**

**Phosphatidylinositol 3-Kinase Facilitates Bile Acid-Induced Ca2+ Responses in Pancreatic Acinar Cells**

L. Fischer1,2, A. S. Gukovskaya1, J.M. Penninger3, H. Friesz2, I. Gukovsky1, S.J. Pandol4

1VA Greater Los Angeles Healthcare System and University of California, Los Angeles, CA, USA; 2University of Heidelberg, Germany; 3Institute for Molecular Biotechnology, Austrian Academy of Sciences, Vienna, Austria

**Background and Aims**: Bile acids induce Ca2+ responses in pancreatic acinar cells; however, the underlying mechanisms are poorly understood. We have recently shown that phosphatidylinositol 3-kinase (PI3K), and in particular its gamma isoform, regulates changes in free cytosolic Ca2+ ([Ca2+]i) elicited in pancreatic acinar cells by neurohormones such as cholecystokinin (CCK). That is, by PI3K-induced inhibition of the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) there is a reduced reloading of Ca2+ into endoplasmic reticulum (ER) stores during CCK stimulation. The present study sought to determine whether PI3K regulates bile acid-induced [Ca2+]i responses in pancreatic acinar cells.

**Results**: In isolated pancreatic acini, pharmacologic inhibition of PI3K with LY294002 or wortmannin markedly inhibited [Ca2+]i responses (measured with Fura-2) induced by tauroliothocholic acid 3-sulfate (TLC-S) and taurochenodeoxycholate (TCDC). Furthermore, genetic deletion of PI3K gamma isoform also decreased [Ca2+]i responses to bile acids. Depletion of CCK-sensitive intracellular Ca2+ pools or application of caffeine, a blocker of inositol 1,4,5-trisphosphate (IP3) receptor, inhibited bile acid-induced [Ca2+]i signals, indicating that bile acids release Ca2+ from agonist-sensitive ER stores via IP3-dependent mechanism. The PI3K inhibitors increased the amount of Ca2+ releasable from the intracellular stores during exposure of acinar cells to bile acids. This suggests that PI3K negatively regulates the SERCA-dependent Ca2+ reloading into ER during bile acid stimulation. Further, in permeabilized acinar cells bile acids inhibited Ca2+ reloading into the ER, and this effect was augmented by phosphatidylinositol 3,4,5-trisphosphate (PIP3), a major product of PI3K. These results indicate that both bile acids and PI3K act synergistically to inhibit SERCA.

**Conclusions**: Our results indicate that PI3K and its product, PIP3, facilitate bile acid-induced [Ca2+]i responses in pancreatic
acinar cells through inhibition of SERCA-dependent Ca\(^{2+}\) re-loading into the ER. The findings have important implications for the mechanism of acute pancreatitis since [Ca\(^{2+}\)]\(i\) increases mediate key pathologic processes in this disorder.

---

**T8**

**Comparative Effects of Cholecystokinin-58 and Cholecystokinin-8 on Calcium Signalling in Mouse Pancreatic Acinar Cells**

D.N. Cridde\(^1\), G.M. Green\(^3\), J.R. Reeve Jr\(^4\), R. Sutton\(^2\), O.H. Petersen\(^1\)

MRC Group, Departments of \(^1\)Physiology and \(^2\)Surgery and Oncology, University of Liverpool, UK; \(^3\)University of Texas Health Science Center; \(^4\)University of California, USA

**Background and Aims:** Various molecular forms of the gastrointestinal hormone cholecystokinin exist, with differences in bioactivity between the well-characterised cholecystokinin-8 (CCK-8) and larger cholecystokinin-58 (CCK-58) recently reported. We have compared the effects of CCK-58 and CCK-8 on cytosolic calcium concentration ([Ca\(^{2+}\)]\(i\)) in pancreatic acinar cells, to ascertain potential differences in their action.

**Methods:** Isolated mouse pancreatic acinar cells were loaded with Fluo 4-AM to measure changes of [Ca\(^{2+}\)]\(i\) induced by CCK-58 and CCK-8, using confocal microscopy. NADH autofluorescence was also monitored as an indication of mitochondrial metabolism. In some experiments whole-cell patch-clamp was used to measure Ca\(^{2+}\)-dependent currents.

**Results:** Application of CCK-58 (1–5pM) induced transient, oscillatory increases of cytosolic [Ca\(^{2+}\)]\(i\) and Ca\(^{2+}\)-dependent currents (34 of 37 cells). These elevations of cytosolic [Ca\(^{2+}\)]\(i\) were associated with a rise of mitochondrial NAD(P)H autofluorescence. In contrast, a supraphysiological concentration of CCK-58 (5nM) induced a single large ‘spike’ increase of cytosolic [Ca\(^{2+}\)]\(i\) that declined to a steady plateau which remained above the basal level at 20 min after application. Withdrawal of extracellular Ca\(^{2+}\) from the bathing solution (with 1mM EGTA) after 10 min exposure to 5nM CCK-58 produced a rapid reversal of the sustained plateau increase in cytosolic [Ca\(^{2+}\)]\(i\), indicating a dependence on Ca\(^{2+}\) entry into the acinar cell (18 of 18 cells). In cells dispersed from the same tissues, CCK-8 induced similar patterns of responses to those of CCK-58, with oscillatory increases of cytosolic [Ca\(^{2+}\)]\(i\) at lower concentrations (1–5pM; 12 of 15 cells) and sustained responses at 5nM (10 of 10 cells).

**Conclusions:** CCK-58 and CCK-8 produced similar dose-dependent Ca\(^{2+}\) signals in isolated cells. CCK-58 was as potent as CCK-8, generating clearly resolved Ca\(^{2+}\) signals in the 1–5pM concentration range. These new data are compatible with the view that CCK-58 is the principal physiological form of this hormone.

---

**T9**

**Oral Administration of a Pancreatic- and Neutrophil-Elastase Inhibitor Ameliorates Caerulein Induced Pancreatitis**

J. Mayerle \(^1\), M. Rutenburg\(^1\), F.U. Weiss\(^1\), W. Halangk\(^2\), M.M. Lerch\(^1\)

\(^1\)Department of Gastroenterology, Endocrinology and Nutrition, Ernst-Moritz-Arndt-University Greifswald, \(^2\)Division of Experimental Surgery, Otto-von-Guericke University Magdeburg, Germany

**Introduction:** Circulating serum levels of neutrophil-elastase (PMN-elastase) are known to correlate with the severity of clinical pancreatitis and are closely associated with the extent of leukocyte transmigration into inflamed tissue. We tested whether prophylactic administration of a novel, orally available peptidyl trifluoromethyl ketone inhibitor of PMN-elastase (ZD-0892), with cross-specificity for pancreatic elastase, can ameliorate experimental pancreatitis.

**Methods:** Male Wistar rats (250–300 g) received gavage tube feeding of ZD-0892 (at a dose of 240 mg/kg per day) at 3 h and 1 h before the induction of acute caerulein pancreatitis for 4 and 12 h. Pancreatic and lung tissue as well as serum was collected for further analysis of clinical and morphological severity markers. Inhibitor specificity and capacity was tested in vitro. Isolated pancreatic acini were incubated with ZD-0892 and protease activity after supramaximal caerulein stimulation was monitored employing specific fluorogenic substrates for trypsin, elastase, cathepsin B and cathepsin L.

**Results:** Oral administration of the elastase inhibitor ZD-0892 significantly reduced the serum level of amylace, the extent of pancreatic edema, as well as leukocyte infiltration in the pancreas and lungs during caerulein-induced pancreatitis. Incubation of isolated pancreatic acini with ZD-0892 and subsequent supramaximal CCK stimulation reduced pancreatic elastase activity to below 20%, whereas neither trypsin, nor cathepsin B nor cathepsin L activity were affected.

**Conclusion:** Prophylactic administration of a novel, orally available PMN-elastase inhibitor with cross-specificity for pancreatic elastase improves the severity of acute experimental pancreatitis. ZD-0892 therefore represents the first orally active drug with treatment potential for patients at risk of developing pancreatitis such as those before ERCP, those with exogenous risk factors (e.g. ethanol abuse) and those with inherited predispositions.
functions in the adult pancreas. Pdx1, Hnf-1α and Ptf1α control the expression of pancreatic specific genes.

**Aim:** To investigate the expression of Pdx1, Hnf-1α and Ptf1α and the expression of key genes under control of these transcription factors during acute pancreatitis.

**Methods:** Cerulein pancreatitis was induced in C57BL/6 mice. At timed intervals the pancreases were processed for western-blot and immunohistochemistry. Cell proliferation was assessed by BrdU uptake. Insulin and amylase mRNA were assessed by qRT-PCR. Insulin was measured by ELISA.

**Results:** In normal pancreas Ptf1α is exclusively expressed in acinar cells. Pdx1 is expressed in the islets and in some acinar cells. Expression of Hnf-1α is high both in the islets and in most acinar cells. Early after pancreatitis (2–3 h), expression of all three transcription factors is greatly reduced. Normal expression levels recover by 24 h for Pdx1 and 4–10 days for Hnf-1α. These changes are associated with significant reductions in amylase mRNA (4 h) and insulin pre-mRNA (24 h) (RQ = 0.39 ± 0.05 and 0.28 ± 0.12 respectively; p < 0.01) which herald a decrease in pancreatic amylase activity (from 26 ± 1.6 to 9.9 ± 1.5 at 7 h; U/mg d.w. p < 0.01) and serum insulin (from 1.8 ± 0.1 to 0.6 ± 0.1 at 48 h; ng/ml p < 0.01). Further analysis of expression by micro fluidic cards (Applied Biosystems) confirms the selective reduction in insulin (RQ = 0.2) and pancreatic digestive enzyme mRNAs (trypsinogen RQ = 0.38) but not Gpx (RQ = 9.8) TGFβ1 (RQ = 2.7) or CFTR (RQ = 2). BrdU staining shows opposite kinetics to that of Hnf-1α expression levels.

**Conclusions:** Following mild pancreatitis, the levels of expression of transcriptional regulators responsible for the terminal differentiation of acinar and β cells are severely reduced. Continuous impairment in these regulatory processes could lead to persistent pancreatic dysfunction.

---

**T11**

**PAR-1 (Protease Activated Receptor-1) Regulates the Expression of MMP-1 (Matrix metalloprotease-1) and the Activation of Human Pancreatic Stellate Cells**

*S. Hozawa, H. Souma, N. Watanabe, H. Higuchi, Y. Yamagishi, M. Kikuchi, T. Hibi*

Division of Gastroenterology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan

**Introduction:** PAR-1 is a G protein-coupled receptor that is cleaved and activated by a ligand such as thrombin. MMP-1 has been recently recognized as a ligand for PAR-1 (Cell 120: 303–13, 2005). PAR-1 antagonism inhibits hepatic stellate cell activation in vitro and protects against fibrosis development in a rodent model of liver cirrhosis (Hepatology 39: 365–75, 2004). PPARα is also thought to be a strong candidate for targeting in the treatment of pancreatic fibrosis, and the activation of PAR-1 may regulate MMP-1 expression. In the current study, we examined whether PAR-1 agonist/antagonist can regulate human pancreatic stellate cell activation and its MMP-1 expression in vitro.

**Methods:** Human pancreatic periacinar fibroblast-like cells (hPFC) which posses characteristics of pancreatic stellate cells (Pancreas 14: 1997) were used in this study. Morphologic changes, proliferation assay, cell motility, mRNA expression of α1(I) procollagen and α SMA were examined in order to explore the activation of stellate cells treated by PAR-1 agonist (TFLLR-NH3) /antagonist (RWJ-58259). Northern blot analysis and gelatin zymography were performed to examine mRNA and protein expression of MMP-1 respectively.

**Results:** Treatment of hPFC by 50 nM of TFLLR-NH3 suppressed the expression of MMP-1 and enhanced the mRNA expression of procollagen and α SMA, while changes of the other parameters for stellate cell activation such as cell shape, motility and proliferation were not observed by the treatment. Treatment by 50 nM of RWJ-58259 has enhanced MMP-1 expression, and has slightly suppressed cell motility and mRNA expression of procollagen and α SMA.

**Conclusions:** It was suggested that PAR-1 agonist/antagonist might regulate the activation of human pancreatic stellate cells as well as their MMP-1 expression. PAR-1 might be a candidate for targeting in the treatment of pancreatic fibrosis in the future.

---

**T12**

**Role of Engulfment of Apoptotic Bodies by Pancreatic Stellate Cells on Pancreatic Fibrogenesis**

*K. Shimizu, J. Tahara, K. Shiratori*

Department of Gastroenterology, Tokyo Women’s Medical University, School of Medicine, Tokyo, Japan

**Background and Aims:** Phagocytosis is essential as a means of removing damaged or senescent cells and protecting against inflammation. Pancreatic stellate cells (PSCs) have been characterized as the major source of extracellular matrix and cytokine production in the pancreas and we have previously reported that PSCs have phagocytic function. The aim of the present study was to investigate whether engulfment of apoptotic cells by PSCs regulates pancreatic fibrogenesis.

**Methods:** Rat acinar cells were cultured for 48 h to induce apoptosis, and after allowing them to interact with rat PSCs for 12–48 h, MTT assay was performed. α-smooth muscle actin (α-SMA) expression was detected by Western blot analysis. Annexin V-FITC and propidium iodide (PI) staining or detection of DNA fragmentation was used to identify cell death. The TGF-β concentration of the culture medium was measured by ELISA.

**Results:** Most aged acinar cells were undergoing apoptosis, and they were engulfed by PSCs. Engulfment of apoptotic acinar cells significantly decreased the number of living cells and α-SMA expression of PSCs. PSCs ingested apoptotic acinar cells showed DNA fragmentation and were Annexin V-positive, suggesting that phagocytosis of apoptotic cells induced apoptosis of PSCs. There was no difference between the concentrations of TGF-β in the medium of the control PSCs and the PSCs that had engulfed acinar cells.

**Conclusions:** Engulfment of apoptotic acinar cells by PSCs may be essential to remove damaged or senescent acinar cells in the pancreas and we have previously reported that PSCs have phagocytic function. The aim of the present study was to investigate whether engulfment of apoptotic cells by PSCs regulates pancreatic fibrogenesis.
**T13**

**Natural Vitamin E Tocotrienols, but Not-Tocopherol, Induce Apoptosis and Autophagy in Pancreatic Stellate Cells (PSCS)**

M. Rickmann, E. Vaquero, X. Molero, J.R. Malagelada
Digestive System Research Unit, Hospital Universitari Vall d’Hebron

**Introduction:** Natural vitamin E comprises tocotrienols and tocopherols. Although -tocopherol is widely used in clinical practice, tocotrienols exert stronger antioxidant, cardioprotective, neuroprotective and tumor suppressive effects. Oxidative stress promotes activation and expansion of PSCs in chronic pancreatitis. Therefore, induction of PSC apoptosis might halt the disease progression.

**Aims:** To investigate the antioxidant and death promoting effects of -tocopherol and a palm oil derived tocotrienol-rich fraction (TRF) in PSCs.

**Methods:** Activated rat PSCs were treated with TRF or -tocopherol for 30 min to 72 h. Intracellular radical oxygen species (ROS) were measured by FACS using DCFH-DA; lipoperoxidation by fluorogenic assay in cells labelled with diphenyl-1-pyrenylphosphine (DPPP); cell number by CyQUANT; apoptosis by DNA fragmentation, mitochondrial membrane potential, and caspase-8, -9 and -3 activities; autophagy by detection of autophagic vacuoles stained with monodansyl cadaverine (MDC) and LC3-II protein accumulation by immunoblot.

**Results:** Both TRF and -tocopherol efficiently reduced ROS levels by 20% and lipoperoxidation by 40% from control. Cell number decreased by 30% after 24 h incubation with TRF but it was not affected by -tocopherol. TRF significantly increased DNA fragmentation (5.71±1.5-fold increase; p < 0.001) and caspase-8, -9 and -3 activities. Apoptotic changes were heralded by a pronounced and early mitochondrial membrane depolarization. Interestingly, TRF did not induce apoptosis in quiescent (oil red positive) PSCs plated on basement membrane-like substrate (Matrigel). In contrast to TRF, -tocopherol showed a modest increase in DNA fragmentation (1.80±0.2-fold increase; p < 0.00 01) without caspase activation and a weaker mitochondrial membrane depolarization. Cells exposed to TRF, but not -tocopherol, developed cytoplasmic autophagic vesicles as shown by MDC auto-fluorescence and accumulation of LC3-II.

**Conclusions:** Tocotrienols, but not -tocopherol, induce a robust cell death program (apoptosis and autophagy) in activated PSCs. Tocotrienols should be strongly considered as a potential therapeutic tool for chronic pancreatitis.

**T14**

**Proapoptotic Action of Tocotrienols in Activated Pancreatic Stellate Cells (PSCS) Involves Mitochondrial Dysfunction but Not Suppression of Nuclear Factor (NF)-κβ Activity**

E. Vaquero, M. Rickmann, X. Molero, J.R. Malagelada
Digestive System Research Unit, Hospital Universitari Vall d’Hebron

**Introduction and Aims:** Tocotrienols represent a class of vitamin E with powerful antioxidant properties. We have shown that tocotrienols exerts antioxidant and apoptotic effects on activated PSCs, the cell type responsible for pancreatic fibrogenesis. We sought to investigate intracellular targets of tocotrienols, which include the mitochondria and NF-κβ, a redox sensitive transcription factor that promotes PSC survival.

**Methods:** Activated rat PSCs were treated from 30 min to 24 h with a tocotrienol-rich fraction (TRF) derived from palm oil. Mitochondrial membrane potential (DeltaPsim) was measured by FACS analysis using DiOC6(3); apoptosis by caspase-3 and -9 activities and DNA fragmentation; NF-κβ by ELISA p50 and p65 quantification in nuclear extracts and by luciferase reporter gene assay.

**Results:** TRF induced a collapse of DeltaPsim that was rapid (within 30 min), persistent (>24 h) and almost as potent as DeltaPsim dissipation induced by the depolarizing agent carbonyl cyanide m-chlorophenylhydrazone. DeltaPsim drop was followed by caspase-9 and -3 activation and DNA fragmentation. TRF apoptotic action was linked to mitochondrial permeability transition pore (mPTP) opening, since the mPTP blocker cyclosporin A abrogated DeltaPsim dissipation, caspase-9 and -3 activation and DNA fragmentation. These results suggest that TRF directly targets mitochondria to trigger PTP opening and the mitochondrial pathway of apoptosis. TRF did not inhibit basal NF-κβ activity and did not prevent NF-κβ activation following TNF-α and PDGF-BB stimulation. Combination of TRF with NF-κβ inhibitors MG-132 and salicylic acid or with the protein synthesis inhibitor cycloheximide induced a 3.82-fold, 2.73-fold and 2.13-fold increase in DNA fragmentation over TRF alone, respectively. Interestingly, natural phytonutrients with NF-κβ inhibitory properties, i.e. curcumin and epigallocatechin-3-gallate, enhanced by 2-fold DNA fragmentation induced by TCT.

**Conclusion:** Tocotrienols stimulate apoptosis of PSCs via mPTP-dependent mitochondrial dysfunction. Proapoptotic effect of tocotrienols does not involve the NF-κβ pathway but it can be enhanced by inhibitors of NF-κβ.
Pancreatic Destruction Influences the Extend of Neural Damage and Growth via the Neurotrophic Factor Artemin in Chronic Pancreatitis

G.O. Ceyhan\textsuperscript{1}, F. Bergmann\textsuperscript{2}, M. Erkan\textsuperscript{1}, U. Hinz\textsuperscript{2}, M.W. Müller\textsuperscript{1}, I.E. Demir\textsuperscript{1}, T. Giese\textsuperscript{3}, M.W. Büchler\textsuperscript{1}, N.A. Giese\textsuperscript{1}, H. Friess\textsuperscript{1}

\textsuperscript{1}Department of General Surgery, University of Heidelberg, \textsuperscript{2}Institute of Pathology, \textsuperscript{3}Unit of Documentation and Statistics, Department of Surgery, \textsuperscript{4}Institute for Immunology, University of Heidelberg, Germany

Introduction: Neural alterations and infiltration of the perineurium by inflammatory cells in association with severe abdominal neuropathic pain are characteristic features of chronic pancreatitis (CP). Artemin, a member of the GDNF family of ligands, was recently shown to eliminate pain and to reverse neurochemical changes after nerve injury in an animal model. Therefore, we investigated whether Artemin and its receptor GFRα3 may play a role in CP pathogenesis.

Methods: The expression of Artemin and its receptor GFRα3 were investigated in CP (n = 66) and normal (n = 22) pancreatic tissues by QRT-PCR and Western blot analysis and correlated with intrapancreatic pathomorphological changes (inflammatory cell infiltration, perineural invasion by immune cells, neural alterations and fibrosis) and clinical parameters (pain). Immunohistochemistry was used to localize Artemin and GFRα3 in the tissues. To detect potential cellular sources of Artemin, primary human pancreatic stellate cells (hPSCs) were isolated and analyzed by QRT-PCR- and immunocytochemistry.

Results: Development of CP was accompanied by intrapancreatic overexpression of Artemin- and GFRα3 (p < 0.001) located in the smooth muscle cells of arteries, Schwann cells and neural ganglia. Increased levels of Artemin-mRNA correlated with the degree of inflammation, perineural infiltration by immune cells, severe pain, neural sprouting, neural hypertrophy and – the extent of fibrosis. Furthermore, there was a positive relationship between the degree of fibrosis and neural growth. α-smooth muscle actin (SMA) positive hPSCs expressed low levels of Artemin mRNA which were up-regulated by exposure to TGF-β1.

Conclusions: (a) The severity of pancreatic destruction correlates with the degree of neural damage in CP. Since TGF-β1 stimulates Artemin expression in hPSCs, we hypothesize that the degree of pancreatic damage is regulating neural alterations in CP. (b) Correlation of endogenous expression of Artemin with pain is mirroring the severity of the inflammatory processes and neural disorder in CP. It does not lead to anti-nociception in CP.
Natural History of Chronic Hereditary Pancreatitis (CHP)

V. Rebours1, M.C. Boutron-Ruault2, C. Férec3, M. Schnee4, P. Hammel5, P. Ruszniewski1, P. Lévy1, Association des Pancréatites Chroniques Héréditaires (APCH)

1Department of Gastroenterology, Beaujon Hospital, Clichy, 2Gustave Roussy Institute, Unité Inserm E3N, Villejuif, 3Inserm-0115, Génétique Moléculaire et Génétique Epidémiologique, Etablissement Français du Sang-Bretagne, Université de Bretagne Occidentale, Brest, 4Department of Gastroenterology, La-Roche-Sur-Yon, France

The prevalence and natural history of CHP in France (population of 60 million), occurring largely due to mutations of cationic trypsinogen gene (PRSS1), remains ill documented.

Aim: To assess genetic, epidemiological, clinical and morphological characteristics of CHP as part of a large extensive national survey.

Patients and Methods: A cohort comprising all French CHP patients was constituted by contacting all French gastroenterologists and pediatricians (response rate: 84%) and genetics laboratories. Inclusion criteria were the presence of PRSS1 gene mutation, or chronic pancreatitis in at least 2 first-degree relatives, or 3 second-degree relatives.

Results: The cohort comprised 78 families and 200 patients (mean number of generations: 3; men 53%, smokers 34%). PRSS1 gene mutations were found in 68% (R122 H: 78%, N29I: 12%, others: 10%). SPINK 1 and CFTR gene mutations were found in 13 and 68% (R122 H: 78%, N29I: 12%, others: 10%). SPINK 1 and CFTR gene mutations were found in 13 and 2%, respectively. Maternal inheritance was observed in 65%. Median age at first symptom and at inclusion in the study, was 10 (1–73) and 30 (1–84) years, respectively. 17% of the patients were symptom-free (penetrance: 83%). CHP manifestations were pancreatic pain (83%), acute pancreatitis (69%), pseudocysts (23%), cholelithiasis (3%), pancreatic calcifications (61%, median age of onset 23 years), steatorrhea (34%), diabetes mellitus (26%) and pancreatic adenocarcinoma (5%, median age 55 years). Exocrine and endocrine insufficiency occurred at 29 and 38 years (median). No differences in clinical and morphological data according to genetic status were observed. Endoscopic treatment and surgery were necessary in 16 and 23% of the patients, respectively. 19 patients died, including 10 from CHP, at a median age of 60 years.

Conclusion: The numbers of French families and patients with CHP include at least 78 and 200 respectively. PRSS1 gene mutations are found in 2/3. Mutation type is not correlated to clinical/morphological expression.

Exocrine Pancreatic Function Test – 13C-Mixed Triglyceride Breath Test

P. Kocna1, Z. Vanickova1, T. Krechler2, M. Lukas2, J. Kocni1, P. Kohout1

1Inst. Clin. Biochem. Lab. Diagnost., 2Fourth Med. Department, First Med. Faculty and Gen. Fac. Hospital, Charles University, 4Thomayer’s Faculty Hospital, Prague, Czech Republic

Introduction: Chronic pancreatitis could be well diagnosed by histopathology, but for clinical purposes, differential diagnostics and patient follow-up we use mainly imaging procedures and non-invasive pancreatic function tests, if available. In this study we report the 4 years experiences with noninvasive test of exocrine pancreatic function – breath test with 13C-mixed triglycerides (MTG-BT). The diagnostic significance has been compared with determination of fecal elastase-1 in relation to new clinically oriented classification of chronic pancreatitis (Buchler and Malfertheiner, Bern 2000).

Aims and Methods: We studied 180 subjects with suspected chronic pancreatitis (CHP). The grading of CHP was evaluated in 169 patients. MTG-BT test was performed with 250mg of Glyceryl-1,3-dioctadecanoate-2-octanoate-1-13C, 13C:12C ratio was analysed by infrared Isomax 4,000 analyser. Cumulative recovery of 13C was calculated by two methods, Body Surface Area and Basal Metabolic Rate. Fecal elastase 1 (FELA) was determined using monoclonal antibody (ScheboTech, Germany).

Results: Cumulative recovery (cPDR) significantly distinguishes severe CHP (grade C3) from all other groups, mild CHP (grade A) is significantly higher compared to other groups of CHP. Precision NDIRS × IRMS was checked 15 times using calibration IRMS reference calibrators the mean difference was 4.9% (Pearson’s coeff. = 0.990). Concordant results of MTG and FELA were found in 79.6%. The highest percentage (70.0%) of disconcordant results (low FELA, normal MTG) was in group CH-B and CH-C3.

Conclusions: Measurement of fecal elastase 1 is simple, non-invasive, robust test which well correlates with extent of tissue damage. MTG-BT is better in evaluation of dynamic and kinetic aspects, real digestive ability and response to stimulation. MTG-BT is, contrary to FELA, suitable to evaluate pancreatic supplementation therapy.

Optimizing Oral Pancreatic Enzyme Substitution Therapy in Patients With Chronic Pancreatitis to Assure a Normal Nutritional Status


Department of Gastroenterology, University Hospital of Santiago de Compostela, Spain

Background: We have previously shown that malnutrition persists in most patients with maldigestion secondary to chronic pancreatitis (CP) despite an adequate clinical response to oral enzyme...
substitution therapy. As hypothesis, objective evaluation of digestion under therapy may allow optimizing the treatment and assuring a normal nutritional status in patients with CP.

**Aim:** To evaluate the usefulness of the $^{13}$C-mixed triglyceride breath test ($^{13}$C-MTG-BT) as a tool for optimizing the therapy of malabsorption related to CP, and to analyze the impact of this therapeutic optimization on the patients’ nutritional status.

**Methods:** Prospective, interventionist, comparative and paired study. A total of 20 consecutive patients (17 male, mean age 55 years, range 43–77 years) with fat malabsorption secondary to alcoholic CP, who had abnormally low circulating levels of retinol-binding protein (RBP) despite at least one year of adequate clinical control of malabsorption (absence of weight loss and diarrhea) by enzyme substitution therapy were included. In all of them, treatment of malabsorption was optimized by increasing the daily dose of oral pancreatic enzymes (enteric-coated mini-microspheres) as much as needed to obtain a normal $^{13}$C-MTG-BT result ($^{13}$CO$_2$ recovery rate >57%). A proton pump inhibitor was associated in case of insufficient response to oral enzymes. Serum levels of RBP (normal 3–6 mg/dl) and prealbumin (normal 21–41 mg/dl) were measured before and one year after optimization of the therapy. Results are shown as mean and 95% confidence interval, and compared by the Student-t test for paired samples.

**Results:** Before optimization, patients were under treatment with oral pancreatic enzymes at a median dose of 20,000 U lipase/meal. A normal $^{13}$C-MTG-BT result was obtained in all patients by increasing the enzyme dose up to 40,000 U (n = 18) and 60,000 U (n = 2) lipase/meal. In addition, three patients (15%) required the association of esomeprazole 40 mg od to normalize the breath test result. By optimizing the therapy, serum levels of RBP increased from 2.4 mg/dl (2.1–2.7 mg/dl) to 3.1 mg/dl (2.8–3.4 mg/dl) (p < 0.001). Similarly, serum prealbumin levels increased from 20.7 mg/dl (18.8–22.6 mg/dl) to 25.2 mg/dl (23.8–26.6 mg/dl) (p < 0.001). Serum RBP and prealbumin levels reached normal values in 14 (70%) and 20 (100%) patients respectively.

**Conclusion:** Therapy of CP-related maldigestion may be optimized by the $^{13}$C-MTG-BT This therapeutic optimization allows assuring a normal nutritional status in patients with CP.

---

**T20**

**Haplotype Analysis of Common PRSS1 R122H Mutations in Northern Germany: No Indication of a Founder Effect**

F.U. Weiss¹, M. Zenker², P. Simon¹, M.M. Lerch¹

¹Department of Gastroenterology, Endocrinology and Nutrition, Ernst-Moritz-Arndt-University Greifswald,²Institute of Human Genetics, University of Erlangen, Germany

**Background:** To date at least 23 point mutations in the cationic trypsinogen gene have been found in association with pancreatitis. The most common point mutation (R122H) in exon 3 of the trypsinogen gene (PRSS1) is detected in approximately 75% of patients with hereditary pancreatitis. Patients and families with this particular mutation have been found world-wide but a large number of affected families originate from Northern Germany. We investigated whether a potential relatedness between these kindreds (‘founder effect’) exists which explains the geographical clustering.

**Patients and Methods:** From 10 HP-families (all R122H), for which patients and non-affected members were available over 2 or more generations, genomic leukocyte DNA was isolated. Using PCR and genomic sequencing we determined R122H associated haplotype blocks by analysis of 8 SNPs (including 4 hSNPs) located in close vicinity of the PRSS1 locus.

**Results:** R122H mutations segregated with at least 7 different haplotypes indicating this specific mutation as a frequent and unrelated event in most of these families. Comparison with data from the HapMap project indicated that the identified R122H-associated haplotypes correspond to the most frequently found haplotypes in the central European population.

**Conclusions:** The high prevalence of HP kindreds in Northern Germany could be accounted for by ascertainment bias in a tertiary referral center and by multiple unrelated mutations in a ‘hot spot’ of the PRSS1 gene. Our results do not support the notion of a founder effect as the cause of HP clustering in Northern Germany.

---

**T21**

**Anti-Oxidant Therapy and Disease Progression in Painful Chronic Pancreatitis**

A. Bagul, A.K. Siriwardena

HPB Unit, Department of Surgery, Manchester Royal Infirmary, Manchester, UK

**Introduction:** The generation of short-lived oxygen free radicals (oxidative stress) has been implicated in the pathogenesis of chronic pancreatitis (CP). Anti-oxidants are endogenous inhibitors scavengers of oxygen free radicals and their levels are depleted in the disease process. There is some evidence that supplementation with exogenous anti-oxidants cause relief of symptoms in CP. This study reports long-term outcome in a cohort of CP patients on oral anti-oxidant therapy.

**Methods:** Patients diagnosed as CP (n = 39) and having their index presentation to a regional hepatobiliary centre in the calendar year 1993 were identified by using ICD (version 9:577.1). These patients had undergone assays for anti-oxidant levels prior to commencing on antox (Pharmanord, UK) preparation comprising of selenium, methionine and ascorbic acid. The patient records were reviewed to confirm that they had either CT/ERP confirming a diagnosis of CP. Data for annual levels of anti-oxidants, opiate use (documented weekly prescriptions to signify ‘regular’ requirement), pain, endocrine/exocrine insufficiency, surgical intervention and mortality over the 10 year study period were recorded. Data are presented as median (range). Non-parametric statistical comparisons of data were made with significance accepted at p < 0.05 level.

**Results:** Serum ascorbic acid levels at recruitment were 14.7 (1.9–28.3) mg/l compared to 15.05 (1.9–27.1) mg/l at 10 year interval. [p = 0.52; Mann-Whitney U test]. Selenium at recruitment were 87 (28–214) gm/l compared to 143 (86–245) gm/l at 10 years [p ≤ 0.0001 Mann-Whitney U test]. The laboratory reference range for ascorbic acid was 4–20 mg/l and for selenium was 83–152 gm/l. At recruitment, 2 patients (5%) were diabetic and by 10 years this rose to 20 (51%) while 26 (78%) presented with pain compared to 25 (75%) at end period (2003), 8 (27%) were on regular opiates at baseline compared to 21 (73%) at end point [p = 0.01; Fisher’s 2-sided].

**Conclusion:** Although long-term anti-oxidant therapy appears to sustain selenium levels the supplementation with anti-oxidants...
T22

Multiple Stents for Calibration of Pancreatic Strictures in Chronic Pancreatitis

Á. Pap, M. Burai, T. Gyökeres
Department of Gastroenterology, MAV Hospital, Budapest, Hungary

Introduction: Pancreatic duct stenting is an established procedure for chronic pancreatitis with dominant stricture at the head region. However, stent-induced lesions, dislocation, occlusion, even sepsis can occur.

Method: During a 6 years period 56 pancreatic stentings in 25 patients were performed in our institution. In 17 cases when multiple stent placement seemed feasible in one setting or after some days of nasopancreatic lavage we used 2–3 10 French tellon plastic stents (MTW) to calibrate the pancreatic stenosis for a long term. Mean age of the 3 females and 22 males was 56.4 (ranges 42–78) years. In 16 cases 1 stent (Group 1), in 11 cases 2 stents, in 6 cases 3 stents (Group 2) were placed over 2 guide wires after pancreatic papillotomy and progressive dilatation of the dominant stricture with 6–10 French dilator. In 8 patients ESWL and/or citrate lavage (18 cases) was performed meanwhile. We regularly followed-up the patients 2 monthly or when the pain relapsed with ultrasonography and amylase measurement.

Results: No complication occurred at stenting. Stents remained in place for 3.3 months and 5.3 months (range 1–18) in Group 1 and Group 2, respectively and removed as indicated by US and the typical postprandial pain with/without amylase elevation. Relapse-free follow-up occurred for 31.6 and 41.6 months (range 8–80) in Group 1 and Group 2, respectively until now. Increasing the stent number prolonged the relapse free period in 2 patients. Relapses (7–7 cases) were provoked by alcohol, smoking and heavy meals and treated mainly by multiple stenting (6 cases) or operation (2 patients).

Conclusion: Multiple pancreatic stents increase the relapse-free follow-up period after endotherapy, however, alcohol intake and smoking have to be prevented. A randomized, well-controlled study is indicated to definitely prove the advantage of multiple pancreatic stents for calibrating the pancreatic strictures.

T23

Experimental Pancreatitis in Cathepsin B Overexpressing Mice

M. Beier1, M. Ruthenbürger1, B. Brandt-Nedelev2, I. Jarosakova, T. Reinheckel2, W. Halangk2, M.M. Lerch1
1Department of Gastroenterology, Endocrinology and Nutrition, Ernst-Moritz-Arndt University, Greifswald, 2Division of Experimental Surgery, Otto-von-Guericke-University Magdeburg, Germany

Background: Premature activation of pancreatic zymogens plays a critical role in the initiation of acute pancreatitis. The lysosomal proteinase cathepsin B (CTSB) has been shown to activate directly trypsinogen in the pancreatic acinar cell. Using CTSB-deficient mice, rather than CTSB expressing wild type mice, nearly abolished the premature intrapancreatic trypsinogen activation characteristic of experimental pancreatitis.

Aim: To investigate whether overexpression of human CTSB in mice accordingly causes increased trypsinogen activation and a more severe course of acute pancreatitis?

Materials and Methods: Pancreatitis was induced in FVB wild type (WT) and FVB CTSB overexpressing mice (CTSB + + +) by seven intraperitoneal injections of caerulein (50 μg/kg body weight). We determined the intraacinar localization of Trypsin (polyclonal rabbit anti Trypsin) and CTSB (monoclonal mouse anti human cathepsin B) in pancreatic tissue sections. Acini were incubated with 100 nM cholecystokinin (CCK) to measure intraacinar trypsin activity and necrosis. Trypsin and trypsinogen activities were measured fluorometrically and in subcellular fractionations. To distinguish total and active CTSB we used the subcellular fractions for western blot analysis and NS-196, a high specific biotinylated CTSB inhibitor.

Results: In the transgenic animals we found a 4 fold increase in CTSB expression, but CTSB + + + animals showed no obvious difference in pancreatic trypsin activity or severity of caerulein induced pancreatitis in comparison to WT animals. Immunofluorescent staining of pancreatic acini revealed a correct intracellular sorting of the transgenic human CTSB which was confirmed by subcellular fractionation. Trypsin and human CTSB were consistently colocalized. Despite the 4-fold increase in total CTSB in CTSB + + + animals with NS-196 assays identified only 25% of total CTSB content to be active.

Conclusion: The transgenic overexpression of hCTSB does not lead to an increase in trypsinogen activation nor to an increase in the severity of pancreatitis. This suggests that only a limited portion of CTSB can participate in trypsinogen activation and this portion is not readily enlarged by simply increasing the content of cellular CTSB.
protein (MAP) kinases ERK, p38 MAPK and JNK was analysed by Western blot with phospho-specific antibodies.

Results: Both nicotine and ethanol treatment of AR4-2J cells caused a rapid activation of ERK1/2 with a maximum at 3 and 5 min after stimulation, respectively. The response to nicotine treatment was maximal at the concentration of 1 mM whereas the maximal effect of ethanol was obtained at a concentration of 100 mM. The combination of both agents showed an additive effect on the phosphorylation of ERK1/2. In contrast, neither nicotine nor ethanol or the combination of both had an effect on p38 MAPK and JNK activation.

Conclusions: This study showed that nicotine and ethanol act additively on the activation of ERK signalling in pancreatic AR4-2J cells. Our results indicate that nicotine may aggravate the effects that are commonly attributed to ethanol during the early stage of acute pancreatitis.

T25
The Recurrence of Alcohol Induced Pancreatitis may be Prevented – An Interim Analysis of an On-Going Prospective Randomised Trial
H. Pelli, H. Lappalainen-Lehto, J. Sand, I. Nordback
Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, Finland

Introduction: Previously we have shown that continuing alcohol abuse is the most important risk factor for recurrent acute pancreatitis (RP) after the first acute alcoholic pancreatitis (Pancreatology, 2005). This random study was aimed to evaluate whether a repeated intervention against alcohol consumption could reduce RP compared to single intervention.

Patients and Methods: Power calculation showed 120 patients (60 per group) to be needed if the expected recurrence rate in the control group was 40% (a = 0.05). One hundred and twenty patients entered the study: 60 in the study group and 60 in the control group. Three patients were withdrawn (1 each because of insufficient data, 1 because of death). The study was carried out in 2 phases: the first lasting 6 months and the second lasting 1 year. During the first phase, patients were randomised to study group (repeated outpatient intervention every 6 months) or to control group. All had a control visit scheduled in 2 years. By December 2005 inclusion had stopped and 86 patients had finished at the time of admission.

Results: The highest levels of IL-10 were noted in patients with mild pancreatitis and uncomplicated course of SAP. During all period of observation the insignificant elevation of IL-10 levels were noted in patients with mild pancreatitis and uncomplicated course of SAP. Starting from the seventh day the obvious rise of IL-10 concentration was noted in patients who subsequently developed septic complications (r = 0.622132; p = 0.006692).

Conclusions: This study showed that nicotine and ethanol act additively on the activation of ERK signalling in pancreatic AR4-2J cells. Our results indicate that nicotine may aggravate the effects that are commonly attributed to ethanol during the early stage of acute pancreatitis.
PFOA (150 mg/kg) or the PPAR-α agonist clofibrate (10–100 mg/kg) were injected s.c. 30 min before the start of the caerulein infusion. Rats were sacrificed 2 after the end of the infusion and tissue samples were excised for the determination of pancreatic oedema, leukocyte activation and release of prostaglandin (PG) E2, 6-keto-PGF1α and thromboxane (TX) B2.

**Results:** Caerulein induced acute oedematous inflammation of the pancreas, associated with a significant accumulation of activated leukocytes in the tissue (160,310 ± 47,910/mg protein) and release of PGE2 (13.4 ± 4.1 pg/mg dry weight), 6-keto-PGF1α (113.9 ± 16.9 pg/mg d.w.) and TXB2 (38.5 ± 9.0 pg/mg d.w.). PFOA had no effect on the inflammatory oedema but significantly (p < 0.05) reduced activated leukocytes (41,330 ± 9,970/mg protein), PGE2 (3.9 ± 4.5 pg/mg d.w.), 6-keto-PGF1α (46.6 ± 13.7 pg/mg d.w.) and TXB2 (12.9 ± 1.7 pg/mg d.w.). Conversely, clofibrate had no significant effect on any of the investigated parameters.

**Summary:** PFOA attenuates the accumulation of activated leukocytes in the pancreatic tissue and reduces the synthesis of prostanoids in caerulein-induced acute pancreatitis. However, unlike its action in cutaneous inflammation, PFOA does not affect oedema formation. Activation of PPAR-α does not appear to be the mechanism of action of PFOA in the pancreas.

---

**T29**

**Markers of Autoimmunity in Children with Chronic Pancreatitis**

G. Oracz1, B. Cukrowska2, B. Oralewska3, J. Ryzko3, J. Socha1

1Department of Gastroenterology, Hepatology and Immunology, 2Department of Pathology, The Children’s Memorial Health Institute, Warsaw, Poland

**Introduction:** The pathogenesis of autoimmune chronic pancreatitis is poorly understood and the frequency of autoimmune stigmata in children with chronic pancreatitis (CP) is unknown. Clinical and epidemiological data were recorded and analyzed. Gamma globulin, immunoglobulin, antinuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), antimitochondrial antibodies (AMA), anti-gastric parietal cell antibodies (APCA), liver-kidney microsomal antibodies (LKM) and antithyroid antibodies (AHA) were measured in all children.

**Results:** Hyperimmunoglobulinemia (>16 g/l) was present in 7 cases. An increase of IgG level was present in 5 children. Autoantibodies were present in 17 children (41.5%). ANAs (>1/80) were present in 11 patients in 8 patients (>1/320), in 6 patients >1/640); ASMA (>1/80) in 12 children (4 patients >1/320, in 1 patient >1/640). APCAs, AMAs, AHAs and LKM were absent in all patients. Combining clinical and biochemical autoimmune parameters, 17 patients had at least 1 autoimmune marker of the disease. In 10 patients with CP and autoimmune stigmata other known causative factors of CP were present. In 7 patients we found gene mutations; in 3 patients anatomic anomalies of pancreatic duct; in 3 children hypertiglycerideremia. Autoimmune disease was present in 4 patients (5%); ulcerative colitis, PSC, dermatomiositis and panniculitis in 1 patient each. There was no difference in age of the disease onset (9.0 years vs. 9.3 years, NS); in the severity of the disease and in the clinical course between children with autoimmune stigmata and patients without autoimmune markers.

**Conclusions:** In children with CP, similarly to adults, there is a high frequency of clinical and biochemical markers of autoimmunity. The results may suggest that the number of CP with autoimmune origin in children is greatly underestimated.
**T30**

The Japanese Diagnostic Criteria for Autoimmune Chronic Pancreatitis; is it Completely Satisfactory?


Department of Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea

**Introduction:** Autoimmune chronic pancreatitis (AIP) is a very attractive disease to clinicians in terms of its dramatic response to the oral steroid therapy in contrast to ordinary chronic pancreatitis. In the year 2002, the Japan Pancreas Society published the diagnostic criteria of AIP and many clinicians around the world use this criteria for its diagnosis. The purpose of this study was to evaluate whether Japanese criteria for the diagnosis of AIP is adequate or not.

**Methods:** We retrospectively analyzed the clinical, radiologic, laboratory and histologic features of 31 patients with AIP who have been successfully treated with oral corticosteroid. All the enrolled patients showed normalization or marked improvement in symptoms, laboratory and imaging findings (CT and ERCP) after steroid treatment.

**Results:** The mean patients’ age was 56 years (range, 32–78 years) and were comprised of 25 males and 6 females. Seven patients who responded to the steroid did not satisfy the Japanese imaging criterion because the extent of irregular narrowing was less than one third of the entire length of main pancreatic duct. Among these seven patients, one patient did not meet the laboratory and histopathologic criteria as well. Another two patients fulfilled Japanese imaging criterion only and showed normal IgG level, negative results of autoantibody measurements and nondiagnostic pancreatic histopathology. Taken together, nine (29%) of 31 patients did not meet the Japanese diagnostic criteria for AIP yet responded to the steroid.

**Conclusions:** Clinicians may miss a substantial portion of AIP patients who may benefit from steroid therapy when the diagnosis is confined to those who satisfy the criteria proposed by the Japan Pancreas Society. It is necessary to convene a worldwide consensus to develop an improved diagnostic criteria for AIP.

**T31**

Corticosteroids are Efficacious in Patients with Lymphoplasmocytic Chronic Pancreatitis (LCP), However Relapses are Frequent

P. Hammel¹, V. Rebours¹, D. O’Toole¹, M.P. Vullierme², A. Couvelard³, O. Corcos¹, F. Maire¹, K. Nahon-Uzan¹, O. Hentic¹, A. Aubert¹, P. Ponsot¹, P. Lévy¹, P. Ruszniewski¹

¹Service de gastroentérologie, hôpital Beaujon, Clichy, France
²Service de radiologie, hôpital Beaujon, France
³Service d’anatomopathologie, hôpital Beaujon, Clichy, France

**Introduction:** LCP (either ‘autoimmune’: AIP or eosinophilic [EP]) are currently well characterized but effects of treatment have been poorly evaluated.

**Aim:** Assess therapeutic management of patients with LCP.

**Patients and Methods:** All patients with LCP were prospectively included between 01–2002 and 08–2005. The diagnosis of LCP was made according to Yoshida’s criteria. Symptomatic patients without untreated biliary stenosis or sepsis received oral corticosteroids.

**Results:** 29 patients (21 M, median age 39 (17–73) years, 25 with AIP, 4 with EP) were included with a median follow-up of 14 (1–55) months. First symptom was abdominal pain, acute pancreatitis, weight loss, and jaundice in 35, 24, 21 and 17% of patients, respectively (1 symptom-free patient). Associated auto-immune or inflammatory bowel diseases was present in 6 (21%) and 4 (14%) patients, respectively. Pathological data obtained from EUS-guided biopsy were available in 12 (41%) patients, showing typical features of AIP in 11 and fibrosis in 1. Prednisolone (1 mg/kg/day for 1 month with progressive tapering over 3 months) was given to the 13 (45%) patients with pain or jaundice. Biliary drainage was necessary in 3 patients. Symptoms and radiological improvement were observed in 12 of the 13 patients (92%) and 58%, respectively. Pancreatic (n = 3) or biliary (n = 5) symptoms relapsed in 8 patients (67%) at time of treatment discontinuation. Second course of prednisolone was given in 7 patients. (2 of whom also required biliary stenting), and biliary stenting alone was performed in 1 patient. Azathioprine or imatinib were prescribed in 2 and 1 patient experiencing at least 2 relapses, respectively, with no further recurrence after 7–10 months.

**Conclusion:** Corticosteroid treatment of LCP was necessary in 45% of patients, occasionally associated with biliary drainage. Treatment efficacy concerning symptoms is high (90%) but relapses are frequent. Immunosuppressive therapy is useful for steroid-dependent patients.

**T32**

Co-Expression Of PDX1 and PTF1A Initiates Full-Scale Pancreatogenesis in Undifferentiated Endoderm

K. Furuyama¹, Y. Kawaguchi¹, A. Fukuda¹, S. Kodama¹, T. Kuhara¹, M. Horiguchi¹, M. Kawaguchi¹, C.V.E. Wright², R. Doo³

¹Department of Surgery and Surgical Basic Science, Kyoto University, Japan; ²Department of Cell and Developmental Biology, Vanderbilt University, USA

**Introduction:** Pancreas organogenesis begins in formation of dorsal and ventral pancreatic buds at the specific sites in the primitive gut endoderm at Embryonic day 9.5 when Pdx1 and Ptf1a, the ‘master genes’ in pancreatogenesis, are co-expressed. However, possible mutual regulation between these two important transcriptional factors is unknown. We have previously shown that transgene-based misexpression of Pdx1 from Ptf1a cis-regulatory sequence (Ptf1a-Pdx1 Tg) restores pancreas tissue to pdx1-null mice that otherwise lack mature exocrine and endocrine cells because of an early arrest in organogenesis. Focusing on these genes, we aimed to reveal the requirement of Pdx1 for the expression of Ptf1a and to investigate the precise mechanism underlying this transgene-based pancreas restoration.

**Methods:** Using Ptf1a cre-mediated cell tracking we analyzed the lineage of Ptf1a-expressing cells in the Pdx1-null endoderm and Ptf1a-Pdx1 Tg rescue mice.
**Conclusion:** Pdx1 is required for the Ptf1a expression at the early dorsal pancreatogenesis but Ptf1a expression is independent of Pdx1 function at later stages. In ventral endoderm, Ptf1a is a down-stream gene of Pdx1. The epithelium of Pdx1-null dorsal ductule can respond the delayed expression of Ptf1a and Pdx1 (Ptf1a-Pdx1 Tg product) and differentiate into full-scale pancreas tissue, suggesting a surprising level of plasticity for pancreatic re-commitment. This finding supports the notion that co-expression of Ptf1a and Pdx1 is essential for pancreatic precursors to initiate pancreatic differentiation program.

---

**T33**

**ADAM17/TACE Influences MMP Expression and Invasion Behavior of Pancreatic Ductal Adenocarcinoma**

J. Ringe1,2, R. Jesenowski2, W. Fleig1, M. Löh2

1Department of Medicine I, University of Halle, 2Department of Medicine II, Mannheim Medical Faculty, University of Heidelberg and German Cancer Center, Heidelberg, Germany

**Introduction:** ADAM molecules are known for their unique potential to combine adhesion, proteolysis, and signaling. Based on our data about the aberrant ‘de novo’ expression of ADAM17/TACE in late PanIN stages and in pancreatic cancer cells and tissues we investigated its functional relevance. Thus, we examined the influence of ADAM17/TACE on invasion behavior and associated molecules including matrix metalloproteases (MMPs) and their inhibitors (TIMPs).

**Methods:** The influence on invasion was analysed by siRNA silencing assays. These were performed by introduction of siRNA duplexes in different pancreatic cancer cell lines. Specific silencing of the ADAM17/TACE gene was confirmed by RT-PCR and by Western blot. Expression of MMP1, MMP2, MMP14 and TIMP1 mRNA was investigates by using RT-PCR. Invasion behavior was investigated using matrigel-coated transwell systems.

**Results:** The highest efficiency of gene silencing was revealed in CAPAN-1 cells as seen on RNA and protein level. ADAM17/TACE gene silencing reduced invasion behavior dramatically compared with CAPAN-1 cells transfected with the control siRNA. MMP14 mRNA was not detectable in ADAM17 silenced cells. Furthermore, MMP1 and MMP2 mRNA were significantly downregulated. In contrast, the inhibitor TIMP1 was downregulated in ADAM17/TACE expressing cells.

**Conclusion:** Our findings provide evidence of aberrant expression of the proteolytically active ADAM17/TACE in PDAC and in its advanced precursor lesions and its critical involvement in invasion process. Introduction of siRNA against ADAM17/TACE caused a complete reduction of MMP whereas the inhibitor TIMP1 was downregulated in ADAM17/TACE expressing cells. Thus, ADAM17/TACE increases the malignant potential of PDACs. Our first data suggest new regulation mechanism and functional pathways of ADAM17/TACE. Further experiments are on the way.

---

**T34**

**Adrenomedullin (ADM) is Induced by Hypoxia and Enhances Pancreatic Cancer Cell Invasion**

S. Keleg1, H. Kayed1, T. Giese2, M.W. Buchler1, H. Friess1, J. Kleege1

1Department of General Surgery, University of Heidelberg, 2Institute of Immunology, University of Heidelberg, Heidelberg, Germany

**Introduction:** Adrenomedullin (ADM) is a peptide synthesized by different cells that act by binding calcitonin receptor-like receptor (CRLR) and members of the receptor activity-modifying protein (RAMP) family. It plays an important role in various functions, such as blood pressure regulation and angiogenesis. In this study, the expression and functional role of ADM and its signalling components were investigated in pancreatic adenocarcinoma.

**Methods:** QRT-PCR, immunohistochemistry, ELISA, ADM silencing, cell growth and invasion assays, induction of and hypoxia were utilized.

**Results:** Median ADM and CRLR mRNA levels were 1.5-fold and 2.5-fold higher, respectively, but a decrease in RAMP-1, 2 and 3 mRNA expression levels was observed in PDAC tissues compared to normal pancreatic tissues. ADM, CRLR, RAMP1 and RAMP2, but not RAMP3, were localized at moderate to high levels in pancreatic cancer cells. ADM serum levels were significantly increased in pancreatic cancer patients compared to controls, with a sensitivity of 80% and specificity of 85% at a cut-off ADM level of 30.6 ng/ml. 8 pancreatic cancer cell lines expressed ADM and CRLR, whereas RAMP1, 2 and 3 were present only in some of the cell lines. ADM was strongly induced by hypoxia and significantly increased invasiveness of 4/5 human pancreatic cancer cells. Blocking of CRLR decreased invasiveness in all tested cell lines, but ADM silencing by siRNA oligonucleotides decreased invasiveness of 1/3 human pancreatic cancer cell line tested. In addition, recombinant ADM up-regulated VEGF in 3/5 cultured pancreatic cell lines, with no significant VEGF changes in response to CRLR antagonist or ADM silencing.

**Conclusion:** ADM is induced by hypoxia, over-expressed in pancreatic cancer, and released into the blood, suggesting that it might serve as a potential tumour marker. ADM increases invasiveness of some pancreatic cancer cells and might influence angiogenesis via VEGF induction, suggesting that blocking this pathway might have therapeutic potential.
**T35**  
**Increased Dietary Fat is Associated With Accelerated Growth of MIA PACA2 Human Pancreatic Cancer Cells in the Pancreas of Nude Mice**  
F. Wang, M.K. Herrington, J. Permert  
Department of Surgery, Karolinska University Hospital at Huddinge, Stockholm, Sweden

**Introduction:** High fat diet (HFD) and obesity have been proposed as risk factors for pancreatic cancer. We hypothesized that the nutrient status associated with HFD is favorable for the growth of pancreatic cancer cells.

**Methods:** MiaPaCa2 human pancreatic cancer cells were transplanted into mice fed regular mouse diet (RD) or HFD (21% fat) (n = 338 Pancreatology 2006;6:323–405  
and HFD-T mice (169 mZq/l) were similar to those seen in RD-C mice (166 ± 23 mZq/l, P < 0.01), suggesting that disposal of circulating FFAs in HFD-fed mice was faster in the presence of the tumor xenograft. The expression of medium-chain acyl-CoA dehydrogenase (MCAD or ACADM) in tumors from HFD-T mice was increased compared to that seen in RD-C mice. In contrast, the FFA levels in HFD-T mice were significantly lower than those in HFD-C mice (P < 0.01), suggesting that disposal of circulating FFAs in HFD-fed mice was faster in the presence of the tumor xenograft. The expression of medium-chain acyl-CoA dehydrogenase (MCAD or ACADM) in tumors from HFD-T mice was increased compared to that seen in RD-C mice. Thus, when the hosts were fed HFD, fatty acid oxidation in MiaPaCa2 cells appeared to be increased.

**Conclusion:** The nutrient status associated with HFD promotes the growth of pancreatic cancer cells.

**T36**  
**Fanconi Anaemia Pathway Mediated DNA Repair in Familial Pancreatic Cancer**  
J. Earl1, L. Vitone1, J. Wilson2, N.J. Jones2, J.P. Neoptolemos3, W.G. Greenhall1

1The Division of Surgery and Oncology, 5th Floor UCD Block, RLUH, 2Cell Regulation and Signalling Research Division, School of Biological Sciences, University of Liverpool, Liverpool, UK

**Introduction:** Familial Pancreatic Cancer (FPC) is a rare autosomal dominant disease, the causative gene(s) of which is unknown. The only FPC gene identified to date is the DNA repair and recombination gene BRCA2, which we have shown to be involved in up to 20% of FPC. BRCA2 is one of 12 genes responsible for the cancer-prone syndrome Fanconi anaemia (FA). Functional defects of the FA pathway have been identified in pancreatic cancer cell lines and germline FANCC and FANC G mutations have been found in patients with pancreatic cancer. Cells mutant for BRCA2, and FA genes show extreme sensitivity to DNA cross-linking agents such as mitomycin C. DNA repair can be assayed, indirectly or directly, in lymphocytes from pancreatic cancer patients and their unaffected relatives.

**Methods and Patients:** The integrity of the FA pathway was determined by FANCD2 mono-ubiquitination assay in pancreatic cancer cell lines. A novel technique was devised that involved the quantification of stalled FANCD2 replication forks after exposure mitomycin C by QPCR using random primers. In addition, the mono-ubiquitination and phosphorylation site of FANCD2 were analysed by DNA sequencing.

**Results:** The familial pancreatic cancer cell line FAMPAC appeared to show reduced levels of the mono-ubiquitinated form of FANCD2 after exposure to mitomycin C. DNA sequence analysis determined that this was not due to mutation in the mono-ubiquitination site of FANCD2.

**Conclusion:** A novel technique was devised to assess the integrity of the FA pathway in cell lines and circulating lymphocytes from affected individuals and possible carriers.
Results: We identified 146 differentially expressed genes from which 78 were over- and 68 genes were under-expressed in pancreatic cancer stroma. Hierarchical clustering using the 146 genes and the gene expression profiles of microdissected pancreatic tumor epithelia identified a subset of pancreatic cancers epithelia displaying gene expression similar to the cancer stroma. Annotation of the 146 genes resulted in the identification of soluble factors from the Wnt and the Notch signaling pathways overexpressed in the stroma from cancer tissue.

Conclusion: In conclusion, gene expression analysis of the stromal compartment could identify new markers and therapeutic targets for the tumor-associated stroma of pancreatic cancer.

T38
Depletion of Heat Shock Protein-70 (HSP70) Initiates Apoptosis in Pancreatic Cancer Cells
A. Aghdassi1, P.A. Phillips2, R. Sharif2, S. Ghafoor2, R. Dawra1, M.M. Lerch1, A. Saluja2
1Department of Gastroenterology, Endocrinology and Nutrition, Ernst-Moritz-Arndt-University Greifswald, Germany; 2Department of Surgery, University of Massachusetts Medical School, Worcester, USA

Introduction: Pancreatic cancer is one of the leading causes of cancer death in Europe and it is characterized by poor response to radiation and chemotherapy. One of the underlying mechanisms is their resistance to apoptosis. It has already been demonstrated that heat shock proteins are capable of interfering in the apoptotic pathway and thus prevent cancer cells from programmed cell death. One of the major heat shock proteins is Hsp70 whose expression can be inhibited by bioflavonoids. In this study expression of Hsp70 in pancreatic tumor cells was assessed and compared to non-transformed pancreatic ductal cells. Secondly apoptosis was examined after inhibition of Hsp70 by the bioflavonoid quercetin or by RNAi.

Methods: Pancreatic cancer cell lines and normal pancreatic ductal cells were incubated with varying concentrations of quercetin or dihydroquercetin (an analogue which does not suppress Hsp70). Cell viability was assessed using the tetrazolium salt WST-8 and Hsp70 expression was shown by western blotting of cell lysates. Apoptosis was determined by analysis of annexin V labelling, caspase activity. This was confirmed by TUNEL staining. Similar results were obtained by using siRNA.

Results: Pancreatic cancer cells showed increased expression of Hsp70 compared to non-transformed ductal cells. Depletion of Hsp70 by quercetin decreased cell viability in cancer cells but not in normal ductal cells. Treatment with quercetin also induced apoptosis in pancreatic cancer cells as shown by significant increase of annexin V positive cells and caspase activity. This was confirmed by TUNEL staining. Similar results were obtained by using siRNA.

Conclusions: Hsp70 plays in important role in apoptosis and selective down-regulation of Hsp70 can enhance apoptosis in pancreatic cancer cells. Targeting Hsp70 may therefore be a potential option in the treatment of pancreatic cancer.

T39
Expression of the Coxsackie and Adenovirus Receptor (CAR) in Pancreatic Cancer and Normal Pancreatic Tissue
C.S. Verbeke1, S. Hamdan2, J. Booth1, H.S. Pandha3, G.E. Blair2
1Department of Histopathology, St James’s University Hospital Leeds, 2School of Biochemistry and Molecular Biology, University of Leeds, 3Postgraduate Medical School, University of Surrey, UK

Background: Pancreatic cancer has a poor prognosis. Because the response to existing therapies is limited, gene therapy may offer a new approach to treatment. Efficient adenovirus infection of target cells depends upon the presence of the Coxsackie and adenovirus surface receptor CAR.

Aim: To evaluate the potential efficacy of adenoviral therapy in pancreatic cancer, we evaluated expression of CAR in human pancreatic cancer cell lines and archival tissues of pancreatic cancer and normal pancreas.

Method: Surface CAR expression in 10 human pancreatic cancer cell lines was analysed by flow cytometry following treatment with a polyclonal rabbit anti-CAR antibody. CAR expression was correlated with the efficiency of transduction of these cell lines with recombinant Ad5CMVEGFP virus. Using the same antibody, immunostaining was performed on tissue microarrays containing 188 pancreatic ductal adenocarcinomas and 68 matched controls.

Results: The level of surface CAR expression varied amongst the pancreatic cancer cell lines, and correlated with their susceptibility to adenoviral transduction. Immunostaining for CAR was absent in 103 (55%) of adenocarcinomas, while moderate and strong staining was observed in 59 (31%) and 26 (14%) cases, respectively. Absence of CAR immunolabelling correlated with poor histological differentiation and clear cell morphology of pancreatic cancer. In normal tissue, strong immunolabelling was seen in the majority of islet cells as well as in peripheral and interlobular pancreatic ducts.

Conclusion: Absence of CAR expression in a considerable proportion of pancreatic cancers and constitutive CAR expression in normal pancreatic tissue may reduce the suitability of adenoviral gene therapy in pancreatic cancer patients.

T40
Engineering Diphtheria Toxin in Pancreatic Cancer Cells: A Promising Tool for Therapy
P. Fogar1, F. Navaglia2, E. Greco2, A. Stranges2, C.-F. Zambon2, A. Falda1, S. Pedrazzoli2, M. Pleban2, D. Basso2
1Departments of 1Medical and Surgical Sciences, Laboratory Medicine, University of Padova, Italy
2Laboratory Medicine, University of Padova, Italy

Introduction: Diphtheria toxin is a promising tool for cancer cell killing. It causes cell death by an apoptosis-mediated pathway catalysing ADP-ribosylation of elongating factor 2. Our aim was to test DT catalytic subunit (fragment A 1–193 a residues) (DTA) efficacy in killing 5 different pancreatic cancer cell lines obtained from...
primary (BxPC3, PANC1, PSN1 and MIAPaCa2) or metastatic (CAPAN1) tumors.

**Methods:** DTA was subcloned in an eukariotic expression vector (pRc) under the control of a constitutive promoter (RSV). The efficiency of chemical transfection (Lipofectamine 2000) was evaluated by FACs analysis, using a FITC-oligonucleotide as tracer. Transcription efficiency was evaluated by FACs analysis and western blotting using a reporter gene (GFP) cloned in pRc. Study design was: (1) transfection of 250,000 cells for 6 h with 4 μg DNA; (2) after 24 h cells were seeded in quadruplicate in a 96-well plate; (3) cell growth was evaluated daily for 3 days by the cell viability XTT test. For each cell line two controls were run in parallel: lipofectamine only treated cells and cells transfected with the empty DNA vector (pRc).

**Results:** More than 50% of cells were efficiently transfected. In four cell lines GFP transcription was recorded: CAPAN-1 did not translate the inserted gene. In agreement this cell line was resistant to DTA gene transfer. A complete growth inhibition was achieved after translate the inserted gene. In agreement this cell line was resistant to DTA gene transfer.

**Conclusions:** DTA expression is lethal for pancreatic cancer cells and this supports the potential use of this toxin for pancreatic cancer gene therapy. The lack of gene translation found in CAPAN-1 cells, a metastatic cell line, might be consequent to the absence of specific transcription factors recognizing the RSV promoter.

---

**T41**

**The Influence of Resection Margins and Treatment on Survival for Patients with Pancreatic Cancer within a Meta-Analysis of Randomized Controlled Trials**


1University of Verona, Italy; 2Cancer Research UK Clinical Trials Unit, University of Birmingham, UK; 3University of Heidelberg, Germany; 4University Hospital Rotterdam, The Netherlands; 5University of Bergen, Norway; 6Teikyo University School of Medicine, Japan; 7Agia Olga Hospital, Athens, Greece; 8University of Liverpool, UK

**Introduction:** It is uncertain whether it is possible to influence the long term outcome of patients with pancreatic cancer and a positive resection margin. A meta-analysis of randomized controlled trials to assess the influence of resection margins and adjuvant chemoradiation or chemotherapy on survival for patients with pancreatic cancer.

**Methods:** Individual patient data were available on 875 patients from four randomised controlled trials. 278 (32%) patients had R1 and 591 (68%) had R0 resections. Kaplan-Meier estimates of survival were compared using log-rank analyses. Poled hazard ratios of the effects of chemoradiation and chemotherapy treatments on the risk of death were calculated separately and across groups according to resection margins status.

**Results:** 698 (80%) had died with median follow-up of 44 months for the alive patients. Resection margin involvement was not a significant factor for survival (hazard ratio = 1.10, 95% CI: 0.94, 1.29, χ² LR = 1.4, p = 0.24). The two and five year survival rates for R0 patients were 33% and 16% and 29% and 15% for R1. Chemoradiation in R1 patients resulted in a 28% reduction in the risk of death (hazard ratio = 0.72, 95% CI: 0.47, 1.10) compared with a 19% increased risk in R0 patients (hazard ratio = 1.19, 95% CI: 0.95, 1.49). Chemotherapy in R1 patients had a 4% increased risk of death (hazard ratio = 1.04, 95% CI: 0.78, 1.40) compared with a 35% reduction in risk in the R0 subgroup (hazard ratio = 0.65, 95% CI: 0.53, 0.80).

**Conclusions:** Adjuvant chemotherapy but not chemoradiation should be the standard of care for patients with either R0 or R1 resections for pancreatic cancer.

---

**T42**

**Phase III Randomised Comparison of Gemcitabine (GEM) with Gemcitabine Plus Capecitabine (GEM-CAP) in Patients With Advanced Pancreatic Cancer**


University of Liverpool, Liverpool, UK

**Introduction:** Both gemcitabine and fluoropyrimidines are valuable treatments for advanced pancreatic cancer. This study was designed to compare the survival of gemcitabine (GEM) with gemcitabine plus capecitabine (GEM-CAP).

**Methods:** Patients with previously untreated histological or cytological proven locally advanced/metastatic carcinoma of the pancreas, performance status ≤2 were recruited. Patients were randomised to GEM (1,000 mg/m2 weekly × 7 q8 weeks, 1 week rest) thereafter weekly × 3 q4 weeks) or to GEM-CAP (gemcitabine 1,000 mg/m2 weekly × 3 q4 weeks, capecitabine 1,660 mg/m2/day for 21 days, 7 days’ rest). Treatment continued until disease progression or intolerable toxicities. The primary outcome measure was survival.

**Results:** Between May 2002 and Jan 2005, 533 patients were randomised to GEM (n = 266) and GEM-CAP (n = 267) arms. Baseline characteristics were balanced (GEM/GEM-CAP) with regards to median age (62/62), stage IVB disease (71%/70%) and WHO performance status (PS) 0–1 (82%/81%). At interim analysis, May 2005, 373 (70%) deaths had occurred. Interim results showed GEM-CAP significantly improved overall survival over GEM alone (Hazard Ratio [HR]: 0.80; 95% CI: 0.65–0.98; p = 0.026). Median survival for GEM and GEM-CAP was 6 months and 7.4 months and 1-year survival rates were 19% and 26% respectively. After adjusting for baseline stratification factors (disease extent and PS), the survival advantage for GEM-CAP remains (HR: 0.77; 95% CI: 0.63–0.95; p = 0.014). Objective response rates were 7% (0CR, 19PRs) and 14% (3CRs, 35 PRs) in GEM and GEM-CAP respectively (p = 0.008).

Grade 3/4 toxicity episodes in GEM and GEM-CAP arms respectively were anaemia (2%/1%), neutropenia (11%/17%), thrombocytopenia (2%/3%), fever (1%/0%), diarrhoea (1%/1%), stomatitis (0%/0%), hand foot syndrome (0%/2%) and vomiting (2%/1%).

**Conclusions:** With 70% death rate, these interim data show a significant improvement in overall survival by the addition of...
T43

Randomised Phase I/II Trial Assessing the Safety and Efficacy of Radio Labelled Anti-Carcinoembryonic Antigen (CEA) Antibodies Given Intra-Arterially (I.A.) or Intravenously (I.V.) in Patients with Unresectable Pancreatic Adenocarcinoma

A. Sultana1, S. Shore1, S. Vinjamuri2, J.E. Evans3, S. Chauhan1, L. Bosonnet1, C. Garvey3, J.P. Neoptolemos1, P. Ghaneh1

1Division of Surgery and Oncology, Departments of 2Nuclear Medicine, 3Radiology, Royal Liverpool University Hospital, Liverpool, UK

Introduction: Advanced pancreatic cancer has a very poor prognosis and novel treatments may improve the outlook. This study aimed to evaluate the safety and tolerability of KAb 201, an iodine 131 (I131) labelled anti-CEA monoclonal antibody. Pharmacokinetics, antigenicity and efficacy of KAb 201 were also assessed.

Methods: Patients with histologically/cytologically proven, advanced adenocarcinoma of the head of pancreas were randomised to i.a./i.v. arms. Following a positive dosimetry scan, a therapeutic dose of KAb 201 was given. Patients were assessed for safety and efficacy and followed up until death.

Results: Between February 2003 and July 2005, 24 patients were enrolled. Nineteen patients (median age 59 years, range 47–66 years; median Karnofsky Performance Status [KPS] 80, range 70–100; stage IVa = 8; stage IV b = 11) were randomised, 9 to the i.v. and 10 to the i.a. arms.

Eighteen patients received the treatment dose (one patient had a negative dosimetry scan). In the i.a. arm, dose limiting toxicity (DLT) was seen at in 2/6 (33%) patients at 50 mCi whereas in the i.v. arm, DLT was noted in 1/6 (17%) patients at 50 mCi, but did not occur at 75 mCi (0/3).

Median overall survival was 6 months (inter quartile range = 3.25–9 months; range = 2–14 months) from study entry, with no significant difference between the i.v. and i.a. arms on Kaplan Meier analysis (log rank p = 0.51). Three patients were alive at the time of this analysis, at 9 (one patient) and 13 months (2 patients).

Patients with KPS scores of 90/100 survived significantly longer than those with KPS of 70/80 (log rank p = 0.035) (provisional results).

Conclusions: Dose limiting toxicity for KAb 201 by the i.a. route was 50 mCi, while DLT was not reached in the i.v. arm. A good performance status was associated with a survival advantage.

Acknowledgement: Xenova Biomedix sponsored this study.

T44

Meta-Analyses of the Management of Locally Advanced and Metastatic Pancreatic Cancer

A. Sultana1, C. Tudur-Smith2, P. Ghaneh1

1Division of Surgery and Oncology, 2Centre for Medical Statistics and Health Evaluation, University of Liverpool, UK

Introduction: There are a large number of randomised controlled trials (RCTs) involving chemotherapy, radiotherapy and combination therapy in the management of advanced pancreatic cancer. There is no consensus on which modality and agents to be used. The aim of these meta-analyses is to examine the following comparisons: (i) chemotherapy vs. best supportive care (BSC), (ii) 5 FU vs. 5 FU combination chemotherapy, (iii) gemcitabine vs. gemcitabine combination chemotherapy, (iv) radiotherapy vs. chemoradiotherapy, (v) chemotherapy vs. chemoradiotherapy, followed by chemotherapy.

Methods: Relevant RCTs were identified by searching databases, trial registers and conference proceedings. The primary end point was overall survival (OS) and secondary endpoints were progression free survival (PFS)/time to progression (TTP), response rate (RR) and adverse events (AE). Meta-analyses of OS, PFS and TTP were summarised using hazard ratio (HR) with RR and AEs summarised using relative risk.

Results: 110 RCTs were identified, and 43 trials involving 7,925 patients selected. Comparing chemotherapy to best supportive care, survival was improved in the chemotherapy group (HR 0.64; 95% confidence interval 0.42–0.98). 5 FU combination chemotherapy did not result in better overall survival compared to single agent 5 FU (HR 0.94; 95% CI 0.82–1.08).

Survival was improved following gemcitabine combination chemotherapy compared to gemcitabine (HR 0.89; 95% CI 0.83–0.96). Indirect comparisons demonstrated gemcitabine combined with a platinum derivative or capcitabine are superior to irinotecan or 5 FU based combinations.

Chemoradiotherapy improved survival over radiotherapy alone (HR 0.70; 95% CI 0.52–0.94). Chemoradiotherapy followed by chemotherapy did not lead to a survival advantage over chemotherapy (HR 0.79; CI 0.32–1.95).

Conclusions: There was a significant survival benefit for chemoradiotherapy over best supportive care, gemcitabine combinations over gemcitabine and chemoradiotherapy over radiation. This supports the use of gemcitabine based combination chemotherapy in the treatment of advanced pancreatic cancer.
Experimental

P1
The Effects of Tuftsin and Splenectomy on the Evolution of Experimental Pancreatitis
J.S. Yan, W.X. Wang, Y.M. Ding, Y. Cheng, W. Zhang, L. Yu
Department of Hepatic-Biliary-Pancreatic Surgery, Renmin Hospital of Wuhan University, Wuhan, China

Introduction: Spleen, a vital component of immune system, is involved in immunoreaction and immunosuppression. Immunomodulator tuftsin, synthesized and secreted from spleen, can improve the immune response. The evolution of acute pancreatitis is associated with immune organ and immune media like tuftsin. In this experiment, we explored whether splenectomy or administration of tuftsin could enhance or ameliorate the severity of acute pancreatitis.

Methods: 120 Sprague-Dawley rats were divided into 5 groups. In group I, splenectomy was performed before acute pancreatitis was induced by intraductal infusion of 4.0% sodium taurocholate, 20 min later after splenectomy and pancreatitis induction, the rats were treated with tuftsin 75 μg/kg i.v. In group II, the animals received saline treatment after sleneectomy and pancreatitis induction, instead of tuftsin. In groups III and IV, either pancreatitis induction or saline treatment after slenenctomy and pancreatitis induction, the rats were treated with tuftsin 75 μg/kg i.v. in group V, the animals received saline treatment instead of tuftsin. Pancreatic samples were collected under anesthesia at 0, 3, 6 and 12 after the last operation or injection for histological assessment.

Results: There were the highest histological scores (9.0 ± 0.8 and 11.5 ± 2.7) in group II at 6 and 12 h, indicating the most severe pancreatic injury. Compared to group II, tuftsin treatment in group I could enhance the severity of pancreatitis significantly (p < 0.05). Interestingly, there was milder pancreatic histological damage in group I (8.5 ± 1.6) than in group III (9.8 ± 0.5) at 12 h time point (p < 0.05). Histological changes of pancreas was not observed in control group and splenectomy group.

Conclusions: Administration of exogenous tuftsin aggravates the histological changes of pancreatitis and splenectomy ameliorates the development of pancreatitis. It indicated that immune organ and immune media, like spleen and spleen secreted tuftsin, may play an important role in the evolution of pancreatitis.

P2
Proteomic Analysis of Microdissected Pancreatic Cancer Specimens Reveals High S100A8 and S100A9 Protein Expression in the Tumour Associated Stroma, but not in Benign or Malignant Epithelia
D. Vimalachandran1, C. Thompson1, R.E. Jenkins2, A. Shekouh1, F. Campbell1, A. Dodson3, W. Prime4, T. Crnogorac-Jurcevic5, N.R. Lemoine6, E. Costello1
1Division of Surgery and Oncology, Royal Liverpool University Hospital, 2Biomedical Sciences Proteomics Facility, Department of Pharmacology and Therapeutics, 3Department of Pathology, 4Cancer Tissue Bank Research Centre, Department of Pathology, University of Liverpool, 5Cancer Research UK Centre for Molecular Oncology Unit, Institute of Cancer, Barts and the London School of Medicine and Dentistry, John Vane Science Building, Charterhouse Square, London, UK

Introduction: Pancreatic cancer remains a devastating disease with a dismal prognosis. It is characterised by an intense stromal response, the role of which remains unclear. We employed laser capture microdissection and proteomics to study the stromal response in pancreatic cancer further.

Methods: Following sectioning and staining of pancreatectomy specimens, laser capture microdissection (LCM) was used to obtain benign and malignant ductal cells and stromal cells surrounding malignant ducts. Proteins were separated using two dimensional gel electrophoresis (2DE) and visualized using silver staining. Gels were compared to identify differential spot expression and mass spectrometry used to identify proteins. The expression of candidate proteins was confirmed and characterised using immunohistochemical and immunofluorescent analysis of pancreatic cancer sections and a custom-built pancreatic cancer tissue microarray.

Results: LCM was used to procure benign ductal (n = 4), malignant ductal (n = 4) and stromal cells (n = 3). Analysis of 2DE gels revealed two spots (S100A9 and S100A8) consistently overexpressed in stromal gels when compared to benign and malignant ductal gels. Immunohistochemical analysis of cancer specimens from the tissue microarray (n = 74) confirmed these findings, demonstrating both proteins to have a similar pattern of expression in stromal inflammatory cells. Immunofluorescent double staining of pancreatic cancer specimens (n = 3 patients) revealed co-expression of S100A8 and S100A9 in these cells, however, a number of cells showed expression of S100A9, but not S100A8.

Conclusions: Overexpression of both S100A8 and S100A9 in pancreatic cancer has been reported previously, however, tissue enrichment strategies were not employed so that conclusions about their cellular origin could not be made. We have elaborated on the expression of these proteins in pancreatic cancer further, by
demonstrating their expression in host stromal cells and not cancer cells. Coexpression of S100A9 and S100A8 in a subset of these stromal cells is a novel finding, and clearly requires further investigation.

P3

Assessment of Isoform Specificity for a Polyclonal Elastase ELISA

F.U. Weiss1, M. Ruthenbürger1, E. Hammer1, U. Völker2, M.M. Lerch1

1Department of Gastroenterology, Endocrinology and Nutrition, Ernst-Moritz-Arndt-University Greifswald, 2Laboratory for Functional Genomics, University of Greifswald, Germany

Introduction: Elastase is secreted from the pancreas and passes through the GI-tract without significant degradation. Exocrine pancreatic function analysis can therefore be performed with Elastase stool tests. Five different isoforms of human pancreatic elastase (PA I, IIA, IIB, IIIA, IIIB) have been identified. Three different polyclonal antisera that are used in a commercial ELISA were investigated for their specific recognition of human elastase isoforms in human pancreatic juice.

Material and Methods: Secreted proteins from human pancreatic juice were analysed by one or two-dimensional gel-electrophoresis, followed by western blot analysis using 3 different polyclonal anti-Elastase antibodies (BIOSERY GmbH, Germany) and MALDI-TOF-MS. Elastase-activity was analysed in immunoprecipitates from human pancreatic juice using a fluorogenic Elastase substrate. Finally, cross-reactivity of the antibodies was tested against pancreatin from pig pancreas.

Results: In 1D Western blots of pancreatic juice all three polyclonal antisera against human Elastase detected a single ~30kDa protein. Immunoprecipitates with these antibodies exhibited elastase activity as determined with the fluorogenic Elastase substrate. In 2D-Westernblots (pH3-10) proteins in the molecular weight range of ~30kDa were separated into a number of spots of different isoelectric points (pI). MALDI-TOF-MS-analysis of these spots revealed the presence of pancreatic Elastase IIIA and IIIB isoforms, but not Elastase II or Elastase I isoforms. Western blot analysis of pancreatin from pig pancreas revealed no cross-reactivity with any of the three antisera tested.

Conclusion: All three commercial antibodies that are used in a polyclonal Elastase ELISA preferentially detect human Elastase Isoforms IIIA and IIIB, and do not cross-react with pig pancreatin. At present differences concerning expression and specific function of PA II or PA III isoforms are still unknown, but we could demonstrate, that PA III isoforms clearly possess Elastase activity as determined by a fluorogenic Elastase substrate. Elastase I is not an enzyme expressed in the adult human pancreas and should therefore not be referred to in commercial test kits for exocrine pancreatic function.

P4

Role of Initial Coagulation in the Protective Effect of Ischemic Preconditioning in Acute Ischemia/Reperfusion-Induced Pancreatitis

Z. Warzecha1, A. Dembinski1, P. Ceranowicz1, M. Dembinski1, J. Cieszkowski1, S.J. Konturek1, B. Kusnierz-Cabala2, J.W. Naskalski2, R. Tomaszewska3, W.W. Pawlik1

1Department of Physiology, 2Department of Clinical Biochemistry, 3Department of Pathology, Jagiellonian University Medical College, Krakow, Poland

Introduction: Previous studies have shown that ischemic preconditioning (IP) exhibits protective and healing effect in ischemia/reperfusion- and caerulein-induced acute pancreatitis, and that beneficial effect of IP seems to be associated with initial clot formation and subsequent activation of fibrinolysis. This observation suggests the important role of primary activation of coagulation in initiation of protective mechanisms of IP. The aim of present study was to assess this hypothesis by inhibition of clot formation by heparin.

Methods: Heparin was administered twice before and during induction of acute pancreatitis at the dose 150 u/kg. IP was performed, 30 min before induction of acute pancreatitis, by short-term clamping of celiac artery (2 × 5 min). Acute pancreatitis was induced in rats by clamping of inferior splenic artery for 30 min followed by reperfusion. Rats were sacrificed after 6 and 24 h of reperfusion. Biochemical and morphological evaluation of pancreatitis severity, activated partial thromboplastin time (APTT) and plasma D-dimer were determined.

Results: Pretreatment with heparin or IP applied alone reduced the severity of acute pancreatitis. Both procedures caused a similar reduction in plasma lipase, amylase and pro-inflammatory interleukin-1β. Also histological signs of pancreatic damage were reduced, whereas the pancreatitis-evoked fall of pancreatic blood flow and DNA synthesis were partly reversed. Combination of heparin plus IP did not only exhibit any additional protective effect against ischemia/reperfusion-induced pancreatitis, but also reduced the protective effect of heparin or IP applied alone. It was manifested by an increase in pancreatic damage and plasma level of lipase, amylase and interleukin-1β, as well as by reduction in pancreatic DNA synthesis. These findings were associated with reduction in the IP-induced increase in plasma D-dimer and anti-inflammatory interleukin-10.

Conclusions: Inhibition of clot formation by pretreatment with heparin abolishes the protective effect of ischemic preconditioning in ischemia reperfusion-induced pancreatitis.
P5

Glucose Stimulates Hypoxia Inducible Factor-1α Expression in Human Pancreatic Cancer Cells

F Wang, J. Permerc

Department of Surgery, Karolinska University Hospital at Huddinge, Stockholm, Sweden

Introduction: Pancreatic cancer cells that are subjected to intratumoral hypoxia and paracrine insulin markedly express hypoxia inducible factor-1 (HIF-1). HIF-1 increases glycolysis in pancreatic cancer cells and thereby accelerates glucose turnover and disturbs energy homeostasis in the host. HIF-1 production in pancreatic cancer cells may also be stimulated by excessive glucose. If so, hyperglycemia, which is a component of metabolic complications in pancreatic cancer, would be expected to enhance HIF-1 expression in pancreatic cancer cells.

Methods: Wild type MiaPaCa2 human pancreatic cancer cells were cultured under normoxic or hypoxic (1% oxygen) conditions with different concentrations of glucose (5.6–22.2 mM). HIF-1α, which is the regulatory subunit of HIF-1, and the glycolytic enzyme hexokinase were measured in these cells using Western blot assay. Glucose consumption and lactate accumulation in the culture media were used as indexes of cell glycolysis.

Results: MiaPaCa2 cells expressed considerable HIF-1α during hypoxia but barely did so during normoxia. Glucose increased HIF-1α expression dose-dependently in hypoxic MiaPaCa2 cells. In the same cells, the expression of hexokinase was correlated with the expression of HIF-1α induced by glucose. The addition of insulin to the culture medium at a final concentration of 1 μM enhanced the stimulating effect of glucose on HIF-1α and hexokinase expression. Insulin also increased glucose consumption and lactate production in hypoxic wild type MiaPaCa2 cells but not in a MiaPaCa2 variant whose HIF-1α gene was functionally silenced by short interfering RNA.

Conclusion: In concert with insulin, glucose stimulates HIF-1 expression and glycolysis in hypoxic pancreatic cancer cells in vitro.

P6

Blockade of Neurokinin-1 Receptor Attenuates CC and CXC Chemokine Production in Acute Pancreatitis and Associated Lung Injury

J. Sun, M. Bhatia

Department of Pharmacology, National University of Singapore, Singapore

Introduction: The neuropeptide substance P (SP) and its receptor neurokinin-1 receptor (NK-1R) play a pivotal role in the pathogenesis of acute pancreatitis and associated lung injury. The present study investigated the involvement of CC and CXC chemokines in SP/NK-1R-related pathogenesis of this condition.

Methods: Acute pancreatitis was induced in mice by hourly intraperitoneal (i.p.) injections of caerulein (50 μg/kg) for 3, 6, and 10 h.

A specific NK-1R antagonist CP-96,345 (2.5 mg/kg, i.p.) was administered either half an hour before or one hour after the first caerulein injection to block the interaction of SP and NK-1R. The temporal and dose-related effects of caerulein hyperstimulation and CP-96,345 treatment on the mRNA and protein expression as well as immunohis-tochemical localization of various CC and CXC chemokines were examined.

Results: MCP-1, MIP-1α and MIP-2 expression was found upregulated early (within 3 h) in both the pancreas and lungs, suggesting they are early mediators in the condition and mediate local as well as distant inflammatory responses. In contrast, RANTES expression was induced only in the pancreas 6 h after AP induction indicating it is more important in mediating local inflammatory responses at a later stage of the condition. Caerulein induction of MCP-1, MIP-1α and MIP-2, but not RANTES expression was found significantly suppressed by administration of CP-96,345, suggesting the former three chemokines are regulated by SP/NK-1R. Additionally, in the pancreas chemokines were immunohistochemically localized to acinar cells and the infiltrating leukocytes, while in the lungs they were expressed by alveolar macrophages, epithelial and endothelial cells.

Conclusions: We therefore identified the CC chemokines MCP-1 and MIP-1α and the CXC chemokine MIP-2 as important mediators involved in SP-related pathogenesis of acute pancreatitis. Substance P, by acting via NK-1R on the chemokine-producing cells, stimulates chemokine production that aggravates local pancreatic damage and its systemic sequelae.

P7

Expression of Protease-Activated Receptor-1 (PAR-1) and Matrix Metalloprotease-1 (MMP-1) in Human Pancreatic Cancer Tissues and Pancreatic Cancer Cell Lines

H. Souma1, S. Hozawa1, K. Kameyama2, T. Shiomi2, N. Watanabe1, H. Higuchi1, Y. Yamagishi1, K. Aiura3, Y. Okada2, T. Hibi1

Department of Internal Medicine1, Pathology2 and Surgery3, Keio University School of Medicine, Tokyo, Japan

Introduction: PAR-1 is a G protein-coupled receptor that is cleaved and activated by ligands including thrombin and trypsin. MMP-1 is recently recognized as a ligand for PAR-1 that promotes invasion and tumorigenesis of breast cancer cells (Cell 2005;120: 303). The aim of this study is to examine the expression and localization of MMP-1 and PAR-1 in pancreatic cancer, and to compare them with biological characteristics of pancreatic cancer cells in vivo and in vitro.

Methods: Pancreatic cancer tissues operatively resected from ten patients in our hospital were classified by their histological type, the amount of interstitial tissue, the pattern of invasion, and presence or absence of distant metastasis. Immunohistochemistry were performed to detect the protein expression of PAR-1, MMP-1 and α-smooth muscle actin (α-SMA) in pancreas tissues. Pancreatic cancer cell lines with various degree of differentiation were used in this study. Northern blot analysis and Gelatin zymography were performed to detect the mRNA and protein expression of PAR-1 and/or MMP-1 in pancreatic cancer cell lines.
**Results:** Cancer cells in pancreatic cancer tissues did express MMP-1 protein. Whereas, α-SMA positive stromal cells around cancer cells showed the strong expression of PAR-1, but did not express MMP-1. Stromal cells in normal pancreas tissues only scarcely showed the expression of PAR-1. The expression of PAR-1 and MMP-1 did not correlate with histological type or potentiality of invasion and metastasis of pancreatic cancer. On the other hands, well-differentiated pancreatic cancer cell lines showed the stronger expression in both PAR-1 and MMP-1 than poorly-differentiated cell lines.

**Conclusions:** PAR-1 was highly expressed in α-SMA positive stromal cells almost exclusively around pancreatic cancer cells. MMP-1 was expressed in pancreatic cancer cells. It was suggested that cell-to-cell interaction between stromal and tumor cells may be related to PAR-1 expression in the stromal cells.

**P9**

**Influence of Stromal Matrix Proteins on Migratory Activity of Pancreatic Tumor Cells**


Department of Surgery, University of Heidelberg, German Cancer Research Center, Heidelberg, Germany

**Introduction:** The ability to migrate is a prerequisite of pancreatic tumor cells for invasion and metastases. Several previous investigations as well as our own studies showed high expression of extracellular matrix proteins in stromal tissue of pancreatic cancer. The relevance of tumor stroma for tumor invasion and metastases of pancreatic cancer was poorly investigated. The aim of the present study was to study the influence of different stromal proteins on migratory activity of pancreatic tumor cells.

**Methods:** The influence of stromal proteins (collagen I, III, IV, fibronectin, vitronectin, vimentin, laminin) on migratory activity of 10 different tumor cell lines was investigated by 3D-time-lapse microscopy and by 96 wells migration assay in collagen I gel.

**Results:** Collagen I matrix induced a spontaneous migration in 8 of 10 tumor cell lines (FAMPAC, Patu8988t, Patu890t, Capan-1, Panc-1, DANG, PaCa44, MiaPaCa2). The percentage of migrating tumor cells varied from 15% (MiaPaCa2) to 87% (FAMPAC). The mean velocity of tumor cell migration varied from 5.6 ± 2.5 μm/h (Panc-1) to 17.8 ± 4.2 μm/h (FAMPAC) depending on the appropriate cell line. The addition of collagen III/IV, fibronectin, vitronectin, vimentin did not significantly change the migration of single tumor cells. However, the migration of all cell lines in collagen matrix was significantly increased after addition of laminin.

**Conclusions:** Extracellular matrix proteins such as collagen I and laminin promote the migratory activity of single tumor cells of pancreatic cancer which could contribute to the high metastatic potential of this tumor type.

**P10**

**Complete Analysis of the Human Mesotrypsinogen Gene in Patients with Chronic Pancreatitis of Uncertain Aetiology**

J. Rosendahl, V. Keim, J. Mössner, N. Teich

University of Leipzig, Department of Medicine II, Leipzig, Germany

**Introduction:** The imbalance of pancreatic proteases and their inhibitors seems to be one major pathogenic step in the development of chronic pancreatitis (CP). Recently, several genes were shown to be associated with CP (PRSS1, SPINK1, CFTR). Mesotrypsin (MT) counts for a minority of human trypsin activity. By degradation of trypsin inhibitors MT may enhance food digestion but lowers the effect of IAPP in maintaining blood glucose levels during acute pancreatitis.
activity of protective proteases within the pancreas. Therefore mutations of the mesotrypsinogen gene might provoke pancreatic autodigestion. Earlier work solely revealed the E32del polymorphism, which is not disease associated.

**Methods:** We analysed all 5 exons and the intron/exon junctions of the MT gene by direct sequencing of 96 unrelated patients with CP (59 patients with idiopathic CP, 37 with hereditary CP without PRSS1 mutations; m = 45, f = 51, mean age 30 years). Arising from their family history and disease course, all patients were suspicious for an unrecognised genetic background.

**Results:** Aside the E32del polymorphism (34 patients), no exonic mutation could be found. The analysis of the splice sites only revealed wild type sequences. The intronic variants IVS3 + 191G > A, IVS3–22C > T, IVS1 + 175G > T, IVS2 + 256A > T, IVS4 + 218T > C and IVS5–66C > G were detected in 2, 1, 57, 59, 12 and 12 patients, respectively.

**Conclusions:** Although actual data qualify the MT gene as a reasonable candidate gene for chronic pancreatitis, we did not find disease associated mutations in 96 patients with a highly suspicion of genetically determined CP.

---

**P11**

**Overexpression of ICAM-1 in Acinar Cells during Acute Pancreatitis. Effect of N-Acetylcysteine**

L. Ramudo1, M.A. Manso1, S. Yubero1, S. Lázaro1, S. Vicente2, I. De Dios1

1Department of Physiology and Pharmacology, 2Toxicology, University of Salamanca, Spain

**Introduction:** Infiltration of leukocytes within pancreas is a common feature in acute pancreatitis (AP), with different cells involved in the production of adhesion molecules and integrins. We investigate the ability of acinar cells to produce ICAM-1 as well as the kinetic of CD11b expression in leukocytes during AP. The involvement of reactive oxygen species upregulating these molecules was examined using N-acetylcysteine (NAC) as antioxidant treatment.

**Methods:** AP was induced in rats by bile-pancreatic duct obstruction (BPDO). N-Acetylcysteine (NAC) (50 mg/kg/day) was administered 1h before and 1h after BPDO. ICAM-1 mRNA expression was analyzed in acinar cells by RT-PCR. The presence of ICAM-1 in acinar cell membrane was evaluated by flow cytometry. ICAM-1 plasma levels were analyzed by ELISA. CD11b expression was analyzed in neutrophils and monocytes by flow cytometry. Mieloperoxidase (MPO) activity was measured in the pancreas.

**Results:** Both in rats treated and non-treated with NAC, ICAM-1 expression (mRNA and protein) was found significantly (p < 0.001) increased in acinar cells at 3h after BPDO. The increased ICAM-1 plasma levels found during AP was not reduced by NAC. CD11b expression significantly (p < 0.05) increased in neutrophils and monocytes from 6h after BPDO. NAC treatment delayed this effect and only a transient increase was found 12h after BPDO. MPO activity significantly (p < 0.001) rose in the pancreas from 6h after BPDO, however, NAC delayed and reduced neutrophil infiltration until 24h after BPDO.

**Conclusions:** Damaged acinar cells offer the first signal for the recruitment of leukocytes during AP by upregulating ICAM-1 expression by antioxidant-resistant mechanisms. By contrast, NAC protects the pancreas and reduces leukocyte infiltration during AP by preventing the expression of CD11b in neutrophils and monocytes.

---

**P12**

**Pressure Induces Extracellular Matrix and Cytokine Synthesis in Pancreatic Stellate Cells via Generation of Reactive Oxygen Species**


Department of Gastroenterology and Metabolism, University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan

**Introduction:** In chronic pancreatitis, pancreatic tissue pressure is higher than that of normal pancreas. We have reported that pressure induces extracellular matrix (ECM) and cytokine synthesis in pancreatic stellate cells (PSCs). Since strong antioxidant, green tea polyphenol epigallocatechin gallate (EGCG) inhibits transformation from quiescent to activated phenotype and synthesis of ethanol-induced ECM and cytokine in PSCs, oxidative stress and reactive oxygen species (ROS) may be important factors in PSC activation. Here we examined whether mechanical stress induces ROS in PSCs, and evaluated the effects of EGCG on pressure-induced ECM and cytokine synthesis.

**Methods:** We used isolated rat PSCs between passage two to three. Pressure was applied to cultured rat PSCs by adding compressed helium gas into the pressure loading apparatus to raise the internal pressure. PSCs were cultured with or without EGCG (25 μM) in the absence or presence of pressure.

**Results:** In the presence of pressure, generation of ROS in PSCs was detected at 30s stimulation period and was much stronger at 60min stimulation period. In incubation with EGCG, generation of ROS in PSCs was strongly suppressed for 60min stimulation. Pressure significantly increased protein levels of type I procollagen and mRNA levels of α-smooth muscle actin, type I procollagen and transforming growth factor-β1 in PSCs. EGCG inhibited pressure-induce increased of these protein and mRNA levels in PSCs.

**Conclusion:** Our result indicated that ROS play as one of key factors in PSC activation, and that antioxidants may be presumably effective as therapeutic reagents against pancreatic fibrosis.

---

**P13**

**The Role of Oxidative Stress in the Early Phases of Acute Pancreatitis in the MT-SSAT Transgenic Rat Model**

M. Merentie, A. Uimari, R. Sinervirta, L. Alhonen

Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Finland

**Introduction:** Oxidative stress is an important factor in the pathogenesis of acute pancreatitis (AP). In the MT-SSAT transgenic
rat model the metallothionein promoter driven SSAT (spermidine/spermine-N1-acetyltransferase) transgene is induced by zinc leading to depletion of higher polyamines mainly in pancreas and to development of AP. In this study the role of oxidative stress in the initial phases of AP in the MT-SSAT rats was analysed. Also the effects of prophylactic polyamine analogue treatment, which prevents AP in this model, on the oxidative stress markers were evaluated.

Methods: Pancreatic specimens were collected 3 and 6 h after induction of AP with zinc. There were 3–5 rats per group. Polyamine levels and SSAT activities in pancreas were measured. Pancreatic oxidative stress was evaluated by determining the amount of lipid peroxidation, measured as malondialdehyde (MDA), and the amount of glutathione. Also the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were measured.

Results: Already within 3 h after zinc induction the marked depletion of higher polyamines (p < 0.01) due to massive increase of SSAT activity was observed in all treated animals. After 3 and 6 hs lipid peroxidation slightly increased (p = 0.08; p = 0.4, respectively). The activity of SOD was somewhat decreased at 6 h time point (p = 0.07) but CAT remained mainly unchanged. Minor depletion of reduced glutathione was observed after 6 h. Prophylactic polyamine analogue treatment had no marked effects on the measured parameters.

Conclusions: The results suggest that oxidative stress has no major role in the early development of AP in MT-SSAT rats and the beneficial effect of polyamine analogue treatment on the prevention of AP is obtained by means other than functions connected to early phase oxidative stress factors. The protective potential of zinc against oxidative stress damage in this model also needs to be clarified.

P15
Transient Ptf1a Misexpression Converts Mouse Embryonic Foregut to Pancreas Tissue Capable of Ameliorating Streptozotocin-Induced Hyperglycemia
T. Kuhara1, Y. Kawaguchi1, K. Furuyama2, A. Fukuda3, S. Kodama1, M. Horiguchi1, M. Koizumi1, M. Kawaguchi1, C.V.E. Wright4, R. Doi1
1Department of Surgery and Surgical Basic Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2Vanderbilt Developmental Biology Program, Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA

Introduction: Previous studies have revealed the functions of several genes involved in pancreatic organogenesis. Above all, Pdx1 and Ptf1a play a pivotal role in specifying pancreatic fate from the undifferentiated gut epithelium. We tested the hypothesis that transiently misexpressed Ptf1a in non-pancreatic Pdx1-expressing foregut epithelium triggers the transdifferentiation or trans-specification into the pancreatic fate and runs autonomously the endogenous differentiation program.

Methods: Foregut, including distal stomach and proximal duodenum was dissected from E11.5 mice. Pancreatic buds were microscopically excised to avoid contamination by endogenous pancreatic tissue, and explants were infected with replication-deficient Ptf1a–adenovirus (Ad-ptf1a) and placed in three-dimensional culture in type I collagen gel. Induction of pancreas-specific genes was evaluated by RT-PCR and immunohistological analysis. Thereafter, cultured explants were transplanted into the subrenal space of the streptozotocin-induced diabetic nude mice.

Results: RT-PCR analysis showed that endogenous ptf1a mRNA was induced suggesting the commitment of pancreatic lineage. By immunohistological analysis, at the higher titer of Ad-ptf1a infection, amylase-producing cells and Cytokeratin-positive duct structures were evaginated from weakly Pdx1-expressing epithelial cells. On the other hand, at the lower titer of Ad-ptf1a infection, insulin-producing cells and single-layered duct structures were contiguous to strongly Pdx1-positive, Cdx2-expressing duodenal epithelial cells. These cells were phenotypically fully matured with multi vesiculated insulin and membrane-bound Glut2. Induced ectopic pancreas secrete a robust insulin in response to the successive changes in glucose concentration of the media. Furthermore, transplantation of these tissues significantly ameliorated the morbidity and hyperglycemia in streptozotocin-induced diabetic mice.
Conclusions: Transient adenovirus-mediated misexpression of Ptf1a induced the trans-differentiation of foregut epithelium, leading to the activation of an endogenous pancreatogenic program. Induced insulin-producing cells are biologically functional in vitro and in vivo. Our results suggest that the easily accessible gut epithelium may be a useful substrate for de novo generation of insulin-producing tissue for diabetes therapy.

P16
How does the Severity of Acute Pancreatitis Correlate with Polyamines Levels in the Blood and the Pancreas?

Introduction: Polyamines (spermidine, spermine and putrescine) are essential to the mammalian cells. Previous studies have shown that after induction of polyamines catabolizing SSAT (spermidine/spermine N1-acetyltransferase), pancreatic polyamine content change in various models of acute pancreatitis, but the effect of pancreatitis severity on these changes has never been studied. In this study we investigated whether polyamine levels in blood and pancreas change according to the severity of acute pancreatitis.

Methods: Sublethal pancreatitis was induced by 2% taurodeoxycholate, while lethal pancreatitis was induced with 6% taurodeoxycholate. Sham operation included laparotomy only. Pancreas and blood specimens were collected for assessing pancreatic histology, SSAT activity, polyamine levels in the pancreas and RBC, and serum amylase activity.

Results: There was no mortality in the sham operation group or the sublethal pancreatitis group, whereas in the lethal pancreatitis group the mortality was 67% and 100% at 24h and 48h, respectively. Serum amylase activity and pancreatic histology also suggested more severe injury in the lethal than sublethal pancreatitis. SSAT was induced in the pancreas during the evolution of pancreatitis. This induction was delayed in lethal pancreatitis compared to sublethal pancreatitis. Pancreatic spermidine decreased by 64% or 54% at 24 h in sublethal or lethal pancreatitis, whereas pancreatic spermine changed minimally. Sham operation induced a decrease in RBC spermidine and spermine levels since 1.5 h. Spermidine decreased by 74% in sublethal and 88% in lethal pancreatitis (p < 0.05 vs. sham). There was 28% and 84% decrease of RBC spermine in sublethal and lethal pancreatitis (p < 0.05 vs. sham) respectively.

Conclusions: Acute pancreatitis is associated with pancreatic SSAT induction and consequent changes in polyamine levels in pancreas and RBC. Because RBC spermidine and spermine changes are early, and dependent on the severity of acute pancreatitis, they need to be further studied in clinical setting.

P17
Characterization of Organ Damage in Severe Acute Pancreatitis Induced by Depletion of Polyamines

Introduction: Polyamines are small organic cations essential for cell growth and function. We have previously shown that depletion of polyamines in transgenic rats with activated polyamine catabolism results in severe acute pancreatitis whereas prior treatment with methylated polyamine analogues prevents the development of pancreatitis. Furthermore, rats receiving the analogues after the induction of pancreatitis are rescued from pancreatitis-associated mortality, by currently unknown mechanism. As multiple organ failure is a major cause of death in early phase of acute pancreatitis, we have examined the systemic complications found in transgenic rats with pancreatitis.

Methods: Pancreatitis was induced by activating the transgene expression via metallothionein promoter with injection of ZnSO4 (10 mg/kg i.p.). The therapeutic effects of α-methylspermidine and bis-α-methylspermine were studied by injecting the analogue (50 mg/kg; 25 mg/kg i.p.) 4 and 8 h after zinc. Samples from pancreas, lung, liver, kidney, small bowel and colon were collected 8, 24 and 48 h after induction. Survival was monitored up to 2 weeks.

Results: Pancreatic edema and serum amylase and alanine transaminase activities increased in rats with pancreatitis. The rats had ascites and at later time points also pleural effusion. Some signs of acute tubular necrosis in the kidney were seen. The animals also had inflammation in the lungs, liver and the small bowel, as assessed by histological analysis and increased myeloperoxidase activity. Analogue treatment did not significantly attenuate abovementioned parameters, despite reducing mortality from 80 to 20% (p < 0.01).

Conclusions: Some signs of organ damage in the kidney, lung, liver and small bowel were observed in this pancreatitis model. However, while polyamine analogues reduced mortality, they did not alleviate the histopathological changes, indicating that their therapeutic effect is likely to be related to damage in other organs not studied here, or to other complications, such as microcirculatory disorders.
P18

Treatment with BX471, a Non-Peptide CCR1 Antagonist, Protect Mice Against Acute Pancreatitis-Associated Lung Injury by Modulating Neutrophils Recruitment

M. He1, R. Horuk2, M. Bhatia1

1Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 2Department of Immunology, Berlex Biosciences, Richmond, Canada

Introduction: Chemokines and their receptors play a key role in the pathogenesis of acute pancreatitis. BX471 is a potent non-peptide CCR1 (CC chemokine receptor-1) antagonist in both human and mouse [1]. The aim of the present study was to evaluate the effect of prophylactic and therapeutic treatment with BX471 on experimental acute pancreatitis in the mouse and to investigate the underlying mechanisms.

Methods: Acute pancreatitis was induced in mice by hourly intraperitoneal injection of caerulein. BX471 was administered either 30 min before or 1 h after starting caerulein injections, and pancreatic inflammation and lung injury were assessed. The expression of ICAM-1 (intercellular adhesion molecule-1), P-selectin and E-selectin was studied by RT-PCR and Immunohistochemistry.

Results: In caerulein-induced acute pancreatitis, treatment with BX471 significantly protected mice against lung injury associated with caerulein induced pancreatitis by attenuating MPO (myeloperoxidase) activity (p < 0.01), an indicator of neutrophil recruitment in lungs and attenuating lung morphological changes in histological sections. Treatment with BX471 had little effect on pancreatic damage judging by plasma amylase, water content and histology examination, although pancreatic MPO activity was reduced in BX471 treated groups (p < 0.01). Blocking CCR1 by BX471 also down-regulated ICAM-1-P-selectin and E-selectin expression at mRNA and protein levels in both lungs and pancreas compared with vehicle-treated groups.

Conclusions: These findings suggest that interfering with neutrophil migration and activation by targeting CCR1 may represent a promising strategy to prevent disease progression in acute pancreatitis.

P19

Periostin Creates a Tumor Supportive Microenvironment in Pancreatic Cancer by Sustaining Fibrogenic Stellate Cell Activity

M. Erkan1, J. Kleeff1, A. Gorbachevski1, C. Reiser1, T. Mitkus1, I. Esposito2, T. Giese3, M.W. Büchler1, N.A. Giese1, H. Friess1

1Department of General Surgery, 2Institute of Pathology, 3Institute of Immunology, University of Heidelberg, Heidelberg, Germany

Introduction: Pancreatic ductal adenocarcinoma creates an excessive tumor desmoplasia by stimulating pancreatic stellate cells. The degree of desmoplasia influences the aggressiveness of the tumor and its responses to chemo- and radiotherapy. The aim of this study was to analyze the role of a recently identified stellate cell specific molecule -periostin- in tumor-stroma interactions in pancreatic cancer.

Methods: Stellate-cancer cell interactions in primary and metastatic lesions of the pancreatic cancer patients treated with and without neoadjuvant chemo-radiotherapy were evaluated by immunohistochemistry and immunoblotting. Survival of patients (n = 41) was correlated to the mRNA expression of periostin by quantitative RT-PCR. In vitro interactions of these cells and the effects of periostin in modulation of cellular responses under hypoxia, serum deprivation and radio/chemotherapy were assessed by growth, clonogenicity and invasion assays as well as immunoblotting.

Results: Stellate cells are the only source of periostin in the pancreas. Periostin mRNA mirrors its protein expression and increases 42-fold in cancer. Patients with periostin mRNA expression higher than the normal range had a tendency for a shorter overall survival (19 months vs. 12 months p = 0.14). In tissues, tumor cells seem to trigger periostin secretion in primary and metastatic sites by stimulating local α-smooth muscle actin-positive cells. In vitro, tumor supernatants, hypoxia and radiation upregulated periostin expression. In stellate cells, periostin increased collagen I production, significantly reduced their invisivaility (p = 0.02) and sustained their active state which was otherwise suppressed by radiotherapy. In cancer cells, periostin increased growth, conferred resistance to serum deprivation and had a dose-dependent effect on invasivity.

Conclusions: Cancer cells increase periostin secretion by stimulating stellate cells. Periostin perpetuates stellate cell activity even under radiotherapy and influences the extracellular-matrix composition, creating a tumor supportive microenvironment. Excessive periostin expression may therefore reflect a more aggressive tumor phenotype.

P20

Effect of Stimulation and Inhibition of Cannabinoid 1 Receptors on the Severity of Caerulein-Induced Pancreatitis in Rats

A. Dembinski1, Z. Warzecha1, P. Ceranowicz1, M. Dembinski1, J. Cieszkowski1, W.W. Pawlik1, R. Tomaszewska2, B. Kusnier-Cabala3, J.W. Naskalski3, P.C. Konturek4

1Department of Physiology, 2Department of Pathology, 3Department of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland; 4Department of Internal Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany

Introduction: Recent studies have shown that stimulation of cannabinoid 1 (CB1) receptor reduces the ischemic myocardial necrosis area, inhibits gastric and intestinal secretion, and protects gastric mucosa against stress ulcers. In the pancreas, usage of CB1 receptor antagonist has prolonged the survival of rats with taurocholate-induced acute pancreatitis. Aim of present study was to check whether the administration of CB1 receptor agonist or antagonist affects the development of edematous pancreatitis.

Methods: Acute pancreatitis was induced in rat by caerulein. A natural ligand for CB1 receptor, anandamide (Ki = 61 nM) was administrated twice (30 min prior to caerulein or saline injection and...
Results: Administration of anandamide or AM 251 did not affect pancreatic condition in animals without acute pancreatitis. In animals treated with caerulein, administration of anandamide increased dose-dependently the severity of pancreatitis. In histological examination, we observed an increase in pancreatic edema and inflammatory infiltration. Also, administration of anandamide augmented the pancreatitis-induced increase in serum level of lipase, amylase, poly-C ribonuclease, and pro-inflammatory interleukin-1β (IL-1). Treatment with AM 251 reduced histological signs of pancreatic damage, serum activity of pancreatic digestive enzymes and serum concentration of pro-inflammatory IL-1 in animals with acute pancreatitis. Pretreatment with AM 251 reversed deleterious effect of anandamide in caerulein-induced acute pancreatitis.

Conclusions: Activation of CB1 receptors by anandamide increases the severity of edematous acute pancreatitis; whereas blockade of CB1 receptors by AM 251 attenuates pancreatic damage in caerulein-induced pancreatitis.

P21

Hyperlipidemia Aggravates Necrotizing Pancreatitis via Altered Nitric Oxide Synthase in Rat

L. Czakó¹, A. Szabolcs¹, L. Tiszlavicz², T. Csont³, A. Pósa³, A. Berkó⁴, Cs. Varga⁴, J. Lonovics¹

¹First Department of Medicine, ²Department of Pathology, ³Department of Biochemistry, ⁴Department of Comp. Physiol., Univ. of Szeged, Szeged, Hungary

Background: The aim of the present study was to investigate whether hyperlipidemia can cause acute pancreatitis or alter its severity. We studied the influence of cholesterol-enriched diet-induced hyperlipidemia on the formation of pancreatic nitric oxide synthase (NOS), oxygen-derived free radicals, endogenous scavengers, heat shock proteins and nuclear factor-κB during acute edematous and necrotizing pancreatitis.

Methods: Male Wistar rats were fed a 3% cholesterol-enriched diet or a normal diet for 16 weeks. Edematous pancreatitis was induced with 3 × 75 μg/kg bw cholecystokinin s.c., and necrotizing pancreatitis with 2 × 200 mg/100 g bw of L-arginine i.p., in separate groups of normal and hyperlipidemic rats. The rats were sacrificed 24 h following the induction of pancreatitis, and the severity of the pancreatitis was assessed by measurement of the plasma amylase and lipase concentrations, and the ratio pancreatic weight/body weight (pw/bw), and via the histology.

Results: The cholesterol diet increased the plasma cholesterol and triglyceride levels by 40% and 90%, respectively. The pancreatic cNOS activity was significantly reduced, while the iNOS activity was significantly increased in the hyperlipidemic rats as compared with the non-hyperlipidemic animals (12.7 ± 5.6 vs. 36.1 ± 1.8 and 10.8 ± 2.8 vs. 1.03 ± 0.1 pmol/min/mg protein). The cholesterol diet did not worsen the levels of serum amylase and lipase, the ratio pw/bw or the histological score in the animals with edematous pancreatitis. In the animals with necrotizing pancreatitis, the serum amylase and lipase levels, the ratio pw/bw ratio and the histological score were significantly increased in the hyperlipidemic animals as compared with the non-hyperlipidemic animals.

Conclusion: Cholesterol-enriched diet-induced hyperlipidemia leads to a decrease in pancreatic cNOS activity and an increase in iNOS activity, which may contribute to the aggravation of necrotizing pancreatitis.

Supported by 5K 503 and BÖ No. 5/2003.

P22

Treatment with a Neutralizing Anti-IL-10 Reverses Crambene Mediated Protection Against Acute Pancreatitis

Y. Cao¹, S. Adhikari¹, M.V. Clément², M. Wallig³, M. Bhatia¹

¹Department of ¹Pharmacology and ²Biochemistry, National University of Singapore, Singapore; ³Department of Pathobiology, University of Illinois at Urbana Champaign, Urbana, Illinois, USA

Background: We have earlier shown that induction of the pancreatic acinar cell apoptosis in vivo by crambene (1-cyano-2-hydroxy-3-butenone – CHB), a plant nitrile, protects mice against acute necrotizing pancreatitis. In the current study, we report a role for IL-10, an anti-inflammatory mediator, as part of the mechanism by which acinar cell apoptosis induced by CHB protects against acute pancreatitis.

Methods: Acute necrotizing pancreatitis was induced in the mouse by administering hourly intraperitoneal injections of a supra maximally stimulating dose (50 μg/kg) of the cholecystokinin analog caerulein for 10 h Neutralizing monoclonal anti-IL-10 antibody (2.5 mg/kg) was administered either with or without crambe (70 mg/kg) 12 h before the first caerulein injection. Severity of acute pancreatitis was evaluated by estimation of serum amylase, pancreatic myeloperoxidase (MPO), water content, MCP-1 level, and morphological examination. Apoptosis in pancreatic sections was visualized by the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end labeling method.

Results: CHB administration to normal mice resulted in persistently increased IL-10 levels in pancreas from 12 to 48 h. While the prophylactic treatment with crambe in the acute pancreatitis model significantly reduced the severity, as evidenced by a significant attenuation of hyperamylasemia, pancreatic edema and, pancreatic MCP-1 levels. Also there was histological evidence of diminished pancreatic injury. The biochemical effects were reversed by the administration of anti-IL-10 together with CHB. There was also greatly reduced apoptosis and enhanced necrosis with inflammation in mice treated with anti-IL-10 and CHB together.

Conclusion: IL-10 plays an important role in the protection against acute pancreatitis by CHB and suggets that anti-inflammatory pathways may be significantly involved in the protective effect of acinar cell apoptosis against acute necrotizing pancreatitis.
P23

Treatment with the CXCR2 Antagonist Antileukinate Protects Mice Against Acute Pancreatitis and Associated Lung Injury
M. Bhatia, A. Hegde
Department of Pharmacology, National University of Singapore, Singapore.

Introduction: Chemokines are believed to play a key role in the pathogenesis of acute pancreatitis. We have earlier shown that treatment with a neutralising antibody against CINC, a CXC chemokine, protects rats against acute pancreatitis associated lung injury. The hexapeptide antileukinate (Ac-RRWWCR-NH2) is a potent inhibitor of binding of CXC chemokines to the receptors (CXCR2). This study aims to evaluate the effect of treatment with antileukinate on acute pancreatitis and the associated lung injury in mice.

Methods: Acute pancreatitis was induced in adult male swiss mice by hourly intraperitoneal injections of caerulein (50 μg/kg/h) for 10 h. Antileukinate (52.63 mg/kg, s.c.) was administered to mice either 30 min before (prophylactic) or 1 h after (therapeutic) starting caerulein injections. Severity of acute pancreatitis was determined by measuring plasma amylase, pancreatic water content, pancreatic myeloperoxidase (MPO) activity and histological examination of pancreas sections. A rise in lung MPO activity and histological evidence of lung injury of lung sections were used as criteria for pancreatitis-associated lung injury.

Results: Treatment with antileukinate protected mice against acute pancreatitis, as evident by reduced hyperamylasemia, reduced pancreatic water content, and pancreatic MPO activity, and confirmed by histological examination of pancreas sections. This treatment was also protective against acute pancreatitis associated lung injury, as evident by reduced lung MPO activity, and confirmed by histological examination of lung sections.

Conclusion: These results show that anti-chemokine therapy may be of value in the treatment of acute pancreatitis and its systemic complications.

P24

Hsulf-1 Regulates Growth and Invasion of Pancreatic Cancer Cells by Interfering with Heparin-Binding Growth Factor Signalling
I. Abiatari, J. Kleeff, J. Li, K. Felix, N.A. Giese, M.W. Büchner, H. Fries
Department of General Surgery, University of Heidelberg, Germany

Introduction: Hsulf-1 is a newly identified enzyme with aryl-sulfatase activity that can regulate the sulfation state of cell surface heparin sulfate proteoglycans, and has the ability to decrease the growth of hepatocellular, ovarian, and head and neck squamous cell carcinoma cells by interfering with heparin-binding growth factor signalling. Since pancreatic cancers over-express a number of heparin-binding growth factors and their receptors, the expression and function of this enzyme in pancreatic cancer was analyzed.

Methods and Results: Pancreatic cancer samples expressed significantly (22.5-fold) increased Hsulf-1 mRNA levels compared to normal controls, and Hsulf-1 mRNA was localized in the cancer cells themselves as well as in peritumoral fibroblasts. Stable transfection of the Hsulf-1 negative Panc-1 pancreatic cancer cell line with a full length Hsulf-1 expression vector resulted in increased sulfatase activity and decreased cell-surface heparin-sulfate proteoglycan sulfation. Hsulf-1 expression reduced both anchorage-dependent and independent cell growth and decreased FGF-2 mediated cell growth in this cell line. In vitro, overexpression of this enzyme increased invasiveness and adhesiveness of Panc-1 cells. An in vivo xenograft nude mouse tumour model revealed a markedly reduced growth potential of Hsulf-1-expressing Panc-1 cells, which correlated with a significantly lower proliferation rate. Hsulf-1-positive nude mouse tumours displayed better development of interstitial matrix structures, with increased blood vessel density within these tumours. In an orthotopic model, Hsulf-1-positive tumours exhibited enhanced local invasiveness.

Conclusion: High expression of Hsulf-1 occurs in the stromal elements as well as in the tumour cells in pancreatic cancer and interferes with heparin-binding growth factor signalling. Hsulf-1-mediated desulfation of heparin sulfate proteoglycans reduces the growth ability of Panc-1 pancreatic cancer cells, but increases the basal invasiveness of these cells, suggesting an important role of this enzyme in pancreatic cancer progression.

P25

Bleeding Pseudoaneurysms: Effectiveness of Pancreatic Resection
E. Zdanyte, A. Sileikis, S. Jurevicius, K. Strupas
Center of Abdominal Surgery, Vilnius University Hospital ‘Santariskiu Klinikos’, Vilnius, Lithuania

Introduction: Optimal treatment of bleeding peripancreatic pseudoaneurysm remains controversial. No evidence-based guidelines are currently available regarding the optimal treatment modality. This study was undertaken to survey the outcome after treatment of bleeding visceral artery pseudoaneurysms associated with acute or chronic pancreatitis, pancreatic pseudocysts or pancreatic tumours.

Methods: 23 patients (20 male, 3 female; mean age 48.8, range 34–75 years) were treated because of bleeding pseudoaneurysms between January 1995 and December 2005. Retrospective analysis was performed in 4 patients treated from 1995 to 2000, and prospective evaluation of 19 patients treated from 2001 was conducted. Data regarding clinical presentation, radiological investigations, etiology and morphological characteristics of pseudoaneurysms, pancreatic pathology and operative procedures were collected. Preoperative diagnoses were compared with postoperative and histological findings.

Results: Chronic pancreatitis was diagnosed in 19 (82.6%) cases, acute pancreatitis – in 3 (13.4%), 12 of them were complicated...
by pancreatic pseudocysts. Neuroendocrine carcinoma was detected postoperatively in 1 (4.3%) patient. Bleeding developed because of splenic artery pseudoaneurysms in 12 cases, pancreaticoduodenal superior/inferior artery – in 6, small aortic branches – in 3, gastroduodenal artery – in 1 and cystic artery – in 1 case. Active bleeding was present in 12 patients, whereas others had evidence of recent hemorrhage. Two bleeding episodes developed and pseudoaneurysms were diagnosed after endoscopic transmural drainage of pancreatic pseudocysts. All patients underwent surgery because of haemodynamic instability or pancreas-related pathology: distal pancreatic resection with splenectomy (9), pancreaticoduodenal resection (6), duodenal preserving pancreatic head resection (3), segmental pancreatic resection (1), ligature of bleeding vessel (3), splenectomy (1). Two patients required subsequent operations because of several re-bleeding episodes after ligature of bleeding vessel. There were no re-bleedings after uncomplicated pancreatic resections. Overall morbidity rate was 21.7%, mortality rate 8.7%.

**Conclusion:** Pancreatic resection is effective in the treatment of bleeding visceral aneurysms with underlying pancreatic pathology.

---

**P26**

**Surgical Treatment of Pancreatic Pseudocysts in Alcoholic Pancreatitis**

V. Yudin, L. Gostev, M. Stavtsev

Surgical department #1, Municipal Clinical Hospital #11, Ryazan, Russia

Treatment of progressive cysts is surgical. We have operated 67 patients with chronic alcoholic pancreatitis. Different modifications of internal cyst drainage have been used. We prefer to perform transcutaneous pancreatojjunostomy. This technique has a copyright #2180530 (obtained 14.05.1999).

Technique essence is in maximum shortening of fistula length. Performing of extremely short anastomosis between duct rupture area and intestine is a technique basis. Cyst cavity, deprived of pancreatic juice, heals on its own. Single-layer matress suture gives a possibility to form pancreatointestinal fistula safely and to eliminate pathologic hypertension in duct system of pancreas.

We have an experience of draining endoscopic cyst in 5 patients; this procedure has been successful and has not required performing other internal pancreatointestinal anastomosis in future. External cyst draining has not been used because of danger of infection, bleeding and forming of external pancreatic fistula. As a result of investigations we have obtained positive results of treatment of patients with pseudocysts of pancreas. Maximum monitoring term of patients, done after performing transcutaneous pancreatoenterostomy, is 6 years. Disease relapse has been noticed in 3 patients. The causes have been as follows: development of cancerous lesion in pancreas, obstruction in the small intestine due to adhesion development, acute pancreatitis due to alcoholism accordingly.

We consider that it is necessary to use transcutaneous pancreatointestinal internal draining as treatment of pancreatic pseudocysts (having a tendency to growth). This method is an alternative to methods described earlier. The benefit of this method is optimum suppression of pathologic intraduct hypertension in alcoholic pancreatitis.

---

**P27**

**Videoscopic Assisted Retroperitoneal Debridement in Infected Necrotising Pancreatitis as a Pilot Study to Introduce a Randomised Controlled Trial**

H.C. van Santvoort, M.G.H. Besselink, T.L. Bollen, M.S. van Leeuwen, B. van Ramshorst, H.G. Gooszen

University Medical Center Utrecht, 1Department of Surgery, 2Department of Radiology, 3Department of Radiology, St. Antonius Hospital Nieuwegein, 4Department of Surgery, 5Dutch Acute Pancreatitis Study Group, The Netherlands

**Introduction:** The current standard for intervention in patients with infected necrotising pancreatitis (INP) is necrosectomy by laparotomy. Mortality and morbidity remain high. As an alternative, videoscopic assisted retroperitoneal debridement (VARD) was introduced as a minimally invasive treatment strategy. No randomized controlled trial (RCT) has yet compared a minimally invasive strategy with laparotomy in INP.

**Methods:** In case of (suspected) INP a retroperitoneal percutaneous drain is placed in the (peri-) pancreatic collection, preferably at least 30 days after onset of disease. If surgery cannot be obviated after a maximum of two percutaneous drainage procedures, the collection is approached via a 5 cm subcostal incision using the drain as guidance and videoscopic assisted debridement is performed. Patients in whom retroperitoneal access is not possible undergo laparotomy. As a prelude to a RCT we analyzed the first patients treated with VARD in the period April 2001 to September 2003.

**Results:** A total of 13 out of 24 patients with INP underwent VARD. Nine complications occurred in 7/13 patients (54%). An additional laparotomy was needed in 4/13 patients (31%). One patient (8%) died. Median preoperative hospital stay was 41 days (range 1–90), total hospital stay 100 days (range 41–240).

PANTER (pancreatitis, necrosectomy vs. minimally invasive step-up approach) is a RCT in which patients with (suspected) INP are randomly allocated to maximal necrosectomy by laparotomy or percutaneous drainage, if necessary followed by VARD. Primary endpoint is the total of major morbidity and mortality. Patients will be allocated from 24 hospitals of the Dutch Acute Pancreatitis Study Group in a 3-year period.

**Conclusion:** Our initial experience indicates that VARD is a feasible technique in a proportion of patients with INP that needs further definition. PANTER is the first RCT to compare a minimally invasive treatment strategy with conventional necrosectomy by laparotomy.

---

**Conclusion:** Pancreatic resection is effective in the treatment of bleeding visceral aneurysms with underlying pancreatic pathology.
P28

Genetic Alterations in Intraductal Papillary Mucinous Tumors of the Pancreas

B. Tihanyi1, T.F. Tihanyi1, L. Nehéz1, K. Díofafy2, J. Lüttges3, Å. Andrén-Sandberg4

11st Department of Surgery, Semmelweis University, Budapest, Hungary; 2Institute of Pathology Christian-Albrecht’s University of Kiel, Germany; 4Stavanger University Hospital, Bergen University, Stavanger, Norway

The intraductal papillary mucinous tumor, IPMT, is one of the mucin producing tumors of the pancreas and thought to originate in the Wirsung-duct or its collateral branches. Recently, intraductal papillary mucinous tumors have attracted attention and the number of reported cases is rapidly increasing. Despite of the more frequent reporting, the natural history of this disease is discussed. While almost 25% of IPMTs are associated with an infiltrating adenocarcinoma capable of regional and systemic metastasis, the biology of these tumors affords patients a relatively good prognosis after surgical resection compared with their counterpart ductal adenocarcinomas.

The favourable clinical behaviour of IPMTs attracts interest into the potential genetic and epigenetic factors that foster this tumor’s evolution and progression from pancreatic ductal epithelium. The knowledge of the genetic alterations in intraductal papillary mucinous tumors is limited.

We present the review of the world literature, where we found frequent LOH at 6q (54%), 8p (31%), 9p (62%), 17p (38%), and 18q (38%). Activating point mutations in the K-ras oncogene have been reported in 60–80% of the malignant IPMT cases, HER-2neu overexpression in 70–80%, the loss of p16 in 100%, loss of Smad-4 in 10–40% of invasive IPMTs, and the accumulation of the p53 gene product in 50–60%. In pancreatic juice K-ras point mutations were detected all of 12 patients, whereas p53 mutations were detected in 42%.

The presented results of the same genetic alterations of the invasive ductal adenocarcinoma showed significant differences (p < 0.005) between the IPMTs and the ductal adenocarcinomas in the following genes: cdx2, Smad4-loss, Dpc4-expression, more than 2 K-ras mutation, p27.

Conclusion: A summary of the world litterature on IPMT give some clues also to the epigenetics of this tumor, which may lead to better diagnosis and treatment options.

P29

Morphological Expression Pattern of PDX-1 and SHH in Human Pancreatic Cancer

S. Stintzing1, K. Quint2, E.G. Hahn2, C. Herold3, O. Dietze1, M. Ocker1, D. Neureiter1

1Institute of Pathology, Paracelsus Private Medical University Salzburg, Austria; 2Department of Medicine I, Friedrich-Alexander-University Erlangen-Nuernberg, Germany

Aims: There is evidence that transcriptional factors of embryonic development are essentially involved in human pancreatic carcinoma. The aim of our study was to investigate the morphological expression pattern of duodenal homebox gene 1 (PDX-1) and sonic hedgehog protein (SHH) in human pancreatic cancer.

Material and Methods: Tumors of patients being resected for invasive ductal adenocarcinoma (UICC pT3 pN0/1) and having a complete 5 year follow-up data were characterized with histochemy (HE) and immunohistochemistry (PDX-1 and SHH) on consecutive slides. The distribution of PDX-1 and SHH was compared with morphological patterns of the pancreatic carcinoma and pancreatic intraepithelial neoplasia (PanIN) as well as with reactive reaction of normal pancreata.

Results: The expression of PDX-1 was found to be distinct and to a greater extend in ductal tumor formations than in solid and dissociated tumor formations. In all tumors only some tumor cells at the invasion front showed positivity for SHH. Furthermore we could see a positive association of PDX-1 and the grade of PanINs. Upregulation of PDX-1 and SHH could be observed in areas of extended pancreatic fibrosis and atrophy. In normal pancreas primarily endocrine cells express PDX-1 and SHH.

Conclusion: The study showed that morphology patterns of human ductal pancreatic carcinoma are associated with PDX-1 expression and not with SHH. Finally, high grade intraepithelial carcinoma showed the strongest expression of PDX-1.

P30

Is Obesity a Risk Factor for Severe Alcoholic Acute Pancreatitis?

D. Štimac, I.K. Zrnić, M. Šmin, M. Novak, S. Milić

Clinical Hospital Rijeka, Rijeka, Croatia

Introduction: Obesity has been associated with worse prognosis in acute pancreatitis (AP) favouring the development of complications. We aimed to evaluate the association between obesity and severity of pancreatitis according to Atlanta criteria in patients with the first attack of alcoholic AP.

Patients and Methods: We reviewed medical records of 584 patients with the first attack of AP admitted to our hospital centre during the last ten years. Among them, 106 patients had acute pancreatitis of alcoholic etiology. Those patients were classified according to the body mass index (BMI) in normal weight (BMI <25 kg/m²) and overweight (BMI ≥ 25 kg/m²) group consisting of 51 and 55 patients respectively.

Results: In a normal weight group 54% (28/51) of patients developed severe AP, while in an overweight group 45% (25/55) of patients presented with the severe form of disease. Accordingly, in patients with alcoholic etiology of AP the risk for development of severe AP was significantly decreased following increase of BMI (relative risk (RR) 0.55; 95% confidence interval (CI) 0.41–0.74).

Conclusion: Although obesity is considered as a risk factor for development of severe AP, our study showed that increased BMI in a group of patients with alcoholic etiology decreases that risk.
Introduction: The incidence and prevalence of acute and chronic pancreatitis seems to be increasing in the Western countries. We investigated the trends in hospital admissions for acute and chronic pancreatitis from 1992 to 2004 and calculated the predicted admissions up to 2010.

Methods: We retrospectively analyzed hospital discharge data accumulated by Pris mant Health Care Information. This organization collects data on all hospitalizations in the Netherlands. All primary admissions for acute and chronic pancreatitis (ICD-9: 577.0 and 577.1) from 1992 to 2004 were identified. For trend analysis, linear and curvilinear regression models were used. During prediction the model with the highest R² was chosen, unless more conservative estimates were attained with a simple linear model not below 5% of the highest R².

Results: In 1992 were 1,785 admissions for acute pancreatitis registered. In 2004 this number raised to 3,120, representing a 75% increase. The linear regression model (R² = 97.1%) predicted 3,344 (95% CI 3,130–3,557) and 3,680 (95% CI 3,444–3,916) admissions for 2007 and 2010, respectively. This reflects a further increase for acute pancreatitis by an additional 18% in 2010 compared to 2004.

In the same 12 years the admissions (>1 day) for chronic pancreatitis showed an 75% increase (from 790 to 1,386 admissions). The cubic regression model fitted best (R² = 91.3%) for the description of the trend in admissions from 1992 to 2004. Using this model, the predicted admission rate for respectively 2007 and 2,010 are 2,246 (95% CI 1,708–2,785) and 3,830 (95% CI 2,446 to 5,234) admissions. The number of single day admissions from 1992–2004 almost tripled from 37 to 105 admissions (184% increase).

Conclusions: Hospital admissions acute and chronic pancreatitis have increased substantially from 1992 to 2004. This trend will probably continue for the near future. Consequently, it can be anticipated that the burden and costs of pancreatitis admissions to the Dutch health care system will further increase.
assessed with a scoring system that includes clinical symptoms and treatment options of chronic pancreatitis. Finally, the new classification system successfully categorized our cohort of patients with chronic pancreatitis according to the disease etiology, the stages and the severity of the disease.

**Conclusions:** The M-ANNHEIM multiple risk factor classification system is simple, objective, accurate and relatively noninvasive and incorporates etiology, different stages of the disease and various degrees of clinical severity. This new classification will be helpful in investigating the impact and interaction of various risk factors on the course of the disease. This system will also facilitate the comparison and combination of interinstitutional data.

---

**P34**

**Human Cationic Trypsinogen (PRSS1) and Trypsinogen Inhibitor Gene (SPINK1) Mutational Screening in a Finnish Hereditary Pancreatitis Family**


1Department of Gastroenterology and Alimentary Tract Surgery, 2Department of Radiology, Tampere University Hospital, Tampere, 3Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Finland

**Background:** A mutation in the third exon (R122H) of the cationic trypsineogen gene (PRSS1) has been associated with hereditary pancreatitis (HP). Few other additional mutations outside the third exon of the PRSS1 gene have also been found to be linked with HP. The most frequent mutations of PRSS1 are N29I and R122H in exon 2 and 3, respectively. R122H mutation has been identified in families with HP in France, Germany, UK, Japan and USA in 52% of patients, N29I in 21% of patients, and the less frequent mutation A16V in 4% of patients. In addition, a mutation (N34S) in the serine protease inhibitor Kazal type 1 gene (SPINK1) has been shown to be associated with HP. In India up to 73% of patients with HP are reported to have this mutation. No previous data on the mutation frequency in the PRSS1 gene has been reported in Finnish or in other Scandinavian population.

**Patients and Methods:** Forty individuals from one Finnish family with HP were initially enrolled in this study. Four died leading to 36 individuals in the final analysis (21 males; 15 females; median age of 38 years). All individuals underwent ultrasound and laboratory tests (glucose, faecal elastase-1 test). Blood samples were taken for mutational analysis of PRSS1 (R122H, N29I and A16V) and SPINK1 (N34S).

**Results:** Ten (28%) individuals of the family were affected by mutation: the most frequent mutation was R122H, affecting 8 (22%) individuals, 2 (6%) individuals were affected by the N34S mutation and none by the other tested mutations (N29I or A16V). Four out of 8 (50%) R122H positive individuals had diagnosed chronic pancreatitis without other known aetiologies compared to 0 out of 28 mutation negative individuals, p = 0.001. Interestingly, 4 out of 5 (80%) male individuals with R122H mutation had also clinical pancreatitis, whereas none (0/3) of the mutation positive females had any signs or symptoms of chronic pancreatitis.

**Conclusions:** In the investigated Finnish family with HP, the PRSS1 mutation R122H seems to be linked with the disease. Although SPINK1 (N34S) was also observed in two individuals, it was not linked with the disease. The penetrance of the disease was high in males having R122H mutation.

---

**P35**

**Risk of Pancreatic Cancer and Hereditary Pancreatitis: the First National Cohort in France**

V. Rebours, M.C. Boutron-Ruault, C. Férec, M. Schnee, P. Hammel, P. Ruszniewski, P. Lévy, Association des Pancréatites Chroniques Héréditaires (APCH)

1Department of Gastroenterology, Beaujon Hospital, Clichy, 2Gustave Roussy Institute, Unité Inserm E3N, Villejuif, 3Inserm-0115, Génétique Moléculaire et Génétique Epidémiologique, Etablissement Français du Sang-Bretagne, Université de Bretagne Occidentale, Brest, 4Department of Gastroenterology, La-Roche-Sur-Yon, France

Hereditary pancreatitis (HP) is caused by a mutation in the cationic trypsinogene gene (PRSS1). Chronic pancreatitis (CP) is a known risk factor of pancreatic adenocarcinoma (PA).

**Aim:** To evaluate the incidence of the pancreatic cancer in an exhaustive French national series of patients with HP and to search for risk factors.

**Patients and Methods:** A cohort comprising all French HP patients was constituted by contacting all French gastroenterologists and pediatricians (response rate: 84%) and genetics laboratories. Inclusion criteria were the presence of PRSS1 gene mutation, or chronic pancreatitis in at least 2 first-degree relatives, or 3 second-degree relatives, in the absence of another cause of pancreatitis (notably, alcohol). Diagnosis of PA was based on histological examination.

**Results:** The cohort included 78 families and 200 patients (patients). It represented 6,673 person years (males: 53%, tobacco intoxication: 34%). At time of inclusion, the median age was 30 years (extremes: 1–84). PRSS1 mutations were detected or not by 68% and 32% respectively (R122H: 78%, N29I: 12%, others: 10%). PRSS1 inheritance was maternal for 65%, 10 PA were diagnosed (6 males) in a median age of 55 years (extremes: 39–78). The SIR of PA for the cohort, the males, and the females was 87 (42–113), 69 (25–150) and 142 (38–225) respectively. At the age 50, 60 and 75 years old, the cumulated risk of PA was 11%, 16% and 49% by males respectively and 8%, 22% and 55% by females. The risk of PA by smokers at age 75 years old increased at 61% and 70% for the males and the females.

**Conclusions:** Patients with HP have a markedly increased risk of pancreatic cancer compared with the general French population (SIR:87). There is no correlation between this risk and the PRSS1 genetic status but the PA is highly linked with the tobacco intoxication, the diabetes mellitus and the absence of acute pancreatitis.
P36

Role of D-dimer in Early Prediction of Severity in Acute Pancreatitis

D. Radenkovic, D. Baijac, N. Ivanovic, N. Milic, A. Karamarkovic, V. Jeremic, P. Gregori, V. Djukic, B. Stefanovic

1Center for Emergency Surgery, Clinical Center of Serbia and School of Medicine, 2Institute for Medical Statistics, School of Medicine, University of Belgrade, Serbia and Montenegro

Introduction: Clinical studies in acute pancreatitis (AP) suggest activation of haemostatic system. Early, accurate and reliable prediction of severity in AP is very important in the clinical practice. Aim of this prospective study was to assess whether early haemostatic abnormalities play a role in prediction of severity in AP.

Methods: From January 2004 until April 2005 a total of 91 patients with AP treated in our Institution were included. Measurement of coagulation, anticoagulation and fibrinolysis parameters: prothrombin time, activated partial thromboplastin time, fibrinogen, antithrombin III, protein C, plasminogen activator inhibitor-1, d-dimer, a2-antiplasmin and plasminogen were done on admission and 24h after. The severity of AP was defined according to the Atlanta classification system. At the end of study, groups of 33 patients with severe and 58 patients with mild form of disease were compared.

Results: On admission, the mean d-dimer concentration was 682 µg/l (sd. 460) for severe and 258 µg/l (sd. 182) for mild disease (p < 0.001). On first day after admission respective values were 835 µg/l (sd. 428) and 336 µg/l (sd. 206 p < 0.001). The sensitivity, specificity, positive predictive, and negative predictive values of the test to show severe AP compared with mild AP on admission (>414 µg/l or 1.7-fold higher than upper limit), were 73%, 91%, 83%, and 82% respectively, and 24h after admission (>525.50 µg/l or 2.1-fold higher than upper limit), were 76%, 82%, 72%, and 82%. The levels of all other measured parameters were significantly different between the patients with severe and mild AP, but all values were within physiological limits.

Conclusions: Prediction of severity is already possible on patients admission to the hospital with high sensitivity and specificity. D-dimer as single test parameter significantly contributes to an improved stratification of patients at risk to develop severe AP and deserves routine clinical application.

P37

The Plasma Renin-Angiotensin System in Human Acute Pancreatitis

R. Pezzilli, B. Barakat, L. Fantini, A. Timpano, A.M. Morselli Labate, R. Connaides

1Department of Internal Medicine and Gastroenterology, 2Department of Emergency, Sant’Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

Background: Activation of the local pancreatic renin-angiotensin system (RAS) has been reported in experimental acute pancreatitis (AP) and no data are available in humans.

Aim: To evaluate the plasma RAS in AP. Patients. Twenty-one patients with AP (13 M, 8 F; mean age 57.9 years, range 20–84) were studied within 24 h of pain onset. None of the patients had arterial hypertension or other known diseases nor were they taking drugs able of modifying the RAS. According to the Atlanta criteria, 14 patients (66.7%) had mild AP and seven (33.3%) the severe AP.

Methods: In all patients, plasma renin activity (reference range 0.2–2.8 ng/ml/h), plasma angiotensin I converting enzyme activity (reference range 65.8–114.4 U/L) and plasma aldosterone concentration (reference range 33–489 pg/ml) were determined immediately after hospital admission using commercial available kits. Serum amylase and lipase activities were also determined. Statistical analysis was carried out using the Mann-Whitney and the Spearman rank correlation tests.

Results: Mean ± SD plasma renin activity, angiotensin I converting enzyme activity, and aldosteron concentration were 0.73 ± 0.84 ng/ml/h, 56.8 ± 30.4 U/L, and 92.2 ± 112.8 pg/ml, respectively.

In particular, the plasma renin activity was above the reference range in one patient (4.8%); the plasma angiotensin I converting enzyme activity was above the reference range in one patient (4.8%) and below the reference range in 15 patients (71.4%); plasma aldosteron concentration was below the reference range in 5 patients (23.8%). No significant relationship was found between serum amylase or lipase activities and plasma renin activity, plasma angiotensin I converting enzyme activity or aldosteron concentration. Furthermore, no significant relationship was found between plasma renin activity, angiotensin I converting enzyme activity and aldosteron concentration and the severity of the disease.

Conclusions: The RAS may be impaired in patients with AP and it does not seem to be related to the severity of the disease.

P38

Enucleation of Endocrine Pancreatic Tumors: 25-Year Experience

S. Pedrazzoli, C. Pasquali, C. Sperti, S. Scappin, G. Liessi

1Department of Medical and Surgical Sciences, Clinica Chirurgica IV, University of Padua, Padova, 2Department of Radiology, Castelfranco Veneto Hospital, Treviso, Italy

Introduction: Enucleation of pancreatic endocrine tumors (PETs) is a simple procedure, but an high pancreatic fistula rate is reported after open and laparoscopic [1] procedure. We report a retrospective evaluation of our series of PETs.

Methods: From 1980 to 2004, 109 patients underwent surgery for PET. Enucleation was performed in 33 patients, while 76 underwent several different procedures; 24 (73%) were insulinoma. Age, sex, site and size of the tumor, associated diseases, hospital stay and complications were retrospectively reviewed by the clinical records. Since 1986 intraoperative ultrasonography (IOUS) was used to choose among different surgical procedures and to verify that the Wirsung duct was intact at the end of the procedure.

Results: Patients (12 males, 21 females) averaged 56.8 years, range 20–86 years. Mean size of the tumor was 1.7 cm and 54.5% were in the pancreatic head; 78.8% of cases had medical associated diseases. Overall hospital stay averaged 12 days (range 6–81 days) and it was reduced to 8.9 days in the last 5 years. Mean period of
gastric suction was 4 days. Sixty percent had an uneventful postoperative course. Complications related to pancreatic surgery were 6/33 (18.2%); 5 pancreatic fistulas (4 low output) and 1 acute pancreatitis, while 5/33 had a general surgery complication (2 leaking due to gastric and duodenal associated operations). Medical complications were recorded in 7 cases. No patient underwent surgery for pancreatic complication; No mortality occurred. Of notice, the fistula rate of the 24 insulinoma was 8% (2/24), and no fistula was registered since 1986 among 17 insulinoma patients in whom IOUS was applied.

Conclusions: In our experience enucleation of PETs can be performed with a very low pancreatic fistula rate provided that IOUS is performed.

Reference:

---

P39

Role and Indications of Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) in Neuroendocrine Pancreatico-Duodenal Tumors

C. Pasquali¹, C. Sperti¹, S. Scappin¹, F. Chierichetti², G. Liessi², S. Pedrazzoli¹

¹Department of Medical and Surgical Sciences, Clinica Chirurgica IV, University of Padua, Padova, ²PET Center Nuclear Medicine, ³Department of Radiology, Castelfranco Veneto Hospital, Treviso, Italy

Introduction: Neuroendocrine pancreatic-duodenal tumors (NPDT) detection by imaging may be a problem. Furthermore, few studies reported the ability of FDG-PET to differentiate benign from malignant NPDT. Aim of this study was to evaluate the role of FDG-PET in patients with NPDT.

Material and Methods: From 1994 to 2004, 70 patients 33 male and 37 female, (mean age 52.2 years, range 18–84) with NPDT (82.8% pancreatic) were investigated with FDG-PET. PET results were compared with CT-scan, MRI, Octreoscan scintigraphy and clinicopathologic features of patients and survival.

Results: Overall PET sensitivity was 55.7%; 79% of malignant tumors, and 17% of benign tumors were detected by FDG-PET. No duodenal tumor was detected by PET scan. Only 16% of primary less than 2 cm in size was localized. In 17.9% of cases PET scan provided new informations able to change therapeutic management. In PET positive patients the additive informations obtained by PET scan when compared with Octreoscan, MRI and CT scan were respectively 40.5% more, 21.7% more and 25.6% more. In malignant neuroendocrine tumors PET positivity was related to short survival. No patient with PET negative malignant tumor died for disease progression in the follow, while 13/30 with PET positive malignant tumor died (p < 0.03).

Conclusions: FDG-PET proved to be a second line technique in NPDTs. PET results improve clinical staging of disease and is related to survival in malignant cases; in 17% of cases may change the therapeutic option. From our experience FDG-PET should be reserved for patients with suspected malignant tumor or pancreatic mass more than 2 cm or MEN 1 cases with at least one visible lesion. PET is useless in duodenal tumors, benign insulinomas and small single pancreatic neuroendocrine lesion.
undergo pseudocyst drainage following an EUS examination; 2 were too small to drain, and in 1 the cyst wall > 1 cm. Cystgastrostomy was performed in 25; 4 with a duodenoscope without EUS assessment, 9 with a radial EUS assessment followed by drainage using a duodenoscope and 12 with EUS assessment and subsequent drainage using a therapeutic linear echoendoscope. The site of drainage was gastric body in 18, antrum in 2, cardia in 1 and duodenum in 1. Endoscopic drainage was successful in 22 of the 25 patients (88%). Nine had complete resolution after one procedure (41%) while 13 required multiple procedures (59%). Of the remaining 3, 1 required an additional percutaneous radiological procedure and 2 required surgical cystgastrostomy for complete resolution. Following the initial endoscopic procedure complications occurred in 7 of the 22 patients (32%) within 30 days. Puncture site bleeding in 3, pancreatitis in 1, secondary cyst infection in 2 and pneumonia in 1. All complications were managed conservatively and there were no deaths. Median follow-up period is 14.5 months. There were no late complications.

Conclusions: Endoscopic cystgastrostomy is an effective treatment for PP in carefully selected individuals. However this is associated with a 32% complication rate.

P42
Clinicopathological Analyses on Surgically Resected Intraductal Papillary Mucinous Neoplasm of the Pancreas
K. Nagai, A. Kida, K. Kami, D. Ito, M. Kawaguchi, R. Doi
Department of Surgery and Surgical Basic Science, Kyoto University, Japan

Introduction: Intraductal papillary mucinous neoplasm (IPMN) is a mucin-producing neoplasm of the pancreas with cystic dilatation of main and/or branch pancreatic duct. Although IPMN is becoming increasingly recognized, the indication for resection of IPMN is still controversy. The aim of this study was to analyze clinicopathological features of surgically resected IPMNs.

Methods: We reviewed 56 patients who underwent surgical resection for IPMNs between 1984 and 2006 in our hospital.

Results: A total of 56 cases, 36 males and 20 females aged between 31 and 85 (median 62.4) were histologically diagnosed as invasive IPMN (n = 20), and non-invasive IPMN (n = 36) including adenoma (n = 16), borderline (n = 8) and carcinoma in situ (n = 12). 70% of the all cases were symptomatic. Although the diagnostic accuracy of endoscopic ultrasonography was very high, it was difficult to distinguish non-invasive IPMNs from invasive IPMNs. Five-year overall survival rates of invasive and non-invasive IPMNs were 57% and 95%, respectively (p = 0.012, log-rank test).

Conclusions: IPMN is thought to have malignant potential and transform from non-invasive IPMN to invasive cancer. The prognosis of invasive IPMNs in the current series was significantly worse than that of non-invasive IPMNs. Because preoperative differentiation of invasive IPMN from non-invasive IPMN was difficult even with the combination of diagnostic imagings, resection aiming cure should be undertaken once a patient with IPMN is indicated for surgery.

Disialotransferrin, Determined by Capillary Electrophoresis, is an Accurate and Rapid Test for Detecting Alcoholic Cause of Acute Pancreatitis
T. Methuen¹, M.-L. Kylänpää², O. Kekäläinen¹, T. Halonen³, E. Tukiainen², S. Sarna⁴, P. Puolakkainen², E. Kemppainen², R. Haapiainen², M. Salaspuro¹
¹Research Unit of Substance Abuse Medicine, ²Department of Surgery, Helsinki University Central Hospital, Helsinki, ³Department of Clinical Chemistry, Kuopio University Hospital, Kuopio, Finland; ⁴Department of Public Health, University of Helsinki, Helsinki

Introduction: Determination of serum disialotransferrin, a specific marker of heavy alcohol consumption, by capillary electrophoresis, is simple, rapid, reproducible and inexpensive. We tested its accuracy in detecting alcoholic cause of acute pancreatitis (AP) by comparing it to AST, ALT, MCV, plasma amylase, CRP and bilirubin.

Methods: Serum samples from 271 consecutive AP patients, admitted to the Helsinki University Central Hospital emergency unit, were analysed.

Results: The median (range) disialotransferrin value was significantly higher (p = 0.001) in AP patients with alcoholic (n = 172) 1.6% (0.3–14.4)% than with biliary (n = 60) 0.7% (0.3–1.3)% or other causes (n = 39) 0.8 (0.3–4.1)%). In ROC analysis, disialotransferrin, as a single analyte, was significantly (p = 0.001–0.0001) more accurate (0.88, from 0.84 to 0.92; AUC, 95% CI), in detecting alcoholic AP as compared to AST (0.57, from 0.51 to 0.63), ALT (0.63, from 0.57 to 0.69), MCV (0.72, from 0.67 to 0.78), amylase (0.74, from 0.67 to 0.78), CRP (0.65, from 0.59 to 0.71) and bilirubin (0.55, from 0.49 to 0.62). Multivariate modelling did not increase its clinical utility. At a disialotransferrin cut off of 1.2%, giving an 8% false positive rate, the positive likelihood ratio was 8.47. Thus, a positive disialotransferrin test at a disialotransferrin cut off of 1.2%, giving an 8% false positive rate, is accurate and rapid single biomarker of the alcoholic cause of acute pancreatitis. This implies that disialotransferrin would be of value in routine diagnostics and, therefore, warrants further testing for its clinical application.

Outcome of Patients Presenting with Suspected Pancreato-Biliary Malignancy
S.D. Mansfield¹, R. Lochan¹, K. Jaques², J. Scott², M.K. Bennett³, C.B. O’Sullivan³, B.C. Jaques¹, D.M. Manas⁴, R.M. Charney¹
¹Hepato-Pancreato-Biliary Surgery, ²Radiology and ³Pathology, Freeman Hospital, Newcastle-upon-Tyne, UK

Methods: Over a 4 year period from October 2000, prospective data was collected on patients referred to our centre with suspected
neoplasia of the pancreas, ampulla, duodenum or lower bile duct. Modes of presentation and treatment were recorded. Final diagnosis was determined histologically, cytotologically or in a minority, by a combination of overwhelming clinical, radiological and biochemical information. Positive histology or cytology was not mandatory prior to resection.

**Results:** Of 493 patients, a final diagnosis of malignancy was present in 430 (87.2%): Ductal carcinoma in 294 (59.4%), ampullary carcinoma in 61 (12.4%), duodenal carcinoma in 13 (2.6%) and carcinoma of the lower bile duct in 34 (6.9%). Of 63 patients without malignancy, 16 had benign tumours and 47 pancreatitis, epithelial hyperplasia, stone disease or no detectable abnormality on further imaging. Of 321 (65.1%) patients presenting with obstructive jaundice, malignancy was present in 299 (93.1%). Of the remaining 172, malignancy was present in 131 (76.1%; p < 0.001 Chi-squared test). Of 112 patients with a distal bile duct stricture undergoing resection, malignancy was present in 100 (89.3%). Of the 12 remaining, 4 had benign tumours and the remainder inflammatory conditions.

In total, 170 patients underwent resection (34.5%). Of ductal carcinomas of the pancreas 62/294 (21.2%) were resected, of ampullary carcinomas 32/61 (52.5%), of duodenal carcinomas 8/13 (61.5%) and of distal bile duct cancers 20/34 (58.8%). Of 16 pancreatic cystic tumours (10 malignant), 10 (62.5%) were resected (4 malignant). Of the 27 patients who underwent resection and had benign disease, 14 had benign neoplasms and 13 had inflammatory conditions or epithelial hyperplasia (7.6% of all resections).

**Conclusions:** Of patients referred with suspected pancreaticobiliary malignancy, 87.2% actually had a malignant tumour of which only 68.4% were ductal pancreatic adenocarcinomas. In the presence of a distal bile duct stricture, the chance of malignancy was 93.1%.

---

**P46**

**Endoscopic Ultrasound (EUS) for Etiological Evaluation of Idiopathic Acute Pancreatitis (IAP)**

**J. Lariño-Noia, S. Seijo-Ríos, J. Iglesias-García, M. Vilariño, J.E. Domínguez-Muñoz**

Department of Gastroenterology, University Hospital of Santiago de Compostela, Spain

**Background:** EUS, by accurately visualizing the pancreatic parenchyma and ducts, and the biliary tree, may provide with important etiological information in patients with IAP.

**Aim:** To evaluate the usefulness of EUS for identifying the potential etiological factor in patients with IAP.

**Methods:** A prospective, consecutive and descriptive study was performed. Among 228 consecutive patients diagnosed with acute pancreatitis (AP) over one year, 24 patients (mean age 56 years, range 20–89, 14 female) were classified as IAP and included in the study. An episode of AP was considered as idiopathic if gallstones, chronic pancreatitis, pancreatic tumours, alcohol abuse, pancreaticotropic drug consumption, and metabolic and renal disorders were excluded by means of a careful clinical history, haematological and biochemical analysis, and abdominal ultrasound. EUS was performed after recovering from AP by lineal and/or radial scanning Pentax FG-38UX and EG-3630UR echoendoscopes. Biliary and pancreatic abnormalities potentially causing AP were evaluated.

**Results:** Time average between recovery from AP and the performance of the EUS was 14 weeks (range 1–26 weeks). Patients have had a median of 2 episodes of AP (range 1–5 episodes). EUS revealed a potential etiological factor of AP in 16 patients (67%), among them biliary microlithiasis (n = 6, 25%), choledocolithiasis (n = 4, 17%), chronic pancreatitis (n = 4, 17%), and pancreatic tumour (n = 2, 8%). EUS did not detect any abnormality in 8 patients (33%).

**Conclusion:** EUS is an accurate technique for the etiological evaluation of IAP, which is able to identify the potential cause of the disease in two out of each third patient. Inclusion of EUS in the etiological diagnostic algorithm of AP should be thus recommended.
The Role of Selective Mesenteric Angiography in the Initial Management of the Vascular Complications of Acute Pancreatitis


1Departments of Surgery and 2Radiology, Glasgow Royal Infirmary, Glasgow, UK

Introduction: The vascular complications of acute pancreatitis are rare, can be associated with significant mortality and optimal management remains uncertain.

Methods: A retrospective review of patients admitted with acute pancreatitis who had undergone mesenteric angiography from 1998 to 2005.

Results: Records for 32 patients were available for analysis. The median age was 54 years (range 33–73) and 62% were male. The aetiology of the acute pancreatitis was gallstones (n = 12), alcohol (n = 12), idiopathic (n = 7) and post-ERCP (n = 1). Twenty-one patients had intervention prior to angiography, most commonly percutaneous or open necrosectomy. Mesenteric angiography was performed in 29 (91%) patients because of acute bleeding (abdominal drains, GI tract, haemodynamic compromise). Four patients required emergency laparotomy for hypovolaemic shock at presentation and following re-bleed underwent angiography or embolisation. Twenty-five patients underwent angiography as their initial intervention. Bleeding was localised in 15 with control in 11. Of these 5 patients required repeat embolisation for re-bleeds and one of these also required surgery thereafter. Surgical intervention was necessary to control haemorrhage in 4 patients who were unsuitable for embolisation. Angiography did not localise bleeding in 10 patients (observation n = 5, laparotomy n = 4, embolisation n = 1). The remaining 3 (9%) cases had pseudoaneurysms of the splenic artery, with no evidence of bleeding, detected on radiological imaging and they underwent successful embolisation. The vessels predominantly involved were splenic and gastroduodenal arteries. Overall, there were no complications of ischaemia related to embolisation and in-patient mortality was 15.6%.

Conclusions: Selective mesenteric angiography is effective in localising the bleeding site but also allows definitive treatment by major vessel embolisation and is not associated with increased ischaemic complications. Angiography should be considered as first line therapy for mesenteric bleeding secondary to acute pancreatitis.

Low Plasma Glutamine Levels in Severe Acute Pancreatitis Justify Glutamine Substitution in Critically Ill Pancreatitis Patients


1Department of Internal Medicine, Municipal Clinic of Lueneburg, Germany; 2European Institute of Oncology, Milan, Italy; 3New York Medical College, Valhalla, NY, USA; 4University of Bonn, Germany

Glutamine stabilizes intestinal barrier function and has been shown to reduce bacterial translocation in acute experimental pancreatitis. Glutamine infusions are frequently given to critically ill patients, but in the case of acute pancreatitis, there so far has been little evidence to justify this treatment. Our aim was to assess the prognostic value of plasma glutamine levels in acute pancreatitis and to establish a rationale for glutamine substitution in these patients.

Patients and Methods: Plasma glutamine levels were measured both on admission and after 72 h in 26 patients with a first attack of acute pancreatitis. They were then considered in the context of the following parameters: age, gender, aetiology, smoking, body mass index, pain intensity, guarding and rebound tenderness, APACHE-II score on admission, Ranson and Imrie scores (72 h) as well as the result of a contrast-enhanced computed tomography (CT; Balthazar score) within 72 h. The association between plasma glutamine levels and patient characteristics was determined using the mean value recorded on admission (384 μmol/l plasma) as the cut-off value.

Results: Low levels on admission were associated only with increased intensity of pain (p = 0.026). Low levels after 72 hs were associated with old age (≥60 years; p = 0.016), increased intensity of pain (p = 0.016), rebound tenderness on admission (p = 0.005), and with a Ranson score ≥4 points (p = 0.016). A decrease of plasma glutamine levels between admission and day 3 was associated with old age (p = 0.026), biliary pancreatitis (p = 0.026), intensity of pain on admission (p = 0.049), Balthazar E4 (p = 0.034), and with both the Ranson and the Imrie score (p = 0.0076; p = 0.0147).

Conclusion: Plasma glutamine levels are decreased in severe pancreatitis which justifies glutamine substitution in such patients when either enteral or parenteral nutrition is indicated.

Epidemiology of Alcoholic Acute Pancreatitis in North Coast Region in Croatia

I.K. Zrnica, M. Radić, R. Mihovilić, K. Baraba, S. Milić, D. Štimac

Clinical Hospital Rijeka, Rijeka, Croatia

Introduction: There is a wide range in the report of incidence of alcoholic acute pancreatitis worldwide. This study was undertaken to establish current incidence, gender distribution and complications in patients with alcoholic acute pancreatitis in our region.
Patients and Methods: We performed a retrospective study of all cases with first episode of acute pancreatitis in our region admitted to clinical hospital centre during 10-year period. Patients with alcoholic etiology were analysed separately.

Results: Acute pancreatitis was associated with biliary disease in 60.7%, alcohol 17.3%, idiopathic etiology 15% and other etiologies 7%. Incidence of alcoholic acute pancreatitis was around 6/10^5 and tended to be stable during 10-year period. Considering gender distribution, there was a statistically significant predomination of men (86%). Considering age, patients with alcoholic etiology are statistically younger than patients with biliary etiology (p < 0.001). Although local complications as well as combination of local and systemic complications occur statistically more often in alcoholic than in other etiologies (p < 0.001), mortality rate is low.

Conclusion: Epidemiological characteristics of alcoholic acute pancreatitis in North Costal region in Croatia do not differ much comparing to the other Mediterranean countries. Gender distribution and occurrence of complications are also compatible to the literature data.

P50 The Analysis of Factors that Affect the Mortality Rate in Severe Acute Pancreatitis

Department of Internal Medicine, Institute of Digestive Disease and Nutrition, Korea University College of Medicine, Seoul, Korea

Introduction: Severe acute pancreatitis occurs in about 20% of patients with acute pancreatitis and can be associated with multigorgan failure and local complications. In patients with predicted severe acute pancreatitis, mortality rates is about 15–30%. The aims of our study was to determine the factors correlated with mortality in patients with severe acute pancreatitis.

Methods: We reviewed 586 consecutive cases of acute pancreatitis from January 2000 to May 2005. Of them, 90 patients who fulfilled the criteria of Atlanta classification for severe acute pancreatitis were enrolled. We collected data by chart review included age, gender, etiology, body mass index (BMI), Modified Glasgow score, APACHE II score, Balthazar CT severity index, and other laboratory parameters performed within 48 hs from initial admission. We analyze the factors that affect the mortality by univariate and multiple logistic regression analysis.

Results: Severe acute pancreatitis was most commonly caused by Alcohol (52%). Of the 90 patients, 20 patients were died and of the 20 death, 9 occurred within the first week (45%). In univariate analysis, acute renal failure (Ser >2.0), serum creatine phophokinase (>1,000 IU/l), Balthazar CT severity index (≥6) and pleural effusion were found to be significant predictors for hospital mortality (p < 0.05). Multiple logistic regression analysis of variables showed that creatine phophokinase (>1,000IU/l), Balthazar CT severity index (≥6) and serum C-reactive protein (>150 mg/l) were independently correlated with hospital Mortality (p < 0.05).

Conclusions: Balthazar CT severity index (≥6), creatine phophokinase (>1,000IU/l) and serum C-reactive protein (>150 mg/l) were the significant predictors for hospital mortality in severe acute pancreatitis.

P51 Diagnosis and Management of Autoimmune Pancreatitis: Our Experience of 6 Cases

D. Ito, K. Kami, K. Nagai, Y. Kawaguchi, R. Doi
Department of Surgery and Surgical Basic Science, Kyoto University, Kyoto, Japan

Introduction: Autoimmune pancreatitis (AIP) has recently been proposed as a novel clinical entity of idiopathic chronic pancreatitis. The increased levels of serum-IgG have often observed in AIP patients and have been speculated to associate with its etiology. Because obstructive jaundice occurs in many cases of AIP, differential diagnosis from the disease indicated for surgical resection is important. We report the diagnosis and management of 6 AIP patients experienced for recent 7 years.

Patients and Methods: Five males and one female. The mean age was 68.7 years. Diabetes mellitus was observed in all patients. Other autoimmune diseases were not observed. Patients were diagnosed by clinical findings, pancreatic/biliary imaging and laboratory data.

Results: Increased levels of serum-IgG and IgG4 were observed in 3 and 2/6 cases, respectively. Three patients showed positive anti nuclear antigen. Tumor markers (CA19-9 or DUPAN 2) were elevated in 3 cases. Dynamic contrast enhanced computed tomography demonstrated the diffusely enlarged pancreas and a capsule-like rim that shows lowdensity in all cases. F-18 fluoro-2-deoxy-D-glucose-psoitoron emission tomography showed accumulative signals in all of the lesions. Although typical pancreatography of AIP shows sclerotic stenos of whole main pancreatic duct (MPD), localized stenosis and caudal dilatation of MPD was observed in one patient. After oral administration of predonisole (PSL), abnormal findings of pancreatic/biliary imaging and laboratary data promptly improved. Although recurrent AIP was observed in 2 patients, low dose PSL therapy was also effective.

Conclusion: We experienced an AIP patient with localized stenosis and caudal dilatation of MPD, which is not a typical finding of AIP. Tumor markers were elevated in 3/6 cases. Co-existence of pancreatic cancer must be taken into consideration when making the diagnosis of AIP. Although PSL therapy is useful for diagnostic therapy in AIP patients, further re-examination or surgical resection must be required immediately when PSL is not effective.
P52
Normal Response Data for the Pancreatic Cancer Quality of Life (QoL) Assessment Questionnaire EORTC QLQ-PAN26

F. Howse1, S. Harris1, E. Hedges1, S. George1, R. Pickering1, C.D. Johnson

Department of 1Surgery and 2Health Care Research Unit, University of Southampton

Introduction: The QLQ-PAN26 is validated for measurement of health related QoL in pancreatic cancer (Fitzsimmons 1999). It can also be used, with additional questions, to assess QoL in chronic pancreatitis (Fitzsimmons 2005). This study reports reference data for responses from normal individuals.

Methods: We collected responses to the PAN26 from randomly selected normal people. Every tenth name was extracted from family doctor registers in three areas (inner city, suburb and rural town). Any person known to be suffering from cancer was excluded. A single mailing asked 300 individuals to complete and return the combined EORTC QLQ-C30 and QLQ-PAN26 questionnaires.

Results: Responses were received from 101 (33%) people, median age 39.5 (range 20–84) years, interquartile range 23–55 years. Responses to the QLQ-C30 were similar to previously published normative data (EORTC). As expected, the responses to symptom-based questions showed a skewed distribution, with most replies indicating absent or very low scores: 83% had no or little abdominal discomfort or bloating; 91% had no, or little worries about future health; worry about low weight was absent, weakness was reported by only 3%, ‘yellowness’ by 2%. Frequency of bowels affected QoL ‘quite a bit’ for 18% and ‘a lot’ for 9% of normal individuals; 13 had ‘quite a bit’ and 3 ‘a lot’ of back pain. Overall health and QoL responses showed 88% and 84% better than the mid point of the scale, median 6, normalised mean score 80.7.

Conclusions: This is the first report of norm reference data for the QLQ-PAN26. More responses are needed from individuals aged over 60, to provide a relevant comparison for pancreatic cancer patients. Normal individuals have better QoL than published findings in pancreatic cancer and chronic pancreatitis.

P53
Second Endoscopic Treatment of Chronic Pancreatitis in Case of Recurrence of Pain after a Previously Successful Endoscopic Treatment

L. Heyries, A. Guilin-Poujol, J. Sahel

Department of Gastroenterology, Hôpital Sainte Marguerite, Marseille, France

Aim: Endoscopic treatment of chronic pancreatitis (CP) improves pain in 66% of cases after a follow-up of 5 years. In case of recurrence of pain, the choice between a new endoscopic or a surgical procedure is debated.

Methods: Between 1989 and 2002, among patients presenting with a painful CP who underwent an endoscopic treatment, 8 had another endoscopic procedure for recurrence of pain: 4 males and 4 females, 26–77 years old (median 38 years). Etiology of CP was alcoholic in 7 cases and idiopathic in 1 case. Patients suffered from chronic pain (n = 4) and/or acute pancreatitis (n = 7). Main pancreatic duct stenosis was present in all cases and was associated with pancreatic stones in 6/8 cases. All cases had a pancreatic stenting with a plastic stent (7–10 Fr, median 10 Fr) during 6–22 months (median 14 months), with exchange every 4 months. In all cases, pain was improved after initial endoscopic treatment. After 5–84 months (median 18 months) another endoscopic treatment was proposed because of recurrence of pain according the same protocol. Duration of pancreatic stenting was 7–17 months (median 11 months).

Results: At the end of pancreatic stenting time, pain was improved in all cases, ductal lesions were improved in 3 cases, unchanged in 4 cases or worsened in 1 case. After a follow-up of 12–18 months (median 29 months) without pancreatic stent, 6/8 patients had no more presented painful attacks; diabetes mellitus was unchanged (n = 2) or worsened (n = 3). Tabacco and alcoholic consumption was unchanged in respectively 6/6 and 3/7 cases. Recurrence of pain was observed in 2 cases: the first patient was treated by a wirsungo-jejunostomy, the second had a third endoscopic treatment (not acceptance of surgical treatment) with good initial results but recurrence of pain after stent retrieval. In those 2 patients, age of diagnosis of CP was 26 and 29 years old, alcoholic consumption was stopped but tobacco persisted; time between retrieval of stent and recurrence of pain was 18 and 22 months.

Conclusion: A second endoscopic treatment improved pain in 6/8 patients. Except a young age of diagnosis of CP, there was no other predictive factors for long term results.

P54
The Evaluation of Limited Pancreatectomy for the Pancreatic Disease

T. Hatori1, A. Fukuda1, S. Onizawa1, N. Kanai1, T. Oohara1, K. Furkawa2, H. Matsuura2, T. Imaizumi2, K. Takasaki2

1Department of Gastroenterological Surgery, Tokyo Women’s Medical University, Tokyo, 2Department of Surgery, School of Medicine, Tokai University, Isehara, Japan

Introduction: It is possible to choose limited pancreatectomy without lymphadenectomy for the localized pancreatic disease such as branch duct IPMN or endocrine tumor. However, limited pancreatectomy has some problems including technical difficulty or higher incidence of the postoperative complications including pancreatic fistulae. The aim of this study is to evaluate the indication and significance of the limited pancreatectomies for the pancreatic diseases.

Methods: A total of 104 patients who underwent limited pancreatectomy for the various pancreatic diseases at Tokyo Women’s Medical University Hospital between 1981 and 2005 were chosen in this study. Of 104 patients, 59 patients were IPMN, 18 were endocrine tumor, 6 were Solid-pseudopapillary tumor, 6 were serous cystadenoma, 5 were MCN, 5 were benign localized stricture of the pancreatic duct and 5 were others.

Results: In 59 patients with IPMN, 44 patients (74.6%) had adenoma or borderline atypia, 14 (23.7%) patients had non-invasive...
carcinoma and one patient (1.7%) had an invasive carcinoma. Brunch duct IPMNs were seen in 40 of 59 patients (67.8%). Malignant tumors were observed in 23 of 104 patients (22.1%). Duodenum-preserving pancreatic head resection (DPHR) was performed in 13 patients, ventral pancreatectomy (VP) in 14 patients, segmental (middle) pancreatectomy in 52 patients and spleen-preserving distal pancreatectomy (SPDP) in 25 patients. Pancreatic fistulae were seen in 8 of 104 patients (7.7%). However, pancreatic fistulae in VP had been seen in 5 of 14 patients (35.7%) for the wide cross section of the cut surface of the pancreatic head. Tumor recurrence in the residual pancreas was detected in only one patient with invasive carcinoma of IPMN. Exocrine and endocrine functions of the pancreas were preserved within preoperative levels in most patients.

**Conclusion:** The limited pancreatectomies were significant procedure to preserve pancreatic function for the localized pancreatic disease such as brunch duct IPMN or endocrine tumor. However, it is an immediate issue to prevent pancreatic fistulae in performing ventral pancreatectomy.

---

**P55**

**Endoscopic Interventions via the Minor Papilla**

T. Gyökeres, M. Burai, Á. Pap

Department of Gastroenterology, MÁV Hospital, Budapest, Hungary

**Introduction:** Endoscopic interventions are demanding on the minor papilla. The main indications are pancreas divisum with acute relapsing or chronic pancreatitis. Recently, it has been used as alternative approach to the pancreatic duct, in case of impossible cannulation of the major papilla.

**Patients and Methods:** During the last 5 years we have performed endoscopic manipulations on the minor papilla in 15 patients. We had 13 men and 2 women, the median age was 56 (30–62) years. Previous pancreatic surgery was performed in 6 patients, while previous endotherapy was applied in 3 patients. The main indications for ERC were: pain in 14, severe weight loss in 4, jaundice in 3 and pancreatic ascites in one patient. Seven patients had pancreatic divisum, others suffered from severe chronic calcifying pancreatitis that occluded the Wirsung duct. We performed endoscopic pancreatic sphincterotomy mainly with needle knife on the minor papilla in 12 patients, in 3 patients cannulation of the minor papilla remained unsuccessful even after precutting in 2. We observed mild or moderate complications in 4 pts. Patients needed 4 (1–12) ERCPs on average.

**Results:** We removed pancreatic duct stones from 5 patients, we placed nasopancreatic catheter for lavage with citrate via minor papilla in 8 patients. Finally we placed 7–10 French teflon stents into the dorsal duct in 9 patients. The median duration of the drainage was 2.5 months. Fourteen pts was followed for on average of 27 (1–60) months. Eleven patients (78.6%) are doing well without symptom recurrence, 1 patient (7%) developed pancreatic ascites in one patient. Seven patients had pancreas divisum, others suffered from severe chronic calcifying pancreatitis that occluded the Wirsung duct. We performed endoscopic pancreas sphincterotomy mainly with needle knife on the minor papilla in 12 patients, in 3 patients cannulation of the minor papilla remained unsuccessful even after precutting in 2. We observed mild or moderate complications in 4 pts. Patients needed 4 (1–12) ERCPs on average.

**Conclusion:** Endotherapy performed via the minor papilla can be successful in patients with chronic pancreatitis as in pancreas divisum. A satisfactory long-term results can be achieved using various endoscopic therapeutic modalities.

---

**P56**

**Postoperative Pancreatic Fistula: An International Study Group (ISG) Definition Compared to Clinically Usable Definition**

A. Gulbinas, M. Stakys, J. Pundzius, G. Barauskas

1Institute for Biomedical Research, 2Department of Surgery, Kaunas University of Medicine, Kaunas, Lithuania

**Introduction:** Postoperative pancreatic fistula (POPF) has been regarded traditionally as the most frequent major complication and is a potentially serious, life-threatening event that may prolong hospital stay and increase costs. After pancreateodudenectomy, the reported rate of POPF is highly variable, ranging from 2% to more than 20%. These differences might be related to the variability of the definitions used. The International Study Group (ISG) developed definition of POPF, graded primarily on clinical impact. Therefore the aim of our study was to compare clinical impact of two definitions of POPF (ISG definition and definition used previously).

**Methods:** Clinical data was prospectively collected from 29 patients undergoing Whipple resection (9 patients), pylorus preserving pancreateodudenectomy (22 patients) and distal pancreatectomy (2 patients). Level of amylase in serum and in drainage fluid was measured on postoperative day 1, 3 and 7. If there was a suspicion of pancreatic fistula an X-ray examination (fistulography) was performed.

Pancreatic fistula was defined either when amylase rich (>1,000 U/l) drainage fluid of more than 10 ml in 24 h was present for more than 3 postoperative days or it was confirmed by radiologic imaging.

**Results:** Eight patients (28%) developed grade A, 4 patients (14%) grade B and 1 patient (3%) grade C POPF according to ISG definition. In total POPF should have been diagnosed in 44% of patients. However only 2 patients (7%) developed postoperative pancreatic fistula according to previously used definition. One of these patients should have been regarded having grade C POPF whereas second patient with fistula confirmed by imaging studies did not fully match newly developed definition of POPF.

**Conclusion:** Clinical significance of grade A and grade B POPF seems to be doubtful. Clinically or radiologically evident fistula not always fully matches ISG criteria of POPF.

---

**P57**

**Psychosomatic Status of Patients with Chronic Pancreatitis**

N.B. Gubergrits, I.V. Shalayeva

Department of Internal Diseases No 1, Donetsk State Medical University, Ukraine

**Aim:** To analyze psychosomatic disorders in patients with chronic pancreatitis (CP).

**Methods:** We observed 30 CP patients in exacerbation stage of the disease. The diagnose proved with characteristic clinical picture, presence of the phenomenon of enzymes ‘deviation’ to blood, decrease of pancreatic excretory function and with structural changes of pancreas as by the sonography. Psychosomatic status was evaluated
with the help of questionnaire, which assesses state of the health, activity and mood of patient. We also estimated type of somatogenic pathology. 30 healthy persons were examined.

Results: Evaluating patients’ psychosomatic status before treatment we revealed reduction of levels of all three studied categories. The clinical picture often was in form of asthenic depression with hyperesthesia, irritable weakness, increased emaciation, tearfulness. In presence of constant intensive pain increasing after meal patients develop asthenization, sitophobia, somatogenic depression, hypochondriac and psychasthenic symptoms. It should be noted that after ordinary therapy of CP with inclusion of Eglonil only 6 patients discharging from a hospital experienced dispirited mood, feelings of melancholy, anxiety, dissatisfaction with results of treatment. Significant improvement of patients’ psychosomatic status was proved with better indices of questionnaire.

Conclusion: There is bilateral relation between CP exacerbation and psychosomatic disorders. It is characteristic for the patients to develop a decrease of the state of health/activity/mood, presence of somatogenic psychopathology. Inclusion of Eglonil into complex therapy of CP reduce intensity of psychosomatic disorders.

P58
The Influence of Antihomotoxic Therapy on Results of Direct Test of Pancreatic Excretory Function in Patients with Chronic Pancreatitis

N.B. Gubergrits, V.Y. Kolkina
Department of Internal Diseases No 1, Donetsk State Medical University, Ukraine

Aim: To study an influence of antihomotoxic therapy on pancreatic excretory function of patients with chronic pancreatitis (CP).

Methods: We observed 60 CP patients, which received rehabilitative treatment during a month after CP exacerbation. The main group includes 30 CP patients, which in addition to the traditional therapy received antihomotoxic preparation Momordica compositum during a month, while comparative group (30 patients) received traditional medicines only. After the rehabilitative course we conducted direct (probe) study of pancreatic excretory function. We also examined 30 healthy persons.

Results: Lipase outflow per h in patients of the main group was amounted to 110,860 ± 2,250 U/l h, in comparative group – 90,140 ± 2,380 U/l h, while in healthy – 120,800 ± 4,640 U/l h. It is important that results in the main group had approached to the normal level, whereas in the comparative group they were reduced significantly. In patients of the main group normal type of pancreatic secretion was determined in 33.3% (10 patients), in the comparative group – 20.0% (6 patients). In presence of severe pancreatic insufficiency there was no improvement of pancreatic excretory function.

Conclusion: Including of antihomotoxic preparation Momordica compositum in rehabilitative course of CP patients contributes to improvement of pancreas functional state in case of mild to moderate pancreatic excretory insufficiency. In patients with severe pancreatic excretory insufficiency the antihomotoxic therapy had low effect, in such cases constant substitution therapy is necessary.

P59
Anthropometric and Protein-Calorie Intake Analysis of Pre-Operative Patients Prior to Pancreatectoduodenectomy

K.S. Goonetilleke1, S. Burden2, A.K. Striwardena1
1Hepatobiliary Unit, 2Department of Nutrition and Dietetics, Manchester Royal Infirmary, Manchester, UK

Introduction: Although several studies have examined peri-operative nutritional supplementation in patients undergoing pancreaticoduodenectomy (PD) all provided support at various timepoints. In theory, the pre-operative period offers a therapeutic ‘window’ for nutritional support. This study undertakes a detailed nutritional assessment in the pre-operative work-up period to identify whether there is a window for nutritional supplementation.

Methods: Data were collected prospectively on 14 patients undergoing PD under the care of an individual hepatobiliary surgeon in a regional HPB service during the calendar year 2005. Data were collected from index admission to discharge on: demographics, clinical course, tumour-related data, anthropometric measurements, calorie intake, operative detail and outcome. All data refer to time from admission to HPB unit.

Results: The median age was 58 (range 32–75) years and 8 were female. Ten were in-patients at admitting hospitals prior to referral with a-pre HPB unit median hospital stay of 10 (1–31) days. Eight (57%) were jaundiced. Median delay from admission to surgery was 36 (9–60) days. Median pre-operative daily calorie and protein intake was 1,945 (1,134–2,942) Kcal and 85 (47–134) g protein respectively. One patient received regular pre-operative nutritional supplementation. Median body mass index (BMI) on admission was 24 and unchanged prior to surgery. Mean percentage change in mid arm circumference (MAC) from index admission to immediate pre-op was –0.46%, and the mean percentage change in triceps skin fold thickness (TSFT) from index admission to immediate pre-op was +3.09%. The mean nutritional risk index was 102 prior to surgery.

Conclusion: The nutrition risk index and percentage changes of anthropometric data do not show a significant pre-operative nutritional impairment in the preoperative staging period prior to undergoing PD to warrant supplemental nutritional intervention during this ‘window’ period.

P60
Resection without Biliary Drainage in Patients Undergoing Proximal Pancreatic Resection

Department of HPB Surgery, Freeman Hospital, Newcastle upon Tyne, UK

Introduction and Aim: Biliary stenting prior to resection is standard management of patients with proximal pancreatic/peripancreatic malignancy. Resection before biliary drainage however is
offered to few patients mainly for logistical reasons. We have attempted to identify patients suitable for resection without drainage and have compared outcomes with those undergoing drainage before resection.

Methods: Between 1/04/04 and 30/09/05, based on their presenting bilirubin levels and other logistical factors, jaundiced patients who might be suitable for resection without drainage were identified. Data on complications and hospital stay were compared with those patients in whom a conventional pathway (with biliary drainage) was used during the same time period. Data was also compared with a group of patients from the preceding 6-months (1/10/03 to 30/9/04).

Results: Nine patients underwent resection without drainage and 49 patients were treated by the conventional pathway. In resection-without-drainage patients, median (range) serum bilirubin level (μmol/l) was 265 (162–391) at time of the operation compared to 43 (4–194) (p ≥ 0.0001) in conventional patients. Median (range) of time from referral to operation, 14 days (6–33) vs. 59 (6–201), was significantly shorter in resection-without-drainage patients vs. conventional patients (p ≤ 0.0001). Median (range) of total hospital stays at 21 days (12–36) vs. 32 (14–144) (p = 0.014), was significantly shorter in resection-without-drainage patients. There was no difference in rates of surgical complications and mortality. Prior to surgery the 49 conventional patients underwent a total of 73 biliary drainage procedures resulting in 7 major complications. Comparison with the group of patients from the previous time period indicated that the conventional group were not disadvantaged.

Conclusions: Management of patients with distal biliary strictures by resection without drainage is safe and gives clear benefit in terms of reducing the waiting time to surgery, the number of biliary drainage procedures per patient and the total hospital stay.

P61

Efficacy of Oral Pancreatic Enzymes in Form of Enteric-Coated Mini-Microspheres for the Treatment of Maldigestion Secondary to Duodenopancreatectomy (DP)

J.E. Dominguez-Munoz, M. Vilarino, J. Iglesias-García
Department of Gastroenterology, University Hospital of Santiago de Compostela, Spain

Background: Maldigestion developing after gastroduodenal and pancreatic resection is a major therapeutic challenge. Enteric-coated pancreatic enzymes in mini-microspheres are the therapy of choice for maldigestion in chronic pancreatitis.

Aim: To evaluate the efficacy of this oral enzyme substitution therapy in patients with maldigestion after DP. Since the distal part of the stomach is also surgically removed, it was also evaluated whether this therapy can be improved by opening the enzyme capsules or by inhibiting gastric acid secretion.

Methods: A prospective, randomized, open, comparative, crossover study was carried out in 18 patients (12 male, mean age 55 years, range 34–76 years) 32 months (range 3–164 months) after DP. Patients with maldigestion were randomized to receive four capsules containing 10,000 U lipase each in form of enteric-coated mini-microspheres (Kreon®, Solvay Pharma, Germany) either closed or open during two consecutive 10-day periods. Esomeprazole 40mg od was added for further 10 days in patients with persisting maldigestion despite enzyme therapy. Fat digestion before (basal) and on the last day of each of the three treatment periods was evaluated by an optimized mixed 13C-triglycerides breath test. The cumulative recovery rate of 13CO2 was considered as the result of the test. Data are shown as mean [95% CI] and compared by the Student-t test for related samples.

Results: Fifteen patients (83%) suffered from fat maldigestion and were included in the study. One of them was finally excluded because of protocol violation. Basal 13CO2 recovery rate was 35.9% [0–56.1%]; (normal >57.0%). Therapy with oral pancreatic enzymes markedly improved fat digestion (13CO2 recovery 57.1% [15.8–84.9%]; p < 0.01 vs. basal), independently of whether capsules were taken closed or open (13CO2 recovery 57.3% [0–94.1%] and 56.9% [31.7–95.2%], respectively; n.s.). Eight patients (57%) normalized fat digestion under enzyme therapy. The addition of esomeprazole in patients with persisting maldigestion increased significantly the efficacy of oral pancreatic enzyme substitution therapy (13CO2 recovery from 40.0% [15.8–55.0%] to 52.2% [22.3–63.3]; p < 0.01), and 3 further patients were able to normalize fat digestion.

Conclusion: Maldigestion develops in most patients after DP. Treatment with oral pancreatic enzymes in form of enteric-coated mini-microspheres is highly effective in this setting. Despite partial gastrectomy, opening enzyme capsules is not required. Inhibition of gastric acid secretion is of major help in patients with insufficient response to oral enzyme therapy.

P62

Is Total Pancreatectomy (TP) Safe and Reasonable?

O. Corcos1, A. Sauvanet2, A. Couvelard3, O. Farges2, V. Rebours1, P. Hammel1, P. Levy4, J. Belghiti5, P. Ruszniewski1
1Service de gastroenterologie, 2service de chirurgie digestive, 3Service d’anatomopathologie, hopital Beaujon, Clichy, France

Introduction: TP may be proposed for diffuse intraductal papillary mucinous tumors of the pancreas (IPMT) or multiple endocrine tumors. Decisions pertaining to TP can be elective when abnormalities are diffuse (e.g., diffuse IPMT), during surgery when resection margins are involved or disease-relapse after partial pancreatectomy. Little data related to survival and morbidity is available.

Patients and Methods: From 1995 to 2005, 23 patients were retrospectively evaluated. Mean age at TP was 56 [27–76] years. Indications were: 1) non-invasive diffuse IPMT (n = 9), invasive cancer (n = 7), multiples endocrine tumors (n = 5, 4 with MEN-1), ductal adenocarcinoma (n = 1) or relapse of endocrine tumor (n = 1); 2) totalisation of pancreatectomy in 6 patients for relapse or incomplete resection performed a median of 2 [0–7] years before. Diagnosis was made preoperatively in all patients except 2. Mortality related to TP and survival were estimated.

Results: Post-operative death was nil. After a median follow-up of 1.4 [0.2–12] years, 16 patients are still alive (5-year actuarial survival = 70%). Seven deaths occurred from relapse of adenocarcinoma, late anastomotic ulcer bleeding or hypoglycemia in 5, 1 and 1 patients, respectively. Reversible hypoglycemic coma and gastrojejunal anastomotic ulcer were observed in 3 (15%) and 5 (20%) patients,

Pancreatology 2006;6:323–405
Disorders in Necrotizing Pancreatitis

S. Chooklin, A. Perejaslov, M. Posyvnych
Department of Surgery, Medical University, Lviv, Ukraine

Disorders of microcirculation are the typical for severe acute pancreatitis. Cytokines and adhesion molecules play an important role in the endothelium activation with the subsequent enhanced of vascular permeability, intravascular coagulation resulted in hemocoencentration and progression of necrotic changes. Protein C system is a part of regulatory system which is limited thrombin generation. Activated protein C (APC) decelerates the coagulation cascade by inactivating factors Va and VIIIa. Unfortunately, the relation of cytokines, adhesion molecules, and APC in patients with necrotizing pancreatitis did not study.

Forty-six patients with necrotizing pancreatitis who admitted in clinic not later than 48 h after disease onset were enrolled in this study. The control group compiled 19 patients with mild acute pancreatitis. The levels of IL-1β, IL-18, ICAM-1, and APC were measured in all patients immediately after admission, at the third and seventh day.

Increased levels of all cytokines and ICAM-1 were noted in both groups of patients with significantly highest levels in patients with necrotizing pancreatitis (p < 0.05) already at the time of admission. By that, the obvious decrease of APC concentration was noted in patients with necrotizing pancreatitis (51.9%). IL-1β and IL-18 concentrations elevated during first week with the subsequent decrease in patient with uncomplicated course of the disease, while in patients with septic complications its levels remain high. The peaked levels of ICAM-1 were noted at the third day that correlated with the decline of APC levels. Levels of IL-1β, IL-18 clear correlated with the ICAM-1 and APC concentrations and with the disease severity.

Thus, IL-1β, IL-18, ICAM-1 and APC implicated in pathogenesis of coagulation disturbances in patients with necrotizing pancreatitis.

P64
Leptin in Human Necrotizing Acute Pancreatitis

J. Bonior¹, J. Jaworek¹, J. Panek², M. Tusinski³, J. Zasada³, D. Karcz², S.J. Konturek², W.W. Pawlik²

¹Department of Medical Physiology Health Care Faculty, ²Chair of Physiology, ³General Surgery II Medical Faculty, Jagiellonian University, School of Medicine Cracow, Poland

Introduction: Leptin, circulating protein involved in the control of body weight and energy expenditure recently received attention as a modulator of immune response of the organism. Experimental studies have shown that leptin protects the gastric mucosa and the pancreas from the acute damage, but the involvement of leptin in the pathogenesis of human necrotizing acute pancreatitis (AP) is unclear.

Methods: The blood samples have been taken from healthy volunteers (control group) and from the patients with necrotizing AP; at admission and during 7 consecutive days of conservative treatment, before surgery to measure the concentration of leptin by radioimmunoassay (RIA). Then all AP patients were subjected to pancreatic surgery and pancreatic tissue samples have been taken. Control samples were obtained from histologically unchanged pancreatic tissue from patients with pancreatic cancer. Gene expression for leptin was determined by RT-PCR. The protocol has been approved by the Jagiellonian University Ethical Committee for Human Researches.

Results: In control group (without necrotizing AP) plasma leptin concentration reached 17.57 ± 5.0 ng/ml. In patients with necrotizing AP plasma leptin concentration at admission was 22.84 ± 7.5 ng/ml, and after 7 days of conservative treatment, before surgery it increased to 35.08 ± 8.0 ng/ml. The plasma leptin concentration returned to the control values in patients during recovery. Leptin gene expression was detected in the control human pancreas and this signal was significantly increased in all tissue samples obtained from the patients with necrotizing AP.

Conclusion: Leptin plasma level could be taken into consideration as one of the markers of acute pancreatitis severity in humans.
Methods: 104 consecutive patients were included in this study. PDE was chosen when the head pancreatic mass was present or pancreatic cancer could not be ruled out (48 patients); otherwise DPPHE was performed (56 patients). Quality of life was measured prospectively three times – before and in the follow up period (median 39 and 66 months after operation) with the European Organization for Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire (QLQ-C30). Pain intensity was quantified using a specially designed pain score. Early postoperative morbidity and mortality were also observed and evaluated in each group of patients.

Results: The total pain score significantly decreased after surgery treatment in the both group of patients. During follow up period median 39 months, global quality of life improved by 30.4% in the DPPHE group and by 23.2% in the PDE group. There were no significant differences during follow up period median 66 months. Postoperative morbidity and mortality was higher in the resection group, but differences were not significant.

Conclusions: Both surgical procedures led to a significant improvement quality of life and pain relief after operation for CP. The EORTC QLQ-C30 was found to be a valid and available test for quality-of-life assessment in patients with chronic pancreatitis.

Abstracts

P66

Percutaneous Drainage of Pancreatic Fluid Collections

S.T. Barbu1, N. Rednic2, M. Cazacu3

1Fourth Surgical Clinic, 2Fourth Medical Clinic, Universalitsu Hatieganu Cluj-papoca, Romania

Introduction: Pancreatic fluid collections (PFC) represent a diverse group of lesions of various causes, significance and treatments, and are reflective of the dynamic nature of pancreatitis.

Purpose: To assess patient’s selection, techniques and results of PFC treatment using ultrasound guided percutaneous catheter drainage (PCD); to find predictors of successful outcome.

Methods: Between 1999 and 2002, PCD was used in 32 patients with PFC complicating pancreatitis (21 alcoholic, 8 biliary, 2 hypertriglyceridaemic, 1 idiopathic). Indications were: 11 acute post-necrotic pseudocysts, 13 abscesses, 4 organized pancreatic necrosis, 3 infected acute fluid collections and 1 tuberculous peritoneal empyema. Twenty-nine patients had unilocular collections, 2 had multilocular abscesses and one had two neighbouring pseudocysts. Multivariate regression analysis determined predictors of PCD success. Variables entered into the analysis included: type, diameter, location, complexity of PFC, and drainage technique (Seldinger or Trocar).

Results: Trocar technique was used in 20 patients and Seldinger technique in 12. Twenty-five patients were successfully treated with PCD alone, without PFC recurrence during the follow-up period (mean = 29 month). Catheter drainage duration averaged 25.1 days. Catheter drainage duration averaged 25.1 days. The tuberculous peritoneal empyema needed a second PCD for a left subphrenic abscess. Surgical treatment was necessary in 6 patients: 2 infected pseudocysts (after 2 and 3 days for inefficient drainage, but with stabilization of sepsis), one multilocular abscess, and all 3 infected acute fluid collections. Pseudocyst recurrence occurred in one patient after 9 months. Trocar drainage technique (p = 0.03) and single PFC (p = 0.04) were independent predictors of successful outcome.

Conclusions: PCD should be considered as the initial therapy in selected patients with PFC, and as a staging method for the resolution of sepsis prior to surgery. Selection of patients, time of PCD performance, and a skilful technique with multiple, adequate size catheters insertion is of critical importance.

P67

Lack of Correlation between Free Plasma Fragment DNA and Disease Outcome in Human Acute Pancreatitis

A. Bagul, W. Newman, A.K. Siriwardena

HPB Unit, Department of Surgery, Manchester Royal Infirmary, Manchester, UK

Background: Release of genomic DNA into plasma as a result of necrotic and apoptotic pathways is a feature of a range of human tumours. Severe acute pancreatitis (AP) is characterized by inflammation but may also be associated with accelerated apoptotic and necrotic pathways. This study uses quantitative real-time PCR to measure free circulating DNA in patients with severe AP.

Methods: DNA was purified and quantified using the RNaseP transcription assay and quantitative PCR in 43 patients with severe acute pancreatitis and compared to plasma DNA from 12 patients with pancreatic cancer and 28 undergoing laparoscopic cholecystectomy. In acute pancreatitis patients, baseline samples were taken on admission and further samples taken at a median of 5 days into the disease course. Data are presented as medians (range) Statistics: where statistical comparisons are made, non-parametric analyses are used and significance is accepted at the p < 0.05 level (stats Direct Ltd).

Results: Plasma DNA levels on admission in patients with acute pancreatitis were lower than in non-cancer controls 0.40 (0.05–0.79) compared to 1.60 (0.45–9.10) [p < 0.001; 95% CI 0.67–1.2; Mann-Whitney U test]. In patients with acute pancreatitis, DNA levels fell during the disease course 0.40 (0.05–0.79) compared to 0.08 (0.00–0.53) [p <0.001; 95% CI 0.05–0.12; Wilcoxon’s signed ranks test]. When entered into a logistic regression model for prediction of mortality, DNA levels were non-significant and only admission logisitic organ dysfunction score was positively correlated with adverse outcome.

Conclusion: This is the first study to use quantitative PCR to measure free plasma DNA in SAP (severe acute pancreatitis). The results show that plasma DNA is lower in patients with acute pancreatitis compared to control and that values fall further during the disease course.
**P68**

**Novel ERCP-Technology for Endoscopic Evaluation of the Pancreatic Duct System**

U. Arnelo1, N. Albini2, L. Enochsson3, S. Meissner3, J. Fhernert1, L. Lundell1

Divisions of 1Surgery and 2Radiology, Karolinska University Hospital, Huddinge, Stockholm, Sweden; 3Department of Surgery, Bisbebjerg Hospital, Copenhagen, Denmark

**Introduction:** Oral cholangioscopy has an established role in the assessment and management of diseases within the biliary system. The current commercially available endoscopic fiberoptic devices require involvement of two endoscopists. This abstract presents a case in which a new and simplified endoscopic fiberoptic device was utilized single-handedly to directly inspect the pancreatic duct system.

**Methods:** The flexible technology is delivered with an outer diameter of 0.9 mm giving an image of transmission of 6,000 pixels. The ocular of the flexible optic is connected to a CCD camera by use of a TV adapter, connected to the camera head. The light source is Xenon. The flexible optic is introduced through the regular guide wire channel of a rotatable spincterotome, allowing for directional control of the optical tip to get a luminal view of the duct system. To facilitate the direct visualization of the duct, the device was perfused with saline.

**Results:** A patient with a recent history of a benign tumor and gastrojejunoscopy presented with a chronic inflammatory reaction possibly resulting from a pancreatic duct rupture. ERP with pancreatoscopy was carried out to delineate the ductal anatomy of the gland. The exact location of the defect in the duct of Santorini could be visualized, clearly mapped out and the leakage of pancreatic juice blocked by an insertion of a plastic stent. The subsequent course has been completely uneventful and total duration of the stent treatment was 12 months. The patient is now in good health.

**Conclusions:** There exists a variety of clinical situations where it is of crucial importance to obtain direct visual information of the macro anatomical structure of the pancreatic duct system. The advent of improving technology will allow direct visualization and inspection of these ducts and illustrates a rapid progress toward elimination of the dual endoscopist approach.

---

**P69**

**Will Embolization of Large Pancreatic Arteries Lead to Acute Pancreatitis?**

Å. Andén-Sandberg1, K. Eriksson1, C. Ansorge1, F. Johannessen2, L. Fjetland2

1Department of Surgery and Radiology, Stavanger University Hospital/Bergen University, Stavanger, Norway

**Introduction:** In pancreatic text-books embolization of the pancreatic parenchyma is reported as a rare etiology to acute pancreatitis, however, seldomly seen – and even more seldomly verified – in clinical settings. As it has been discussed arterial emboli as a cause of otherwise idiopathic acute pancreatitis we have followed-up cases of pancreatic embolization on purpose.

**Results:** In five patients a bleeding duodenal ulcer was stopped by embolization of pancreatic arcades from both superior and inferior pancreaticoduodenal arteries. In one case an aeurysm central in the pancreatic body, fed by arteria pancreatica magna, was embolized. The success of the embolization was verified by postembolization arteriographic pictures showing no arterial blood flow in the embolized vessels and, in the cases of bleeding duodenal ulcers, clinically stopped bleeding with no further need for blood transfusions.

**Conclusions:** Limited embolization of the pancreas in humans is not enough to cause acute pancreatitis.

---

**P70**

**Proinflammatory Cytokines Levels in Patients with Acute Alcoholic Pancreatitis with Different Forms of the Disease**

J. Panek1, D. Karcz1, J. Bonior2, J. Zasada1

1Second Surgical Department, 2Department of Medical Physiology, Faculty of Health Care, Collegium Medicum Jagiellonian University, Cracow, Poland

**Introduction:** Proinflammatory cytokines play a fundamental role in the local and systemic inflammatory response in the initial stages of acute pancreatitis and in the development of severe form of the disease.

**Material and Methods:** We assessed the systemic release of proinflammatory and tried to characterize differences between the patients with mild and severe forms of the disease. In the study 43 patients with mild form of acute alcoholic pancreatitis (MAAP) were compared with 11 patients with severe acute alcoholic pancreatitis (SAAP). Serum levels of TNF-α, IL-1β, IL-6, IL-8, and IL-12p40 were measured every second day after admission during one week.

**Results:** The TNF-α level was similar in all days of analysis in patients with MAAP but lower comparing to the patients with SAAP. The level of IL-1β was not detectable in patients with MAAP and SAAP. The level of IL-6 peaked on admission in both groups, but in the patients with SAAP the obtained values were significantly higher (p < 0.05) significantly decreased during the period study (respectively: p < 0.05, p < 0.001). The level of IL-8 on admission day was slightly higher in the group of patient with MAAP and during the next days significantly decreased (p < 0.0009). Increased insignificantly levels of this interleukine during the next days of investigation were seen in SAAP. The mean levels of IL-12p40 were insignificantly higher in patients with SAAP during the study period. Simultaneously the total white blood cells, neutrophils and monocytes were significantly higher in patients with severe form of the disease.

**Conclusions:** We hypothesized that a therapy aimed at particular elements of white blood cells will bring therapeutic advantages.
Basic

P71

Immunogenetical Aspects of Pathogenesis of Chronic Alcoholic Pancreatitis (CAP)
Y.A. Zagorenko, N.B. Gubergrits
Department of Internal Diseases #1, Donetsk State Medical University, Ukraine

Introduction: The hypothesis of immunogenetical predisposition to CAP is still poorly studied.

Aim: To study the role of immunogenetical predisposition as a risk factor for development of different clinical variants of CAP.

Methods: We observed 92 patients and 30 healthy persons in our clinic. Excretory and incretory function of the pancreas, results of ultrasonography and immunogenetical examination (antigens AB0, Rh, HLA) were studied. Immunogenetical testing was also conducted in 456 healthy.

Results: It was proved that risk of CAP development is higher in presence of blood group A(II), Rh-antigens combination CDe, HLA haplotype A10–Cw6 and HLA antigens A1, Bw40, B13, B18, B27, DR4. HLA antigen Cw4 has a protective role in regard to development of CAP. With the help of cluster analysis we divided CAP patients in two groups depend on clinico-pathogenetical variant of the disease. First variant – parenchymatous pancreatitis. Such patients had in phenotype – HLA haplotype A10–Cw6 and antigen B18, in clinic – intensive pain syndrome, significant phenomenon of enzymes’ ‘deviation’ to blood, rare incidence of pancreatic juice outflow disorders and of significant impairment of pancreatic excretory function, but high incidence of upper obstructive type of pancreatic secretion and ultrasound signs of pancreas edema on the background of moderate fibrosis. Second CAP variant – indurative-obstructive. It was characteristic for such patients the presence in phenotype of HLA antigens Bw40, B13, B27, DR4, mild pain syndrome, absence of the phenomenon of enzymes’ ‘deviation’ to blood, decreasing of excretory and incretory function of the pancreas as clinically, as by results of direct and indirect tests of pancreatic secretion, high incidence of pancreatic juice outflow disorders as by results of functional tests and ultrasonography, and high incidence of calcinates and of significant pancreatic fibrosis.

Conclusion: It is obvious that there is an immunogenetical predisposition for development of different clinico-pathogenetical CAP variants.

P72

not submitted

P73

Evolution of Pancreatic Phospholipase A2
T.J. Nevalainen
Department of Pathology, University of Turku, Finland

Ten distinct secreted human and murine phospholipases A2 (PLA2) have been cloned. Secreted PLA2s are expressed in distinct cell types throughout the body. Group IB PLA2 is synthesized by pancreatic acinar cells and secreted into the duodenal lumen to digest dietary phospholipids. Homologous enzymes have been cloned from a number of other sources including snake venoms and marine invertebrates. In order to study the phylogenetic relationships of secreted PLA2s, I compared the molecular primary structures of PLA2s cloned from various vertebrate and invertebrate species.

The protein sequences of 30 secreted PLA2s were obtained from the NCBI GenBank data base and aligned by the CLUSTAL W program, and the phylogenetic tree was constructed using the PHYLIP program. Three distinct clades of PLA2 molecules emerged. First, human and mouse group IIA, C, D and F PLA2s together with human group V and Gaboon viper venom group IIIB PLA2s formed a distinct group. Second, group III (bee venom) and numerous PLA2s from unrelated sources including metazoans and eukaryotic and prokaryotic single cell organisms grouped together. Third, in the clade including human pancreatic group IB PLA2 there were PLA2s from the venom of Indian cobra (Naja naja naja), fish (bream Pampus major) hepatopancreas, echinoderm (starfish Acanthaster planci and Asterina pectinifera) spines and pyloric ceca, respectively, and cnidarian (sea anemone Adamsia carciinopadios) tentacles and acothia.

The functions of invertebrate PLA2s are largely unknown but may include roles in the eicosanoid metabolism, the capture and digestion of prey and the antimicrobial and toxic defense of the animal. It is noteworthy that cnidarians have been found in fossils form the Precambrian era more than 550 million years before present. It can be speculated that vertebrate pancreatic group IB PLA2 may represent an evolutionally ancient molecule with a conserved digestive function.

P74

Hepatic Stellate Cells – Selective Carrier Mannose 6-Phosphate Modified Albumin (M6P(28)-HSA), the Attempt to Prove the Existence of the Analogy for the Pancreatic Stellate Cells in Chronic Pancreatitis
H. Nechutova1,2, P. Dite1, M. Hermanova3, L. Pac2
1Department of Internal Medicine-Gastroenterology, University Hospital, 2Department of Anatomy, Medical Faculty, Masaryk University, 3Department of Pathological Anatomy, University Hospital, Brno

Introduction: Pancreatic stellate cells (PSC) present dynamic cellular type responsible for many specific histopathomorphological processes (mainly fibroproliferation) during chronic pancreatitis. After the introductory (mechanic, chemical …) stimulus the cells become...
activated, undergo radical cellular changes (structural, proteosynthetic, mitotic . . . of communication and interaction trends . . .). The prime impacts of these acts are the overproduction of the collagen structures (decrease of their degradation), and production of signal substances for the interaction with nearer or distant surroundings, i.e. representative of inflammation . . . It supports and multiplies pathological changes in the pancreatic parenchyma.

But still, there is not definite solution how to slow the steps of scarring etc. or interrupt them. So we need antifibrotic therapy, targeted on PSC, and ideally arranged as specific drug delivery for the PSC (to avoid side-effects in other tissue and organs).

Beljaars et al. Presented in 1999 the role of the albumin modified with mannose 6-phosphate as a potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells. Now we are testing the existence of this specific affinity for the pancreatic stellate cells.

Methods: We used the peroperative samples of human pancreatic parenchyma (patients with chronic pancreatitis). For the identification of pancreatic stellate cells the slices were stained for vimentin, desmin, αSMA, GFAP. M6P-modified bovine serum albumin (BSA) was incubated with this human pancreatic tissue and albumin was stained by appropriate antibody.

Results: Now we have collected more than 15 samples of human chronic pancreatitis tissue, and for this time, more than half showed preferential affinity of M6P-modified bovine serum albumin (BSA) to pancreatic stellate cells in the case of human chronic pancreatitis.

Conclusion: This specifically modified albumin should be used as at least the component of the specific carrier in planned drug targeted antifibrotic therapy in pancreas.

P75
Stimulation of Pancreatic Enzyme Secretion by Luminal Ghrelin. Involvement of Enteropancreatic Reflex
K. Nawrot-Porabka¹, J. Jaworek¹, A. Leja-Szpaki, M. Macko¹, J. Szklarzcyk¹, M. Kot¹, S.J. Konturek², W.W. Pawlik³
¹Department of Medical Physiology Health Care Faculty, University of Science, Cracow, Poland

Introduction: Ghrelin, a peptide produced by the stomach has been reported to affect the pancreatic exocrine functions but the mechanism of pancreatosecretory action of this peptide is unclear.

Aim: (1) To evaluate the effect of intraduodenal (i.d.) infusion of ghrelin on pancreatic amylase outputs under basal conditions and following the stimulation of pancreatic secretion with dipeptidyl peptidase IV (DPP IV) (2) to determine the involvement of neural mechanisms and CCK in that process.

Methods: The studies were carried out on Wistar rats. The animals were surgically equipped with silicone catheters, one of them was inserted into pancreatic-biliary duct, the other one into duodenum. Following i.d. administration of ghrelin at doses of 0.1, 1.0 or 10.0 μg/kg pancreatic amylase concentration was measured. To determine the role of vagal nerves in the effects of luminal ghrelin on exocrine pancreas, bilateral vagotomy was performed. To assess the involvement of sensory nerves in above effects capsaicin was given at total dose of 100 mg/kg for 10 days before the study. The CCK-1 receptor blocker, lorglumide at dose of 1 mg/kg was administered to the rats before ghrelin application. The CCK plasma level were measured by RIA.

Results: Ghrelin given intraluminally at doses of 1 or 10 μg/kg increased pancreatic amylase outputs under basal conditions or following the stimulation of pancreatic secretion with DPJ. Bilateral vagotomy, deactivation of sensory fibers or pretreatment of the rats with lorglumide completely abolished all stimulatory effects of luminal ghrelin on amylase secretion. Ghrelin at doses 1 or 10 μg/kg significantly increased CCK plasma level under basal conditions or following the stimulation of pancreatic exocrine secretion with DPJ.

Conclusion: Activation of vagal reflexes and CCK release could be implicated in the stimulatory effect of luminal ghrelin on the pancreatic exocrine functions.

P76
Gluing Bowel on to the Pancreas: Biocompatibility on Adhesive Properties of Six Preparations
T. Läämsä, H.T. Jin, J. Sand, I. Nordback
Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, Finland

Introduction: The use of tissue adhesives between pancreas and bowel has been little studied. Aim of this study was to investigate biocompatibility and adhesive properties of fibrin sealants and cyanacrylate derivatives in pancreatico-jejunal attachment.

Methods: Pancreatico-jejunal attachment was created with adhesives in overall 138 Sprague-Dawley rats with three cyanacrylate derivatives (Histoacryl, Dermabond and Glubran) and three fibrin sealants (Bioglu, Tisseel Duo Quick and Quixil). Pancreatic specimens and blood samples were harvested 1, 3, 7 and 21 days after gluing for histological determination and amylase activity measurement. Pancreatico-jejunal attachment created with adhesives underwent tensile strength measurement. Samples were also taken from 5 unoperated base line rats and from 24 sham-operated rats. Six preparations and sham-group both induced a similar increase in amylase activity at the day 1 with normalization by the day 3. Sham-operation did not induce pancreatic acinar cell changes. Each tissue adhesive induced changes in pancreatic histology both at the site of gluing and at a site far away from the exposure area. There was more acinar necrosis at day 21 with cyanacrylates than with fibrin sealants (1 vs. 0, [histological scoring 0–4]) which, however, also included acinar vacuolization and necrosis. The tensile strength was lowest on day 3 (0.17N) and reached the maximum mostly by day 7 (0.81N). Histoacryl and Quixil induced lower tensile strength than the other adhesives at day 7 and 21, respectively (0.35N; 0.05 N).

Conclusions: All of the tissue adhesives studied showed histotoxicity on the pancreas. Even though tensile strength was adequate with four of the six preparations tested, harmful tissue effects might compromise the use of the substances in pancreatic surgery.
**P77**

**Effect of Chronic Pretreatment of the Suckling Rats with Bacterial Lipopolysaccharide on Pancreatic Morphology and Functions**

J. Jaworek¹, M. Macko¹, A. Leja-Szpak¹, J. Szklarczyk¹, J. Bonior², K. Nawrot-Parabka¹, M. Kot¹, A. Dembinski², Z. Warzecha³, J. Stachura³, R. Tomaszewska³, S.J. Konturek¹, W.W. Pawlik²

¹Department of Medical Physiology Health Care Faculty, ²Chair of Physiology, ³Department of Cell Morphology, Medical Faculty Jagiellonian University School of Medicine Cracow, Poland

**Introduction**: Lipopolysaccharide (LPS) released from the bacterial cell wall contributes to the pathophysiology of bacterial infection, but the significance of endotoxemia induced in the early period of life on the pancreatic structure and functions is yet unknown.

**Aim**: To investigate the effects of endotoxemia induced in suckling rats on pancreatic morphology, pancreatic exocrine and endocrine secretions, antioxidative properties of pancreatic tissue and cytokine production in the young and in the adult animals.

**Methods**: Suckling rats (30g) were injected with LPS of Escherichia coli (1, 5 or 10 mg/kg/day) for 5 consecutive days. Control rats received physiological saline. First group of rats was sacrificed after 5-days treatment with LPS, second-2 month later. Blood samples were taken to determine amylase, lipase, tumor necrosis factor alpha (TNFα), interleukin 10 (IL-10) or insulin blood levels. The acinar cells and leukocyte infiltration of the pancreatic tissue has been detected and mild edema of the acinar cells and leukocyte infiltration of the pancreatic tissue has been observed. The other parameters have not been significantly changed in this group of animals, as compared to the adult rats injected in early period of life with physiological saline instead of LPS.

**Conclusions**: Endotoxemia induced in the suckling rats resulted in the marked increase of antioxidative enzyme SOD activity in the pancreatic tissue of these animals.

**Results**: In the suckling rats LPS treatment produced edema, and leukocyte infiltration of the pancreas, marked decrease of lipase blood level, accompanied by significant increases of TNFα, IL-10 and insulin blood levels, as compared to the suckling rats injected with physiological saline. Two months after LPS treatment the marked rise of SOD in the pancreatic tissue have been detected and mild edema of the acinar cells and leukocyte infiltration of the pancreatic tissue has been observed. The other parameters have not been significantly changed in this group of animals, as compared to the adult rats injected in early period of life with physiological saline instead of LPS.

**Conclusion**: Endotoxemia induced in the suckling rats resulted in the marked increase of antioxidative enzyme SOD activity in the pancreas of adult animals. That was accompanied by mild edema and leukocyte infiltration of the pancreatic tissue of these animals.

**P78**


P. Hegyi¹, V. Venglovecz², I. Ignáth³, B. Öszvári¹, T. Takács¹, J. Lonovics¹, A. Várro², Z. Rakonczay Jr¹

¹First Department of Medicine, ²Department of Pharmacology and Phamacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary

**Introduction**: Since 1979, more than a thousand papers have reported the use of bicarboxyethylcarboxyfluorescein (BCECF) for making measurement of intracellular pH (pHi) using microspectrofluorometric systems (MSFS). Looking critically at these papers, some odd and unexplained things can be observed. For example, there are big differences in the resting pHi of the cells, even in similar cells, in similar conditions and described in the same paper. Despite the huge development of imaging systems (IS), only few imaging measurements can be found in the literature to monitor real-time changes of pHi. Our aim was to compare MSFS (LIFE SCIENCES) and IS (OLYMPUS CellR).

**Methods**: pHi was measured in isolated pancreatic duct cells using the fluorescent dye BCECF. The nigericin/high K⁺ method was utilized to set the cells pHi to the same level and the fluorescence ratios (F490/F440 from which the pHi is calculated) were compared in MSFS and IS.

**Results**: Using IS, we found that there were groups of cells which were already saturated with the dye while others were not causing inhomogeneity between the fluorescent intensities of regions of interests (ROIs). These differences in the saturations lead to non-desirable deviations in ratios. Using MSFS, the inhomogeneity is not possible to detect, therefore, this can result in inaccurate calculation of pHi. However, the usage of IS allow to assign ROIs with almost identical homogeneities of fluorescence intensities. With using an IS one is capable of assigning 4–6 homogeneous ROIs at the same time.

**Conclusion**: An imaging set-up should be preferred to measure pHi, and more importantly, this change in practise would lead significantly less number of animals sacrificed for scientific experiments.

Supported by OTKA, MTA, OM.

**P79**

**Morphological and Functional Changes of Small Intestine in Patients with Chronic Pancreatitis (CP)**

N.B. Gabrielits, Y.V. Linevskiy, Y.A. Zagorensko

Department of Internal Diseases #1, Donetsk State Medical University, Ukraine

**Introduction**: Due to progress of pancreatic excretory insufficiency patients with CP have developed intestine dysbiosis and further – secondary enteritis. Inflammation of small intestine mucosa leads to accomplishing of maldigestion with malabsorption. Atrophy of small intestine mucosa is a cause of reduced efficacy of substitution therapy.
**Aim:** To study enzymatic indices of small intestine digestive function in patients suffering from CP.

**Methods:** We examined 32 patients with CP. Aspiration biopsy of mucous membrane of small intestine was taken. Amylolytic (by Smyth-Roe) and lipolytic (by Ugolev-Nurks) enzyme activity, the activity of lactase (by Linevsky), saccharase (by Ugolev-lezuitova), maltase (by Linevsky), glycine-L-leucinedipeptidase (by Ugolev-Timofeeva), monoglyceridlipase (by Ugolev-Chernyakhovskaya) and alkaline phosphatase (by Fomina-Mihlin) were examined in homogenate of taken mucosa samples. An absorptive function of small intestine was evaluated by test with D-xylose. Patients were underwent relaxation duo-denography with tight contrast enhancement of the duodenum.

**Results:** X-ray examination of duodenum showed smooth internal outlines of pars descendence (as a result of pancreatitis) and ‘snow storm’ symptom on a residual contrast enhancement, i.e. signs of duodenitis. Histology of jejunal biopitates revealed dystrophy of villi epithelium, its marked leucopedesis and different grades of atrophy. Amylolytic and lipolytic activity of membrane and cavitary digestion was increased, also a reduced production of monoglyceridlipase was determined. An increased production of saccharase, reduced level of glycine-L-leucinedipeptidase in mucous membrane, growth of alkaline phosphatase contents were obtained. Insufficiency of small intestine absorptive function, bacterial proliferation as a result of unusual microorganisms existence in small intestine were revealed.

**Conclusion:** Secondary disorders of small intestine enzyme production are developing (compensatory increase or decrease due to mucosa atrophy) in patients with CP as far, as reduction of intestinal mucosa absorptive function. The disorders observed in small intestine should be taken into account in planning medical management of patients with CP.
Healing Effect of Ghrelin in Experimental Caerulein-Induced Acute Pancreatitis

Z. Warzecha¹, A. Dembinski¹, P. Ceranowicz¹, J. Cieszkowski¹, S.J. Konturek¹, B. Kusnierz-Cabal¹, J.W. Naskalski², R. Tomaszewska³, W.W. Pawlik¹

¹Department of Physiology, ²Department of Clinical Biochemistry, ³Department of Pathology, Jagiellonian University Medical College, Krakow, Poland

Introduction: Ghrelin, a 28-amino-acid polypeptide was originally isolated from the stomach. Recent studies have shown that pretreatment with ghrelin exhibits protective effect in the gut. Administration of ghrelin reduces gastric mucosal damage evoked by ethanol, as well as inhibits the development of experimental pancreatitis. However, this protective effect requires administration of ghrelin before exposure to damaging factor and thus has a limited clinical value. The aim of present study was to assess the influence of ghrelin administered after development of acute pancreatitis on the severity of this disease and pancreatic regeneration.

Methods: Acute pancreatitis acute pancreatitis was induced by caerulein given i.p. 5 times at a dose 50 μg/kg/dose with 1 h intervals. Ghrelin was administered twice a day at the dose 8 nmol/kg/dose, starting 24 h after last injection of caerulein. Rats were sacrificed 0 h or 1, 2, 3, 5, 7 and 10 days after cessation of caerulein administration.

Results: Administration of caerulein led to the development of acute edematous pancreatitis and maximal severity of this disease was observed 24 h after induction of pancreatitis. Treatment with ghrelin reduced morphological signs of pancreatic damage such as pancreatic edema, leukocyte infiltration and vacuolization of acinar cells, and led to earlier complete regeneration of the pancreas. Also biochemical indexes of the severity of pancreatitis such as serum activity of lipase, amylase and poly-C ribonuclease were significantly reduced in animals treated with ghrelin. These effects were accompanied with an increase in pancreatic DNA synthesis and a decrease in serum level of pro-inflammatory IL-1β. Moreover administration of ghrelin improved pancreatic blood flow.

Conclusions: (1) treatment with ghrelin exhibits therapeutic effect in caerulein-induced experimental acute pancreatitis; (2) this effect is related, at least in part, to the improvement of pancreatic blood flow; reduction in pro-inflammatory IL-1β and stimulation of pancreatic cell proliferation.
**P82**

**Beneficial Effect of Zerumbone on Cholecystokinin-Octapeptide-Induced Acute Pancreatitis in Rats**

A. Szabolcs, L. Tiszlavicz, J. Kaszaki, Cs. Varga, A. Pósa, A. Berkó, J. Lonovics, T. Takács

1First Department of Medicine, 2Department of Pathology, 3Institute of Experimental Surgery, 4Department of Comparative Physiology, University of Szeged, Hungary

---

**Introduction:** Zerumbone is a natural substance extracted from the rhizome of Zingiber zerumbet. In vitro the drug was found to inhibit activation of NF-κB, iNOS and COX-2 and to exert antioxidant effects. Our experiment was designed to investigate the effect of zerumbone pre-treatment on cholecystokinin-octapeptide (CCK-8)-induced acute pancreatitis in rats.

**Methods:** Male Wistar rats weighing 220–270 g were used in this study. Animals were divided into 4 groups. Group ZP received 20 mg/kg zerumbone followed by injections of 100 μg/kg CCK-8 with 1 h differences. Group P received an ip. injection of DMSO followed by pancreatitis induction. Group C was treated with physiological saline instead of zerumbone and CCK-8. Four hour after the last injection rats were exsanguinated through the abdominal aorta.

**Results:** The serum amylase and lipase activities and pancreatic weight/bodyweight ratio were significantly elevated in group P and ZP compared to group C, but compared to group P the elevation of these parameters was significantly smaller in group ZP. The elevation of pancreatic iNOS activity, IL-6 and TNF-α concentration was significantly reduced in group ZP vs. group P. Pancreatic iNOS activity was reduced in group P but not in group ZP vs. group C. Serum ASAT concentration was significantly higher in group Z and ZP vs. group C. A beneficial effect of zerumbone on the histological parameters of pancreatitis was not observed.

**Conclusion:** The in vivo protective effect of zerumbone in the model of CCK-8-induced pancreatitis was demonstrated for the first time in this study. Zerumbone significantly reduced the changes of several parameters of the disease. The influence of the substance on the liver function needs further investigation.

---

**P83**

**Prostaglandin E2 Promotes the Expression of Monocyte Chemotactic Protein-1 in the Pancreatic Acinar Cell AR42J: Interaction between Macrophages and Pancreatic Acinar Cells in Pancreatitis**


Pancreatitis Research Laboratory, Department of Visceral & Transplant Surgery, University Hospital Zürich, Zürich, Switzerland

**Introduction:** MCP-1 is a strong chemoattractant for monocytes/macrophages and lymphocytes, and plays an important role in the recruitment of monocytes/macrophages in inflammatory tissues, including in chronic pancreatitis.

In a model of chronic pancreatitis (WBN/Kob) rat we recently demonstrated that the expression of MCP-1 was significantly diminished in COX-2 inhibitor treated rats as compared to untreated rats. There was a markedly decreased level of PGE2 and decreased pancreatic infiltration rate of macrophages. We investigated the role of prostaglandins (PGE2) in the interaction between macrophages and acinar cells.

**Methods:** To simulate interactions of macrophages and acinar cells, we used a mouse macrophage cell line (RAW 264.7) and a rat acinar cell line (AR42J).

**Results:** We first identified the cells which express MCP-1 in the rat model, and localized it predominantly in acinar cells. To test whether PGE2 might have any effects in acinar cells we verified that PGE2 receptors EP1, EP4 are actually expressed in AR42J cells. These cells produced MCP-1, and the expression of MCP-1 was regulated by TNFα and PGE2. In the presence of PGE2, TNFα-dependent expression of MCP-1 was significantly higher than with TNFα alone. TNFα regulated the expression of itself in this cell line. In the presence of PGE2, expression levels of TNFα were also further enhanced. PGE2 demonstrated chemotactic activity on macrophages (RAW264.7). Activated macrophages induced the secretion of pancreatic-associated protein in the AR42J cell. The presence of acinar derived molecules caused a markedly increased directional migratory response in macrophages.

**Conclusions:** Taken together, pancreatic acinar cells secrete MCP-1, and the expression of MCP-1 in pancreatic acinar cells was modulated by PGE2. The latter is predominantly secreted by infiltrating macrophages. This mechanism might be a key step in establishing the chronic phase of pancreatitis. Prostaglandins, the down stream products of COX influence this process.

---

**P84**

**Genomic Profiling of Ductal Adenocarcinoma of the Pancreas Using SCOMP**

N.H. Stoecklein1, A. Lübke1, M. Baudis2, A. Erbersdobler2, S.B. Hosch1, C.A. Klein3, W.T. Knoefel1,4

1Klinik für Allgemein- und Viszeralchirurgie, Universitätsklinikum Düsseldorf, Düsseldorf, 2Institut für Pathologie, Universitätsklinikum Eppendorf, Hamburg, Germany; 3Division of pediatric Oncology/Hematology, University of Florida, Gainesville FL, USA; 4Institut für Immunologie, LMU München, München, 5Former Address: Klinik und Poliklinik für Allgemein-, Viszeral- und Thoraxchirurgie, Universitätsklinikum Eppendorf, Hamburg, Germany

**Introduction:** Little is known about the genomic aberrations of ductal pancreatic adenocarcinoma (PDAC). In order to gain a more comprehensive overview of the genomic aberrations in PDAC, we performed comparative genomic hybridization (CGH) of laser-microdissected tumor cells in a relatively large PDAC tumor collective. The results were correlated with clinical follow-up data.

**Methods:** The procedures for tumor cell isolation by laser-microdissection, whole genome amplification and CGH were performed according to the SCOMP protocol optimized for formalin fixed and paraffin embedded tissues (Am J Pathol 2002) on 52 PDAC
Results: The mean chromosomal aberration rate of all tumors was 12.25 (3-22). The most frequent gains were at 8q21-24 (36-40%), 13q21-22 (39%), 3q25-27 (35%), 4q22-31 (31-25%), 12q15-21 (25%), 5q14-23 (20%) and the most frequent losses were at 17p11-13 (58-65%), 18q12-22 (38-58%), 1p31-36 (23-46%), 6q16-27 (23-40%), 19p13-19q13 (38-30%). The patients were grouped according to their survival (> and <18 months). In the group of patients with poor prognosis, (< 18 months, n = 37) gains at 7p, 19q, 20q and losses at 4p and 6p were significantly more frequent. In multivariate analysis, losses in the region of 6p and gains in the region of 19p were prognostic factors as well as lymphatic metastasis.

Conclusion: Our study provides a comprehensive overview of the genomic aberrations observed in primary PDAC and points to chromosomal regions that might harbor genes responsible for the aggressive phenotype of PDAC tumors.
some improvements on surgical outcome occurred in patients who received surgery chemotherapy and/or radiotherapy, the impact on long term survival was minimal owing to the intense resistance of pancreatic cancer to apoptotic stimuli.

Although there is growing knowledge about the mechanisms of apoptosis, the role of many regulating proteins in pancreatic cancer is still unclear.

**Methods:** To elucidate the role of apoptotic genes in pancreatic carcinoma, we analyzed the gene expression of microdissected pancreatic cancer samples of 19 patients and microdissected normal pancreatic ductal epithelial cell samples of 14 patients using DNA gene expression analysis based on the Affymetrix U133 GeneChip set. Validation of the data was carried out by quantitative RT-PCR.

**Results:** Out of the 44,000 probe sets of the U133 GeneChip, we identified 142 probe sets coding for 90 different apoptosis associated genes. We analysed the differential expression of these 142 probe sets using the SAM software with a cut off value of 2 for the fold change and 5% for the q-value and found 8 genes upregulated and 4 downregulated in PDAC. In accordance to our findings, a pathophysiological role in tumorescense was already described for some of the upregulated (i.e. BIRC 5/Survivin, DcR3, FAP-1) and downregulated genes (i.e. Bcl-2, TNF-R1) in pancreatic cancer before. However, the other differentially regulated genes have a so far unknown role in the carcinogenesis of human pancreatic carcinoma. To confirm our results we validated the differential expression by means of quantitative RT-PCR.

**Conclusion:** Using gene expression profiling we were able to identify apoptosis associated genes differentially expressed in pancreatic adenocarcinoma, which might contribute to pancreatic cancer development and apoptosis resistance. These genes might be good candidates for new therapeutic strategies.

---

**P88**

**Treatment with Caerulein and Substance P Induces Chemokine Synthesis in Pancreatic Acinar Cells**

R.D. Ramnath, M. Bhatia

Department of Pharmacology, National University of Singapore, Yong Loo Lin School of Medicine, Singapore

**Introduction:** Chemokines are believed to play a key role in the pathogenesis of acute pancreatitis. We have earlier shown that pancreatic acinar cells produce the CC chemokine MCP-1 in response to caerulein hyperstimulation. We have also known that, in mice with pancreatitis, there are elevated levels of substance P (SP) and an increased number of NK-1 receptors in pancreatic acinar cells. In the present study, we investigated the effect of caerulein and SP on pancreatic acinar cells.

**Methods:** Pancreatic acinar cells (in vitro) were incubated with either caerulein, SP or caerulein and SP simultaneously. After incubation, the supernatant was used for chemokine detection whereas the pellet was used for nuclear factor-κB (NF-κB) detection.

**Results:** We observed that CC chemokine MCP-1, MIP-1α and CXC chemokine MIP-2 were produced when acinar cells were stimulated with caerulein. Furthermore, pancreatic acinar cells produced MCP-1, MIP-1α and MIP-2 when treated with SP alone. Moreover, acinar cells treated with both caerulein and SP caused a significant increase in the chemokine levels, which was significantly higher when compared to caerulein or SP treatment alone. Also, acinar cells stimulated with combined treatment of caerulein and SP caused a significant increase in NF-κB activation when compared to the treatment with caerulein (p < 0.018) or SP (p < 0.02) alone. These results suggest that both caerulein and SP are acting through NF-κB pathway to induce chemokine synthesis. To further confirm this, acinar cells were treated with NEMO-binding domain (NBD) peptide, a selective inhibitor of NF-κB activation. Treatment with NBD significantly attenuated the stimulation in chemokine synthesis caused by treatment with both caerulein and SP.

**Conclusions:** This study shows that caerulein and substance P induce chemokine synthesis through NFκB pathway in pancreatic acinar cells.

---

**P89**

**The Focal Adhesion Kinase FAK, but Not PYK2, Plays a Central Role for Growth Factor and CCK Stimulated Signaling in Pancreatic Carcinoma**

A. Pace, M. Müller, M. Bläker, A.W. Lohse, A. de Weerth

I. Medizinische Klinik und Poliklinik, Universitätsklinikum Hamburg-Eppendorf, Germany

**Introduction:** The structurally related focal adhesion kinases FAK and PYK2 are involved in key signal transduction pathways in normal and malignant tissues. In spite of their structural homology, they have distinct roles in key cellular events such as cell adhesion, regulation of the cell cycle and apoptosis. In contrast to PYK2, FAK promotes cell-survival and proliferation. Pancreatic carcinoma is one of the most frequent GI-cancers with a very poor prognosis. There is rising evidence for FAK to be implicated in pancreatic cancer. The aim was to study the expression of focal adhesion kinases, their activation by HGF and CCK and their involvement in intracellular pathways in pancreatic cancer.

**Methods:** FAK and PYK2 were detected in PANC-1 by immunoprecipitation (IP), their activation assessed by western blotting with phosphotyrosine antibodies. The involvement with other proteins was assessed by coimmunoprecipitation. Their expression in human pancreatic cancer tissues was assessed by PCR and immunohistochemistry.

**Results:** FAK, but not PYK2 is expressed in PANC-1 cells and in pancreatic cancer specimen as shown by WB, PCR and immunostaining. The stimulation with HGF and CCK stimulated the tyrosine phosphorylation (TyrP) of FAK. HGF and CCK induced an association between FAK and the pp60Src. Furthermore the GF and CCK stimulation led to the activation of one of FAK’s downstream targets, ERK 1/2. The addition of the specific Src-inhibitor PP2 inhibited FAK’s TyrP and ERK 1/2 activation.

**Conclusion:** Similar to the expression in other malignant tissues and cell lines, we found FAK but not PYK2 expression in PANC-1 cells and pancreatic cancer specimen. FAK gets activated upon growth factor and CCK stimulation. This TyrP is dependent on Src. Moreover, the HGF and CCK induced ERK activation is Src dependent.

These data suggest that FAK mediates mitogenic signaling in pancreatic carcinoma, implying a central role in pancreatic carcinogenesis for FAK.
**P90**

Bile Acid and Alcohol Metabolite Potentiate Second Messenger – Dependent Intracellular Ca$^{2+}$ Release Resulting in Necrotic Death of Pancreatic Acinar Cells when ATP is Absent

J.A. Murphy$^1$, D.N. Cridle$^1$, J.P. Neoptolemos$^2$, A.V. Tepkin$^1$, O.H. Petersen$^1$, R. Sutton$^2$, MRC Group

$^1$The Physiological Laboratory, $^2$Division of Surgery and Oncology, University of Liverpool, UK

**Introduction:** A sustained rise in the cytosolic [Ca$^{2+}$] causes premature intracellular digestive enzyme activation. Bile salts and alcohol metabolites produce such pathological [Ca$^{2+}$] elevations. Here we evaluate the role of the second messengers inositol triphosphate (IP$_3$), nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic-ADP-ribose (cADPr) and their receptors mediating Ca$^{2+}$ release from endoplasmic reticulum stores.

**Methods:** Mouse pancreatic acinar cells loaded with a Ca$^{2+}$-sensitive fluorescent dye were examined by confocal microscopy. IP$_3$, NAADP or cADPr were added extracellularly via a patch pipette. Taurolithocholic-3-sulphate (TLCS) or palmitoleic acid ethyl ester (POAEE) were added on top of cADPr and their receptors mediating Ca$^{2+}$ release from endoplasmic reticulum stores.

**Results:** IP$_3$ (1–2 μM) induced typical local [Ca$^{2+}$] rises in the granular pole. Extracellular TLCS or POAEE transformed this response into prolonged (>30 s), global [Ca$^{2+}$] elevations (TLCS 20 of 24 cells; POAEE 24 of 29) prevented by the IP$_3$ receptor antagonist caffeine (20 mM). Similar transformations occurred when TLCS (27 of 28), but not POAEE (15 of 15), were added on top of cADPr (10–15 μM) and when POAEE (17 of 19), but not TLCS (11 of 12), were added on top of NAADP (50 nM). Prolonged, global transformation consistently induced cellular necrosis, which was prevented by adding ATP (8 of 8). No cellular necrosis was observed without Ca$^{2+}$ signal globalisation.

**Conclusion:** Low concentrations of a bile salt or a non-oxidative alcohol metabolite can induce pancreatic acinar cell injury by sensitising Ca$^{2+}$ release channels to physiological second messenger actions. This causes pathological Ca$^{2+}$ release, mitochondrial depolarisation and necrotic cell death, which can be prevented by adding ATP intracellularly. These experiments suggest that internal Ca$^{2+}$ release channels may be suitable therapeutic targets in acute pancreatitis.

This work was supported by the Medical Research Council, UK. JAM holds an Amelie Waring Clinical Research Fellowship from CORE.

---

**P91**

Overexpression Of Smad6 Enhances Pancreatic Fibrosis Induced By Chronic Pancreatic Injury In Transgenic Mice

T. Miyamoto

Department of Gastroenterology and Metabolism, University of Occupation and Environmental Health, Kitakyusyu, Hukuoka, Japan

**Introduction:** We have developed transgenic (Tg) mice overexpressing Smad6 in the pancreas and demonstrated that acute pancreatitis induced in these mice by feeding choline-deficient, ethionine-supplemented diet was severer than that in control C57BL/6N (Wt) mice (Kaku M et al: Gastroenterology 2005;128:A379). In the present study, we examined whether overexpression of Smad6 has any influences on pancreatic fibrosis after chronic pancreatic injury.

**Method:** Chronic pancreatic injury was induced in Tg and Wt mice by 6 h intraperitoneal injections of 50 μg/kg body weight cerulein, three times a week for 4 weeks. Pancreatic fibrosis was evaluated by histological examination after H&E and Azan staining, image analysis, and by measuring hydroxyproline contents in the pancreas. In addition, serum amylase activity and amylase and trypsinogen contents in the pancreas were determined.

**Results:** Histological grade of fibrosis and fibrotic area by image analysis in Tg mice were significantly higher than those in Wt mice after induction of chronic pancreatic injury. Hydroxyproline contents in the pancreas in Tg mice were significantly greater than those in Wt mice at week 1 (3.4 ± 0.2 vs. 2.3 ± 0.6 mg/g pancreas, p < 0.05) and week 2 (4.9 ± 0.1 vs. 3.5 ± 0.1 mg/g pancreas, p < 0.05). There were no significant differences of amylase activity in serum and the pancreas between the two groups throughout the observation periods. Pancreatic trypsinogen contents in Tg mice at baseline were similar to those in Wt mice, but significantly increased in Tg mice after induction of pancreatic injury compared with those in Wt mice, from week 1 to 4 week (2.3 ± 0.2 vs. 1.2 ± 0.2 μg/mg DNA, p < 0.05).

**Conclusion:** Our present results demonstrate that overexpression of Smad6 in the pancreas enhances the development of pancreatic fibrosis induced by chronic pancreatic injury, probably due to decreased exocytosis of trypsinogen.

---

**P92**

Elevated Serum Level of the Complement Regulator Protein CD59 is a Predictor of Organ Failure in Acute Pancreatitis

O. Lindström$^1$, L. Kylänpää$^1$, H. Jarva$^2$, P. Puolakkainen$^1$, E. Kemppainen$^1$, R. Haapiainen$^1$, H. Repo$^3$, S. Meri$^2$

$^1$Department of Surgery, $^2$Institution of Haartman, $^3$Department of Medicine, University of Helsinki, Helsinki, Finland

**Introduction:** Complement activation has been shown in the plasma of patients with acute pancreatitis (AP). CD59 is a small protein that down-regulates activation of complement. In this study, we analysed AP patients during the first week after admission, for the serum levels of the complement membrane regulator protein CD59.
The main aim was to determine whether release of soluble CD59 could predict the development of organ failure in AP.

Methods: Thirty-nine patients with AP admitted to Helsinki University Central Hospital were included into the study. Patients were retrospectively categorised into those with mild AP (grade 0) or severe AP according to Atlanta classification. Patients with severe AP were further subcategorised into those who had only local complications and who recovered without organ dysfunction (grade 1), and into those who developed organ dysfunction (grade 2). Thirteen patients with grade 0, 14 with grade 1 and 12 with grade 2 attacks were included. The patients with organ dysfunction (grade 2) were compared to patients without organ dysfunction (grade 0 and 1). Blood samples were collected at admission and on days 1 and 3–7 post admission. Mann-Whitney U-test was used for comparison between the groups.

Results: At admission, the serum CD59 concentration was significantly higher (p = 0.002) in grade 2 patients (median 104.2, range 26.1–186.3) than in grade 0 and 1 patients (median 37.3, range 30.3–75.9 and median 38.6, range 19.9–96.1). The serum CD59 concentration remained also significantly higher in grade 2 patients than in grade 0 and 1 patients during the hospitalisation on days 1 and 3–7 (p = 0.001 and p = 0.002). Also, the overall serum CD59 concentrations were significantly higher in patients with organ dysfunction (p < 0.001) than in patients without organ dysfunction.

Conclusion: Elevated serum levels of complement regulator protein CD59 seem to predict organ failure in severe AP.

P94
A New Treatment for Pancreatic Cancer by An Oncolytic HSV-1 Vector with Gemcitabine
K. Kami1, M. Wada1, S.-I. Miyatake2, R. Doi1
1Department of Surgery and Surgical Basic Science, Kyoto University, Kyoto, and 2Department of Neurosurgery, Osaka Medical College, Osaka, Japan

Introduction: We have previously reported about a combined therapy of an oncolytic replication-competent HSV-1 vector (d120.survE) using survivin promoter and radiation for pancreatic cancer. We constructed a HSV-1 vector (d120.survE) which replicates selectively in survivin expressing cells. Survivin expresses in cancer cells specifically, and is considered to be a chemo-resistant factor for pancreatic cancer cells. We assessed the ability of d120.survE and/or gemcitabine to inhibit the growth of pancreatic cancer cells.

Methods: (1) A transgene (TK promoter-LacZ-transcriptional enhancer-survivin promoter-ICP4) was cloned into tk gene of the mutant HSV-1 d120 which lacks ICP4 gene, to generate d120.survE. (2) In vitro studies; Transgene (ex. ICP4, LacZ) expression was examined by RT-PCR and X-gal staining. The ability of d120.survE to replicate and the cytotoxic effect of d120.survE in cells treated with and without gemcitabine were examined. (3) In vivo studies; Nude mice harboring AsPC-1 tumors subcutaneously received once on day 1 intraperitoneal injection of d120.survE with and without intraperitoneal injection of gemcitabine (1mg/body) once per week. The tumor sizes were measured every 5 days.

Results: (1) Transgene was expressed in the cells infected with d120.survE. The ability of d120.survE to replicate and the cytotoxic effect of d120.survE were correlated with survivin promoter activity of the host cells, and they were augmented after gemcitabine treatment. (2) Subcutaneous tumors treated with d120.survE were significantly smaller than control tumors by day 70 post-infection. Tumors treated with gemcitabine and d120.survE were significantly smaller than tumors treated with d120.survE alone. Transgene expression was observed in tumors treated with d120.survE, but not in normal tissues from mice treated with d120.survE.

Conclusion: A combined therapy of d120.survE and gemcitabine may be a useful treatment for pancreatic cancer.
P95
Role of NO in the Protection Against Acute Pancreatitis and Associated Lung Injury by CXCR2 Antagonist Antileukinate
A. Hegde, A.D. Ang, M. Bhatia
Department of Pharmacology, National University of Singapore, Singapore

Introduction: Various chemokines and endogenous inflammatory mediators released from the inflamed pancreas can result in the overproduction of nitric oxide (NO). NO is involved in the pathophysiology of acute pancreatitis. Antileukinate (Ac-RRWWCR-NH₂) is a potent inhibitor of binding of CXC chemokines to the receptors (CXCR2) and shown to protect rodents against acute inflammation. This study aims to evaluate the effect of antileukinate treatment on CXC chemokine macrophage inflammatory protein-2 (MIP2) and NO levels in acute pancreatitis in mice.

Methods: Acute pancreatitis was induced in adult male swiss mice by hourly intraperitoneal injections of caerulein (50 μg/kg/h) for 10 h. Antileukinate (52.63 mg/kg, s.c.) was administered to mice either 30 min before (prophylactic) or 1 h after (therapeutic) starting caerulein injections. Plasma and pancreas homogenates were assessed for MIP-2 with a sandwich ELISA. Nitrite/nitrate (NOX) levels as an indicator of NO production were determined in plasma by enzymatic reaction and spectrophotometric analysis.

Results: Caerulein treatment resulted in a significant increase in plasma, pancreas, and lung MIP-2 and plasma NOX levels compared to the corresponding levels in control mice treated with normal saline. Prophylactic and therapeutic administration of antileukinate showed a trend to reduce MIP-2 levels especially in pancreas and lung. There was a significant reduction in NOX levels in plasma with therapeutic antileukinate treatment.

Conclusion: These results suggest that antileukinate therapy may protect against acute pancreatitis and associated lung injury by an NO-dependent mechanism.

P96
Proteome and Transcriptome Analysis Reveals an Isolated Protease Deficiency in the Pancreas in Patients with Autoimmune Pancreatitis
R. Faisnner¹, D. Koczan², P. Bewerunge³, B. Brors³, G. Klöppel⁴, R. Lösel⁴, B. Sipos⁵, M. Löhr¹

¹Molecular Gastroenterology, Department of Medicine II, Institute of Immunology, University of Rostock, ²Division of Intelligent Bioinformatics Systems, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, ³Department of Pathology, University of Kiel, Germany, ⁴Department of Clinical Pharmacology, Medical Faculty Mannheim, University of Heidelberg, Mannheim

Introduction: Autoimmune pancreatitis (AIP) represents a newly identified entity complementing the spectrum of inflammatory pancreatic diseases with unknown etiology and antigens. The aim of this study was to determine new target antigens and differentially regulated genes/proteins with transcriptomics and proteomics.

Methods: Gene expression profiling was done using total RNA from fresh-frozen pancreatic tissue samples from four patients with autoimmune pancreatitis and five patients with alcoholic chronic pancreatitis, which were hybridized to Affymetrix HG U133A Gene Chip. For proteome analysis, two-dimensional polyacrylamid gel electrophoresis (2D-PAGE) was run for each of eleven autoimmune pancreatitis, six chronic alcoholic pancreatitis and two normal pancreas specimens on IPG strips of pH 4–7. Gel image analysis was performed (PDQuest) and differentially regulated proteins were excised from the gel, digested, and submitted to mass spectrometry (MALDI-TOF-MS: Bruker Reflex II). Profiles from peptide spectra were run against the database (e.g. Mascot).

Results: Most of the overexpressed genes in AIP are involved in inflammation and autoimmune response. Expression of 61 gene products, including several proteases (anionic and cationic trypsinogen, mesotryptsinogen, chymotryptsinogen, elastase) was downregulated in autoimmune pancreatitis. In 2D-PAGE, we found as well a significant reduction of serine protease precursors (including trypsinogen) in all patients suffering from autoimmune pancreatitis. By immunohistochemistry, the selective lack of trypsinogen in AIP material could be confirmed in histologically normal appearing acini from the same tissue sample.

Conclusion: Patients with autoimmune pancreatitis have an isolated defect in pancreatic proteases, namely trypsinogen. This may be used as a specific diagnostic test for autoimmune pancreatitis.

P97
Functional Assessment of MDR-1 and MRP-1 Activity in Human Pancreatic Cancer
K. Diófalvi¹, B. Tihanyi², L. Nehéz³, E. Schäfer³, T. Micsik³, R. Schwab³, A. Pap³, T.F. Tihanyi²

¹MÁV Hospital First Department of Surgery, ²First Department of Surgery Semmelweis University, ³Rational Drug Design Labs, Budapest, Hungary

Introduction: Malignant pancreatic tumours respond badly for chemotherapy. Those methods that can show information about the chemo-resistance of these tumours are not available in the clinical practice at present. It is known that the tumour cells express more multidrug-resistance (MDR)-transporters, than the normal ones. ABC (ATP-binding-cassette) transporters have also been suggested to play a role in resistance to chemotherapy in different malignancies. Follow-up studies show significant correlation between the rate of MDR expression and the prognosis of the disease.

Method: Eighteen intraoperative samples were collected from different pancreatic cancer. The malignancy was proven by intraoperative aspiration cytology in every case. The epithel cells were harvested by collagenase cell-separation and subsequently dyed and examined in calcein-assay. The results of the MDR expression were measured in flow-cytometer. Cut-off value of normal MDR activity was based on previous AML-results which demonstrated to correlate with clinical resistance to the therapy (Br J Haematol 2001;112: 308–314).

Results: More than 50% of cell viability was detected in 10 of 18 samples. We found elevated MDR-1 activity in 6, and elevated...
MRP-1 activity in 4 cases, suggesting these results could influence the management of the chemotherapy.

**Conclusion:** The MDR examination described above was able to show the potential chemoresistant-cases, and could support the individual chemotherapy-plans. 30% of our cases showed markedly elevated transporters dependent chemo-resistance. This method can be an important tool in the future tumour diagnostic and the oncological therapy.

---

**P98**

**Kinetical Expression of CD45 on Pancreatic Acinar Cells During Acute Pancreatitis**

I. De Dios, L. Ramudo, S. Lázaro, S. Yubero, A. García-Montero, M.A. Manso

Department of Physiology and Pharmacology, University of Salamanca, Spain

**Introduction:** CD45 is a member of the protein tyrosine phosphatase family expressed in haematopoietic cells, which play an important role in regulating immune responses. We investigate CD45 expression on pancreatic acinar cells during acute pancreatitis (AP) and examine its role in the inflammatory response developed by these cells.

**Methods:** AP was induced in rats by bile-pancreatic duct obstruction (BPDO). N-Acetylcysteine (NAC) (50 mg/kg/day) was administered 1 h before and 1 h after BPDO. CD45 was assessed in control acinar cells by immunohistochemistry. CD45 mRNA expression was evaluated in acinar cells by RT-PCR. At protein level, CD45 was analyzed together with TNF-α by flow cytometry using a double labelling PE/Cy5-anti-CD45/PE-anti-TNF-α. Phospho p-38 MAPK was analyzed in cytosolic extracts of acinar cells by Western blot.

**Results:** Acinar cells constitutively express CD45 mRNA, whose levels significantly (p < 0.05) decreased from 12h after BPDO. In parallel with a marked increase in phospho-p38 MAPK and TNF-α production a significantly (p < 0.01) decreased expression of CD45 was found on acinar cell surface from 6h after BPDO onwards. NAC treatment reduced MAPK activation, prevented TNF-α production by acinar cells and reduced and delayed the decrease in the CD45 acinar expression induced by AP.

**Conclusions:** CD45 is constitutively expressed in acinar cells. It seems to play a role in negatively regulating cytokine production during AP.

---

**P99**

**Not submitted**

---

**P100**

**Characteristics of Caerulein-Induced Pancreatitis in Different Strains of Mice**

B. Brandt-Nedelev1, J. Mayerle2, I. Jarosckova1, T. Wartmann1, A. Aghdassi2, H. Lippert1, M.M. Lerch2, W. Halang1

1Division of Experimental Surgery, Otto-von-Guericke-University Magdeburg, 2Department of Gastroenterology, Endocrinology and Nutrition, Ernst-Moritz-Arndt-University Greifswald, Germany

**Introduction:** Transgenic and knock-out mice are frequently used to investigate acute experimental pancreatitis. Recently we found different trypsinogen isoenzyme patterns in mice of various genetic backgrounds. Here we studied the extent of pancreatic and extrapancreatic injury in three strains of mice after induction of caerulein pancreatitis.

**Methods:** In different strains of mice (FVB/NHanHsd (FVB); C57BL/6JOldHsd (BL6); Hsd:ICR (CD-1)) acute pancreatitis was induced by 7 h injections of caerulein (50 µg/h, i.p.). Control mice received i.p. saline. Amylase and lipase were enzymatically determined in serum and pancreatic tissue. Pancreatic oedema was calculated as ratio of pancreatic wet weight to body mass. Myeloperoxidase (MPO) activity was photometrically measured in lung and pancreas samples after 8 h and 24 h, respectively. A histological score in H&E staining to determine pancreatic injury was performed. Serum cytokine levels (TNFα, IFNγ, IL-6, IL-10, IL-12 and MCP-1) were monitored.

**Results:** After supramaximal caerulein stimulation we did not detect a significant difference between the mice strains in the ratio of pancreatic wet weight to body mass. Highest levels of serum amylase and lipase were observed after 8 h. In comparison to CD-1 and FVB mice BL6 mice displayed significantly lower levels of lipase and amylase (40% lower levels), which was not due to lower pancreatic expression. MPO activities as marker of neutrophil infiltration were significantly lower in pancreatic tissue of BL6 mice compared to CD-1 and FVB mice. MPO activities in lung tissue were significantly increased vs. controls already at 8 h. Here, FVB and BL6 mice showed lower levels compared to CD-1 mice. This finding was supported by increased MCP-1 and IL-6 levels in CD-1 mice.

**Conclusions:** Our results demonstrate that each of the three strains of mice showed individual characteristics in the time course and extent of local and systemic pancreatic injury of experimental pancreatitis. In BL6 mice, frequently used as background strain for knock-out mice, pancreatic injury was determined as less severe after caerulein-hyperstimulation. This needs to be taken into account for further investigations employing animal models.
Clinical

P101
Pancreatic Pseudocysts: Factors Influencing Effectiveness of Endoscopic and Surgical Treatment

E. Zdanye, S. Jurevicius, A. Sileikis, K. Strupas
Center of Abdominal Surgery, Vilnius University Hospital ‘Santariskiu Klinikos’, Vilnius, Lithuania

Introduction: The innovative less invasive endoscopic techniques challenge the traditional surgical management of pancreatic pseudocysts. Surgical approaches allow internal decompression, resection of diseased pancreas as necessary with low morbidity, mortality and recurrence rates. Endoscopic drainage provides a good alternative or supplement to a surgical treatment. The purpose of our study was to evaluate factors influencing therapeutic outcomes in cases of endoscopic and surgical management of pseudocysts.

Methods: Prospective study of 106 patients (mean age 44.9) with symptomatic and/or bigger than 5 cm pseudocysts treated from 2000 to 2004 was conducted. Thirty nine patients underwent endoscopic drainage (40) procedures: wall incisions (15), transmural drainage (24), pancreatic duct sphincterotomy (1). Sixty eight patients had surgical procedures: cystojejunostomy (29), cystogastrostomy and cystoduodenostomy (1), pancreatic resection (24), external drainage (5), pancreaticojejunosuy (9). The impact of endoscopic vs. surgical management, gender, age, surgical risk according to ASA, nutritional status, etiological and morphological peculiarities of pseudocysts on the treatment outcomes was investigated.

Results: No significant difference between the effectiveness of endoscopic and open operations was found. A lesser experience of surgeon (p = 0.008), female gender (p = 0.008) and incision (vs. transmural drainage) (p = 0.0055) reduced operative effectiveness, pancreatic necroses increased the number of postoperative complications (p = 0.04) in endoscopic group. In the open surgery group advanced age (p = 0.04), male gender (p = 0.03), ASA grade III (p = 0.04), localization of pseudocyst in the pancreatic body/tail (p = 0.04) increased number of intraoperative complications. An average patient age (p = 0.03) and pseudocyst volume (p = 0.04) was greater and localization of pseudocyst in the pancreatic body/tail more frequent (p = 0.003) in patients with postoperative complications.

Conclusions: Endoscopic drainage is an effective alternative to surgical treatment of pseudocyst. Surgical management is necessary in cases of pseudocyst without close opposition to gastrointestinal wall, pancreatic necrosis, underlying pancreatic/pancreatic duct pathology when pancreatic resection and/or duct drainage operations are indicated.

P102
Similarities and Differences in the Mucin Expression of the Pancreatic Ductal Adenocarcinoma and Intraductal Papillary Mucinous Tumor of the Pancreas

B. Tihanyi1, T.F. Tihanyi1, L. Nehéz1, K. Diófalvi2, J. Lüttges3, Á. Andrén-Sandberg4
1First Department of Surgery, Semmelweis University, Hungary; 2Department of Surgery, MAV Hospital, Budapest, Hungary; 3Institute of Pathology Christian-Albrecht’s University of Kiel, Germany; 4Stavanger University Hospital, Bergen University, Stavanger, Norway

Despite advancing knowledge of the molecular biological aspects of the pancreatic ductal adenocarcinoma, less has been known of the pathologic molecular alterations in IPMTs, intraductal papillary mucinous tumors. The evolution of tumor in IPMT follows molecular pathways that similar, but distinct from pancreatic ductal adenocarcinoma. The favourable clinical behaviour of IPMTs attracts interest into the potential genetic and epigenetic factors that foster this tumor’s evolution and progression from pancreatic ductal epithelium. We present the result of a world literature review. It has been shown that the expression of MUC1 (membrane-bound type mucin) and MUC2 (intestinal type secretory mucin) is different in different pancreatic tumors.

MUC1 mucin is highly expressed in invasive ductal adenocarcinoma of the pancreas and it is also related to malignant character of the IPMT carcinomas. The MUC5AC alone is related to benign potential. In contrast MUC2 and MUC5 mRNA are highly expressed in invasive IPMT but rarely expressed in ductal adenocarcinoma (p < 0.005). Patients with IPMT that are positive for MUC2 mRNA or MUC5AC mRNA expression have better survival compared with patients who lack such expressions. The degree of MUC2 expression can correlate with the malignant potential of those IPMTs, that expressing MUC2.

MUC1 mucin expression may be related to the invasiveness or metastatic behaviour of IDC, whereas the lack of MUC1 expression may be related to the less invasive characteristics of IPMT-dark cell type tumor. The expression of MUC2, an abundant extracellular mucin with high viscosity, by a majority of IPMT-dark cell type tumors may be correlated with the site restricted growth of tumors that display lower levels of invasion and metastasis.

Conclusion: The difference between the mucin expression pattern in ductal pancreatic cancer and in the different IPMTs shows that this pattern may play role in the biological behaviour of pancreatic tumors and their malignant potential.
**P103**

**Evaluation of VEGF Gene Polymorphisms in Pancreatic Adenocarcinoma and Chronic Pancreatitis**

R. Talar-Wojnarowska¹, A. Gasiorowska¹, B. Smolarz², H. Romanowicz-Makowska², A. Kulig², E. Malecka-Panas³

¹Department of Digestive Tract Diseases, Medical University, ²Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother’s Memorial Hospital, Lodz, Poland

**Introduction:** Vascular endothelial growth factor (VEGF) is a potent stimulator of angiogenesis, necessary for microvasculature development and important for growth and spread of pancreatic tumours. Functional polymorphism of VEGF gene at position C-460T and G + 405C may influence VEGF serum level.

**Methods:** VEGF gene polymorphisms at position C-460T and G + 405C were evaluated in 22 patients with pancreatic adenocarcinoma (PA), 41 with chronic pancreatitis (CP) and 40 healthy volunteers. VEGF genotypes were studied by allele-specific polymerase chain reaction in DNA isolated from blood samples. The serum concentrations of VEGF were measured by an enzyme-linked immunosay (R&D Systems, USA). The associations of the VEGF genotypes with tumour characteristics at diagnosis were examined.

**Results:** We found an increased frequency of the homozygous +405G/G VEGF genotype in patients with PA (54.5%) compared with CP (24.4%) and control group (20%) (p < 0.01). In contrast, the distribution of genotype and allele frequencies of the −460C/T polymorphism in the PA patients did not differ from those in the CP and control groups. Plasma levels of VEGF were significantly higher in PA patients (mean cytokine level: 756pg/ml) compared with CP (24.4%) and control group (137pg/ml; p < 0.01). In our study, there was no association between VEGF levels and VEGF gene polymorphisms. VEGF C-460T and G + 405C polymorphisms were also unrelated to the tumour size, histologic grade, regional or distant metastases or patients sex and age.

**Conclusion:** These preliminary results indicate that VEGF genotype may play important role in pancreatic carcinogenesis. Further studies are needed to investigate its possible association with PA prognosis.

---

**P104**

**What is the Best Method for Establishing Tissue Diagnosis in Patients with Suspected Pancreatic Tumours?**

A. Sultana¹, H. Ahmad¹, J.M. Grabham¹, C.M. Halloran¹, L. Bossonnet¹, J.C. Evans², M. Lombard², H.L. Smart², I. Gilmore³, M. Rayay¹, M. Hartley¹, R. Sutton¹, J.P. Neoptolemos¹, P. Ghaneh¹

¹Division of Surgery and Oncology, ²Department of Radiology, ³Department of Gastroenterology, Royal Liverpool University Hospital, Liverpool, UK

**Introduction:** Tissue diagnosis in patients with surgically unresectable pancreatic malignancy is critical in decision making for chemotherapy/clinical trials. Currently there is no consensus on which is the best diagnostic approach. The aim of this study was to assess the diagnostic yield of different methods in patients with suspected pancreatic tumours at a single tertiary referral centre.

**Methods:** Patient demographics, diagnostic approaches and histology details of patients with pancreatic tumours were obtained from a prospectively maintained database (January 1997–December 2005). The yields of the diagnostic approaches were evaluated.

**Results:** A total of 763 patients were identified. Of the 387 patients who had histology/cytology, 203 underwent endoscopic retrograde cholangiopancreatogram (ERCP), 36 endoscopic ultrasound (EUS), 46 CT guided biopsies, 33 ultrasound guided biopsies (USG) 46 laparoscopic ultrasound (LUS), and 23 operative biopsies. CT guided biopsy sensitivity was 84%, accuracy 84% and negative predictive value 12.5%. The figures for USG biopsy were 86.4%, 84.8% and 17% respectively. Similar rates were noted for EUS (78.8%, 80.6%, 30%) and operative biopsy (73%, 74%, 14%). However, the diagnostic yield with ERCP overall was lower (57.5%, 58.9%, 6%) as was LUS (55%, 57%, 9%). The figures for a revised method (cytosal) for processing ERCP specimens (after January 2001) revealed 61.8% sensitivity, 59.3% accuracy and 1.8% negative predictive value. All modalities were 100% specific with a 100% positive predictive value.

**Conclusions:** The diagnostic yield for CT, USG and EUS guided biopsies were similar. The diagnostic yield of ERCP is slightly lower than the other methods. However, it is still the most relevant as it is both diagnostic and therapeutic. Considerable effort must be made to obtain specimens during ERCP. The other approaches should be used to complement it, on a case-by-case basis.
in family history were found in 36 patients (26%) and closer analysis showed that gastric cancer (11 cases) and gynecological cancers (7 cases) were most dominated cancers. BRCA1 mutation was found in two patients out of 70 patients blood testing. For patients who are younger 55 and/or have positive family history for other cancers MLH1, CDKN2, LKB1/STK11 gene mutation was analysed. None of those patients carried these mutations.

Conclusions: Smoking is the only risk factor with significant difference from control group. Pancreatic cancer heredity in Latvia population is low (2%). BRCA1 mutation may predispose to pancreatic cancer. Gynaecological cancer was one of most often found cancers in family history, and two of analysed patients carried this mutation.

P106
Validation of Hospital Discharge Data for Acute and Chronic Pancreatitis in the Netherlands
B.W.M. Spanier1, D. Schreuder1, M.G. Dijkgraaf2, M.J. Bruno1
1Department of Gastroenterology, 2 Department of Clinical Epidemiology and Biostatistics, AMC, Amsterdam, The Netherlands

Introduction: In the Netherlands, the Prismant Health Care Information is frequently used for epidemiological studies. This organization collects data on all hospitalizations in the Netherlands. We analysed the reliability of hospital discharge data used by Prismant to classify admissions for acute pancreatitis (AP) and chronic pancreatitis (CP).

Methods: We retrieved all outgoing discharge data from our hospital to Prismant with ICD-9 codes relating to pancreas disease (577*) from 1/1/2002 until 12/31/2003. For validation of the diagnoses we conducted a retrospective study of multi-disciplinary reports.

Results: 284 patients were reported, relating to 483 admissions with 523 pancreas disease-related discharge diagnoses. Of the 523 diagnoses, 112 were coded as AP, 250 as CP and 92 as pseudocysts. The remaining 69 discharge codes were classified as other (un)specified diagnoses, 112 were coded as AP, 250 as CP and 92 as pseudocysts. The remaining 69 discharge codes were classified as other (un)specified diseases of the pancreas. Discharge diagnoses regarding AP were correct in 78% (87/112). Due to miscoding, an additional 40 admissions were identified as AP amounting to a total of 127 admissions. Ultimately this leads to an underestimation of the total number of admissions for AP of 40%, for CP of 4.4% (112/250). Discharge diagnoses regarding CP were correct in 91.2% (226/250). Due to miscoding, an additional 44 admissions were identified as CP amounting to a total number of 260 admissions. This leads to an overestimation of the total number of admissions for CP of 4.3% (250/260). Overall, 74.2% (388/523) of the pancreatitis-related hospital discharge codes on the basis of individual admissions were correct, implying 25.8% were false.

Conclusion: There is a substantial miscoding of discharge diagnoses of AP and CP on the level of individual hospital admission. By itself this would lead to a considerable overestimation. However, due to additional miscoding within each diagnostic entity, part of this overestimation is compensated. As a consequence, the numbers of Prismant discharge diagnoses for AP and CP are slightly underestimated.

P107
Endoscopic Characteristic of Upper Gastrointestine in Patients with Chronic Alcoholic and Chronic Relapsing Pancreatitis
N.V. Shirinskaya
Omsk Medical Diagnostic Center, Omsk, Russian Federation

Introduction: Revealing of endoscopy features of damage of gullet, stomach and duodenum in patients with a chronic pancreatitis of various etiology.

Methods: Eighty patients with chronic relapsing pancreatitis (CRP) and 32 with chronic alcoholic pancreatitis (CAP) were studied.

Results: More often in patients with CAP erosive changes of mucosa of stomach and duodenum were diagnosed 87.5%, frequency of their detectability increased in the development of disease. Papillitis was diagnosed in 84.4% more often in patients who were ill longer than 11 years. Duodeno-gastral reflux was revealed in 62.5%. Endoscopically positive gastro-esophageal reflux diagnosed in 34.4%. Most often it was marked in patients with CAP after 6–10 years. More seldom in CAP patients ulcers were found (6.2%), and only in persons with average terms of illness (6–10 years).

Endoscopically patients with CRP showed changes of major papilla duodeni mucosa. Changes of stomach and duodenum mucosa varied from surface gastroduodenitis (80%) up to erosive gastritis (5%) and duodenum ulcers (18.7%). Duodeno-gastral reflux was present in 75% patients with CRP and in the development of disease with growth of detectability read from 57.4% up to 100%. Gastro-esophageal reflux was revealed in 23.7% patients with CRP, and the number of patients with this symptom did not depend on the duration of the disease.

Conclusions: Most often in patients with CAP erosive changes of mucosa and surface gastroduodenitis were found. In patients with CRP papillitis and duodeno-gastral reflux were revealed more often.

P108
The Incidence of Acute Alcoholic Pancreatitis Follows the Changes in Alcohol Consumption in Finland
J. Sand, A. Välikoski, I. Nordback
Department of Gastroenterology and Alimentary Tract Surgery Tampere, Finland

Alcohol is the best known cause of acute pancreatitis in Finland, accounting around 70% of cases. Between 1970 and 1989 the incidence of pancreatitis increased in association with increased alcohol consumption from 47 to 73/100,000 inhabitants. During 1987–2001 alcohol consumption increased from 8.2 to 9.0 litres / inhabitant with a temporary decrease during early 90s in Finland. Our aim was to investigate whether hospitalizations and mortality for pancreatitis changed according to these changes in alcohol consumption. Hospitalizations for liver cirrhosis and gallstone disease were studied for comparison.

Methods: The annual data of alcohol consumption and hospital discharge data and number of deaths for pancreatitis, liver cirrhosis and gallstone disease between 1987 and 2001 were obtained from...
Patients aged 70 or more had a positive LR of 10.89 and negative LR 0.12. For patients younger than 70 years of age, the positive LR was 6.09 and negative LR 0.13. For patients with severe AP, CAPAP is not as good a predictor as MCP-1 but its relatively high concentration in patients with severe AP makes it easier to create a quick test for clinical use.

Conclusions: The overall incidence of hospital discharges due to pancreatitis and liver cirrhosis, as well as alcohol intake increased during the study period. The discharges for acute alcohol pancreatitis decreased with short delay after a temporary decrease in alcohol consumption during period of depression in the early 90s.

P109
CAPAP and MCP-1 are Good Predictors for Severe Acute Pancreatitis
S. Regnér, S. Appelros, C. Jansen, A. Borgström
Department of Surgery, Malmö University Hospital, Lund University, Malmö, Sweden

Introduction: Early prediction of severity of acute pancreatitis (AP) is important for clinical as well as research reasons. Much effort has been dedicated into finding a good biochemical marker for the severity of AP. The aim of our study was to evaluate markers reflecting protease activation and inflammation according to prediction of severity.

Method: In this prospective study 150 patients admitted to Malmö University Hospital with acute pancreatitis were included. CAPAP (the activation peptide of procarboxypeptidase B), aCAP (active carboxypeptidase B) and MCP-1 (monocyte chemotactic protein-1) were analysed in serum samples collected at admission. ROC curves were used for determination of cut offs for the markers. Patients were followed up clinically and classified according to Atlanta. Likelihood ratios (LR) for prediction of severity were calculated

Results: The levels of CAPAP, aCAP and MCP-1 were significantly higher in patients with severe AP than in patients with mild disease (p < 0.05). MCP-1 was the best predictor having positive LR 6.09 and negative LR 0.13. For patients younger than 70 years of age the positive LR for MCP-1 was 10.89 and negative LR 0.12. For patients aged 70 or more MCP-1 and s-CAPAP had a positive LR of 6.09 and negative LR 0.13. For patients younger than 70 years of age, the positive LR was 6.09 and negative LR 0.13. For patients with severe AP, CAPAP is not as good a predictor as MCP-1 but its relatively high concentration in patients with severe AP makes it easier to create a quick test for clinical use.

Conclusions: The overall annual incidence of pancreatitis discharges in the population increased from 68 to 102/100,000 (from 37 to 60 in women and from 96 to 146 in men). In acute alcoholic pancreatitis there was an increased incidence in both middle-aged (45–65 years) women (from 5 to 25) and men (from 60 to 120). Interestingly the discharged rate for acute alcohol pancreatitis decreased with 2 to 3 years delay following decreased alcohol consumption during 91–94. Mortality for pancreatitis did not change during the study period. The discharges due to liver cirrhosis increased both in women (from 13 to 28) and men (from 52 to 90), without temporary decreases as in alcoholic pancreatitis. An increase in gallstone disease discharges was found in women between 15 and 44 years and in men.

Conclusions: The overall incidence of hospital discharges due to pancreatitis and liver cirrhosis, as well as alcohol intake increased during the study period. The discharges for acute alcohol pancreatitis decreased with short delay after a temporary decrease in alcohol consumption during period of depression in the early 90s.
infused in all patients. The contamination of necrotic foci was determined using percutaneous CT-guided fine needle aspiration.

At the time of admission the Ranson, APACHE II, and Balthazar score was not different in both groups. Contamination of the necrotic focuses was confirmed in 22 (35.5%) patients of the first group and in 5 (14.3%) patients of the second group. Besides that, the further spreading of necrotic process was established in 25 (40.3%) patients of the first group and only in 2 (5.7%) patients of the second group. All patients with infected necrosis and 12 patients with sterile necrosis were operated. By that, all patients of the first group required the wide laparotomy due to the widespread necrosis. In patients of the second group the operation contented with drain of abscesses.

Thus, the intra-arterial infusion of antibiotics is effective for the prevention of septic complications in patients with necrotizing pancreatitis.

P113
Liver Function Tests in Patients with acute Alcoholic Pancreatitis with Different Degrees of Severity in the Early Phase of the Disease
J. Panek, D. Karcz, J. Zasada, M. Tusinski
Second Surg Department Collegium Medicum, Jagiellonian University, Cracow, Poland

Introduction: Usually, the recognition and assessment of liver damage and recovery in acute pancreatitis (AP) is done with the help of the liver enzyme profile (ALT, AST), enzyme γ-glutamyltransferase, and with the products of liver synthesis and metabolism (bilirubin and albumin).

Material and Methods: Thirty 6 patients with a mild form of alcoholic AP (MAAP) and 11 with the severe form of alcoholic AP (SAAP) were involved in the study. Serum levels of ALT, AST, γ-glutamyltransferase, bilirubin and albumin were measured on the day of admission and on the 3rd, 5th and 7th day of hospitalization.

Results: Differences between the mean levels of ALT, AST and bilirubin in the groups with MAAP and SAAP were statistically insignificant at admission day. The mean value of transaminases decreased insignificantly in both groups during the period study. The mean serum albumin levels were significantly higher throughout the study in the patients with MAAP as compared to patients with SAAP. Ratio of AST: ALT was significantly higher in patients with SAAP at admission day. The mean level of γ-glutamyltransferase was significantly higher in patients with SAAP.

Conclusions: Liver injury is related to amount of alcohol abuse. Different factors are responsible for liver damage. The most important seems are the FFAE-s.

P112
Successful Conservative Treatment of 50 Consecutive Postoperative Pancreatic Fistulas
S. Pedrazzoli1, C. Pasquali1, C. Sperti1, S. Scappin1, G. Liessi2
1Department of Medical and Surgical Sciences, Clinica Chirurgica IV, University of Padua, Padova, 2Department of Radiology, Castelfranco Veneto Hospital, Treviso, Italy

Introduction: Pancreatic fistula is a feared complication after pancreatic surgery, with a mortality rate up to 28% due to retroperitoneal sepsis and hemorrhage [1]. We performed a retrospective study of the incidence of pancreatic fistula, and of the results of treatment, of patients who underwent pancreatic surgery.

Methods: From January 1994 and January 2006, 335 patients underwent pancreatic surgery. Twelve total pancreatectomies were exclude living 323 patients. Drains were removed within 8 days whenever possible. A pancreatic fistula was diagnosed on the basis of drainage of more than 50 ml of amylase-rich fluid (>5,000 IU)/day. A conservative treatment, based on interventional radiology, aimed to create a straight external fistula was applied to all patients.

Results: Out of 323 patients: 135 underwent surgery for a pancreatic or periampullary cancer, 67 for endocrine pancreatic tumors, 45 for a cystic tumor of the pancreas, 16 for IPMN, 40 for chronic pancreatitis, 15 for pseudocyst post SAP, 16 for several different diseases. A pancreatic fistula was diagnosed in 50 patients (15.5%). The fistula rate was 8.8% (11/124) after pancreateoduodenectomy, 11.9% (11/92) after left pancreatectomy, 53.8% (14/26) after central pancreatectomy, 41.1% (7/17) after DPPHR, 20.7% (6/29) after enucleation, 5.5% (1/18) after pancreaticojjunostomy, 20% (1/5) after subtotal pancreatectomy, 0% after cystojejunostomy (0/9). All underwent conservative treatment. A patient in dialysis for renal insufficiency developed a pancreatic fistula after pancreatic surgery. Twelve total pancreatectomies were performed. By that, all patients of the first group required the wide laparotomy due to the widespread necrosis. In patients of the second group the operation contented with drain of abscesses.

Thus, the intra-arterial infusion of antibiotics is effective for the prevention of septic complications in patients with necrotizing pancreatitis.

Abstracts
**Methods:** Forty three patients (pts) entered: A: Healthy controls without alcohol consumption. Groups B-D: heavy alcohol consumption (>80 g/day pure alcohol); B: Pts without AAIP. C: Pts with one attack of AAIP. D: >3 AAIP. Biopsies during upper endoscopy were taken from duodenal mucosa, total mRNA extracted and reverse transcribed and analyzed by real time PCR for α-defensins HD-5 and -6 and β- defensins hBD-1, -2, and -3. Standard curves for each defensin were performed allowing quantification of gene copy numbers.

**Results:**: Expression of the HD-5/6 was significantly higher in the duodenum compared to the other defensins. HD-6 showed slightly higher expression in patients with more than 3 episodes of recurrent alcoholic pancreatitis, while HD-5 and hBD-1 were not significantly changed. There was only a very low copy number of hBD-2 and -3. In patients with one episode of alcohol induced pancreatitis there was a not significant upregulation of hBD-2 expression indicating inflammation. Discussion: Chronic alcohol intake did not inhibit duodenal defensin expression. The slight upregulation of HD-6 might be the consequence of increased number of Paneth cells. In conclusion, in chronic alcoholism and acute alcoholic pancreatitis duodenal defensin expression is not diminished and only slightly upregulated.

---

**P115**

**Pancreatic and Biliary Abnormalities in Patients with Autoimmune Pancreatitis (AIP) at MRI and Endoscopic Ultrasounography (EUS)**

D. O’Toole1, M.-P. Vullierme2, V. Rebours3, L. Palazzo1, A. Aubert1, P. Ponsot1, K. Nahon-Uzan1, A. Couvelard4, P. Levy2, P.V. Vilgrain2, P. Ruszniewski2, P. Hammel1

1Departments of Gastroenterology, 2Radiology and 3Anatomy, Beaujon Hospital, Clichy, France

**Introduction:** Although AIP is increasingly recognised in the absence of histology or a well-defined associated autoimmune disease (~ 40% [1]) the diagnosis remains difficult.

**Aims:** Examine for pancreatic and biliary signs of AIP at MRI and EUS in a cohort of patients (pts) with AIP followed in a single centre.

**Methods:** Retrospective study of 24 pts (15 men) with AIP (diagnosis based on either histology, or pancreatitis without a definable cause associated with another autoimmune disease and/or a favourable response to corticosteroids) using MRI and pancreatic and biliary EUS.

**Results:** (1) MRI – changes on T1 included: a hypointense zone (n = 14), a pseudotumour (n = 5); on T2: a hyperintense ring (n = 11); parynchymal pseudocyst (PC) (n = 5). Ductal changes at MR-pancreatography were – main pancreatic duct (MPD): not visualised (n = 15, (63%); segmental, n = 13) or a narrowed irregular MPD (n = 6); dilated segments (n = 5; >10 mm in 1 pt); – secondary ducts: moderate dilatation (n = 5). Stenosis of the CBD was observed in 8 pts. (2) EUS – the pancreas was diffusely enlarged in 8 pts with a heterogeneous or hypoechoic echo pattern in 6 and 1 pts, respectively. Calcifications were observed in 1 pt. A focal hypoechoic mass was noted in 13 pts (54%) (head: 9, isthmus: 2 and body/tail: 2). Stenosis of the MPD was observed in 15 pts (63%); diffuse, n = 13) with hyperechoic walls in 11 pts (diffuse in 10). Segmentary or diffuse dilatation of the MPD was observed in 4 and 1 pts, respectively. Peri-pancreatic inflammation was noted in 4 pts (2 with vascular encasement and 4 intra-pancreatic PCs).

CBD stenosis was found in 13 pts (54%) with signs of cholangitis in 8 of these. Lymph nodes >10 mm were visualised in 6 pts.

**Conclusion:** (1) Abnormalities of the MPD appear constant in AIP observed at MRI (88%) or EUS (75%): including non-visibility or an extremely narrowed duct, and these changes are rare in pancreatitis from other causes. (2) Other frequent changes at MRI and EUS supporting the diagnosis of AIP include a pseudotumoral aspect and cholangitis.


---

**P116**

**PIBF (Progesteron Induced Blocking Factor) is Elevated in Pancreatic Cancer Patients**

T. Micsik1,5, B. Polgár1, K. Ráski2, F. Péterffy2, E. Schäfer3, K. Diófai6, F. Hollós6, Á. Papi3, L. Kopper3,5, R. Schwab3, J. Szekeres-Barthó1, I. Peták1,6

1Department of Microbiology and Immunology, Medical University, Pécs, 2Diagnostikum Rt/Ltd, Budapest, 3Department of Gastroenterology, 4Cooperative Research Center, Department of Pathology, Semmelweis University, 5Department of Surgery, MAV Hospital Budapest, Hungary

**Introduction:** The protein of PIBF (progesteron induced blocking factor) is synthesised and secreted by progesterone stimulated lymphocytes and placenta. The effects of PIBF cause multiple protective factor for the embryo development through a Th1 > Th2 shift. On the other hand the evolving environment of decreased cellular immunity and NK cell activity ends up in a favorable condition for tumor development and progression. Recent findings showed elevated PIBF-levels of rapidly proliferating cancer cell lines and epithelial carcinomas were stained positively with PIBF antibodies. Our aim was to investigate PIBF levels of pancreatic malignancies.

**Methods:** Urine samples were obtained from 25 patients with pancreatic cancer (16 without metastasis and 9 with metastasis), while controls were taken from healthy people (19 cases) after written consent. PIBF-levels were determined by a single plate competitive ELISA and afterwards statistical analysis was performed.

**Results:** We found higher PIBF levels in the urine of patients suffering in pancreatic cancer, than in the urine samples of healthy patients. The difference was statistically significant (p = 0.000035; mean: 127 ng/ml vs. 28 ng/ml) between the healthy and malignant cases and there was a trend (p = 0.06, 97 vs. 176 ng/ml) towards higher PIBF levels in pancreatic cases with metastases.

**Conclusion:** The investigated clinical urine samples of different pancreatic cancer cases showed elevated levels of PIBF with ELISA. Higher levels could be determined in metastatic cases. Further analysis and immunohistochemical studies are in progress to evaluate the role of PIBF as a tumormarker in case of pancreatic cancers.
P117

**Obesity Correlates with Early Hyperglycaemia in Patients with Acute Pancreatitis who Developed Organ Failure**

P. Mentula, L. Kylänpää, P. Puolakkainen, E. Kempppainen
Second Department of Surgery, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland

**Introduction:** Hyperglycaemia in early acute pancreatitis (AP) is a prognostic sign of severe attack. Obesity, another risk factor for severe AP, is associated with impaired glucose regulation and might contribute to hyperglycaemia in severe AP. We hypothesized that obesity is related to early hyperglycaemia in patients with AP.

**Methods:** Thirty consecutive patients with severe AP complicated with organ failure and 87 control patients with AP (23 severe AP and 64 mild AP) but without organ failure were retrospectively studied. All patients were admitted to Helsinki University Central Hospital between 1998 and 2001. Plasma glucose level was measured at admission. History of diabetes prior to attack of AP and patients’ height and weight measured at admission were obtained from patient records.

**Results:** Body mass index (BMI) was slightly higher in patients with organ failure than in control patients, median 26.2 kg/m² (inter quartile range 24.9–30.0) vs. 25.2 kg/m² (22.9–27.9), p = 0.033. Plasma glucose level correlated with BMI in patients with organ failure, r = 0.495, p = 0.005, but not in control patients, r = 0.108, p = 0.32. Seven (23%) patients who developed organ failure and six (7%) control patients had history of type II diabetes, p = 0.037. In organ failure group, patients with history of diabetes had higher BMI than that of non-diabetic patients (median 31.1 kg/m² vs. 25.4 kg/m², p = 0.049). In logistic regression model of admission glucose level, history of diabetes and BMI, admission glucose level was the only independent predictor of organ failure.

**Conclusions:** Not only diminished insulin secretion due to necrotizing AP but also obesity associated impaired glucose tolerance may contribute to early hyperglycaemia in patients with AP. Multivariate analysis indicates that obesity may not be an independent risk factor of organ failure but it is associated with impaired glucose tolerance and type II diabetes which may predispose to systemic complications in AP.

---

**P118**

**Insulin Resistance in Diabetes Secondary to Chronic Pancreatitis**

M. Manca, M. Migliori, L. Bastagli, U. Azzolini, R. Pezzilli, R. Casadei, L. Gullo
Institute of Internal Medicine, Sant’Orsola Hospital, University of Bologna, Italy

**Introduction:** It is known that diabetes is a frequent complication of chronic pancreatitis (CP) and its prevalence increases with the progression of the disease. The pathogenesis of this complication essentially consists of the reduction of insulin production caused by pancreatic fibrosis. Nevertheless, some authors reported that insulin resistance may have an important role. The aim of our study was to investigate whether insulin resistance is involved in the pathogenesis of diabetes in chronic pancreatitis.

**Methods:** We studied 16 patients with chronic pancreatitis and diabetes; they were 15 males and 1 female, mean age 50 years, range 31–70 years. The etiology of pancreatitis was alcoholic in 15, all but one were on insulin therapy, 2 had a family history of diabetes and 5 were overweight. Seven healthy subjects were also studied as controls. We measured basal insulin, glucose and c-peptide; plasma c-peptide pre- and post-glucagon and pre- and post-breakfast. HOMA-IR, which measures insulin sensitivity, was calculated as follows: [fasting serum insulin (µU/ml) × fasting plasma glucose (mmol/l)]/22.5.

**Results:** We found a reduced basal c-peptide plasma concentration in chronic pancreatitis patients (mean 0.76 mg/dl) vs. normal control subjects (mean 1.16 mg/dl) and a reduced response of c-peptide to both glucagon stimulus (CP patients, mean +72.73%; normal controls, mean +125.93%) and breakfast (CP patients, mean +84.30%; normal controls, mean +241.36%). HOMA-IR showed 25% of CP patients had reduced sensitivity to insulin, against 14% of healthy controls.

**Conclusion:** The results confirm that in chronic pancreatitis diabetes is mainly due to a reduced insulin production. In a minority of cases, however, insulin resistance may also have a role.

---

**P119**

**Clinical Relevance of Immunohistochemical and Molecular Study of Epidermal Growth Factor Receptors (EGFR) in Human Pancreatic Tumours**

A. Lozano-León, B. Vieites, J. Lariño-Noia, J. Iglesias-García, J. Forteza, J.E. Domínguez-Muñoz
Foundation for Research in Digestive Diseases, 1Department of Gastroenterology, 2Department of Pathology, University Hospital of Santiago de Compostela, Spain

**Introduction:** EGFR has been reported to be overexpressed in pancreatic cancer (PC). Mutations in the tirosin-kinase (TK) domain of EGFR appear to be associated to the response to some chemotherapeutic agents, but the clinical relevance of EGFR mutations and overexpression in pancreatic tumours is far to be known.

**Aim:** To evaluate the clinical relevance of immunohistochemical and molecular study of EGFR in patients with pancreatic tumours.

**Methods:** Tumour samples were obtained from 52 consecutive patients (mean age 64 years, range 29–77, 29 male) who underwent a pancreatic resection for a malignant tumour from January 1999 and December 2005. Genomic DNA from tumours was obtained according to recommendations of Puregene Kit (Gemtra Systems®) for the sequencing analysis of EGFR. Mutations and polymorphisms of EGFR (exons 19–21) were determined by nested polimerase chain reaction (PCR). A mouse monoclonal antibody (Dako-Cytomation) was used for immunohistochemical analysis. Membrane staining intensity and patterns were evaluated by means of a 0 – 3+ scale, in which scores 2+ and 3+ were considered as EGFR overexpression. For 2+ and 3+ scoring, complete membrane staining of more than 10% of tumour cells had to be observed.

**Results:** Final histological diagnosis was ductal adenocarcinoma in 34 patients, ampullary tumour in 8, endocrine tumour in 7...
patients, and squamous carcinoma, anaplastic carcinoma and mucinous cystoadenocarcinoma in one patient each. In duodenal adenocarcinoma, only one mutation (exon 21) and two polymorphisms (exons 19 and 21) were demonstrated in the TK domain of EGFR. No molecular anomaly was found in other tumours. EGFR overexpression was demonstrated in none of the duodenal adenocarcinoma and in 9 (50%) of the remaining tumours. No relationship could be found between EGFR overexpression and clinical and histological data (age, sex, smoking, response to standard chemotherapy, tumour staging at surgery, tumour differentiation grade and survival time).

Conclusion: Mutations and polymorphisms in the TK domain of EGFR, as well as EGFR overexpression, are very uncommon in duodenal pancreatic adenocarcinoma. In other pancreatic and ampullary tumours EGFR overexpression has a limited clinical relevance.

P120
Family History of Cancer and Tobacco Exposure in Index Cases with Pancreatic Ductal Adenocarcinoma
R. Lochan, K. Jaques, A.K. Daly, H.L. Reeves, R.M. Charnley
1Hepato-Pancreato-Biliary Unit, Freeman Hospital, 2Clinical and Laboratory Sciences, 3Clinical Medical Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

Introduction: To examine gene-environment interaction in patients with pancreatic adenocarcinoma by correlating history of cancer in first-degree relatives with tobacco smoking in the index cases.

Methods: Patients with pancreatic ductal adenocarcinoma were prospectively identified and consented. Details of tobacco exposure in index cases and history of cancer in first-degree relatives (FDR) was among the data collected. Cumulative tobacco exposure in patients with and without a family history of cancer in FDR was analysed.

Results: Between June 2005 and Jan 2006, 94 patients diagnosed with pancreatic ductal adenocarcinoma were identified, of which 91 consented. A history of any cancer in FDR was present in 34 and absent in 34 patients. Family history was uncertain in 17 and unavailable in 6 patients. Of the 34 patients with a family history of cancer – 21 had 1, 9 had 2 and 4 had 3 first-degree relatives with cancer. One patient with Li-Fraumeni syndrome was excluded. The mean age of patients with a family history of cancer in FDR was 65 (46–88) years in patients with FDR with cancer and 64 (44–88) years in those without. Present, past and non-smokers accounted for 10, 16, 7 and 11, 15, 8 patients in the FDR positive and FDR negative groups respectively. Total pack-years of tobacco smoking (mean range) were significantly lower (p = 0.047) and in smokers, although the latter association was not statistically significant (p = 0.17). However, 3 of the 4 patients who had multiple relapses, were smokers. Neither the prognostic scores (APACHE II, Ranson, Imrie) nor the result of a contrast-enhanced computed tomography (Balthazar score) correlated with the recurrence rate.

Conclusion: The relapse rate in patients with AIP is low. A change of aetiology is very unlikely. There is no progression from acute to chronic pancreatitis. Patients are advised to stop smoking and to undergo appropriate imaging procedures 3 months after the attack in order to identify the eventual presence of a PCA.

P121
The Natural Course of Acute Idiopathic Pancreatitis
PG. Lankisch, N. Breuer, A. Bruns, B. Weber-Dany, P. Maisonneuve, A.B. Lowenfels
1Department of Internal Medicine, Municipal Clinic of Lueneburg, Germany; 2European Institute of Oncology, Milan, Italy; 3New York Medical College, Valhalla, NY, USA

The aim of our study was to determine the natural course of acute idiopathic pancreatitis (AIP).

Patients and Methods: We identified 106 patients with a first attack of AIP admitted to our hospital from 1987 to 2004. All living patients were interviewed by telephone and questionnaire. For patients who died the cause of death was obtained from their general practitioners.

Results: The aetiology was not idiopathic in two patients in whom a pancreatic carcinoma (PCA) diagnosed during follow-up was responsible for the first attack.

Conclusion: The relapse rate in patients with AIP is low. A change of aetiology is very unlikely. There is no progression from acute to chronic pancreatitis. Patients are advised to stop smoking and to undergo appropriate imaging procedures 3 months after the attack in order to identify the eventual presence of a PCA.

P122
Faecal Elastase I Performance – Ist Use in Diagnosis of Chronic Pancreatitis
1Fourth Department of Internal Medicine, 2Institute of Clinical Biochemistry, Charles University, 3Department of Internal Medicine, Hospital of Merciful Sisters, 4Department of Internal Medicine, Thomayer Faculty Hospital, Prague, Czech Republic

Introduction: For the diagnosis of chronic pancreatitis, we currently use the imaging methods. These imaging methods don’t actually...
tell us anything about the function of the pancreas. The aim of our study was Faecal elastase I determination in patients with chronic pancreatitis.

**Method:** Faecal elastase I (mean values in ug/g of stool) is a simple, non-invasive method which correlates well with the damage of pancreatic tissue, stemming from chronic pancreatitis. Faecal elastase I was determined by a microplate ELISA method using monoclonal antibody to human pancreatic protein.

In our studies we have used a newly proposed classification system, which was created in Bern, 2000 (Büchler and Malfertheiner). This new system encompasses morphological and functional changes. This classification system is broken down into different level after the damage of the pancreas.

**Results:** The Faecal elastase I occurring in the stool was taken from 196 patients who had been diagnosed with chronic pancreatitis. The occurrence of Faecal elastase I was classified according to the levels assigned by the classification system.

The control group used in this study had 144 patients diagnosed with a different kind of disease.

The results demonstrate a very good correlation of Faecal elastase I with the grading of the newly proposed classification system of chronic pancreatitis. Patients with the highest levels of the damage of the pancreas had a significantly lower occurrence of Faecal elastase I in comparison with the non-pancreatic control group.

**Conclusion:** Faecal elastase I performance plays the important role in the diagnosis of the severe cases of chronic pancreatitis and in the follow-up of the patient with the intermediate damage of the pancreas.

---

**P124**

**Long Term Follow-up on Quality of Life Measurement after Frey Procedure vs. Pylorus-Preserving Pancreatoduodenectomy for Chronic Pancreatitis**

Á. Issekutz, R. Makay, T. Belágyi, A. Oláh

Petz Aladár County Hospital, Győr, Hungary

**Background:** The aim of this retrospective non-randomized study was to compare two surgical procedures in the treatment for chronic pancreatitis. Comparison of long term effects of limited pancreatic head resection according to Frey and complete pancreatic head resection as pylorus-preserving pancreatoduodenectomy (PPPD) by measuring Quality of life (QoL) is presented.

**Material and Method:** During the last 8 years 56 consecutive patients were included into this retrospective study. As individual surgical indication PPPD was chosen when major complicated pancreatic head mass was present or pancreatic cancer could not be ruled out (18 patients); otherwise Frey procedure (FP) was performed (38 patients). QoL was measured during follow-up using our simplified ESPAC QoL Questionnaire.

**Results:** Median follow-up was 82 months (62–106). The response rate was 84%. Although the QoL improved in all parameters at both groups after surgery, we found statistically significant difference (*p* < 0.05) regarding pain relief in favour of PPPD in contrast to FP. The complete pain relief was 78% in the PPPD group vs. 42% in the FP group, as occasional pain was detected in 17% and 53%, respectively. The rate of patients with regular pain was similar (5%).

**Conclusions:** Our retrospective study suggests that in advanced cases of chronic pancreatitis PPPD gives better result in the aspect of pain after long-term follow-up. Based of our data, inspite of extended intervention PPPD may be the preferred solution in advanced cases.

---

**P123**

**Treatment of Abdominal Compartment Syndrome with Subcutaneous Anterior Abdominal Fasciotomy in Severe Acute Pancreatitis**


Helsinki University Central Hospital, Helsinki, Finland

**Background:** Hospital mortality in patients with severe acute pancreatitis remains high. Some of these patients develop abdominal compartment syndrome, which contributes to organ dysfunction and outcome. The treatment of the syndrome by the open abdomen method is associated with considerable morbidity and resource utilization.

**Methods:** A novel technique of subcutaneous anterior abdominal fasciotomy is described for the first time in two patients with severe acute pancreatitis. Utilizing 2–3 transverse 2 cm long skin incisions placed in midline subcutaneous tissue was incised and the anterior abdominal fascia divided vertically. The incision was carried out with scalpel and scissors under visual control. The fasciotomy was completed under laparoscopic control using a transparent trocar, laparoscopic scissors and suction tip. On visual inspection, the peritoneum remained intact throughout the procedure in both cases but there was minor oozing of ascites to the operation field that did not seem to have any significant part in decreasing the intra-abdominal pressure. The fasciotomy extended from about 1 cm caudal to the xiphoideum to about 1 cm cranial to the symphysis pubis. The width of the fascial diastasis measured at the incision levels was 8–10 cm in both patients.

**Results:** Following the procedure, the intra-abdominal pressure decreased from 30 mmHg immediately to 23 mmHg and to a sustained level of 12–14 mmHg in the first patient, and from 35 mmHg immediately to 23 mmHg and to a sustained level of 14–22 mmHg in the second.

**Conclusion:** The subcutaneous anterior abdominal fasciotomy can potentially avoid the risk of peritoneal contamination associated with open decompressive laparotomy and persistent open abdomen that could increase the risk of infection of the peripancreatic necrosis. The procedure is a promising method for effective abdominal decompression with sustained effect and avoiding the morbidity associated with the alternative open abdomen techniques.
Histological Evaluation of Chronic Pancreatitis by Endoscopic Ultrasound (EUS)-Guided Fine Needle Biopsy (FNB)

J. Iglesias-Garcia1, J. Lario-Noia1, I. Abdulkader2, J. Forteza2, J.E. Dominguez-Munoz1

1Departments of Gastroenterology and 2Pathology, University Hospital of Santiago de Compostela, Spain

Background: Histological diagnosis of chronic pancreatitis has been classically limited to the study of surgical specimens. Since pancreatic biopsies are rarely done in the context of chronic pancreatitis, histological characteristics at different stages of the disease are unknown.

Aim: To evaluate the histological characteristics of chronic pancreatitis in samples obtained by pancreatic EUS-FNB, and the relationship with EUS findings.

Methods: Fourteen consecutive patients (14 male, mean age 66 years, range 17–81 years) with known chronic pancreatitis, who underwent EUS-FNB for the study of pancreatic masses were included. EUS-FNB was performed under conscious sedation with a 22G needle guided by the linear scanning Pentax FG-38UX endoscope. EUS criteria for the diagnosis of chronic pancreatitis were evaluated: parenchymal criteria included hyperechoic foci, hyperechoic strands, lobularity, cysts and calcifications; and ductal criteria included dilation, duct irregularity, hyperechoic duct margins, visible side-branches and intraductal calcifications. Following histological features were evaluated: presence of acini, ductal epithelium, fibrotic tissue (collagen) and inflammatory infiltration.

Results: Adequate tissue sample for histological evaluation was obtained in all cases. Infiltration by inflammatory cells was observed in all tissue specimens. Samples included pancreatic acini in five cases (35.7%), in number of 2–13 acini each. In the remaining 9 cases (64.3%) the presence of ductal epithelium together with fibrotic tissue was observed. Biopsies including pancreatic acini were those obtained from patients with mild to moderate EUS changes of chronic pancreatitis (up to 5 EUS criteria). On the contrary, biopsy samples from more severe cases (8–10 EUS criteria) were those showing only ductal epithelium with fibrotic component.

Conclusion: EUS-guided pancreatic biopsy allows evaluating histological changes of all stages of chronic pancreatitis. This may be an important advance for a better knowledge of the disease.

Decline of Fecal Elastase-1 Levels in Elderly Individuals Without Know Gastrointestinal Diseases or Diabetes


1AI Virtanen Institute, 2Department of Internal Medicine, KYS, Kuopio, 3Hospital of Pieksämäki, Pieksämäki, 4Department of Surgery, KYS, Kuopio, Finland, 5Department of Internal Medicine UKSH Kiel, Germany, 6Department of Ophthalmology, District Hospital, 7Chair of Pediatrics, University of Poznan, Poland

In elderly people, an increased incidence of dyspeptic symptoms is observed. This increase is assumed to be caused at least in part by inadequate pancreatic enzyme secretion due to atrophy, fibrosis, sclerosis and lipomatosis of the pancreas. These changes correlated significantly with age suggesting that pancreatic enzyme secretion may be reduced even in healthy elderly people. The aim of the study was to evaluate the pancreatic secretion capacity in elderly people without gastrointestinal diseases, alcohol or diabetes medical history.

Methods: 106 volunteered subjects aged 60–69 years (n = 31); 70–79 years (n = 38) and over 79 years (n = 37) as well as 62 controls (19–26 years) were included into the study. Inclusion criteria – age over 60 years, normal health status. Exclusion criteria: diabetes, known gastrointestinal disease/surgery, secondary malabsorption syndrome, or alcohol disease. Pancreatic secretion was determined by measuring fecal elastase-1 in the stool via an enzyme-linked immunosorbent assay (ScheBo® Biotech) – the most sensitive indirect stool test. Fecal elastase levels below 200 µg/g stool were considered to be pathological.

Results: From all the test persons, 21.7% had fecal elastase levels below 200 µg/g (mean ± SEM: 112.1 ± 12.5 µg/g). Fecal elastase levels of the test persons (401.5 ± 23.3 µg/g; n = 106) were significantly lower than in controls (693.5 ± 45.2 µg/g; n = 62; p < 0.0001). There was no statistical differences between the three age groups (p > 0.05), but there was a significant negative correlation between age and fecal elastase levels (R = -0.4420; P < 0.0001).

Conclusions: Fecal elastase levels seem to decline with age suggesting pancreatic insufficiency. Since a considerable percentage of elderly subjects have abnormal pancreatic secretion, pancreatic function testing should become a standard in the medical care of elderly patients with dyspeptic symptoms or weight loss.

Applying of Mini-Invasive Technique in the Complex Management of Necrotizing Pancreatitis

Y. Havrysh, A. Perejaslov, S. Chooklin, R. Chukla

Department of Surgery, Medical University, Lviv, Ukraine

High mortality among patients with necrotizing pancreatitis despite surgery has led to development various mini-invasive technique, such
Abstracts

P128
The CT Severity Index is Not a Good Predictor of Patient Outcome in Acute Severe Pancreatitis Treated by Drainage Placement

O.W. Hamer1, B. Salzberger2, S. Lang2, G. Retzl1, S. Feuerbach1, J. Schömerich2, T. Brünnler2

1Department of Radiology, 2Department of Internal Medicine, University of Regensburg, Germany

Introduction: Acute pancreatitis is a severe condition still associated with high mortality rates. Stratification of patients according to their probable outcome is necessary for optimal planning of therapy. The purpose of this retrospective study was to determine the reliability of the Computed Tomography Severity Index (CTSI) regarding determination of prognosis of patients suffering from acute pancreatitis treated by drainage.

Methods: By searching the radiological, surgical and internal medicine databases we identified all patients with the diagnosis acute pancreatitis treated by drainage between 1992 and 2004 at a university hospital. Clinical, surgical and laboratory data, APACHE II score, SAPS II as well as characteristics of drainage therapy were collected by reviewing patient charts and radiological reports. CTSI was determined by reviewing the CT examination at the time of first drainage placement. Endpoints for patient outcome were mortality and length of hospital stay. Statistical analysis was performed using Cox regression and binary logistic regression. A p-value of <0.05 was considered statistically significant.

Results: Eighty-two patients were identified. Two patients were excluded from statistical analysis because CT examinations could not be provided. Seventy-three of 80 patients (91%) suffered from necrotizing pancreatitis, 7 of 80 patients (9%) from interstitial pancreatitis with severe surrounding fat necrosis. The CTSI ranged between 4 and 10. Sixty-five of 80 patients (81%) were referred to an intensive care unit. The APACHE II scores of these patients ranged between 1 and 38, the SAPS II between 15 and 94. The APACHE II scores and SAPS II correlated statistically significantly with mortality and length of hospital stay (p < 0.005 to p = 0.024). In contrast, there was no statistically significant correlation between outcome parameters and CTSI.

Conclusion: In this retrospective analysis, CTSI was not a good predictor of patient outcome.

P129
Acute Alcohol Induced Pancreatitis in Helsinki and Malmö. Are there Differences?

M. Haataja1, S. Andtskär1, B. Lindkvist1, S. Appelros1, A. Borgström2, A. Juuthi1, M.-L. Kylänpää-Bäck2

1Department of Surgery in Malmö, University of Lund Sweden; 2Helsinki Faculty of Medicine, Institute of Clinical Medicine, Department of Surgery, Helsinki, Finland

Background: The annual incidence of acute pancreatitis (AP) ranges from 7–73/100,000. The two dominant risk factors for AP are gallstones and alcohol. In Malmö gallstone-related AP has increased markedly whereas alcohol-related AP has decreased. In Finnish reports alcohol is the most common causative factor in AP. However, Finnish reports usually include recurrences. The aim of this study was to investigate first attacks of alcohol-induced AP in Malmö and in Helsinki, over a 3-year period. The main parameters investigated were, risk factors, severity, mortality, time between onset of symptoms and admission and CRP levels in serum on admission.

Methods: Clinical records for all patients with a first attack of AP in Malmö and in Helsinki from 2000 to 2002 were validated retrospectively. Evidence for diagnosis was reconsidered and plausible cause was assessed.

Results: Altogether 354 first attacks were identified in Helsinki and 264 in Malmö. The main aetiological factor was alcohol in Helsinki and gallstones in Malmö. The mean age of patients with alcohol induced pancreatitis (n = 164, 135 Helsinki/29 Malmö) was significantly higher in Malmö 51.1 vs. 45.9 years p = 0.035. Time to admission for alcohol induced cases were simlar in both cities 29.2 h in Malmö and 34.7 h in Helsinki p = 0.44. CRP on admission for cases with AP were however significantly higher in Helsinki 90 mg/l vs. 29 mg/l p = 0.023.

Conclusions: Alcohol-related AP dominates in Helsinki even in first attacks. This is most likely a result of greater alcohol abuse in Helsinki than in Malmö. Results in Malmö are in phase with earlier reports with a decreasing and low incidence of alcohol as a riskfactor. The greater amount of severe cases of AP in Helsinki is partly explained by selection bias. Alcoholinduced pancreatitis seem more agressive in Helsinki. Different drinking habits can be one explanation. Genetic
polymorphism for inflammatory mediators could explain the CRP differences.

**P130**

**Experiences with 89 Biliary Self Expandable Metal Stents**

**T. Gyökeres, M. Burai, Gy. Keleti, T. Tihanyi, Á. Pap**

Department of Gastroenterology, MÁV Hospital, Department of Surgery, St. László Hospital, Department of Surgery, Semmelweis University, First Department of Surgery, Budapest, Hungary

**Introduction:** Pancreatobiliary malignancies usually cause biliary obstruction during their course. The palliative management of inoperable patients is relatively well established. In jaundiced pts without duodenal obstruction the endoscopic biliary drainage is the method of choice. The self expandable biliary metal stents have been proven as cost-effective solution of the malignant stricture in pts with relatively good (as many as 6 months) life expectancy.

**Patients and Methods:** We have treated 89 pts with inoperable biliopancreatic tumors using self expandable metal stents (Microvasive/Gianturco uncovered stents) during the last 5 years. Localisation of the tumors were: 41 pancreatic, 31 choledochal/gallbladder, 9 papilla of Vater and 8 Klatskin-type cancers. We assessed the survival, stent patency, need for reinterventions and further oncologic treatment.

**Results:** Early (within 4 weeks) death occurred in 6 pts, mainly due to pulmonary embolism. Only 15 pts got chemotherapy later on. In the pancreatic tumor group 31/41 pts were followed, 19 pts died, 12 are still alive. The median survival of pts was 100 (1–413) days, while stent patency was 74 (1–413) days. Previous plastic biliary drainage were performed in 14 cases. Further interventions were needed after metal stent insertion in 10 pts, 7 of them presented with stent occlusion, 3 had duodenal obstruction, which required surgery or endoscopic intestinal stenting. In the biliary, papillary and Klatskin tumors the median survival was 200, 254, 72 days, the stent patencies were 198, 254, 72 days respectively. Reinterventions (restenting or gas-troentero anastomosis) were necessary in 14 patients. Further interventions were needed after 198, 254, 72 days respectively. Reinterventions (restenting or gas-troentero anastomosis) were necessary in 14 cases. Further interventions were needed after 198, 254, 72 days respectively. Reinterventions (restenting or gas-troentero anastomosis) were necessary in 14 cases. Further interventions were needed after 198, 254, 72 days respectively. Reinterventions (restenting or gas-troentero anastomosis) were necessary in 14 cases. Further interventions were needed after 198, 254, 72 days respectively. Reinterventions (restenting or gas-troentero anastomosis) were necessary in 14 cases. Further interventions were needed after 198, 254, 72 days respectively. Reinterventions (restenting or gas-troentero anastomosis) were necessary in 14 cases.

**Conclusions:** The survival proved to be poor in the pancreatic and Klatskin tumors. More pts should get chemotherapy with pancreatic tumor. Anticoagulation should be taken into consideration among these pts with obvious hypercoagulability. Altogether 32.9% (24/73) of followed pts needed further palliative interventions (mainly restenting) during their remaining life.

**P131**

**Fecal Pancreatic Elastase-1 Profile in Patients Underwent Stomach Resection**

**N.B. Gubergrits, M.I.B. Takhar**

Department of Internal Diseases No 1, Donetsk State Medical University, Ukraine

**Aim:** To study state of pancreatic excretory function in patients after stomach resection.

**Methods:** We examined 40 patients, which underwent stomach resection by Billroth-II due to complicated stomach and/or duodenal ulcer in 1–12 years prior to our research. We conducted endoscopy to all patients, which reveals chronic gastritis of gastric stump and in 21 cases even with anastomosis. We did not enroll patients with peptic ulcer of the gastric stump to our research. Level of fecal pancreatic elastase-1 assessed with labkits ‘Schebo’ (Germany).

**Results:** Pancreatic excretory function was decreased in 32 of the patients (80.0%). Eight patients (20.0%) had level of fecal pancreatic elastase-1 higher than 200 mcg/g, i.e. had its normal level. Twenty four patients (60.0%) had reduced pancreatic elastase-1 level, but not less than 150 mcg/g, i.e. they had mild pancreatic insufficiency. Five patients (12.5%) had moderate pancreatic insufficiency (fecal pancreatic elastase-1 level from 100 to 150 mcg/g). Moderate to severe impairment of pancreas function were revealed in those patients, who had stomach resection more than 8 years prior to our research, moreover most of them had chronic pancreatitis even before the operation.

**Conclusion:** Patients after stomach resection develop reduction of pancreas excretory function predominantly of mild or rarely moderate to severe grade of pancreatic insufficiency.

**P132**

**Modern Approaches to Rehabilitative Treatment of Chronic Postgastroresectional Pancreatitis**

**N.B. Gubergrits, M.I.B. Takhar**

Department of Internal Diseases No 1, Donetsk State Medical University, Ukraine

**Introduction:** Chronic pancreatitis (CP) develops in 20–25% of the patients after stomach resection. This phenomenon is connected to disorders of physiological interrelations of stomach secretion and excretory function of the pancreas. Treatment of postgastroresectional CP is not adequately explored.

**Aim:** To study an efficacy of antihomotoxic therapy in rehabilitative treatment of chronic postgastroresectional CP.

**Methods:** We observed 60 patients, which underwent stomach resection over 3 years ago due to complicated stomach ulcer. Postgastroresectional CP were diagnosed in 38 of the patients. After discharging from a hospital these patients received a combination of antihomotoxic preparations: Nux vomika, Mucosa compositum and Momordica compositum. Before and after the rehabilitative treatment we studied the follow: intensity of clinical manifestation, uroamylase outflow, coefficient of endogen pancreozymin induction, lipase outflow per hour in duodenal content, psychosomatic status of patients.
Results: During the rehabilitation it was observed a significant reduction of the intensity of pain and dyspeptic syndromes. Under the antihomotoxic therapy there were reached reliable decrease of uroamylase outflow over 30 and 60 min after standard test meal and of coefficient of pancreozymin induction over 60 min after the standard meal as well. Lipase outflow per hour over month of rehabilitative therapy reliably increased, but still stay under the normal level though. The patients had their psychosomatic status improves, that had been expressed in better results of self-evaluation psychological testing (assessment of state of health, activity and mood).

Conclusion: Antihomotoxic therapy is effective for rehabilitative treatment of postgastroresectional CP, contributes to the reduction of clinical manifestation of the disease, to reduction of the phenomenon ‘deviation’ to blood, to improvement of excretory pancreatic function and psychosomatic status of the patients.

Results:

During the rehabilitation it was observed a significant reduction of the intensity of pain and dyspeptic syndromes. Under the antihomotoxic therapy there were reached reliable decrease of uroamylase outflow over 30 and 60 min after standard test meal and of coefficient of pancreozymin induction over 60 min after the standard meal as well. Lipase outflow per hour over month of rehabilitative therapy reliably increased, but still stay under the normal level though. The patients had their psychosomatic status improves, that had been expressed in better results of self-evaluation psychological testing (assessment of state of health, activity and mood).

Conclusion: Antihomotoxic therapy is effective for rehabilitative treatment of postgastroresectional CP, contributes to the reduction of clinical manifestation of the disease, to reduction of the phenomenon ‘deviation’ to blood, to improvement of excretory pancreatic function and psychosomatic status of the patients.

P133

**Novel Radiotherapeutic Management of Painful Flare-ups in Chronic Pancreatitis**

L. Guarner, X. Molero, B. Navalpetro, J. Giralt, J.-R. Malagelada

Digestive System Research Unit, Radiation Oncology Service, Hospital Universitari Vall d’Hebron, Barcelona, Spain

Patients with chronic pancreatitis may present repeated painful flare-ups of pancreatitis and even unrelenting pain. Current management options are limited to analgesics and surgery, in selected cases. We reasoned that anti-inflammatory radiotherapy, which appears to be effective in other inflammation based painful disorders of the body, might prove valuable to severely symptomatic patients with chronic pancreatitis.

Patients and Methods: We studied prospectively over a 4-year period till November 05, the efficacy of single dose anti-inflammatory radiotherapy in 12 consecutive patients with chronic pancreatitis (ethanol related in 7, idiopathic in 4 and cystic fibrosis in one) who fulfilled the following criteria: either 2 flare ups of pancreatitis in the previous 6 months (all 12 patients) and/or continuous pain for more than 3 months (2 of the 12). Median age was 41 years (range 32–80), there were 9 males and 3 females; diagnosis of chronic pancreatitis (6 months to 16 years, median 5 years) and number of prior attacks (2 to >15, median 6). Treatment consisted in a single dose of radiation of 8Gy. Before and after radiation we assessed: exocrine function by fecal elastase, endocrine function by c peptide, quality of life (EuroQol) and clinical outcomes. Response was defined as no further pain or flare-ups of pancreatitis.

Results: During follow up (median 33 months, range 1–48 months) 10/12 patients had no further pain or flare-ups. One patient required a second radiation dose after 1 year and has been well since (26 months), 1 patient did not respond and had a pancreaticcojejunoscopy at 1 month. Before radiation 4 patients had exocrine (fecal elastase <100 μg/g) and 2 endocrine (c peptide <0.50 ng/ml) dysfunction. Post treatment 1 additional patient developed exocrine (at 25 months) and endocrine (at 13 months) insufficiency. The responder group (11/12) gained 4–20 kg in weight during follow up (median 6 kg) and EuroQol improved significantly from 0.572 before till 0.817 after treatment (p < 0.01).

Conclusion: Radiation treatment of severely symptomatic chronic pancreatitis is effective and could potentially substitute or delay surgery.

P134

**Acute Pancreatitis Among Adults in England 1997–2004**

G. David, A. Al-Sarira, S. Singer, D.J. Corless, J.P. Slavin

Department of General Surgery, Leighton Hospital, Crewe, UK

Objectives: Hospital admissions for acute pancreatitis (AP) have been increasing since 1960. We have analysed the recent data to see if the trend continued.

Methods: Hospital Episode Statistics (HES) data were obtained from The Department of Health. Data was imported to a Microsoft Access database for analysis. AP was identified based on The International Classification of Diseases code – 10th revision [K85]. The study period extended from April 1997 to March 2004.

Results: Emergency hospital admissions with AP increased steadily from 29.5 per 100,000 population in 1997–98 to 39.3 per 100,000 population in 2003–2004. The average age at presentation in 1997–98 was 57 years (range 16–99) and average age in 2003–04 was 56 years (range 16–102). This difference was statistically significant (p < 0.01). The percentage of males admitted during the period was 51% with no difference observed during the study period. The in-hospital mortality declined from 7.1% in 1997–98 to 5.8% in 2003–04 (p < 0.01). Only 19% of the study population was over 75 years of age, and the mortality in this group was 18.6%. The median hospital stay was 7 days (IQR 4–11 days) during the study period.

Conclusion: Hospital admissions with AP appear to be increasing. The average age at presentation has decreased by one year with slight male preponderance. The in-hospital mortality has decreased over the years with the elderly (above 75 years) having the highest mortality rate. The median length of stay has remained the same.

P135

**The Prevalence of Pancreatic Serine Protease Inhibitor Kazal type 1 (SPINK1) and Cationic Trypsinogen gene (PRSS1) Mutations in Polish Patients with Chronic Alcoholic Pancreatitis and Pancreatic Cancer**

A. Gasiorowska1, R. Talar-Wojnarowska1, B. Smolarz2, H. Romanowicz-Makowska1, A. Kulig2, E. Malecka-Panas1

1Department of Digestive Tract Diseases, Medical University of Lodz, 2Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother’s Memorial Hospital, Lodz, Poland

Background: Only 5–10% of heavy drinkers develop chronic alcoholic pancreatitis (CAP). We have only limited information

Abstracts

Pancreatology 2006;6:323–405
P136
Prevalence of Diabetes Mellitus Secondary to Pancreatic Diseases (Type 3c)

N. Ewald1,2, C. Kaufmann1, A. Raspe1, H.U. Kloer1, R.G. Bretzel1, R.D. Hardt1

1Third Medical Department and Polyclinic University Hospital Giessen and Marburg, Giessen, Germany
2Department of Internal Medicine II, University of the Saarland, Homburg/Saar, Germany

Introduction: Diabetes mellitus secondary to pancreatic diseases is believed to be a very rare condition. Recently a rather high prevalence of exocrine pancreatic insufficiency has been observed in the general population and especially in diabetic subjects. Thus we investigated the prevalence of diabetes mellitus due to pancreatic diseases (type 3c).

Methods: In this study we investigated 1,922 patients diagnosed with diabetes mellitus who were submitted to our hospital for treatment during the last 24 months. Patient data were diligently studied and patients were reclassified diligently according to the diabetes classification as proposed by the American Diabetes Association.

Results: 157 cases among 1,922 diabetic subjects could be classified as type 3c diabetes mellitus (8.2%). Among these were 120 patients diagnosed with chronic pancreatitis (76.4%), 12 with hereditary hemochromatosis, 14 with pancreatic cancer and 7 with cystic fibrosis. Thus, diabetes mellitus due to chronic pancreatitis occurred in this collective in 6.2% of all diabetic subjects. Misclassification of these patients was very common. Only 45.8% were initially classified correctly. 43.3% were initially misclassified as type 2 diabetes, 6.7% as type 1 diabetes and 4.1% were not classified at all. In 17.5% of all cases alcohol abuse could be diagnosed.

Conclusions: Chronic pancreatitis as a cause of diabetes mellitus (type 3c) seems more common than generally believed with a prevalence of 6.2% among the subjects studied here. Since the awareness for the diabetes type is poor, misclassification is quite frequent. A common problem seems to be the differentiation between type 2 and type 3 diabetes mellitus. Yet, the correct classification of diabetes mellitus is very important, since there are special therapeutic options and problems in patients with diabetes secondary to pancreatic diseases.

P137
Palliative Treatment of Obstructive Jaundice in Patients with Adenocarcinoma of the Pancreatic Head or Distal Biliary Tree: Stentimplantation or Hepaticojejunostomy – A Clinical Analysis

M. Distler, F. Rueckert, F. Dobrowolski, R. Gruetzmann, H.D. Saeger

Department of Surgery, University Hospital Carl Gustav Carus, Technical University of Dresden, Germany

Background: Only 20–30% of the patients with malignant pancreatic neoplasm or malignancy of the distal biliary tree are curative resectable at time of diagnosis. Therefore palliation plays an important role. Questionable is whether in this palliative setting the therapy of obstructive jaundice should be non-surgical or surgical.

Patients and Methods: We retrospectively analyzed our patients, regarding to the way of treatment and associated complications as well as survival time. We generated 3 groups: Group 1: only endoscopically placed plastic stent of the bile duct. Group 2: preoperative stenting and in case of incurability a palliative hepaticojejunostomy was performed. Group 3: only hepaticojejunostomy without preoperative stenting. For Group 1 we determined the frequency of rehospitalisation in cause of recurrent jaundice. In these cases a new endoprothesis was placed endoscopically.

Results: Overall 342 patients were treated (median age 63 years). The symptom of obstructive jaundice was shown in 261 (76%) patients. 247 patients were sectioned in to the mentioned groups: Group 1 n = 138 (56%), Group 2 n = 68 (28%), Group 3 n = 41 (16%).

The 30 days lethality for the groups 1, 2, 3 was 2.2%, 0%, 2.4% with a rate of complication 6.5%, 19.1%, 21.9% respectively. For the only endoscopically treated patients the mean interval for changing the stent was 70.8 days (±32).

The median survival time for the Group 1 patients is significantly (p < 0.001) shorter than patients of Group 2 (5.1 vs. 9.4 month). None operated patients was rehospitalized in case of recurrent obstructive jaundice.

Conclusion: Hepaticojejunostomy can be performed with a sufficient operative result and an acceptable rate of complication.
P138
One-Step, EUS-Guided Drainage of Pancreatic Pseudocysts: Experience in 36 Patients
S.M. Denley, C. Shearer, C.R. Carter, C.J. McKay
Pancreatic Unit, Glasgow Royal Infirmary, Glasgow, UK

Introduction: EUS-guided trans-gastric drainage of pancreatic pseudocysts has until recently utilised a single stent, limiting use to carefully selected patients. We report our experience in 36 patients using a one-step, two stent procedure under EUS guidance.

Methods: All patients had persistent, symptomatic cysts. Aetiology was acute pancreatitis in 23 and chronic pancreatitis in 13 patients. Thirteen cysts were infected. ERCP or duct stent placement was either unsuccessful (7 cases) or demonstrated no communication (14 cases). In 11 cases ERCP was not attempted. In the remaining 4 cases, cysts persisted despite pancreatic duct stenting. Cyst drainage was carried out under EUS control using a therapeutic echoendoscope with a 3.8 mm working channel and bridge. Two or more pigtail stents were inserted after balloon dilatation of the cyst-gastrostomy.

Results: Cysts resolved at the first procedure in 26 cases, with one patient undergoing 24 h of lavage via a naso-cyst catheter. A further 5 patients underwent a second procedure, 4 for placement of a naso-cyst catheter and lavage, 1 for further dilatation of the cyst-gastrostomy. All 5 resolved after a second procedure. In 3 patients formal open surgery was required, one 42 days later for incomplete drainage, the other two on the same day for procedure related complications. Two patients died following surgery for a persistent, infected cyst. A third death occurred due to liver failure following successful cyst drainage.

Conclusions: One-step EUS-guided drainage with the deployment of two or more stents is a feasible option with 31 of 36 patients having complete cyst resolution. The procedure is not without complications, particularly when cysts are large, infected or contain significant necrosis. Many of these complications can be overcome with the use of cyst catheter lavage.

P139
Socio-Economic Deprivation Reduces Survival in Pancreatic Cancer
J.J.S. Brown1, B. Ashton2, R.M. Charney1, D. Forman3, B.C. Jaques1
1Hepato-Pancreato-Biliary Unit, Freeman Hospital, Newcastle upon Tyne, 2University of Sunderland, 3Northern and Yorkshire Cancer Registry, Leeds, UK

Introduction: To investigate the impact of deprivation on outcome in patients with pancreatic cancer, data was collected from the NYCRIS (Northern and Yorkshire Cancer Registry) database for new patients registered from January 1998 to December 2002.

Methods: The IMD2000 score (a validated socio-economic deprivation tool) was attributed to each patient. Five quintile groups of similar size were generated with graded deprivation profiles (quintile 1 deprived; quintile 5 affluent), higher scores representing greater deprivation. The difference between the rank of socioeconomic deprivation and average survival in days was also calculated for the cohort of patients who underwent surgery and those who had no surgical intervention.

Results: From a total of 3,976 patients the five quintiles had between 739 and 804 patients, the difference of the mean deprivation score between all groups being significant, p < 0.0001, using Student t test. The mean survival in days, for all treatment modalities between quintile 1 (120 days) and quintile 5 (155 days), was significantly different, p = 0.0003 and a graduated survival between quintiles 1, 2, 3, 4 and 5 was seen.

The mean deprivation scores for those undergoing surgery (n = 229) and those not undergoing surgery (n = 3,640) were 28.31 and 31.64 respectively, p = 0.002. The mean survival of the surgical cohort in the most deprived quintile (n = 33) was 329 days (95% CI 198–460) and the least deprived (n = 53), 424 days (95% CI 320–527), p = 0.26.

Conclusions: Whilst the cohorts represent a varied casemix, socio-economic deprivation appears to have a major effect on survival in patients with pancreatic cancer and also correlates with treatment by surgical intervention. Further work is required to determine the underlying cause of this effect.

P140
Timing of Surgical Intervention in Necrotizing Pancreatitis: A 10 Year Consecutive Case Series and Systematic Review
M.G.H. Besselink1, E.J.P. Schoenmaeckers1, E. Buskens2, B.U. Ridwan3, M.R. Visser3, V.B. Nieuwenhuijs1, H.G. Gooszen1
1Departments of Surgery, 2Julius Center for Health Sciences and Primary Care, 3Microbiology, University Medical Center Utrecht, The Netherlands

Introduction: It is advocated to perform the first surgical intervention for (suspected) infected necrotizing pancreatitis (NP) in the third or fourth week after onset of disease.
Methods: Retrospective study of 445 acute pancreatitis patients in a tertiary referral center over a 10 year period and a systematic review of studies comprising 25 or more patients with NP published in the previous decade.

Results: Fifty-three patients underwent surgical intervention for NP. 42 (79%) of whom had been referred. Median timing of first intervention was 28 days. Eighty-three percent had infected necrosis. Fifty-five percent had organ failure. The mortality rate was 36%. Sixteen patients were operated within 14 days, 11 patients from day 15–28 and 26 patients on day 29 or later (29+ subgroup). APACHEII, Ranson scores and preoperative organ failure and intensive care stay were comparable between the three subgroups. The 29+ subgroup received prophylactic antibiotics for a longer period of time (4, 17 and 25 days, p = 0.0001) and Candida and antibiotic resistant organisms were more often cultured from the (peri-) pancreatic necrosis (1, 1 and 9, p = 0.022). Mortality was decreased in the 29+ subgroup (75, 45 and 8%, p = 0.0001). During the second half of the study, necrosectomy was further postponed (43 vs. 20 days, p = 0.062) and mortality decreased (47 vs. 22%, p = 0.085). Nine studies with a total of 879 patients were included in the systematic review. Median NP surgical volume was 8.3 patients per year (5.3–15.6), timing of surgical intervention 27 days (3–31 days) and mortality 25% (6–56%). An association was observed between (postponed) timing and (decreased) mortality (r = -0.741, p = 0.022).

Conclusions: Postponing necrosectomy in NP until after the fourth week is associated with decreased mortality, prolonged use of antibiotics and increased incidence of Candida and antibiotic resistant organisms.

P141

Effective Management of Pancreatic Cancer is Dependent on the First Symptoms

M. Benes, P. Wohl, J. Spicak

Department of Gastroenterology and Hepatology, Ikern, Prague, Czech Republic

Introduction: The prognosis of pancreatic cancer is dismal, and its effective treatment is dependent on correct and early diagnosis and staging.

Aims and Methods: The aim of our multicentric study was to analyze the effectiveness of the diagnostic process reflected in adequate treatment. We prospectively analyzed the initial symptoms, length of the diagnostic process, and use of imaging methods with consequent application of adequate therapy.

Results: A total of 110 patients with pancreatic cancer being managed within the last 12 months were enrolled. The most frequent first symptom was dyspepsia (43 patients), followed by pain (38) and jaundice (29). While, overall, hyperglycemia was documented at the beginning of the diagnostic period in 30 patients (27.2%), it was present in 23 (77%) of those with dyspepsia. Radical resection was performed in 26 (23.6%) patients, exploratory laparotomy in 16 (14.5%), palliative surgery in 38, and chemotherapy in 21 patients. Mean time from the onset of the first symptoms to presentation (time I) was 38 ± 30 days. Mean time of the diagnostic process to specific treatment (time II) was 3.5 ± 3.4 months (1–24 months). Mean time I in patients presenting with dyspepsia was 44.8 ± 25.1 days, in patients presenting with jaundice or pain 30.2 ± 28.9, and in those who underwent resection 26.1 ± 24.3 days. Mean time II in these groups was 3.9 ± 4.5, 2.7 ± 2.1, and 2.1 ± 1.4 months, respectively. Ultrasound was the most frequently used imaging method (180 examinations) followed by CT (127), ERCP (110), endosonography (35), and MR (7). The mean number of imaging methods used per patient was 4.6 ± 1.5 (2–11). Fine needle biopsy was performed in 18 patients. The most frequent algorithm of use of imaging methods was ultrasound followed by CT and ERCP.

Conclusion: The decision-making process undertaken preoperatively is not sufficiently effective, as reflected by the inadequately high rates of exploratory laparotomy. The length of the process of diagnosis is dependent on the first symptoms, which can influence resectability. The diagnostic process was significantly shorter in resected patients. Dyspepsia with hyperglycemia have been shown to serve as a warning sign, and when this combination appears at an adequate age, intensive search for pancreatic cancer should be initiated.

P142

Natural History of Alcoholic Chronic Pancreatitis in North-Western Romania

S.T. Barbu, M. Cazacu

Fourth Surgical Clinic, University of Medicine and Pharmacy ‘Iuliu Hatieganu’ Cluj-Napoca, Romania

Introduction: Alcohol consumption is the main cause of chronic pancreatitis (CP). Several series reported in Romania have CP complications as the main indication for surgery. Operations for pain account for only 10–34%.

Purpose: To describe the natural history of patients with alcoholic CP from North-Western Romania, to compare it with reports from Western countries, and to find causes for differences.

Methods: We performed a retrospective record analysis and a subsequent prospective follow-up of 99 patients with alcoholic CP, 72.8% of the 136 CP patients operated between 1991 and 2000. Using medical records and questionnaires we obtained data on disease evolution before surgery. CP average duration was 15.6 years (7.3 years postoperative mean follow-up).

Results: Patients were mostly men (93%), with a mean age of 42.9 years at the time of operation and of 33.7 years at the disease onset. Indications for surgery were: intractable pain (12%), complications (83%) and suspicion of malignancy (5%). Twenty-eight patients had two or three concomitant complications. During the evolution, 35 patients needed two or four operations for CP complications. At the end of follow-up, calculations were present in 63.6%, secondary diabetes in 35.4%, and steatorrhea in 32.3% of patients. Liver cirrhosis and pulmonary tuberculosis were associated in 12 and 8 patients. All patients achieved pain relief in advanced CP. Using correlation tests, we found that continuing consumption of alcohol (r = 0.61, p = 0.00014) and drinking more than 200 g ‘tzuica’/day (r = 0.67, p = 0.00001) are strong risk factors for complications, especially acute pancreatitis episodes and pseudocysts.

Conclusions: Alcoholic CP in North-Western Romania is characterized by early onset, and occurrence of multiple severe complications that need repeated surgery. Continuing alcohol consumption and use of more than 200 g ‘tzuica’/day (home made plums whiskey, containing incomplete distillation compounds) are statistically the causes of this severe evolution.
Abstracts

P143
Value of Routine Clinical Tests in Predicting the Development of Infected Pancreatic Necrosis in Severe Acute Pancreatitis

G. Barauskas1, Z. Dambrauskas2, A. Gulbinas3, J. Pundzius3
1Department of Surgery, 2Institute for Biomedical Research, Kaunas University of Medicine, Kaunas, Lithuania

Introduction: Infectious complications account for approximately 80% of deaths in severe acute necrotizing pancreatitis. Currently, guided Fine Needle Aspiration (FNA) is the only invasive mean of early and accurate diagnosis of infected necrosis, however invasive procedures are of risk for infection with bacteria resistant to first line antibiotics. Therefore, our aim was to prospectively determine the value of routine diagnostic tests (WBC count, CRP and α-amylase) to determine the minimal expectation of infected necrosis and thereby preventing unwarranted ultrasound guided aspirations.

Methods: This was a prospective, nonrandomized study. The study population consisted of 52 consecutive patients. Data related to infectious complications was collected and statistical analysis performed to determine the value of routine diagnostic tests. Discriminant function analysis was used to determine discrimination between two occurring groups with infected necrosis and sterile necrosis. The particular cut-off point or criterion value to practically discriminate infected necrosis cases from sterile necrosis cases was evaluated using ROC curve analysis. Logistic regression was performed to determine the risk of infected necrosis if the either WBC or CRP or both values exceeds the defined cut-off.

Results: CRP value and WBC count significantly differ in groups of patients with acute pancreatitis and infected necrosis and acute pancreatitis with sterile necrosis. A cut-off value 81 mg/l for CRP and 13 × 10^9/l for WBC could be used to discriminate infected necrosis cases from sterile necrosis cases after the 3rd week from the onset of severe acute pancreatitis.

Conclusion: Simple routine clinical test (i.e. WBC count, CRP value) are helpful in predicting the development of infected pancreas in SAP after 3rd week of illness. Thus the timing of ultrasound-guided aspirations and selection of patients for these interventions could be optimized based on these findings.

P144
Cachexia in Patients with Resectable Pancreatic Cancer

J. Bachmann1, B. Fröhlich1, C. Dimitriou1, W. Hildebrandt1, M.W. Büchler1, H. Friess2, M.E. Martignoni1
1Department of General Surgery, University of Heidelberg, 2German Cancer Research Center, Heidelberg, Germany

Introduction: Pancreatic cancer is one of the four leading causes of cancer-related deaths in Western countries. In pancreatic cancer many patients develop a dramatic weight loss during the progression of their disease and this is an important factor in the mortality of cancer patients. The aim of the study was to show the impact of cachexia in patients with pancreatic cancer.

Material and Methods: Within 18 months in our department presented 247 patients with a ductal adenocarcinoma of the pancreas. The data of all patients were collected in a prospective database. Every patient was asked for the permission for taking the patient’s history.

Results: Of 247 patients with ductal adenocarcinoma 37% presented with weight loss of more than 10% of the original weight.

Fifty percent of cachectic patients are at time of operation in the tumorstage II, 46% in stage IV (metastatic disease); in the noncachectic group 63% are in the stage II, 31% in a metastatic stage (p = 0.089).

The survival in resected patients is shorter in cachetic than in the noncachetic patients.

Cachectic patients, who could not be resected had a median survival of 134 days; was a resection possible the median survival was about 332 days. In the noncachetic resected patients the survival was about 458 days, could no resection be performed, the survival was shortened to 287 days (p = 0.027).

The 30-days mortality showed a trend to more complications in the cachectic group (p = 0.06).

Conclusion: Cachexia has an impact on the clinical course of patients undergoing surgery for pancreatic cancer. Cachectic patients present more often in an advanced tumor stage. If a resection can be performed, the survival is better in the noncachetic vs. the cachectic patients (458 vs. 332 days respectively).

P145
Stenting of the Pancreato-Enteric Anastomosis after Pancreatic Head Resection: How Long will the Stent be in Place?

Å. Andrén-Sandberg, K. Eiriksson, C. Ansorge, J. Havnen1
Department of Surgery and Radiology1, Stavanger University Hospital/Bergen University, Stavanger, Norway

Introduction: The best way to create the pancreato-enteric anastomosis in the reconstruction after a pancreatic head resection remains to be defined. However, one small step ahead may be to define if and how long stenting of the anastomosis is of value. We are evaluating if stenting to be able to create a secure anastomosis is of value, and in a first step how long a non-sutured stent remains in place.

Methods: In 66 consecutive pancreatic resections (Stavanger and Bergen) a baby-feeding catheter no 3–6 was put into the duct of the pancreatic remnant as far as possible, usually 6–9 cm, and cut 10–15 mm outside the remnant. After a back row of 2–4 fine (5–0), single, absorbable sutures mucosa-to-duct the catheter was introduced into the antimesenteric jejunum loop and a front row of the same number and size of sutures were put mucosa to duct. The catheter was not sutured in place, and it was tried to put as much as possible of it in the pancreatic remnant. The pancreato-jejunosotomy also had a back and a front row of single fine stitches, usually 4–0, serosa to ‘pancreatic capsule’.

Results: In 19 patients without intraabdominal complications there was at least one postoperative CT with the stent in place and a later with the catheter in place; all CTs were taken for clinical reasons.
and none for the present evaluation. The median postoperative time the catheter was last seen in place was 19 days, and the median postoperative time it was first seen dislodged was 30 days. There was a tendency for the larger ducts and catheter sizes to dislodge earlier than the smaller. There was no catheters in place in patients investigated with CTs 12 months after the operation or more.

**Conclusion:** A non sutured stent used for defining the duct at suturing of a pancreato-jejunal anastomosis may be expected to remain in situ 3–4 weeks postoperatively in uncomplicated cases.

---

**P146**

**Treatment of Infected Pancreatic Necrosis by Complete Debridement, and Closed Conventional Drainage**

M.A. Hilal, B. Zidan, F. Howse, A.H. Mirnezami, N.W. Pearce, C.D. Johnson

University Surgical Unit, Southampton General Hospital, Southampton, UK

**Introduction:** After necrosectomy, conventional drainage, continuous lavage or open drainage may be used. In reviews (D’Egidio 1991, Isaji 2000) it is stated that mortality with conventional drainage is higher than after closed lavage or open packing. Published data report different indications for operation and different goals of therapy. The aim of this study is to report the results of closed drainage after necrosectomy for infected necrosis.

**Methods:** Thirty two consecutive patients (median age 55 years, 25 men and 7 women) with infected necrosis were treated by extensive necrosectomy and closed drainage preferably after the third week of illness. Complete debridement was obtained in one procedure if possible. The aetiology of pancreatitis was biliary in 19 patients, alcoholic in 9 and ERCP in 4. CT was performed in all patients with a median Balthazaar CT severity index of 7.

**Results:** Mean time between the acute attack and the necrosectomy was 34.5 days (range 19–60). The mean operative time was 155 minutes; median number of drains used was 4. In 29 patients debridement was obtained after one procedure, in 3 patients the pancreatic bed was packed due to diffuse oozing and reviewed after 48h, one of these patient needed one additional laparotomy and 2 patients needed a further 2 laparotomies for completion of debridement. Three patients died (9%); 8 major complications (25%) occurred, of which 3 needed surgical treatment (re-operation rate after abdominal closure 10%). The mean hospital stay was 53.6 (range 25–120) days, with a mean postoperative ITU stay of 4 days (range 1–22).

**Conclusions:** Our results are similar to reported mortality of 8.3% with this technique (Fernandez del Castillo, 1998). Conventional drainage following aggressive necrosectomy is a valid option in the treatment of pancreatic necrosis and can offer excellent results.

---

**P147**

**Correlation of Calcium and Sodium Changes Induced by Bile Acids and Calcium-Releasing Secretagogues in Pancreatic Acinar Cells**

S.V. Voronina, O.V. Gryshchenko, O.V. Gerasimenko, O.H. Petersen, A.V. Tepikin

Physiological Laboratory, University of Liverpool, Liverpool, UK

The ability of bile acids to trigger acute pancreatitis has been confirmed in a number of studies, but the cellular mechanism of such bile-mediated injury is not clear. We studied the effects of bile acids on pancreatic acinar cells.

Sodium and calcium changes in isolated pancreatic acinar cells were investigated using confocal microscopy and patch clamp technique. Sodium Green fluorescence, calcium-dependent chloride current and fluorescent Ca²⁺ probes were used for simultaneous sodium and calcium measurement. We found that calcium oscillations, induced by small physiological doses of ACh and CCK were accompanied by large sodium responses. Interestingly, the recovery rate of cytosolic sodium level was substantially slower then the recovery of cytosolic calcium level. This provides the conditions for the integration of sodium transients.

In perforated patch clamp experiments we found that taurolithocholic acid 3-sulfate depolarised pancreatic acinar cells. Using whole cell patch clamp we resolved sodium current, calcium current and chloride current. We found no information on the effects of bile acids on intact pancreatic ductal epithelia. The aim of this study was to investigate the effect of various bile acids on pancreatic duct cells.

**Introduction:** Exposure of the pancreas to bile acids is considered to be one of the possible causes of acute pancreatitis. However, no information is available on the effects of bile acids on intact pancreatic ductal epithelia. The aim of this study was to investigate the effect of various bile acids on pancreatic duct cells.
intracellular acidification.

Results: The exposure of intact ducts to all three primary bile acids induced a marked increase in [Ca^{2+}], and chenodeoxycholate caused the largest response. The effect of chenodeoxycholate from the luminal side was more pronounced than from the basolateral membrane. Furthermore, glycin conjugated bile acids, which are not membrane permeable, resulted in Ca^{2+} signals when administered from either side of the ducts. Calcium signals could still be triggered by bile acids in a Ca^{2+}-free external solution, but preincubation of the ducts with an intracellular Ca^{2+}-chelator, BAPTA-AM, totally abolished the effect of bile acids. Basolateral administration of chenodeoxycholate or glycochenodeoxycholate caused a reversible intracellular acidification.

Conclusion: These results suggest the presence of bile acid transporters on guinea pig pancreatic duct cells. The transport of bile acids into duct cells leads to an increase in [Ca^{2+}], which is probably mediated via the release of calcium from intracellular stores. The bile acid-induced acidification of pancreatic ductal epithelia may play a role during acute pancreatitis.

Supported by OTKA, MTA, OM.

P151
Not submitted

P152
Antisecretory Factor (AF) Inhibits Pancreatic Exocrine Secretion via Neural Mechanism
K. Nawrot-Porabka, J. Jaworek, A. Leja-Szpak, M. Macko, M. Kot, S.J. Konturek, W.W. Pawlik, S. Lange, B. Sternby
1Department of Medical Physiology Health Care Faculty, 2Chair of Physiology Medical Faculty, School of Medicine Jagiellonian University, Cracow, Poland; 3Department of Clinical Bacteriology Goteborg University, 4Department of Medicine University of Lund, Sweden

Introduction: AF (antisecretory factor) is an endogenous protein with the ability to regulate cellular fluid and ion transport over the cell membrane. This protein is expressed in most tissues of the human body including mucosal epithelium of the gastrointestinal tract. It has been reported that AF shows the anti-inflammatory properties as good as anti-secretory activities in the intestine, but the effect of AF on exocrine pancreas hasn’t been investigated yet.

Aim: To evaluate the effect of intraperitoneal (i.p.) administration of AF on pancreatic protein and amylase outputs under basal conditions or following the stimulation of pancreatic secretion with diversion of pancreatic-biliary juice (DPJ) and to determine the involvement of sensory nerves in that process.

Methods: The secretory studies were carried out on anesthetized male Wistar rats, weighing 300g. The animals were surgically equipped with silicone catheters, one of them was inserted into pancreato-biliary duct, the other one-into duodenum. Deactivation of sensory nerves was performed by application of capsaicin to the rats (100 mg/kg, 10 days before the study). Following i.p. administration of antisecretory factor at doses of 1.0 or 10.0 μg/kg, the samples of pancreatic juice were collected in 15 min aliquots, from the rats with intact or capsaicin-deactivated sensory nerves. Pancreatic amylase and protein outputs were measured in each sample.

Results: AF at dose of 1.0 μg/kg i.p. failed to affect pancreatic basal secretion while dose of 10.0 μg/kg markedly decreased pancreatic amylase and protein outputs. In the experiments with DPJ
Antineoplastic effects of protein-bound polysaccharide PSK for the treatment of pancreatic cancer

R. Jesnowski, M. Löhr
German Cancer Research Center, CCU Molecular Gastroenterology, Heidelberg, Germany

Background: Pancreatic carcinoma is a particularly aggressive tumor with a poor prognosis and at time of diagnosis, the tumor is generally in an advanced stage no longer suitable for resection. Thus alternative therapy modalities are urgently needed. Polysaccharide-K (Krestin) is a unique protein-bound polysaccharide derived from the fungus Coriolus versicolor, which has been used successfully as a chemoimmunotherapy agent in the treatment of various cancers in Asia for over 30 years. However, there is only one publication concerning the treatment of pancreatic cancer patients with PSK. Moreover, it has been shown, that PSK reduces the invasiveness of a pancreatic tumor cell line by downregulating TGFβ1 and MMP expression.

Methods: We now were interested, if the reduction of the invasive potential by PSK is a general phenomenon in pancreatic tumor cells. For this we treated a panel of different pancreatic tumor cell lines with PSK and subsequently analyzed changes in gene expression using RT-PCR. Moreover, the activity of MMP5 was analyzed using zymography, proliferation of the cells after PSK treatment was analyzed by WST-1 assay.

Results: Treatment of the pancreatic tumor cells with PSK for up to 6 days resulted in a short term induction of the cell cycle inhibitor p21/WAF1. In contrast, PCNA and cycD1 expression gradually decreased during the PSK treatment, reaching maximal repression after about three days. PSK did not change MMP expression, neither on the RNA level nor on the protein level. Moreover, PSK dose dependently decreased the proliferation of all pancreatic tumor cells investigated, reaching a plateau of about 40% decrease of proliferation at a concentration of 250 μg/ml PSK.

Conclusion: Our results demonstrate a direct antineoplastic effect of PSK on pancreatic tumor cells by decreasing the proliferation of the cells, pointing on the possibility for the use of PSK in the treatment of pancreatic cancer.
if self-duplication of the jeopardized cell or transdifferentiation from other cell type has occurred in this process.

Methods: Elastase-CreERTM;ROSA26 mice were injected with tamoxifen. Mice just after injection (pulse group) and 2, 4, 6 month later (chase group) were analyzed. Elastase-CreERTM;ROSA26;PDX-1lox/lox embryos were injected as above and analyzed. After the tamoxifen pulse, acute severe pancreatitis is made by cerulein injection. Analysis was performed at 1, 7, 14 days after last injection.

Results: The proportion of labelled cells is not different between pulse and chase group, indicating that adult exocrine cells are formed by self-duplication in normal situation. PDX-1 inactivation results in diminishing amylase expression and affects cell proliferation. The labelled ratio in PDX-1 inactivation mice is far less than that of control mice at 6 months after injection. The proportion of labelled cells is stable even after cerulein-induced severe pancreatitis.

Conclusion: Adult exocrine tissue is maintained by self-duplication in normal situation. PDX-1 is required for exocrine function and the maintenance of adult exocrine tissue. Survived acinar cells promote self-duplication and maintain exocrine tissue if they are damaged by severe acute pancreatitis.

P156
Development of a Transgenic Mouse Model for Hereditary Pancreatitis

T.S. Athwal, C. Merriman, N. Vlatkovic, J.P. Neoptolemos
Division of Surgery and Oncology, University of Liverpool, Liverpool, UK

Introduction: Pancreatitis is a common disease of the pancreas which can be acute (AP) or chronic (CP). The incidence of AP is 50–100 per 100,000 with a mortality of 6–10%. CP often results in long-term disability and is associated with a 2–19 fold increased risk of pancreatic cancer. Insights into the pathogenesis of pancreatitis followed the discovery of mutations in the cationic trypsinogen gene (PRSS1) associated with Hereditary Pancreatitis (HP). Two common mutations in PRSS1 are R122H and N29I, which are thought to result in increased autoactivation or resistance to autolysis of trypsin. This initiates an intracellular digestive enzyme cascade leading to pancreatic autodigestion and pancreatitis. A transgenic model of HP can therefore be used to study both AP and CP.

Method: The human PRSS1 cDNA was initially cloned by RT-PCR from normal pancreatic RNA using specific primers. Mutants of the PRSS1 gene were generated by PCR mutagenesis and confirmed by sequencing. The cDNAs were sub-cloned downstream of the TRE (Tet responsive element) in vector pBGI-G, thus producing an inducible transgene construct. To achieve expression solely in the acinar cells of the pancreas, rtTA (reversed Tet transcriptional activator) was sub-cloned into the pBEG vector so that its expression would be under the control of an acinar cell-specific elastase promoter. Transgene constructs were microinjected into the pronucleus of fertilized mouse eggs, to generate transgenic strains pBEG-Elastase: Opt rtTA, pBGI-G: PRSS1, pBGI-G: R122H, and pBGI-G: N29I. Candidate founder animals were then identified using Southern screening.

Results: Founder animals have been identified for the R122H and N29I strains with screening of potential candidates for the final two strain in progress.

Conclusion: Ultimately, studies of these transgenic animals will give us a better understanding of the molecular events involved in pancreatitis, resulting in improvements in prevention and treatment for this disease.
Author Index

Abdulkader, I. 390
Abiatar, I. 351
Adhikari, S. 350
Aghdassi, A. 339, 380
Ahmad, H. 382
Aiura, K. 344
Albiin, N. 368
Alhonen, L. 346, 348
Al-Sarira, A. 393
Alteheld, B. 360
Amano, H. 340
Ammerpohl, O. 338
Andrén-Sandberg, Å. 353, 359, 368, 381, 397
Andtskär, S. 391
Ang, A.D. 378
Ansgore, C. 368, 397
Appelros, S. 383, 391
Arneko, U. 345, 368
Asaumi, H. 346
Ashston, B. 395
Athwal, T.S. 401
Aubert, A. 336, 386
Azzolini, U. 387

Babu, M. 355
Bachmann, J. 335, 397
Bagul, A. 332, 367
Bain, M. 373
Bajec, D. 356
Bakkevold, K.E. 340
Baraba, K. 360
Barakat, B. 356
Barauskas, G. 363, 397
Barbu, S.T. 367, 396
Bartel, M. 335
Bassi, C. 325, 340
Bauk, D. 338
Bastagi, L. 387
Baudis, M. 374
Beier, M. 333
Belghiti, J. 365
Beliygi, T. 389
Belina, F. 366
Benes, M. 396
Bennett, M.K. 358
Berberat, P.O. 325
Bergmann, F. 330
Berkão, A. 350, 373
Berry, D. 325
Besselink, M.G.H. 352, 395
Bewerunge, P. 378
Bhatia, M. 344, 349, 350, 351, 375, 377, 378
Bimmer, D. 373
Bissoli, S. 324
Blanc, G.E. 339
Bläuer, M. 376
Bollen, T.L. 352
Bonior, J. 366, 368, 371, 400
Booth, J. 339
Borghström, Å. 383, 391
Borka, K. 326
Bortlik, M. 388
Bossonnet, L. 341, 382
Bourton-Rauault, M.C. 331, 355
Brandt-Nedelev, B. 333, 380
Braunschweig, T. 338
Bretzel, R.G. 394
Breuer, N. 388
Brors, B. 378
Brown, J.J.S. 395
Brunke, G. 385
Brümler, T. 391
Bruno, M.J. 354, 382
Bruns, A. 388
Bücherl, M.W. 325, 330, 337, 340, 349, 351, 397
Burai, M. 333, 363, 392
Burden, S. 364
Buskens, E. 395
Butturini, G. 325, 340
Caletti, A. 325
Campbell, F. 342
Cao, Y. 350
Capitano, A. 345, 374
Carter, C.R. 360, 395
Casadei, R. 387
Cacazau, M. 367, 396
Ceranowicz, P. 343, 349, 372
Ceyhan, G.O. 330
Charnley, R.M. 357, 358, 364, 388, 395
Chau, I. 340
Chauhan, S. 325, 341
Cheng, Y. 342
Chierichetti, F. 324, 357
Choi, E.K. 336
Choi, J.H. 361
Cholkin, S. 334, 366, 384, 390
Chukla, R. 390
Chun, H.J. 361
Cieszkowski, J. 343, 349, 372
Clavier, P.-A. 373
Clément, M.V. 350
Cooke, J. 325
Coreos, O. 336, 365
Corinaldesi, R. 356
Corless, D.J. 393
Costello, E. 342, 372
Couvelard, A. 336, 365, 386
Cridle, D.N. 327, 376
Crnogorac-Jurcevic, T. 342
Csont, T. 350
Cukrowska, B. 335
Cunningham, D. 340
Czakó, L. 350
Daly, A.K. 388
Dambrauskas, Z. 397
David, G. 393
Davies, C. 340
Dawra, R. 339
Dembinski, A. 343, 349, 371, 372
Dembinski, M. 343, 349
Demir, I.E. 330
Denley, S.M. 395
Dem, A. 375
Dervenis, C. 340, 359
Dietze, O. 353
Dijkgraaf, M.G. 382
Dijkgraaf, M.G.W. 354
Dimiroula, C. 397
Ding, Y.M. 342
Diófávali, K. 353, 379, 381, 386
Dios, I. 364, 379
Distler, M. 375, 394
Dite, P. 369
Djukic, V. 356
Dobrowolski, F. 394
Dodson, A. 342
Dor, R. 336, 347, 358, 361, 378, 400
Domínguez-Muñoz, J.E. 331, 359, 365, 387, 390
Doobe, C. 324, 360
Dosedeo, J. 331, 388
Dunn, J. 340
Earl, J. 338
Eiriksson, K. 368, 397
Emchsson, L. 368
Erbersdobler, A. 374
Erkan, M. 330, 349
Esposito, L. 349
Evans, J.C. 382
Evans, J.E. 341
Ewald, N. 394
Faissner, R. 378
Falda, A. 339
Fantini, L. 356
Farges, O. 365
Fashe, T.M. 348
Feick, P. 333
Fekete, A. 375
Felix, K. 351
Férec, C. 331, 355
Feuerbach, S. 391
Filiczsky, I. 375
Finger, T. 324, 360
Fischer, L. 326
Fjetland, L. 368
Fleig, W. 337
Fogar, P. 339
Forman, D. 395
Fortezza, J. 387, 390
French, J.J. 364
Friess, H. 325, 326, 330, 335, 337, 349, 351, 397
Fröhlich, B. 397
Fronke, J. 366
Fukuda, A. 336, 347, 362, 400
Furkawa, K. 362
Furuyama, K. 336, 347, 400
Garcia-Montero, A. 379
Garvey, C. 341
Gasiorowska, A. 381, 393
Gasparics, R. 375
George, S. 325, 362
Gerasimenko, O.V. 398
Gerloff, A. 333
Ghaffoor, S. 339
Ghanem, P. 341, 382
Giese, N.A. 330, 335, 349, 351
Giese, T. 330, 335, 337, 349
Gilmore, I. 382
Giralt, J. 393
Gonzalez, A. 333
Goonetilleke, K.S. 364
Gooszen, H.G. 352, 395
Gorbachevski, A. 349
Gostev, L. 352
Grabham, J.M. 382
Author Index

Hozawa, S. 328, 344
Hyvönen, M. 348
Idziak, J. 390
Iglesias-Garcia, J. 331, 359, 365, 387, 390
Iglesias-Rey, M. 331
Ignáth, I. 326, 371, 398
Imaiizumi, T. 362
Imrie, C.W. 325, 360
Ingold, H. 325
Ito, D. 358, 361
Ivancevic, N. 356
Ivankiv, T. 384
Jänne, J. 348
Jansen, C. 383
Jaques, B.C. 358, 364, 395
Jaques, K. 358, 364, 388
Jarosakova, I. 333, 380
Jarva, H. 377
Jaworek, J. 366, 370, 371, 399, 400
Jeeckel, H. 340
Jeen, Y.T. 361
Jenkins, R.E. 342, 372
Jeremic, V. 356
Jesenowski, R. 337
Jesnowski, R. 400
Jin, H.T. 348, 370
Johannesen, F. 368
Johnson, C.D. 325, 362, 398
Jones, N.J. 338
Julius Špick 384
Jurvic, C. 351, 380
Juuthi, A. 391
Juvenon, P. 390
Kalthoff, H. 338
Kameyama, K. 344
Kami, K. 358, 361, 378
Kanai, N. 362
Karamarkovic, A. 356
Karcz, D. 357, 366, 368, 385
Kaszaki, J. 373
Kaufmann, C. 394
Kawaguchi, M. 336, 347, 358, 400
Kawaguchi, Y. 336, 347, 361, 400
Kayed, H. 337
Keim, V. 324, 345
Kekäläinen, O. 358
Kegel, S. 337
Keleti, Gy. 392
Kempainen, E. 358, 377, 386, 389
Khamdišcanov, A. 345
Kida, A. 358
Kiehne, K. 385, 390
Kikuchi, M. 328
Kim, C.D. 361
Kim, J.C. 336
Kim, J.S. 361
Kim, M.H. 336
Kim, Y.S. 361
Kleeff, J. 325, 337, 349, 351
Klein, C.A. 374
Klinkenbijl, J.H.G. 340
Kloer, H.U. 394
Klöppel, G. 338, 378
Knoefel, W.T. 374
Kocna, P. 331, 388
Koczan, D. 378
Kodama, S. 336, 347, 400
Kohout, P. 331, 388
Koizumi, M. 347
Kolkka, V.N. 364
Konturek, P.C. 349
Kopper, L. 386
Kot, M. 370, 371, 399
Kreczler, T. 331, 388
Kuhara, T. 336, 347, 400
Kulig, A. 381, 393
Kusnierz-Cabala, B. 343, 349, 372
Kylänpää-Bäck, M.-L. 358, 377, 386, 391
Labate, A.M. Morselli 356
Lämsä, T. 347, 348, 370
Lang, S. 391
Lange, S. 399
Lankisch, P.G. 324, 360, 388
Lappalainen-Lehto, H. 334
Lariño-Noia, J. 359, 387, 390
Lau, H.Y. 377
Laukkarinen, J. 347
Lázaro, S.A. 346, 379
Lee, B.J. 361
Lee, H.S. 361
Lee, S.K. 336
Lee, S.S. 336
Lee, S.W. 361
Leja-Szpak, A. 370, 371, 399
Lemoine, N.R. 342
Leppäniemi, A. 389
Lerch, M.M. 327, 332, 333, 339, 343, 380
Li, J. 351
Liessi, G. 324, 356, 375, 385
Lindkvist, B. 391
Lindstrøm, O. 377, 389
Linevsky, Y.V. 371
Lippert, H. 380
Lochan, R. 358, 388
Logue, J.A. 360
Löhr, J.M. 354
Löhr, M. 337, 378, 400
Lohse, A.W. 376
Lombard, M. 382
Lonovics, J. 326, 350, 371, 373, 398
Lössl, R. 378
Lowenfels, A.B. 324, 360, 388
Lozano-León, A. 387
Lübke, A. 374
Lukas, M. 331, 388
Lundell, L. 368
Lüttgens, J. 338, 353, 381
McCarber, D.H.A. 360
McKay, C.J. 360, 395
Macko, M. 370, 371, 399
Madaria, E. de 354
Maire, F. 336
Maisonneuve, P. 324, 360, 388
Makay, R. 389
Malagelada, J.R. 327, 329, 393
Malleck-Panas, E. 381, 393
Manas, D.M. 358, 364
Manca, M. 387
Manes, K. 359
Mansfield, S.D. 358, 364
Manso, M.A. 346, 379
Martignoni, M.E. 397
Martínez, J. 354
Matsuura, H. 362
Mayerle, J. 327, 380
Meissner, S. 368
Mentula, P. 386
Merentie, M. 346, 348
Meric, S. 377
Merlos, A. 327
Merriman, C. 401
Methuen, T. 358
Michalski, C. 335
Micsik, T. 379, 386
Miettinen, P. 390
Migliori, M. 387
Mihovilić, R. 360
Mikkonen, J. 347
Milic, N. 356
Milić, S. 353, 360
Mirnezami, A.H. 398
Mitkus, T. 335, 349
Miyamoto, T. 346, 377
Miyatake, S.-I. 378
Molero, X. 327, 329, 393
Mössner, J. 345
Müller, M. 325, 376
Müller, M.W. 330
Murphy, J.A. 376
Nagai, K. 358, 361
Nahon-Uzan, K. 336, 386
Author Index

Valle, J. 340
Van Leeuwen, M.S. 352
Van Ramshorst, B. 352
Van Santvoort, H.C. 352
Vanickova, Z. 331, 388
Vaqueiro, E. 329
Varga, Cs. 350, 373
Van Ró, A. 326, 371, 398
Venglovecz, V. 326, 371, 398
Verbeke, C.S. 339
Vicente, S. 346
Vieites, B. 387
Vilariño, M. 331, 359, 365
Vilgrain, P.V. 386
Vimalachandran, D. 342
Vinjamuri, S. 341
Visser, M.R. 395
Vitone, L. 338
Vlatkovic, N. 401
Völker, U. 343
Voronina, S.V. 398
Vullierme, M.-P. 336, 386
Wada, M. 378
Walkowiak, J. 390
Wallig, M. 350
Wang, F. 338, 344, 345
Wang, W.X. 342
Wartmann, T. 380
Warzeha, Z. 343, 349, 371, 372
Watanabe, N. 328, 344
Watanabe, S. 346
Weber-Dany, B. 324, 360, 388
Weerth, A. de 376
Weiss, F.U. 327, 332, 343
Wellmann, A. 338
Wente, M.N. 340
Weström, B.W. 399
Wilson, J. 338
Wohl, P. 396
Wright, C.V.E. 336, 347, 400
Yamagishi, Y. 328, 344
Yan, J.S. 342
Yan, L. 372
Yu, L. 342
Yubero, S. 346, 379
Yudin, V. 352
Zagorenko, Y.A. 369, 371
Zak, A. 388
Zambon, C.-F. 339
Zarantonello, F. 325
Zasada, J. 357, 366, 368, 385
Zdanyte, E. 351, 380
Zenker, M. 332
Zhang, W. 342
Zidan, B. 398
Zöller, M. 345
Zrnić, I.K. 353, 360