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Abstracts

Basic Pancreatic Cancer

1 Evaluation of Lentiviral Vectors for Human Pancreatic Cancer Gene Therapy

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Aims: (1) To examine the suitability of lentiviral vectors (LV), based on the equine infectious anaemia virus (EIAV) and human immunodeficiency virus type-1 (HIV-1) as a gene delivery system for pancreatic cancer gene therapy and compare their efficiency. (2) To deliver and determine the long-term stability of CapG, a cytoskeleton regulating protein, identified in our laboratory to be differentially expressed in PDAC cells compared to benign ductal cells.

Methods: Vectors (Oxford Biomedica UK) were produced by co-transfection of 293T cells with (1) a packaging construct, (2) a transfer construct encoding the enhanced green fluorescence protein (EGFP) and (3) a plasmid expressing the vesicular stomatitis virus glycoprotein (VSV-G) envelope protein. Vectors were concentrated by ultracentrifugation and titered on 293T cells. Optimised production conditions resulted in efficient titers of 10^8 tu/ml. Particle/ infectivity ratio was estimated by viral RNA assays. The efficiency of transduction was evaluated by FACS analysis for detection of EGFP. Growth curves were constructed to investigate possible interference of the vector (cells transduced with a control vector) with normal cell growth (non-transduced cells). The CapG gene was cloned into the EIAV system harbouring the Neomycin resistance gene for selection. Cell death is due exclusively to viral replication and cell lysis.

Results: Five pancreatic cancer cell lines (Panc-1, MiaPACA, Suit-2, BxPc3 and CFPAC1) were successfully transduced by both HIV and EIAV vectors. Low MOIs of 2, 3 and 10 for both vectors resulted in transduction efficiencies ranging from 3% to 47% of the vector (cells transduced with a control vector) with normal cell growth (non-transduced cells). The CapG gene was cloned into the EIAV system harbouring the Neomycin resistance gene for selection of transduced cells and overexpression was determined by immunoblotting.

Conclusions: LV vectors are capable of permanent integration into the host’s DNA and there has been no toxicity associated with their use. We have shown that two such vectors (EIAV and HIV-1 based) have resulted in efficient and stable transduction of pancreatic cancer cell lines. Current work involves evaluation of the vectors for infection of primary pancreatic cancer cells and also investigation of the effects of CapG overexpression. We believe that LV vectors will make useful tools in pancreatic cancer research but also offer potential for pancreatic cancer gene therapy approaches.

2 Oncolytic Virotherapy as a Novel Strategy for Pancreatic Cancer

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In an attempt to increase the anti-tumor effect and efficiency of gene expression and delivery, various groups have experimented with replication-competent viruses. Adenovirus E1 gene products, in addition to transactivating other early gene promoters, prepare the cellular environment for optimal viral replication by associating with a number of key cell cycle proteins. Replication-selective viruses may overcome the limitations of gene transfer of conventional adenoviral vectors. Viral replication in a small fraction of tumor cells leads to amplification and extension of the anti-tumor effect of gene expression. Cell death is due exclusively to viral replication and cell lysis.

We have developed a novel gene therapy that targets genetic alterations in pancreatic cancer using oncolytic replication-selective adenoviruses in tumor cells. E1B-55kDa-deleted adenovirus (AxE1AdB) can selectively replicate in TP53-deficient human cancer cells but not cells with functional TP53. Consecutive injection with AxE1AdB markedly inhibited the growth of human pancreatic tumors in severe combined immunodeficiency disease (SCID) mice. Furthermore, AxE1AdB displayed the ability to enhance gene expression as a virus vector. It is reported that uracil phosphoribosyl transferase (UPRT) overcomes 5FU resistance. The therapeutic advantage of a replication-selective adenovirus that expresses UPRT (AxE1AdB-UPRT) was thus evaluated in an intraperitoneal-disseminated tumor model. Combined treatment with 5FU and AxE1AdB-UPRT dramatically reduced the disseminated tumor burden without causing toxicity in normal tissues. We also clarified the process of AxE1AdB-inhibited tumor angiogenesis through the preserved E1A region: an adenoviral E1A protein binds to pRB, forcing the quiescent cell into the S-phase. We constructed a double-mutant, replication-selective adenovirus (AxDAdB-3) containing a mutation in the RB-binding motif of the E1A region and a deletion of large E1B-55kDa. AxDAdB-3 swiftly induced cancer cell death in vitro and showed a potent antitumor effect in vivo. These results strongly suggest that AxDAdB-3 possesses a wider therapeutic potential than previously believed, given that most pancreatic cancers have abnormalities in both the TP53 and RB pathways.
Optimised Retroviral Vectors for Suicide Gene Therapy in Pancreatic Cancer

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Abstracts

**Background:** The success of gene-directed enzyme prodrug therapy (GDEPT) is highly dependent on efficient and tumour-specific expression of the prodrug-activating enzyme. Towards this aim, we have developed the promoter conversion (ProCon)-retroviral vector system. Upon transduction of target cells with ProCon vectors the retroviral promoter is replaced by a tissue-specific promoter, which regulates the expression of the transgene in these cells. Unfortunately, tissue specificity of retroviral vectors often is associated with reduction in viral titer and/or shut down of transgene expression. To overcome these problems, several modifications were introduced into MLV-based retroviral ProCon-vectors, resulting in both 2–3 log increase in viral titer and substantially enhanced expression rates.

**Methods:** A modified cytochrome P450/2B1-containing ProCon-vector (pPCCWmCMV) harbouring the murine CMV promoter in place of the 3’LTR U3-region was constructed. Although this promoter is not specific for pancreatic tumour cells, it is strongly active and was thus chosen for proof of principle studies. Various pancreatic tumour derived cell lines were stably infected with PCCWmCMV, or a conventional LXSN-based retroviral vector carrying the cytochrome P450/2B1-gene (LCSN), respectively. Western blot analyses as well as enzymatic and cytotoxicity assays were performed to compare expression levels, enzymatic activity and bioactivity of cytochrome P450/2B1 in PCCWmCMV-infected cells and LCSN-infected cells.

**Results:** Significantly enhanced cytochrome P450/2B1-expression levels and a higher enzymatic activity of expressed cytochrome P450/2B1 enzyme were observed in PCCWmCMV-infected pancreatic tumour cells as compared to LCSN-infected cells. The cytochrome P450/2B1-mediated sensitivity of PCCWmCMV-infected tumour cells towards ifosfamide was significantly higher than of LCSN-infected cells.

**Conclusions:** Efficient killing of pancreatic tumour cells with an improved ProCon-vector in vitro was demonstrated. The in vivo therapeutic efficacy of these ProCon-vectors on pancreatic tumours in mice is currently under investigation.

In vivo Somatostatin Receptor sst2 Gene Transfer Strongly Impairs Pancreatic Tumor Progression by Inhibiting Tumor Angiogenesis

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**Background:** We recently described human somatostatin receptor sst2 as a candidate tumor suppressor gene for pancreatic cancer. When restored ex vivo, sst2 expression dramatically reduced in vivo pancreatic tumor growth. The present study was conducted to determine whether sst2 gene delivery in vivo would impair human pancreatic tumor progression, and to identify antitumoral bystander mechanisms elicited by sst2 gene transfer.

**Methods:** Capan-1 pancreatic adenocarcinoma-derived cells were injected subcutaneously in athymic mice to generate tumors. Sst2 gene was transferred intratumoraly using linear polymers of ethylenimine (PEI). Tumor growth was monitored every two days up to three weeks post gene transfer.

**Results:** Tumor growth progression and proliferation index were significantly reduced (p < 0.05) in mice receiving PEI:sst2 cDNA complexes, compared to control groups. Iterative intratumoral sst2 administration resulted in long term tumor progression inhibition. We estimated that 2 to 5% of the tumor was positive for sst2 after gene transfer, suggesting a strong sst2-related antitumoral bystander effect that was characterized. RT-PCR, immunostaining and ELISA analysis demonstrated that sst2 gene expression resulted in intratumoral somatostatin production. Using siRNA, we demonstrated that in vivo sst2 antitumoral effect was dependent on somatostatin production. Treating mice with anti asialo GM1 antiserum did not impaired sst2 antitumoral activity, strongly suggesting that NK cells were not involved in sst2-mediated bystander effect. We next found that in tumors receiving sst2 gene transfer: microvessel density was markedly reduced and VEGF mRNA and protein levels were dramatically inhibited compared to control groups. Blocking the ability of sst2-transferred tumors to express and secrete somatostatin by the in vivo administration of siRNA against somatostatin restored VEGF expression and tumor progression.

**Conclusions:** We demonstrated that pancreatic tumor progression was strongly impaired following in vivo sst2 gene delivery that resulted in local production of its ligand, somatostatin, which was essential to the antitumoral effect observed. In vivo gene delivery inhibits VEGF expression and tumor angiogenesis. Therefore, using to target VEGF and its various pathways that enhance the angiogenic process in pancreatic ductal adenocarcinoma might ultimately be of great therapeutic benefit in patients with unresectable disease.
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Combination of TRAIL-Bax Gene Therapy and Chemotherapy for the Treatment of Pancreatic Cancer
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Pancreatic adenocarcinoma is the fifth leading cause of cancer death in Europe and in United States. To date, there is no effective therapy and the prognosis is still worst even when surgical treatment can be accomplished. Here, we investigated the anti-tumour and anti-metastasis effects of a recombinant adenovirus expressing the pro-apoptotic gene TRAIL or Bax in combination with Gemcitabine. This study was realized in vitro and in vivo on BxPC3 and Panc1 pancreatic tumour models. For gene expression targeting in tumour cells, TRAIL and Bax were under the control of Gal4-VP16 regulatory system and telomerase promoter (hTERT). In vitro, our data showed a high gene expression of TRAIL and Bax and a significant tumour cell killing and apoptosis induction in treated pancreatic tumour cells. In vivo, loco-regional administration of recombinant adenoviruses demonstrated no apparent cytotoxicity in normal tissues. However, the number of metastasis and the tumour volumes were significantly reduced after combined administration of Ad-TRAIL and Ad-Bax. This effect was more pronounced with Gemcitabine combination. In addition, the survival duration was longer in animals that received TRAIL-Bax plus Gemcitabine was greater than the sole TRAIL-Bax association. In conclusion, these results showed that treatment of pancreatic cancer using the combined Bax and TRAIL significantly reduced the tumour growth and metastasis development. Moreover, combined TRAIL and Bax plus Gemcitabine was more effective with a prolonged animal survival. Together, these results suggest that pro-apoptotic gene therapy (TRAIL-Bax) combined to chemotherapy (Gemcitabine) could be a promising alternative for pancreatic cancer.

6
Expression Profile of Pancreatic Cancer Cell Conditioned Myoblasts: A 5,000 Muscle Genes Microarray Analysis
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Background: We verified: (1) whether pancreatic cancer (PC) cell lines (MIAPaCa2, CAPAN-1, BXPC3) conditioned media (CM) alter glucose metabolism of mouse myoblasts C2C12 (MYO); (2) the gene expression profile of control and CM MYO using a microarray experiment with a platform of 5,000 skeletal muscle cDNA.

Methods: MYO were incubated with control or CM for 6, 24 and 48 hrs. For the microarray experiments control and CAPAN-1 CM MYO were used to obtain total RNA, extracted after 16 and 26 hrs: 15μg were labelled with Cy3 and Cy5 fluorochromes by direct incorporation (RT).

Results: After 24 hrs lactate increased in all CM MYO [control MYO = 0.48 ± 0.07 mmol/L, mean increment ± SEM; CAPAN-1-CM = 1.10 ± 0.07 (t = 6.19, p < 0.001); PANC-1-CM = 0.8 ± 0.18 (t = 4.48, p < 0.01); MIAPaCa2 2-CM = 1.2 ± 0.13 (t = 3.97, p < 0.01); BXPC3-CM = 1.16 ± 0.13 (t = 3.16, p < 0.05)]. HK1, GSK3A, PYGM, PKM2, ENO3, ALDOA, GAPDH, PKFM, PDH, IDH2,3,G, succinylCoA synthetase, SDHD and MDH2 expression did not vary. The expression level at 16 or 26 hrs in comparison with baseline control MYO gave the following results: 23 genes were over-expressed, 8 exerting known biological functions: RPS16, fibrillarin, APOBEC2, DCL2-associated anathogene, ALY, PXR1, PAFAH1B1 and cathepsin G; 24 genes were downregulated, 9 exerting known biological functions: RPS12, thymosin beta 10, troponin T1, IGF2R, RPL14, RPL8, PET112-like, actin alpha and sorcin. The expression levels found in 16 or 26 hrs CM MYO, in comparison with that of 16 and 26 hrs control MYO, was as follow: a total of 43 genes were overexpressed in CM MYO (among which IDH3B, RPL22, RPS3A, RPS21, propionyl CoA carboxylase), and 22 were underexpressed (among which VAMP5).

Conclusions: One or more PC compound/s induces MYO production of lactate, through a mechanism independent from genes involved in glycolysis or glycogen synthesis. PC CM enhances the expression of proteolytic enzymes, as cathepsin G, and reduces the expression of ribosomal proteins, possibly favouring protein degradation with respect to synthesis, thus underlying cancer cachexia. A downregulation of the expression of IGF2R, which is associated with GLUT4, and of VAMP5 might interfere with glucose transport when MYO conditioning persists.

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Extracellular Signal-Regulated Kinase Cascade Activation by Protein Kinase C Epsilon: Differential Effects on Pancreatic Cancer Cell Growth Depending on Activation Kinetics
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Background: Protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) have been implicated in the effects of regulatory peptides on proliferation. We studied how ERK was activated by PKC following regulatory peptide or phorbol ester stimulation and we also investigated the effect of ERK activation on proliferation in Panc-1 cells.

Methods: Panc-1 cells transfected with CCK1 receptors were treated with 10nM cholecystokinin (CCK), 50nM neurotensin (NT) or 100nM phorbol 12-myristate 13-acetate (PMA). DNA synthesis
was studied by measuring tritiated thymidine incorporation. PKC isoforms were selectively inhibited with 20nM Gö6983 (a, b, g, d) or 200nM Ro-32-0432 (a, b, g, e), their translocation was detected by subcellular fractionation followed by immunoblotting. ERK cascade activation was detected with phosphoERK immunoblotting and inhibited with 20μM PD98059. PKC-Raf1 complexes were detected by immunoprecipitation and western blotting. Expression of cell cycle genes was studied by immunoblotting.

**Results:** PMA and CCK inhibits, NT stimulates DNA synthesis. These effects are inhibited by Ro-32-0432 but not by Gö6983. PMA, CCK and NT cause cytosol-membrane translocation of PKCe, and ERK activation that is inhibited by Ro-32-0432 but not by Gö6983. PKCe is detected in Raf1 immunoprecipitates. ERK activation is prolonged following PMA and CCK, whereas transient after NT treatment. PMA, CCK and NT all activate both cyclinD1 and p21CIP1; each of these effects is inhibited by PD98059. However, the increase in p21CIP1 protein level is more pronounced after PMA and CCK, coinciding with sustained ERK activation.

**Conclusions:** PKCe activation induced either by PMA or by regulatory peptides results in ERK activation with different kinetics. Both inhibitory and stimulatory cell cycle components are activated and the outcome appears to depend on the kinetics of ERK activation: prolonged activity is in favour of growth arrest via the p21CIP1 tumour suppressor.

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**The Biological Role of p120ctn Isoforms in Pancreatic Cancer**

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**Background:** p120ctn is the prototypic member of the Armadillo repeat domain family involved in cell-cell adhesion and signal transduction. Owing to alternative splicing and multiple translation initiation codons, several p120ctn isoforms can be expressed from a single gene. Previously we have shown that upregulation, cytoplasmic redistribution and nuclear import of p120ctn is associated with a more malignant phenotype of pancreatic cancer. Here we have investigated the biological role of p120ctn isoforms in pancreatic cancer cells.

**Methods:** In PaTu 8889-T pancreatic cancer cells endogenous p120ctn expression was suppressed with 21-nucleotide siRNA duplexes and proliferation was determined by BrdU incorporation. Expression of p120ctn isoforms was determined by RT-PCR and western blotting and immunofluorescence analysis. Biological characteristics from two different pancreatic cancer cell lines derived from the same primary tumour (PaTu 8889-S and PaTu 8889-T) were correlated to the expression pattern of p120ctn isoforms.

**Results:** In pancreatic carcinoma tissue p120ctn mRNA levels were increased. Silencing of p120ctn with siRNA duplexes reduces pancreatic cancer cell growth by 40%. PaTu-T and PaTu-S cells differentially express different p120ctn isoforms. The expression of p120ctn isoforms containing exon A, rather than B or AB, correlates with a higher proliferation rate. Expression of isoform 1 AB correlates with increased cell migration. Isoform 1 AB appears to induce a branching phenotype. PaTu 8889-S cells express a newly identified isoform, transcribed from a downstream ATG. We refer to this new isoform as isoform 5AB.

**Conclusions:** We found conclusive evidence for a direct involvement of p120ctn in malignant tumor cell proliferation. Both, p120ctn – defective tumor cell contacts and p120ctn – mediated growth signals appear to contribute to the aggressive spread of pancreatic cancer. The expression of Exon A of p120ctn is responsible for an increased proliferation rate of pancreatic tumour cells. In ongoing experiments we will evaluate whether siRNA silencing of Exon A might be useful as a therapeutic strategy for pancreatic cancer.

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**An Orphan Medicinal Drug for the Treatment of Pancreatic Cancer**

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**Background:** The prognosis of pancreatic cancer is poor, and conventional chemotherapeutic regimens are mostly ineffective. Therefore, we developed a new treatment modality, which enables local conversion of the prodrug ifosfamide at the tumour site with encapsulated cells overexpressing the prodrug-converting enzyme cytchrome P450/2B1.

**Methods:** Human HEK293 cells have been genetically modified to express the cytchrome P450/2B1 enzyme. Single cell clones were subjected to enzymatic assays and Southern blot analyses to identify a candidate clone with high P450/2B1 activity and genetic stability. The clonal P450/2B1-expressing cells were encapsulated in cellulose sulphate capsules of 700 μm in diameter to protect these cells from the host immune system. The ability of the encapsulated cells to mediate killing of surrounding pancreatic tumour cells was investigated in toxicity assays in vitro as well as in in vivo models of pancreatic tumour bearing mice.

**Results:** A modified HEK293 cell clone showing high CYP2B1 activity as well as genetic stability was identified and encapsulated. Cells of several pancreatic tumour lines were effectively killed by the activated ifosfamide released from the co-cultivated encapsulated cells. A significant reduction of tumour mass could be observed following intratumoural injection of encapsulated cells expressing P450/2B1 into pre-established pancreatic tumours in immune deficient mice.

**Conclusions:** The cell therapeutic approach utilised by Austrianova was designated as an ‘Orphan Medicinal Product’ by the European Agency for the Evaluation of Medicinal Products (EMEA) and recognised as the first ‘Advanced therapy medicinal product’. Based on this we will commence an international multi-centric clinical trial by the end of this year.
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Antitumor Activity of the Rapamycin Analog CCI-779 in Pancreatic Cancer Cells

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Introduction: The mammalian target of rapamycin (mTOR) plays a central role in the cell proliferation. Dysregulation of mTOR signaling occurs in diverse human tumors, and can confer higher susceptibility to inhibitors of mTOR. In this study, we investigated whether or not CCI-779, an mTOR inhibitor, has antiproliferative effects in vitro and in vivo models of pancreatic cancer.

Methods: First, we examined the expression pattern of mTOR signaling pathways such as p-mTOR, and p-p70S6K in 6 human pancreatic cancer cell lines (AsPC-1, BxPC-3, KMP-3, KMP-4, Panc-1 and Suit-2) by immunoblot. Next, we examined the cytotoxicity of CCI-779 (0–200 nM) in the 6 cell lines in vitro as single agent and in combination with gemcitabine (10 nM). Finally, the antitumor effects of CCI-779 were examined using in AsPC-1 and Suit-2 xenograft models. After 1 × 106 cells inoculation to right flank (AsPC-1) and intraperitoneal cavity (Suit-2) of nude mice, (1) CCI-779, (2) gemcitabine, (3) CCI-779 with gemcitabine, and (4) vehicle alone were administered by i.p. injection. Tumor volume (AsPC-1) and survival time (Suit-2) of each mouse was measured.

Results: mTOR and p70S6K were activated in all of the 6 cell lines examined. With respect to the growth inhibitory effects of CCI-779, AsPC-1 and KMP-3 cells were highly sensitive to the treatment, and BxPC-3 and Suit-2 cells were slightly resistant. Intriguingly, CCI-779 had additive effects with gemcitabine in the CCI-779 resistant cell lines, BxPC-3 and Suit-2. In vivo, single treatment of CCI-779 induced 75% regression of tumor volume (AsPC-1) and combination treatment of CCI-779 and gemcitabine prolonged survival by 3.8 fold (Suit-2), compared with controls.

Conclusion: These results suggest that mTOR may be a good target for pancreatic cancer therapy and the mTOR inhibitor, CCI-779 is an important new agent to investigate in the treatment of pancreatic cancer.

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Bisphosphonic Acid Acts as Gamma/Delta T Cell Activating Antigen and has Direct Cytotoxic Activity Against Pancreatic Carcinoma Cells

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Background: T cells bearing the Gamma9/Delta2 T cell receptor (TCR) constitute two to ten percent of peripheral blood T lymphocytes. They have recently raised much interest as non-MHC restricted effector cells against a variety of tumors. Gamma/Delta T cells are known to be stimulated by phosphoantigens without the need of professional antigen presenting cells. Furthermore, it is described that incubation with phosphoantigens increases their proliferation rate rapidly.

Materials and Methods: Apoptotic and anti-proliferative effects of two bisphosphonates (pamidronate and zoledronic acid) against eight different ductal pancreatic carcinoma cell lines were measured by Annexin-V/PI stain and MTT assay. Gamma/Delta T cells were enriched from peripheral blood of healthy donors and expanded by stimulation with anti-CD3 and IL-2. Cytotoxic activity of Gamma/Delta T cells of age of 14 days was tested against these cell lines. In the next step, we pulsed tumor cells prior to the 51Cr release assay with bisphosphonates.

Results: Zoledronic acid induced even at lower concentrations inhibition of proliferation. Incubation with a 3 μM solution inhibits proliferation to 11–93%. Cell lines susceptible for this treatment had a higher apoptosis rate. Gamma/Delta T cells showed cytotoxic activity against pancreatic cell lines (cell lysis of 24–37% at an effector to target ratio of 80:1). Inhibition of proliferation correlated significantly with susceptibility against Gamma/Delta T cells (p < 0.003). Pulsing of target cells with zoledronic acid prior to the cytotoxicity assay increased the lysis rate (35–62%).

Discussions: Zoledronic acid has even at concentrations which could be achieved by normal dosage an anti-proliferative and apoptotic effect. Cell lines which are susceptible for bisphosphonates were also susceptible for treatment with Gamma/Delta T cells. The efficacy of Gamma/Delta T cells could be further enhanced by pulsing tumor cells with bisphosphonates.

Conclusion: At least for some pancreatic carcinoma cells a bisphosphonate-based therapy may be useful, particular in combination with adoptive transfer of Gamma/Delta T cells.

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CapG, an Actin Capping Protein, is Expressed in Malignant Pancreatic Ductal Adenocarcinoma Cells

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Background: Previously, we have used proteomics to identify differentially expressed proteins in microdissected benign and malignant ductal cells in pancreatic ductal adenocarcinoma. One of these, CapG, was upregulated in malignant samples. CapG binds to and caps the fast-growing end of filamentous actin, preventing filament elongation. Studies by others suggest a role for CapG in cell motility, possibly through modulation of membrane ruffling at the leading edge of motile cells.

Discussions:
Methods: Our initial identification of CapG was based on mass spectrometric analysis of protein spots from two-dimensional electrophoresis (2-DE) gels. To confirm this, 2-DE gels were immunoblotted using a polyclonal anti-CapG antibody. Immunohistochemistry (IHC) was also performed on a pancreatic cancer tissue array. Antibody specificity was assessed by pre-incubation of the antibody with recombinant his-tagged thioredoxin/CapG fusion protein, with analysis by immunoblotting and IHC.

Results and Discussions: Immunoblotting and 2-DE confirm our proteomics identification. CapG was represented on 2-DE gels of tissue and cell lines in either one or two predominant spots with immunoblotting revealing another one (or two) fainter spots, the identities of which are unknown. Their lower pI values are consistent with modifications including phosphorylation, which has been reported for CapG previously. Analysis of the tissue array confirmed that CapG is expressed in malignant ductal cells and is mostly undetectable in benign ducts (the differences were significant) and in the stroma. Strong staining was seen in macrophages, in which CapG has been reported to represent one percent of total protein. Pre-incubation of the antibody with the fusion protein essentially removed detectable staining in immunoblotting and in IHC. Wound healing and Boyden chamber migration assays are presently being carried out on control and CapG-overexpressing clones of pancreatic cancer cell lines, to examine the role that CapG might play in motility-related processes.

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Abstracts

Comparison of K-Ras Antisense Oligonucleotides and siRNAs Strategies in Human Pancreatic Cancer
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Human pancreatic cancer, one of the most malignant neoplasms, is usually diagnosed at advanced stage. Furthermore, it is often difficult to resect curatively, which results in a worst prognosis because its poor sensitivity to chemotherapy and/or radiotherapy.

Point mutations of the K-ras gene are detected in >90% of human pancreatic cancers and may play an important role in tumorigenesis. Antisense therapeutics with phosphorothioate oligonucleotides (PS-ODN) and short interfering RNAs (siRNA) targeting specific gene expression, represent a promising strategy for pancreatic cancer treatment. The aim of the present work was to address a comparative study concerning the antitumour activity of synthetic PS-ODN and siRNA antisense targeting specific K-ras mutation (K-ras mut12).

The siRNA was delivered as a synthetic 19–22 bases and as a recombinant vector for stable expression (pK.ras-siRNA). The antitumour activity was evaluated (i) in vitro, in cell culture with different human pancreatic cancer cell lines (BxPc3, Panc1, Capan1). The endogenous Ras expression was determined by RT-PCR and Western-blotting. Antiproliferative and cytotoxic effects were evaluated by [3H] thymidine incorporation and MTT tests. (ii) In vivo, antisense (PS-ODN) and siRNAs were delivered by electroporation into pancreatic tumours xenografts on athymic mice. The tumour growth was evaluated by tumour volume measurement during 5 weeks. Our results demonstrate that the antisense PS-ODN targeted specific K-ras mutation oncogene transcripts and reduced their expressions. The antitumour effect observed in vitro and in vivo was less than 50%.

The siRNAs appear to be more efficient with a long lasting effect in cell culture. The best results were obtained with the recombinant vector expressing siRNA (K-ras mut12) which induced an efficient and specific down-regulation and a persistent oncogene expression. The tumour growth of the pancreatic cancer cells (K-ras mut12) targeted with specific siRNA was dramatically inhibited in vitro as well as in vivo.

In conclusion; these results indicate that the siRNA strategy is more efficient than the antisense oligonucleotides one and should be more investigated as an innovative rationale for targeting pancreatic cancer therapy.

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The siRNA was delivered as a synthetic 19–22 bases and as a recombinant vector for stable expression (pK.ras-siRNA). The antitumour activity was evaluated (i) in vitro, in cell culture with different human pancreatic cancer cell lines (BxPc3, Panc1, Capan1). The endogenous Ras expression was determined by RT-PCR and Western-blotting. Antiproliferative and cytotoxic effects were evaluated by [3H] thymidine incorporation and MTT tests. (ii) In vivo, antisense (PS-ODN) and siRNAs were delivered by electroporation into pancreatic tumours xenografts on athymic mice. The tumour growth was evaluated by tumour volume measurement during 5 weeks. Our results demonstrate that the antisense PS-ODN targeted specific K-ras mutation oncogene transcripts and reduced their expressions. The antitumour effect observed in vitro and in vivo was less than 50%.

The siRNAs appear to be more efficient with a long lasting effect in cell culture. The best results were obtained with the recombinant vector expressing siRNA (K-ras mut12) which induced an efficient and specific down-regulation and a persistent oncogene expression. The tumour growth of the pancreatic cancer cells (K-ras mut12) targeted with specific siRNA was dramatically inhibited in vitro as well as in vivo.

In conclusion; these results indicate that the siRNA strategy is more efficient than the antisense oligonucleotides one and should be more investigated as an innovative rationale for targeting pancreatic cancer therapy.

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Growth Factors and Extracellular Matrix Proteins Protect Pancreatic Cancer Cells from Death through Activation of Membrane NAD(P)H Oxidase
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Background and Aims: Growth factors and extracellular matrix (ECM) proteins are 2 major environmental factors protecting pancreatic cancer cells from death. We report here that anti-apoptotic effect of both of these factors is mediated by reactive oxygen species (ROS) produced by NAD(P)H oxidase.

Methods: Human pancreatic carcinoma MIA PaCa-2 and Panc-1 cells were cultured with and without serum (FBS) or insulin-like growth factor (IGF-I), on fibronectin, laminin or polyHEMA (non-adherent substrate preventing cell attachment). We measured intracellular ROS by FACS analysis of cells labeled with DCF, and NAD(P)H oxidase activity in cell homogenates by lucigenin-derived chemiluminescence. Apoptosis was characterized by DNA fragmentation, phosphatidylserine externalization, caspase-3 activation, cytochrome c release, and mitochondrial depolarization. Mitochondrial DNA-depleted Rho0 cells were generated by long-term culture with ethidium bromide.

Results: Both the ECM proteins (fibronectin and laminin) and growth factors (FBS and IGF-I) increased intracellular ROS and stimulated NAD(P)H oxidase activity. ROS production was blocked by the NAD(P)H oxidase inhibitor diphenylene iodonium (DPI). In Rho0 cells depleted of mitochondrial NADH dehydrogenase, the stimulatory effect of growth factors and ECM proteins on cellular ROS and NAD(P)H oxidase activity was the same as in parental MIA PaCa-2. These data indicate that the source of ROS induced by the ECM proteins and growth factors is a non-mitochondrial NAD(P)H oxidase. Nox-4 (but not Nox-1 and Nox-2) isoform of NAD(P)H oxidase was expressed in both cell lines. Inhibiting ROS by pharmacologic agents: DPI, the superoxide scavenger tiron, and antioxidant like growth factor (IGF-I), on fibronectin, laminin or polyHEMA (non-adherent substrate preventing cell attachment). We measured intracellular ROS by FACS analysis of cells labeled with DCF, and NAD(P)H oxidase activity in cell homogenates by lucigenin-derived chemiluminescence. Apoptosis was characterized by DNA fragmentation, phosphatidylserine externalization, caspase-3 activation, cytochrome c release, and mitochondrial depolarization. Mitochondrial DNA-depleted Rho0 cells were generated by long-term culture with ethidium bromide.

Results: Both the ECM proteins (fibronectin and laminin) and growth factors (FBS and IGF-I) increased intracellular ROS and stimulated NAD(P)H oxidase activity. ROS production was blocked by the NAD(P)H oxidase inhibitor diphenylene iodonium (DPI). In Rho0 cells depleted of mitochondrial NADH dehydrogenase, the stimulatory effect of growth factors and ECM proteins on cellular ROS and NAD(P)H oxidase activity was the same as in parental MIA PaCa-2. These data indicate that the source of ROS induced by the ECM proteins and growth factors is a non-mitochondrial NAD(P)H oxidase. Nox-4 (but not Nox-1 and Nox-2) isoform of NAD(P)H oxidase was expressed in both cell lines. Inhibiting ROS by pharmacologic agents: DPI, the superoxide scavenger tiron, and antioxidant N-acetylcysteine, or by overexpression of MnSOD stimulated apoptosis in MIA PaCa-2 and Panc-1 cells. Apoptosis was associated

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with mitochondrial dysfunction, activation of effector caspases, DNA fragmentation, and phosphatidylserine externalization.

Conclusions: ECM proteins and growth factors stimulate ROS production in pancreatic cancer cells through a non-mitochondrial NAD(P)H oxidase. This enzyme is a potential therapeutic target to induce apoptosis in pancreatic cancer.

15 Intracellular Targeting of Boron Compounds to Solid Tumors by Transferrin-PEG Liposomes, for Boron Neutron-Capture (BNCT) Therapy

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The successful treatment of cancer by boron neutron-capture therapy (BNCT) requires the selective delivery of relatively high concentration of 10B compounds to malignant tumor tissue. This study focuses on a new tumor-targeting drug delivery system for BNCT that uses small (less than 200nm in diameter), unilamellar mercaptoundecahydrododecaborate (BSH)-encapsulating, transferrin (TF)-conjugated polyethylene glycol liposomes (TF-PEG liposomes). Their tissue distributions in mouse colon 26 carcinoma (Colon 26)-bearing mice after i.v. injection were compared with those of free BSH. Then antitumor activities of TF-PEG liposomes were done in combination with thermal neutron irradiation in Colon 26 tumor-bearing mice. TF-PEG liposomes showed a prolonged residence time in the circulation and low uptake by the reticuloendothelial system (RES) in Colon 26 tumor-bearing mice, resulting in enhanced accumulation of 10B into the solid tumor tissue. TF-PEG liposomes maintained a high 10B level in the tumor for at least 72 hours after injection. This high retention of 10B in tumor tissue indicates that binding and concomitant cellular uptake of the extravasated TF-PEG liposomes occurs by TF receptor and receptor-mediated endocytosis, respectively. On the other hand, the plasma level of 10B decreased, resulting in a tumor/plasma ratio of 6.0 at 72 hours after injection. Therefore, over 72 hours after injection of TF-PEG liposomes was selected as the time point of BNCT treatment. Administration of BSH-encapsulating TF-PEG liposomes and irradiation with 2 × 1012 neutrons/cm2 for 37 min produced tumor growth suppression and improved long-term survival compared with free BSH. Thus, intravenous injection of TF-PEG liposomes can increase the tumor retention of 10B atoms, which were introduced by receptor-mediated endocytosis of liposomes after binding, causing tumor growth suppression in vivo upon thermal neutron irradiation. These results suggest that BSH-encapsulating TF-PEG liposomes appears to have a potential.

16 Molecular Analysis of the RAS-RAF Pathway in Pancreatic Ductal Adenocarcinomas: Lack of Mutations in the BRAF Gene

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Background: Davies et al. (Nature 2002;417:949) initiated a systematic screen of human cancer cell lines and tumors for mutations in members of the RAS-RAF-MEK-ERK-MAP signaling cascade. They found that the BRAF gene, which encodes a cytoplasmic serine/threonine kinase regulated by RAS, was mutated at high frequency in malignant melanomas and at lower frequencies in many other tumors. Pancreatic ductal adenocarcinomas exhibit the highest incidence of activating KRAS2 mutations observed in any human cancer, and it was therefore of interest to examine how this pattern would relate to BRAF mutations.

Patients and Methods: A material of 51 pancreatic adenocarcinomas (46 surgically resected tumors, 5 needle biopsies from inoperable patients) diagnosed at our department was reviewed by a pathologist, and 41 of the tumors could be classified as infiltrating ductal adenocarcinomas. Cancerous cells were isolated by laser-assisted microdissection of formalin-fixed, paraffin-embedded tissue sections. exon 1 of the KRAS2 gene and exons 11 and 15 of the BRAF gene were PCR-amplified and sequenced.

Results and Conclusions: Thirty-seven ductal adenocarcinomas (90%) were positive for KRAS2 mutations in codon 12 (51% GAT, 22% GGT, 15% GTT, 2% GCT), while four were negative. No changes in BRAF sequence were detected in any of the samples. We therefore conclude that mutations in the hotspot exons 11 and 15 of the BRAF gene generally are absent from pancreatic ductal adenocarcinomas.

17 Pancreatic Cancer Cachexia is Mediated via IL-6 Release of the Tumor and Mononuclear Cells

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Introduction: Cachexia occurs in end stage diseases like HIV, COPD, Crohn’s disease and in many malignancies. In pancreatic cancer cachexia is responsible for approximate 20% of the cancer related deaths. Although much research is done on this subject it is still unclear, whether cachexia is an answer of the body to the tumor or if cancer cells directly induce cachexia.

Patients and Methods: In this study we examined the interaction between pancreatic cancer cells and mononuclear cells of...
non-cachectic and cachetic patients and of healthy donors. Patients who underwent a Whipple resection for pancreatic cancer were prospectively examined whether cachexia was present or not. A DNA microarray analysis was done to screen for cachexia associated factors in specimens of non-cachectic and cachetic patients. Since IL-6 was identified, additionally RT-PCR and immunohistochemistry for IL-6 in pancreatic cancer tissue was done. Preoperatively mononuclear cells (MNC) were extracted and then co-cultured with pancreatic cancer cell lines T3M4 (IL-6 positive) and Panc-1 (IL-6 negative) to further investigate the influence of cancer cell related IL-6 on immune cell related cytokine secretion.

**Results:** DNA microarray analysis revealed IL-6 as the most elevated factor and therefore we focused further experiments on this cytokine. Quantitative PCR confirmed elevated levels of IL-6 in patients with pancreatic cancer and cachexia or non-cachetic patients. By immunohistochemistry IL-6 was almost exclusively located in the pancreatic cancer cells in patients with cachexia. Additionally we could demonstrate significantly elevated IL-6 serum levels of cachetic vs. non-cachetic pancreatic cancer patients. The co-culture of MNC with two pancreatic cancer cells lines revealed only a slight activation in normal and non-cachetic pancreatic cancer patients. In contrast when MNC of patients with cachexia were co-cultured with pancreatic cancer cells a 200-fold increase in IL-6 production in MNC was observed.

**Conclusions:** IL-6 is elevated in the serum and tissue of pancreatic cancer patients with cachexia but not in non-cachetic patients. MNC of patients with cachexia are sensitized and can be triggered by a IL-6 positive cancer cells to produce high amounts of IL-6, which may be a major critical step for the development of cachexia in pancreatic cancer patients.

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**18 Pathophysiology of Cachexia in Pancreatic Cancer**

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Cachexia is a progressive wasting syndrome, resulting in massive loss of both adipose tissue and skeletal muscle mass. Cachexia is very common in patients with pancreatic cancer, with 85% losing weight, and this is evident even at the time of diagnosis (average weight loss 14% pre-illness weight). Weight loss is progressive until death, which usually occurs at weight loss of 30%. Cachexia reduces the response of cancer patients to drug therapy and accounts for the short survival time (average is only 4.1 months). Wasting of skeletal muscle is probably the most important factor regulating morbidity and mortality.

Pancreatic tumours that induce cachexia produce a sulphated glycoprotein, called proteolysis-inducing factor (PIF), that acts directly to stimulate muscle protein breakdown, through inducing an increased expression of the ubiquitin-proteasome proteolytic pathway. This pathway is considered to be the most important in intracellular protein degradation, and expression is elevated in skeletal muscle of pancreatic cancer patients with a weight loss greater than 10%. Administration of PIF to normal mice produces about 10% weight loss in 24 h arising entirely through the loss of skeletal muscle mass. PIF not only increases protein degradation in skeletal muscle, but also inhibits protein synthesis (by 50%). The combined effect results in muscle atrophy.

The catabolic action of PIF is attenuated by the polyunsaturated fatty acid eicosapentaenoic acid (EPA). EPA prevents muscle breakdown by interfering with key regulatory steps leading to increased gene expression of proteasome subunits and ubiquitin-conjugating enzymes. One of the most important steps inhibited by EPA is the nuclear accumulation of the transcription factor nuclear factor-kB (NFkB). EPA has no effect on the inhibition of protein synthesis by PIF. Clinical studies in patients with pancreatic cancer show EPA to effectively attenuate further development of weight loss. When combined with a high energy, high protein, nutritional supplement weight gain was seen, and this was entirely due to an increase in lean body mass. These results show that it is possible to intervene therapeutically in the development of cachexia in pancreatic cancer patients, and this results in an improved quality of life and survival.

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**19 Polymorphonuclear Cells Increase the Adhesion of Circulating Pancreas Carcinoma Cells to Endothelium**

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**Introduction:** Abdominal surgical trauma provokes an inflammatory reaction in which polymorphonuclear cells (PMN) are activated with the release of reactive oxygen species (ROS). We hypothesise ROS promote circulating tumour cell – endothelial cell interactions.

**Materials and Methods:** A reproducible human in vitro tumour cell adhesion model was developed to quantify adhesion of PanC-1 and BxPC-3 pancreas carcinoma cells to monolayers of microvascular endothelial cells.

PMN were obtained from fresh whole blood by Hypaque-Ficoll isolation. To investigate the influence of PMN on tumour cell adhesion, endothelial monolayers were pre-incubated for 12 hours with PMN or PMN stimulated with PMA (phorbol 12-myristate-13-acetate) followed by tumour cell adhesion.

The main ROS produced by PMN is superoxide anion radical. To study the role of this ROS in tumour cell adhesion, superoxide dismutase (SOD), a scavenger for superoxide anion radical, was added in this assay.

**Results:** Basal tumour cell adhesion was between 5 and 10% for PanC-1 and between 25 and 30% for BxPC-3. Pre-incubation with PMN increased tumour cell adhesion. Activation of PMN with TPA gave additional enhancement resulting in over 250% tumour cell adhesion compared to basal adhesion for PanC-1 (p < 0.01) and over 150% for BxPC-3 (p < 0.01). Addition of SOD significantly decreased adhesion (p < 0.01).

**Conclusions:** Activated PMN enhance pancreas carcinoma cell – endothelial cell interactions in vitro. ROS play a major role in this enhanced adhesion and therefore can be responsible for enhanced tumour recurrence after surgical trauma. Insight in the mechanism
of enhanced tumour cell adhesion after surgery may lead to the development of tools to prevent this pathway of post-surgical recurrence.

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Reactive Oxygen Species Enhance Adhesion of Pancreatic Tumor Cells to the Peritoneum
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Introduction: Post-operative intra-abdominal tumor recurrence is a significant clinical problem causing severe morbidity and mortality after ‘curative’ resection of pancreatic carcinoma. Peroperative peritoneal trauma activates a cascade of peritoneal defense mechanisms responsible for postoperative intra-abdominal tumor recurrence. Reactive oxygen species (ROS) play a pivotal role in the post-operative inflammatory reaction.

Aims of the Study: This study explores the influence of ROS on the adhesion of human pancreatic tumor cells to human mesothelial cells of 10 patients undergoing elective surgery in an in vitro adhesion assay.

Methods: A reproducible in vitro assay to study adhesion of tumor cells to a monolayer of human mesothelial cells was used. The human pancreatic tumor cell line Panc-1 and primary human mesothelial cells of 10 patients were used. The superoxide radical was produced using the enzymatic reaction of Xanthine with Xanthine oxidase (X/XO). This was verified with a ferricytochrome-c reduction assay. Human mesothelial monolayers were incubated with superoxide or hydrogen peroxide prior to adhesion of the tumor cells. The scavenger’s superoxide dismutase (SOD) and catalase were used to study the possibility to reduce tumor cell adhesion.

Results: Ferricytochrome-c reduction showed a significant production of superoxide radicals using X/XO. This could be inhibited with SOD. Pre-incubation of the mesothelial cells with superoxide radicals resulted in a significant increase (69.5 ± 41.7%; p < 0.001) in adhesion of Panc-1 in all patients. SOD/catalase could reduce this increase by 56.7% ± 20.4 (p < 0.01). Pre-incubation with hydrogen peroxide resulted in a 82% (±33.1%; p < 0.01) increase in adhesion of Panc-1, which could be reduced by 61.8% (±34.4%; p < 0.05) with catalase.

Conclusions: The ROS released during the post-operative inflammatory reaction play an important role in the adhesion of pancreatic tumor cells to the mesothelium and therefore ROS can be responsible for the enhanced post-operative intra-abdominal tumor recurrence. Clinical use of scavengers should be considered in future anti-cancer strategies.

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Screening Tyrosine Kinase Inhibitors Targeting Pancreatic Cancer: Validation of Assays on Platelet Derived Growth Factor Receptor
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Introduction: Changes in the expression of platelet-derived growth factor receptor (PDGFR) have been described in gastrointestinal tumours, and correlated with more aggressive behaviour. This finding suggests that blockade of PDGF-dependent growth pathways may be an effective strategy to inhibit growth of these tumours.

Our aim was to set-up and characterise a non-radioactive TK assay platform to screen potential drug-candidate compound libraries designed against the ATP binding site of PDGF receptor, representing a uniform functional target.

Methods: An assay based on detection of phosphorylated substrate was established. Recombinant PDGFR alpha derived from baculovirus transfected insect-cell (SF9) expression systems (ProQnase) and a synthetic substrate polyGluTyr (Sigma) was used. Detection was performed utilising anti-phosphotyrosine monoclonal antibody (Clone P9-66 Sigma) conjugated with HRP peroxidase and OPD as peroxidase substrate. Absorbances were measured with ELISA reader and correlated with the phosphorylated substrate thus the enzyme activity.

Results: Following optimisation and standardisation based on individual determination of kinetic parameters and calculation of Ki values, 2 reference PDGFR inhibitors were tested. One of them had a more stable and reproducible inhibitory effect of 90% that was well-comparable to the literature data and thus seems optimal for positive control in future experiments.

Conclusion: The present ELISA based non-radioactive TK assay offers a reproducible, sensitive, simple and rapid method to measure TK activity and enables large-scale screening of PDGFR inhibitors.

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Suicide Gene Therapy of Pancreatic Cancer with Cytosine Deaminase: A Promising in vitro Tool
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Background: Gene therapy is a novel approach for pancreatic cancer (PC) treatment, and the insertion in the host genome of suicide genes seems somewhat promising. Among them cytosine deaminase (CD) is able to convert the 5-fluorocytosine (5-FC) into 5-fluourouracile (5-FU). Our aims were to ascertain in vitro whether CD or CD joint
to 5-phosphoribosyltransferase (5PRT) gene transferred to a series of pancreatic cancer cell lines allows their killing by 5-FC treatment.

**Methods:** Three pancreatic cancer cell lines (CAPAN-1, MIA PaCa-2, PANC-1) were chemically transfected with two plasmid vectors containing both a neo-selectable marker gene and a mammalian constitutive promoter (RSV) and CD (pRSV-CD) or CD + 5PRT (pRSV-PRT) from *Saccharomyces cerevisiae*. Stably transfected cell lines were selected by G418 treatment. Each parental, pRSV-CD and pRSV-PRT transfected cell lines were treated with 5-FC at the dosages of 0, 0.1, 0.5, 1, 5 and 10 mM for 1, 3, 6, 8, 10, 13 and 15 days.

**Results:** pRSV-PRT/PANC-1 cell line growth was significantly inhibited at 0.5 mM 5-FC just after 6 treatment days ($F = 18.3$; $p < 0.001$). The vector pRSV-CD conferred to PANC-1 cells a lesser sensitivity to 5-FC, which inhibited cell growth only after 8 treatment days at dosage of 5 mM ($F = 33.4$; $p < 0.001$). MIA PaCa 2 cells transfected with both vectors become sensitive to 5-FC treatment at 5 mM after 13 days ($F = 5.2$; $p < 0.05$). 5-FC at any dosage and for any time of treatment was ineffective on transduced CAPAN-1 cell growth.

**Conclusions:** The suicide gene CD seems useful to confer 5-FC sensitivity to some pancreatic cancer cell lines. The co-transfection of CD and 5PRT enhances 5-FC sensitivity, possibly because the enzyme encoded by 5PRT may in part counteract the 5-FU degradation observable in some pancreatic cancer cells.

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**Abstracts**

**23**

**The Neurotrophic Factor Artemin is Promoting Invasion in Pancreatic Cancer**

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**Background:** Invasion of pancreatic nerves by pancreatic cancer and its extension to the extrapancreatic-nerve-plexus are still poorly understood events. It leads to retropancreatic tumor extension, precludes curative resection, promotes local recurrence and has a negative impact on survival. To understand the process of neural invasion, Artemin – a neurotrophic factor – and its receptors (RET-GFRα3) were studied in pancreatic cancer.

**Patients and Methods:** Tissues from 31 patients undergoing resection for pancreatic cancer, 10 liver-metastases and 19 healthy organ donors were investigated by western-blot-analysis, immunohistochemistry and QRT-PCR. Artemin was also determined in the pancreatic cancer cell-lines Colo-357, Mia-PaCa-2, BxPc-3, SU-8686, Panc-1, Capan-1, Aspc-1 and T3M4. The influence of Artemin on proliferation was analyzed via a MTT-test and its effects on the invasion was investigated by using the BioCoat-Matrigel invasion chambers.

**Results:** Artemin-mRNA expression was 1.8 fold increased in pancreatic cancer (44.6 ± 7.5 copies) compared with normal pancreas (24.3 ± 6.6). The highest and lowest levels of Artemin-mRNA in pancreatic cancer cell-lines were found in T3M4 and Panc-1, respectively. Western-blot-analysis revealed increased Artemin and its receptor-complex levels in pancreatic cancer, compared to normal pancreas. By immunohistochemistry, cancer samples exhibited strong Artemin, RET and GFRα3 immunoreactivity in arteries, nerves, tubular-complexes and in pancreatic cancer cells. In normal pancreas however, Artemin-immunostaining was only present faintly in arteries. Artemin caused no stimulation or inhibition of cell proliferation in MTT-cell-proliferation-assay in non of the pancreatic cancer cell-lines. However, Artemin increased the invasion capacity of pancreatic cancer cell-lines up to three to five times. In pancreatic cancer liver-metastases, Artemin was found to be strongly immunoreactive in cancer cells and arteries.

**Conclusions:** These findings demonstrate for the first time that, Artemin promotes pancreatic cancer cell invasion, but not proliferation. Its strong presence in metastatic cancer cells suggests that Artemin might also has an influence of the formation of metastases.
The Trophic Effect of Insulin on Pancreatic Cancer Cells is Mediated by Hypoxia Inducible Factor 1a (HIF-1a)

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Background: Insulin in the interstitial fluid of pancreas appears to act as a trophic factor on pancreatic cancer cells. Pancreatic cancer cells may also be regulated by factors produced in the tumor cells. Recently, pancreatic cancer cells were found to express hypoxia inducible factor 1a (HIF-1a), a transcription factor regulating multiple signaling pathways in cells. In this study we investigated whether the trophic effect of insulin on human pancreatic cancer cells (MiaPaCa2) was mediated by HIF-1a.

Materials and Methods: Two new MiaPaCa2 cell lines were created, in which the expression of HIF-1a was stably inhibited by short interfering RNAs (siRNAs) delivered by lentiviral vectors. Regular MiaPaCa2 cells and those transduced with control vectors were used as two control cell lines. The four cell lines were incubated under hypoxic conditions (1% O2 and 5% CO2) for 6 hours in media were used as two control cell lines. The four cell lines were incubated with (100pM–1uM) or without insulin. After the incubation, HIF-1a expression was assessed by Western blotting and cell proliferation was determined by thymidine incorporation.

Results: HIF-1a was expressed in control cell lines and this expression was enhanced by insulin in a dose-dependent manner (P < 0.05). In addition, the insulin-induced HIF-1a expression in control cells was associated with increased expression of hexokinase and aldolase (P < 0.05). In contrast, HIF-1a expression was inhibited in the two cell lines transduced with siRNA constructs targeting the HIF-1a gene, and this suppression was not relieved in the presence of insulin. Furthermore, when the HIF-1a gene was inhibited, hexokinase and aldolase expression was low and was not increased by insulin. Finally, insulin-induced proliferation was seen in control cells but not in cells with inhibited HIF-1a expression (P < 0.05).

Conclusion: The data suggest that the trophic effect of insulin is mediated by HIF-1a in pancreatic cancer cells in vitro.

Clinical Pancreatic Cancer

5-Lipoxygenase is Up-Regulated while 15-Lipoxygenase is Down-Regulated in Human Pancreatic Intraepithelial Neoplastic (PanIN) Lesions and Pancreatic Adenocarcinoma

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Background: Pancreatic cancer has an abysmal prognosis because of late diagnosis and lack of therapeutic response. Pancreatic Intraepithelial Neoplasias (PanINs), the precursor lesions, should be a target for chemoprevention. 5-lipoxygenase (5-LOX) is pro-tumorigenic and 15-lipoxygenase (15-LOX) is anti-tumorigenic. 5-Lipoxygenase (5-LOX) has been shown to be up-regulated in pancreatic cancer cells in vitro experiments.
and overexpressed in pancreatic cancer but not normal ductal cells. Nothing is known about 5-LOX in PanINs or 15-lipoxygenase-1 (15-LOX-1) in pancreatic cancer, so these were investigated.

Methods: 5-LOX was immunostained in human tissues (11 pancreatic adenocarcinoma, 11 chronic pancreatitis, 10 normal pancreas), carcinogen (BOP)-treated hamsters, EL-Kras transgenic mice and controls. Expression of 15-LOX-1 was investigated by RT-PCR and western blotting in pancreatic cancer cell lines and by immunohistochemistry in human tissues (12 normal, 12 pancreatic adenocarcinomas and 12 chronic pancreatitis). Cell proliferation and cell cycle were studied after treatment with the 15-LOX metabolite 13-S-HODE.

Results: Intense 5-LOX staining was seen in cancer cells and PanIN lesions of all surgical specimens but not in ductal cells of normal human pancreas. PanINs in BOP-treated hamsters and EL-Kras mice were also stained, whereas normal ductal cells were negative. In contrast, 15-LOX-1 strongly stained in normal ductal cells, tubular complexes and centroacinar cells, while no staining was seen in islets, cancer cells or PanIN lesions. Western blotting showed absence or very weak expression of 15-LOX-1 in all pancreatic cancer cell lines tested. Treatment with 13-S-HODE inhibited growth of pancreatic cancer cells.

Conclusions: This study shows that the pro-tumorigenic 5-LOX is up-regulated in PanINs from human and animal models of pancreatic cancer. In contrast, expression of antitumorigenic 15-LOX-1 is suppressed in pancreatic cancer and already in PanINs. These findings provide evidence that 5-LOX and 15-LOX-1 play key roles in pancreatic carcinogenesis. Lipoxigenases are attractive targets for the prevention and treatment of pancreatic cancer. 5-LOX inhibitors for chemoprevention or inducing 15-LOX-1 expression may be valuable new antitumorigenic tools in the fight against pancreatic cancer.

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Frequency of K-ras Mutations in PanIN Lesions – A Meta-Analysis

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Background: Early diagnosis of pancreatic ductal carcinoma represents a diagnostic challenge to the clinician. Molecular analyses have demonstrated mutations in the K-ras gene at codon 12 in the majority of pancreatic ductal carcinomas, and K-ras has also been detected in hyperplastic duct lesions thought to be preneoplastic. However, the frequency of K-ras mutation varies, ranging from 0% to 40%.

Methods: In order determine whether the K-ras mutation rate increases parallel to the degree of cellular atypia in hyperplastic duct lesions, we performed a meta-analysis of published reports providing information on K-ras mutations in hyperplastic and dysplastic lesions in the pancreas. The duct lesions were reclassified according to the WHO nomenclature for pancreatic intraepithelial neoplasia (PanIN), and the molecular methods for detecting K-ras were reviewed.

Results: The results reveal a step-wise increase of ras mutations in PanIN lesions found in patients with pancreas cancer (36% in PanIN 1a lesions, 45% in PanIN 1b lesions and 80% in PanIN 2-3 lesions) and a low prevalence in PanIN lesions found in patients with chronic pancreatitis (10%) or in the normal pancreas (11%). Similar trends were observed in studies using either the straightforward PCR, or in studies using the mutation-enriched technique (ME-PCR), but the later technique was associated with higher detection rates of k-ras mutation in PanIN IA and PanIN IB. In chronic pancreatitis, k-ras positivity increases with duration of the disease.

Conclusion: The remarkable high frequency and increase of K-ras mutations in pre-neoplastic duct lesions may serve as screening tool for detecting patients at increased risk of pancreas cancer, in addition to other markers such as p53 mutations or cellular atypia.

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The Risk of Malignancy of Intraductal Papillary Mucinous Tumours of the Pancreas (IPMT) Depends on the Duration of the Disease

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Introduction: IPMT have a high risk potential to malignant transformation (20–70%). However, this risk has only been evaluated in transversal studies and the longitudinal risk, depending on time elapsed from diagnosis, is unknown.

Patients and Methods: All patients (pts) with IPMT histologically proven, or ‘highly probable’ on imaging techniques [cystic lesion(s) communicating with the main pancreatic duct (MPD)] were included. The duration of disease was defined by the delay between the first sign of IPMT (pancreatic pain, acute pancreatitis, pancreatic insufficiency, jaundice, or incidental finding) and surgery or diagnostic of invasive carcinoma or duration of follow-up in patients not operated on without signs of dysplasia or carcinoma and considered as having a benign form. Actuarial risk of at least low grade dysplasia (≥LGD), at least high grade dysplasia (≥HGD) and invasive carcinoma (IC) was calculated according to Kaplan-Meier method. Actuarial curves according to sex, acute pancreatitis, size of the lesions and involvement of MPD were compared using the Log-rank test.

Results: 106 pts (M/F: 49/57; median age at first symptom: 61 years) were included with histologically proven or ‘highly probable’ IMPT in 76 and 30, respectively; initial signs included: pain (n = 28), acute pancreatitis (n = 27), jaundice (n = 6), steatorrhoea (n = 4), other (n = 2) and incidental finding (n = 39). IPMT involved the MPD in 53 pts (15 with MPD diameter >10mm) with or without involvement of branch ducts (BD), and BD-exclusive in 3 pts (4 of whom with BD diameter >10mm). Median duration of disease was 21 (0–241) months. Median follow-up of pts not undergoing surgery was 18 (0–78) months from diagnosis. Actuarial risk of
stage ≥LGD, ≥HGD and IC were 50%, 33% and 22.5% at 5 years, and 67%, 49% and 29% at 10 years, respectively. The involvement of MPD was the only parameter associated with malignancy risk (actuarial risk of lesion ≥HGD: 45% and 50% at 2 and 5 years respectively, versus 10% and 15% in pts with involvement of BD only, p < 0.001). Female sex, acute pancreatitis, size of dilated MPD or BD were not associated with malignancy, but the latter result could be due to lack of statistical power.

Conclusions: The study demonstrates for the first time the risk of malignancy according to the duration of evolution of IPMT. This risk is low in pts with IPMT confined to BD, supporting a non-systematic surgical approach in these patients.

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IL10-1082 and TNFα-863 Polymorphisms Favor the Onset of Chronic Pancreatic Diseases and of the Associated Diabetes, but not Pancreatic Cancer Outcome

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Background: Cytokines genes polymorphisms have been suggested to favor cancer onset in response to triggering factors and drive tumor progression. Our aims were: (1) to evaluate IL-1β-31, IL-1RN (VNTR intron 2), CTGF-447, IFNγ-874, TNFα-1031, TNFα-863, TNFα-592, IL-10-1082, IL-10-819, IL-10-592 genes polymorphisms in patients with pancreatic cancer (PC) or chronic pancreatitis (CP) in comparison with controls (CS); (2) to ascertain whether any of the above genes polymorphisms were associated with any clinical aspect of PC.

Patients and Methods: 60 with PC (28 metastatic and 32 locally advanced; 16 had normal glucose tolerance, 7 glucose intolerance and 37 frank diabetes mellitus); 30 with CP and 78 CS. Genomic DNA was extracted from whole blood; all cytokines genes polymorphisms was PCR amplified and, excepted for IL-1RN, were RFLP analysed.

Results: Only IL-10-819 and IL-10-592 were in complete linkage. A higher frequency of IL-1β-31 T/T genotype was found in CP (57%) as compared to CS (35%) or PC (42%). In PC (43%) and CP (41%) with respect to CS (31%) a higher frequency of IL-10-1082 A/A genotype was found (χ² = 11.43, p < 0.05). None of the studied polymorphisms was correlated with tumor stage or grade. Considering PC and CP all together (χ² = 15.7, p < 0.01), or PC alone (χ² = 21.4, p < 0.001), an association was found between TNFα-863 A allele and diabetes mellitus. Considering PC patients, TNFα-1031 T/T was associated with a slightly higher frequency of metastases (79%) after surgery, in comparison with C/T genotype (55%). Survival was correlated only to tumor stage (χ² = 14.48, p < 0.01), but not to any cytokine studied polymorphism.

Conclusions: IL-1β-31 and IL-10-1082 gene polymorphisms might drive the onset of pancreatic cancer or chronic pancreatitis in response to triggering events, as already described for gastric or prostate cancer; TNFα-863 gene polymorphism seems that mainly involved in favoring diabetes development in patients with PC. Cytokines gene polymorphisms do not seem involved in affecting PC patients survival: only tumor stage was confirmed to predict the outcome of these patients.

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Incidence of Other Cancers in a Cohort of Families with Increased Rates of Pancreatic Cancer

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Background: Pancreatic cancer (PC) has been reported to be hereditary in about 10% of cases. Little is known about other cancers in these families except in rare syndromes including FAMMM, BRCA2, FAP, PJS, HNPCC, and others.

Objectives: To measure the incidence rate of other cancers in a cohort of 55 kindreds at high-risk for PC.

Methods: We identified 55 large kindreds in the Utah Population Database (UPDB) at increased risk for PC; each kindred had at least 5 cases. The UPDB is a unique population-based registry with complete ascertainment that contains genealogical and cancer data on over 6 million individuals. Among the kindreds, 219 PC cases were identified.

Pedigrees of the 55 kindreds were searched for other cancers; pedigree size ranged from 1,900 to 30,000 individuals and included between 5 to 7 generations. The Utah Cancer Registry, a SEER Registry, provided the age-specific cancer incidence data (the statewide rate per 100,000). Members of the cohort were followed for cancer risk beginning in 1966. Age-specific cancer incidence rates were computed for the kindreds and were compared to UCR. Twelve other cancers, chosen for their commonality or association with PC, were observed: prostate, breast, colon, lung, bladder, lymphoma, melanoma, ovary, thyroid, brain, esophageal, and cervix.

Results:

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Expected</th>
<th>Observed</th>
<th>O/E ratio W/ 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>629</td>
<td>913</td>
<td>2.9 (2.71–3.09)</td>
</tr>
<tr>
<td>Breast</td>
<td>378</td>
<td>657</td>
<td>3.5 (3.24–3.76)</td>
</tr>
<tr>
<td>Colon</td>
<td>167</td>
<td>371</td>
<td>2.2 (5.43–6.37)</td>
</tr>
<tr>
<td>Lung</td>
<td>162</td>
<td>267</td>
<td>1.6 (1.41–1.79)</td>
</tr>
<tr>
<td>Bladder*</td>
<td>69</td>
<td>285</td>
<td>4.2 (3.71–4.69)</td>
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<tr>
<td>Lymphoma*</td>
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<tr>
<td>Melanoma</td>
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<td>Ovary*</td>
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<td>Thyroid*</td>
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<td>Brain*</td>
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<td>Esophageus*</td>
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<tr>
<td>Cervix*</td>
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</table>

*Data to follow for presentation.

Conclusion: Families with increased incidence of PC show increased incidence of prostate, breast, colon, lung, bladder, lymphoma, melanoma, ovarian, thyroid, brain, esophageal, and cervical cancer.
**32**

**Preoperative Endoscopic Ultrasound Overestimate Tumor Size in Patients with Pancreatic Adenocarcinoma After Neoadjuvant Chemoradiation**


Pancreas Tumor Study Group, Institut Paoli-Calmettes and Université de la Méditerranée, Marseille, France

**Background:** Accuracy of endoscopic ultra-sound (EUS) staging in pancreatic head ductal adenocarcinoma (PHDA) is demonstrated. In patients treated with preoperative chemoradiation (PCRT), efficiency of EUS staging is controversial. The aim of this study was to compare preoperative EUS staging of PHDA with pathologic examination, respectively in patients who received PCRT and in patients who didn’t received PCRT before surgical resection.

**Patients and Methods:** Forty-three consecutive patients underwent a pancreaticoduodenectomy (PD) for PHDA from November 1996 to October 2003. Twenty-five (59.5%) received a PCRT followed by a PD (group I) and 17 (40.5%) underwent a PD alone (group II). All patients were staged by EUS before resection and prediction of tumor size was considered as correct within 10 mm of pathologic findings.

**Results:** EUS predicted accurately tumor size in 9 group I-patients (36%) and in 14 group II-patients (82%) (p < 0.05). EUS overestimated tumor size in 12 group I-patients (48%) and in 2 group II-patients (12%) (p < 0.05%). EUS predicted accurately lymph node involvement in 2 group I-patients (66%) and in 5 group II-patients (50%) (p = NS).

**Conclusions:** EUS accuracy appears to be significantly altered in patients with PHDA treated by PCRT. EUS restaging after PCRT is consequently useless for surgical decision.

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**Assessment of Pathological Response After Preoperative Chemoradiation and Surgery in Pancreatic Adenocarcinoma**


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**Purpose:** Benefits provided by preoperative chemoradiation (CRT) in pancreatic ductal adenocarcinoma (PDA) are still controversial. However, in most reports from referral centers, local control and survival improvement appears to be provided in selected patients. The aim of this retrospective study was to analyze radiation-induced pathologic effects of preoperative CRT in patients with resectable PDA and precise long-term outcome of responding patients.

**Methods and Materials:** From November 1996 to October 2003, 61 patients received a preoperative CRT for a resectable PDA. Pancreatic tumor location was: head in 49 patients and body in 12 patients. Twenty one patients (34.5%) weren’t operated because of disease progression and 40 patients (65.5%) underwent a pancreatic resection including 32 pancreaticoduodenectomy (80%) and 8 distal pancreatectomy (20%).

**Results:** Major pathologic response was noted in 9 patients including 3 complete response and was found only in patients with tumor of the pancreatic head. Local control rate was similar in patient with and without major pathologic response. Survival in patients with major response was significantly higher than in non-responder patients or with minor response.

**Conclusions:** Major tumor downstaging can be provided by preoperative CRT in patients with resectable celiac PDA. Survival appears to be significantly improved in selected patients.

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**Biochemical Cyst Fluid Characteristics of Intraductal Papillary Mucinous Tumours of the Pancreas (IPMT): Utility in Confirming a Diagnosis of Malignancy**


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Preoperative diagnosis may be difficult in patients (pts) with IPMT, and histological confirmation is not always obtained. Diagnostic accuracy of cyst-fluid analysis has been established in pancreatic cystadenomas. Whether published thresholds previously assessed for the diagnosis of mucinous lesions may be transposed to IPMT remains unknown.

**Aims:** Assess the accuracy of biochemical markers aspirated in IPMT fluid for the diagnosis and prediction of malignancy in pts with this disease.

**Patients and Methods:** IPMT-fluid was obtained in 47 pts (histological confirmation: 68%, unequivocal diagnosis on imaging techniques: 32%), by endoscopic ultrasound fine-needle aspiration (EUS-FNA), aspiration during ERCP or peroperatively in 77%, 17% and 6% of pts, respectively. IPMT were benign and malignant in 75% and 25% of the pts, respectively. Cyst-fluid amylase, lipase, carcinoembryonic antigen (CEA), CA 19.9, CA 72.4 and M1 mucin levels were determined.

**Results:** Median amylase and lipase values (range) were 10,600U/ml (0–424,500) and 12,800U/ml (10–520,000), respectively. Amylase and lipase concentrations were <5,000U/ml in 39% and 29% of pts, respectively. Median (range) tumour marker values were: CEA: 100ng/ml (1–230,000); CA 19.9: 5,400U/ml (2–250,000) and CA 72.4: 8U/ml (0–3,600). Median mucines M1 was 56U/ml (0–750,000).

Marker profiles were identical to those observed in mucinous cystadenomas in 23%–38% of pts. High CEA levels (threshold > 400ng/ml*)
were found in 10/37 (27%) of benign and 2/7 (29%) malignant forms (ns). High CA 72.4 (>40 U/ml) were found in 7/33 (21%) and 2/6 (33%) of benign and malignant lesions, respectively (ns). Only CA 19.9 was predictive of malignant forms of IPMT (threshold >20,000 U/ml; benign 3/35 [9%] vs malignant 9/12 [75%]); using this cut-off value, CA 19.9 had a sensitivity, specificity, positive and negative predictive values of 75%, 91%, 75% and 91%, respectively.

**Conclusion:** Biochemical markers in IPMT fluid are suggestive of mucinous tumour in less than 40% of pts with IPMT. Pancreatic enzymes concentration is low in one third of cases. CA 19.9 is the most accurate marker in predicting malignancy.

*Thresholds for mucinous cystadenomas/cystadenocarcinomas.

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**Detection of p53 Mutations in Pancreatic Cancer and Chronic Pancreatitis**

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**Background:** Mutations in the p53 tumour suppressor gene are present in 40–76% of pancreatic ductal adenocarcinoma (PDAC). Detection of mutant p53 is far more specific than mutant K-ras in PDAC. Data on the presence of p53 mutations in the pancreatic juice of PDAC patients is, however, limited.

**Patients and Methods:** Pancreatic juice was obtained from a consecutive series of 50 patients with PDAC, 49 with chronic pancreatitis and 51 controls with gallstones. Analysis for p53 mutations utilised the yeast functional assay, to identify functional p53 mutations. Exons 5 to 8 are amplified, linked and transformed into yeast. Mutant p53 produces red colonies, from which DNA can be extracted for direct sequencing. K-ras mutations were detected using real time PCR.

**Results:** Mutant p53 was identified in the juice of 23 (45%) of 51 patients with PDAC. Fifteen of the 23 patients with PDAC also had mutant K-ras (65%). Two of 49 (4%) chronic pancreatitis patients had p53 mutations. None of the 51 controls (20% mutant K-ras) had p53 mutations (specificity = 96%). Matched tissue was available in 23 PDAC cases. In 9 (70%) of 13 cases with p53 mutations in tissue, mutations were found in pancreatic juice and in 2 of 10 cases with no p53 mutation in tissue a p53 mutation was identified in juice. Of the two chronic pancreatitis patients, one died from post-operative complications (mutant K-ras) and the other has been followed up for 5 years with no sign of malignancy (wild type K-ras in pancreatic juice and wild type p53 in matched tissue).

**Conclusion:** Combined p53 and K-ras analysis of pancreatic juice may be used as part of the screening strategy for PDAC in high-risk families, in combination with detailed imaging of the pancreas.

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**Diagnostics and Surgical Treatment of Patients with Malignant Ampullary Tumors**

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There were 114 patients (64 male and 50 female) with carcinomas of papilla of Vater, treated in our clinic in the period of 1995–2002 years. Mean age was 57.8 years (range 25–87 years). In 63 patients (52.3%) curative operations were performed, including 27 pancreatoduodenal resections (PDR) and 36 local resections (LR). In 20 patients from PDR group we performed Whipple manipulations.
and in 7 patients - Traverso-Longmire. Since 2000 year manipulations after Traverso-Longmire are preferable in cases when tumor don’t invade the anterior-superior surface of the pancreatic head. LR included 23-papillary tumors and 13- extended papillectomy. In 51 patients we performed palliative manipulations, including 4 laparotomy with biopsy. In 26 patients miniminvasive palliative manipulations were performed including 17 preoperative biliary decompressions. 1 patient after pylorys-preserving PDR died. Common 30-days postoperative mortality in radical surgery group made up 1.6%, 30-days mortality in PDR-group was 3.7%. 1 year survival was 77.8% in PDR group and 100% in LR group. Actuarial 3-year survival was 55.6%, 82.6% in PDR-group was 3.7%. 1 year survival was 77.8% in PDR group and in 7 patients - Traverso-Longmire. Since 2000 year manipulations after Traverso-Longmire are preferable in cases when tumor don’t invade the anterior-superior surface of the pancreatic head. LR included 23-papillary tumors and 13- extended papillectomy. In 51 patients we performed palliative manipulations, including 4 laparotomy with biopsy. In 26 patients miniminvasive palliative manipulations were performed including 17 preoperative biliary decompressions. 1 patient after pylorys-preserving PDR died. Common 30-days postoperative mortality in radical surgery group made up 1.6%, 30-days mortality in PDR-group was 3.7%. 1 year survival was 77.8% in PDR group and 100% in LR group. Actuarial 3-year survival was 55.6%, 82.6% in PDR-group was 3.7%. 1 year survival was 77.8% in PDR group and 100% in LR group. Actuarial 3-year survival was 55.6%, 82.6% in PDR-group was 3.7%. 1 year survival was 77.8% in PDR group and in 7 patients - Traverso-Longmire. Since 2000 year manipulations after Traverso-Longmire are preferable in cases when tumor don’t invade the anterior-superior surface of the pancreatic head. LR included 23-papillary tumors and 13- extended papillectomy. In 51 patients we performed palliative manipulations, including 4 laparotomy with biopsy. In 26 patients miniminvasive palliative manipulations were performed including 17 preoperative biliary decompressions. 1 patient after pylorys-preserving PDR died. Common 30-days postoperative mortality in radical surgery group made up 1.6%, 30-days mortality in PDR-group was 3.7%. 1 year survival was 77.8% in PDR group and 100% in LR group. Actuarial 3-year survival was 55.6%, 82.6% in PDR-group was 3.7%. 1 year survival was 77.8% in PDR group and 100% in LR group. Actuarial 3-year survival was 55.6%, 82.6% in PDR-group was 3.7%.
Endoscopic Ultrasonography (EUS) vs Helical CT for Locoregional Staging of Pancreatic Cancer: A Prospective Comparative Trial Using Histology as Gold Standard

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Accuracy of helical CT for locoregional staging and resectability of pancreatic cancer is limited. Development of EUS has provided with high accurate images of the pancreas and surrounding tissues and organs, which may be highly useful for cancer staging. Comparative trials between CT and EUS for pancreatic cancer staging using histology as the gold standard are scarce. Therefore, we aimed at evaluating the accuracy of EUS for local (T) and lymphatic (N) staging of pancreatic cancer in comparison with contrast-enhanced helical CT scan, using histological analysis of the surgical resection as the gold standard.

Methods: 15 consecutive patients (mean age 62 years; range 41–85, 10 male, 5 female) who were operated upon for resectable pancreatic cancer were prospectively included. The preoperative EUS was performed under conscious sedation by the lineal scanning Pentax FG-38UX echoendoscope, by a single operator who was blinded for the result of helical CT scan. The intraoperative and histological findings were used as the gold standard. The T and N stages were classified according to the TNM classification system (AJCC-1998). Data are shown as percentages and compared by the chi-square test.

Results: 10 patients (67%) were histologically classified as T4 stage and 5 (33%) as T3. None were T2 or T1. With regards to the N stage, 14 patients (93%) suffered from N1 disease and only 1 (8%) from N0 disease. Helical CT made a correct TN-staging in only 4 patients (27%), compared to 12 patients (80%) correctly staged by EUS (p < 0.01). T staging was accurately done in 53% of patients by helical CT compared to 87% by EUS (p < 0.05). The remaining cases were understaged (no overstaging occurred). Seven patients (47%) by CT and only three (20%) by EUS were understaged with respect to lymph node infiltration.

Conclusions: EUS shows a significantly higher accuracy than helical CT for locoregional staging of pancreatic cancer. Routine application of EUS to pancreatic cancer staging should prevent useless surgery for non-resectable disease.

FPC Families Unlinked to the 4q32-34 Locus

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¹The Department of Surgery, RLUH, Liverpool, UK, ²Department of Medical Biometry and Epidemiology, ³Department of Clinical Genetics Phillips University of Marburg, ⁴Department of Internal Medicine, Ruhr University of Bochum, ⁵Department of Surgery, Phillips University of Marburg, Germany

Background: Familial Pancreatic Cancer (FPC) is a rare autosomal dominant disease, the causative gene of which is unknown. However, the locus 4q32-34 has been identified in one large American kindred as a potential locus for the disease. This locus contains over 100 characterised and putative genes including genes involved in DNA repair (HMGB2), apoptosis (PPID) and cell cycle control.

Patients and Methods: The European registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) was established in order to study families with pancreatic disease and we have recruited 113 families with pancreatic cancer. We also collaborate with the FaPaCa group who has additional FPC families. The 4q32-34 locus has been haplotyped in affected and unaffected individuals in 41 EUROPAC and FaPaCa families that fulfil our criteria for FPC. The locus was haplotyped with 9 mapping pairs between D4S413 and D4S415 from section 13 of the Genethon map of chromosome 4. These mapping pairs define an allele and haplotypes are assigned to each individual. High Mobility Group Box 2 (HMGB2) and cyclophilin D (PPID) genes have been sequenced in 10 individuals from 10 different FPC families on the EUROPAC register.

Results and Conclusions: We have excluded linkage to the 4q32-34 locus in 7 of these families. Of the remaining 34 families linkage cannot be excluded due to either the lack of biological samples or that the disease status of individuals cannot be confirmed. A total of 55 haplotypes have been identified as possibly being linked to the disease and a further 75 haplotypes have been ruled out. LOD score analysis will enable us to determine the likelihood of this locus being linked in any of our families. Potential disease causing mutations or nucleotide polymorphisms have not been identified by DNA sequencing of HMGB2 and PPID genes in FPC patients.
Hemorrhage After Duodenopancreatectomy

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Postoperative hemorrhage (PH) after duodenopancreatectomy (DP) is frequently lethal. The aim of this study was to delineate guidelines of management.

Between August 1994 and July 2003, 172 patients underwent DP for cancer. Twenty six patients had an institutional protocol (IP) with standard dose chemoradiation (CRT) and four patients had an extended institutional protocol (EIP) with high dose CRT. Sixteen patients (9.3%) were reoperated for PH. Hemorrhage occurred in 23% of irradiated-patients (4 EIP, 3 IP) and 6% of non irradiated-patient (Confidence Interval (CI) [1.8–6.5]). Sentinel bleeding (SB) was noted in 8 patients (50%) with a mean delay of 10 days after DP. Overall mortality after hemorrhage was 56%. Mortality rates of patients with EIP or IP were respectively 100% and 0%. Mortality rates of patients with or without SB were similar. Mortality rates of axillary (hepatic artery, mesenteric vessels) or lateral (pancreas remnant, splenic vessels) bleeding were respectively 88% and 25% (CI [1.6–18.6]). Completion of pancreatectomy was achieved in 75% without re-bleeding. Preoperative high dosed CRT increase risk of fatal PH. Since SB occurs before massive hemorrhage prompt reoperation could reduce mortality. Completion of pancreatectomy was essential during reintervention. Axial bleeding support a high mortality. Moving to the left the pancreatogastrojejunostomy could avoid contact of pancreatic juice with axial vessels in case of pancreatic leakage. Ligature of the gastroduodenal artery during DP had to leave a stump of at least 1 cm to facilitate the hemorrhage control without ligature of common hepatic artery.

Impact of Reconstruction Method on Outcome of Pancreatoduodenectomy in Pancreatic Cancer Patients
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Local recurrence is one of the most frequent forms of recurrence of pancreatic cancer, even after curative resection. In contrast, local recurrence is rare in bile duct or ampullary cancer. Since the type of recurrence and prognosis differ depending on the type of cancer, these factors should be taken into consideration when the type of reconstruction is chosen. We compared Billroth-I (B-I) and Billroth-II (B-II) type of reconstruction in terms of post-operative and long-term morbidity, and the overall prognosis in pancreatic cancer patients.

Methods: Fifty-four pancreatoduodenectomies were performed in patients with ductal adenocarcinoma of the pancreas from 1994 to 2002. B-I was performed in 27 consecutive patients before 1999 and thereafter B-II was employed in another consecutive 27 patients.

Results: Clinicopathological factors, including histology, nodal status, and stage were not different between the two groups. The time before naso-gastric tube removal was 11.1 ± 9.2 days for B-I and 4.0 ± 2.5 days for B-II patients (P < 0.01). The time before oral ingestion was 27 ± 9.3 days for B-I and 18 ± 6.3 days for B-II patients.
patients (P < 0.01). Seven complications were encountered in B-I patients (2 pancreatic fistula, 2 anastomotic stricture, 2 anastomotic failure, 1 ileus), whereas 3 anastomotic failures occurred in B-II patients. There was no difference in disease-free survival and overall survival between the two groups; however, a bypass operation was required for gastrointestinal obstruction due to recurrence in 6 patients in the B-I group, but only 1 patient in the B-II group (P < 0.01). PTC was performed for biliary obstruction due to recurrence in 6 patients in the B-I group, but only 2 patients in the B-II group.

Conclusion: Our results suggest that a B-II type of reconstruction may have some advantages over the B-I type of reconstruction in terms of post-operative oral ingestion, and avoiding bypass operation at the time of recurrence.

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Increased Expression of Hexokinase II and Inducible Phosphofructokinase in Human Pancreatic Adenocarcinoma Expressing Hypoxia-Inducible Factor-1α
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Background: Energy wasting and negative energy balance is frequently seen as a metabolic complication in many malignant diseases including pancreatic cancer. In pancreatic cancer, the metabolic disorder adds significantly to the poor prognosis of the disease. Mechanism of the metabolic disturbance is not fully understood.

The transcription factor hypoxia inducible factor-1α (HIF-1α) is expressed when cells are exposed to hypoxic conditions. Following HIF-1α expression, glycolysis is enhanced to facilitate survival of the cells in hypoxic conditions. In addition, HIF-1α is also expressed in a variety of malignant tumors. The HIF-1α expression appears to be essential for the neoplastic cells to survive in the host. However, it is unclear whether the HIF-1α expression in tumor cells is involved in the concomitant metabolic disturbance seen in the patients with the tumors. In this study, we investigated the expression of HIF-1α in pancreatic adenocarcinoma and its relation to the expression of glycolytic enzymes in the same tumor.

Patients and Methods: Tumor specimens were obtained from 17 patients with pancreatic adenocarcinoma (M/F: 9/8, median age: 75 (44–81)). HIF-1α, hexokinase II (HXII) and inducible phosphofructokinase (iPFK) expression were determined by Western blot. The expression of HIF-1α was then assessed in relation to the expression of HXII and iPFK.

Results: HIF-1α was expressed in 82% (14/17) of the patients examined. The expression of HIF-1α was associated with a high expression of HXII and iPFK. In contrast, in the HIF-1α negative tumors the expression of HXII and iPFK were either low or absent. Furthermore, low tumor HIF-1α expression (n = 3), was associated with low expression of HXII and iPFK.

Conclusions: HIF-1α is usually expressed in pancreatic adenocarcinoma. The association of HIF-1α expression and the increased expression of HXII and iPFK in the tumor specimens suggests that HIF-1α play a role in increased glycolysis of the pancreatic cancer cells.

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Indium-111-Pentetreotide Scintigraphy and Somatostatin Receptor Subtypes 2 Expression as New Prognostic Factors for Malignant Well Differentiated Endocrine Tumors: A Retrospective Study about 98 Patients
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Background and Aims: Malignant endocrine tumors are difficult to manage because of variable disease outcome and new prognostic factors are requested. These tumors frequently express somatostatin receptors (sst), rationale for the clinical use of somatostatin analogues for tumor localization by somatostatin receptor scintigraphy using Indium-111-pentetreotide (octreoscan) and in therapy. Aim of the study was to evaluate the correlation: (i) between result of octreoscan scintigraphy and expression of somatostatin receptors subtypes; (ii) between octreoscan scintigraphy, sst receptor expression and prognosis.

Patients and Methods: Among the 461 patients who had an octreoscan in our institution between 1994 and 2002, 98 patients with a well-differentiated malignant endocrine tumor (digestive n = 54 including 28 pancreatic tumors, pulmonary n = 24 or unknown primary n = 17) were appraised (sex, age, site of metastases) into two groups depending on the positive (n = 48) or negative (n = 50) labelling at octreoscan scintigraphy. Response to somatostatin analogue treatment and survival were obtained for all patients as well as a systematic pathologic re-examination of endocrine tumors with the expression of sst1 to sst5 assessed by immunohistochemistry. Survival differences between patients with 'positive or negative' octreoscan were tested by a Log-Rank test and Cox model.

Results: The lack of tracer uptake at the octreoscan scintigraphy appeared as a poor prognosis factor (p = 0.007) for overall survival by Kaplan-Meier test (and in multivariate analysis, as well as the age, the presence of hepatic metastasis and absence of humoral syndrome). The tracer uptake (positive octreoscan scintigraphy) correlated with the tumor expression of somatostatin receptor sst2 (p < 0.001) but not with that of sst5, sst3, sst1 and sst4 receptors. In a bivariate analysis tumor sst2 receptor expression significantly correlated with better prognosis and was more sensitive to predict the answer to analogue treatment than octreoscan.

Conclusions: It’s the first study underlying the prognostic interest of octreoscan scintigraphy in malignant endocrine tumors. Sst2 expression correlates with tracer uptake and it’s better than octreoscan to predict the clinical response to somatostatin analogues treatment. The place of octreoscan scintigraphy and sst2 receptor expression in the therapeutic strategy of these tumors has to be discussed.
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Locally Advanced Pancreatic Cancer Treated with Radiation and 5-Fluorouracil: A First Step to Neoadjuvant Treatment?

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Background: A retrospective analysis was performed, in two institutions, of patients with histologically proven locally advanced pancreatic cancer without distant metastases. The aim of this analysis is to assess whether chemoradiation might provide survival benefit in patients with locally advanced disease.

Methods: Forty-five patients from the Erasmus MC (EMC), Rotterdam, received 5-FU and radiotherapy whereas 38 patients from the Academic Medical Center Amsterdam (AMC) did not. Radiotherapy consisted of 50GY external upper abdomen radiation in two courses, concomitant with intravenous 5-FU 25mg/kg/24 hours continuously on the first 4 days of each treatment course.

Results: The treatment protocol was completed in 38 out of 45 patients without complications. Radiological response was evaluated 38 patients. Eleven patients (29%) showed a partial response, stable disease in 11 (29%) and progressive disease in 16 (42%) patients. A second look operation was performed in 8 out of 11 patients with a radiological response, and in three patients the tumor could be resected. The median overall survival time for the EMC group (n = 45) was 9.8 months which compares favorable to a 7.6 months median survival when no treatment was given (AMC group, p = 0.046).

Conclusions: Treatment with 5-FU and radiotherapy might benefit some patients with locally advanced pancreatic cancer, however overall survival remains poor. Although biased by patients selection these results justify further study of neoadjuvant chemoradiation to determine the role of neoadjuvant therapy in the management of locally advanced pancreatic cancer.

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LOH Analysis in Sporadic Pancreatic Cancer to Identify Novel Familial Pancreatic Cancer Genes

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Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) accounts for 40,000 deaths per year in Europe, for which the genetic basis is unknown. Our aim is to identify tumour suppressor genes that are inactivated in pancreatic cancer and are targets for therapy and diagnosis. We are looking for a gene that is mutated in the germline of patients with a familial predisposition for pancreatic cancer and which is frequently mutated in sporadic pancreatic cancers. As a marker we are looking for regions of loss of heterozygosity (LOH).

Patients and Methods: The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) has identified over 80 families with an autosomal dominant genetic predisposition to pancreatic cancer (FPC). No gene has been identified in these families, but by analogy with other cancer syndromes it is most likely to be a tumour suppressor and is likely to be affected by somatic mutations in sporadic cases of pancreatic cancer. So we expect to see loss of the non-mutated allele in the sporadic cases (LOH). By identifying regions of LOH in sporadic cases we, in collaboration with the German Familial Pancreatic Cancer registry (FaPaCa), can focus on these loci as regions of interest in the familial form of the disease.

Results and Conclusions: Tumour and normal DNA was successfully isolated from paraffin-embedded tissue for 111 patients. The quality and quantity of extracted DNA was verified by the techniques of UV Spectrophotometry and Real-Time PCR. Comparative hybridisation using whole genome BAC microarrays from the Sanger centre is being used to identify possible regions with LOH.

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Long Term Outcome of Biliary and Duodenal Stenting in Palliative Treatment of Patients with Unresectable Pancreatic Adenocarcinoma

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Biliary stents are an effective method for palliation management of malignant biliary strictures. More recently, some studies have shown that duodenal stents provide safe and efficacious palliative options in duodenal stenosis. Currently, patients with unresectable pancreatic cancer have improved survival with use of chemotherapy or chemoradiotherapy but the long term outcome of stents in such patients is not well-known.

Aim: Evaluate the necessity, the feasibility and the long term effectiveness of stents in palliative treatment of biliary and duodenal obstruction in patients with unresectable pancreatic cancer.

Patients and Methods: All consecutive patients with unresectable pancreatic cancer, seen between 01/99 and 09/03 were retrospectively studied. Patients with surgical bypass were excluded. All biliary and duodenal obstruction were reviewed. A self-expandable metallic enteral stent was employed if duodenal obstruction was symptomatic (confirmed by endoscopy and barium study). Evaluated outcomes included technical success, stent reobstruction and survival.
**Results:** 144 patients (78 M, 66 F), median age 64 years (32–90), were followed with unresectable pancreatic cancer [locally advanced (60%) or metastatic (40%)], located in the head (64%) or in the corpus or tail (36%). The actuarial median survival was 10 months (0.7–29.3) (metastatic: 8 months, locally advanced: 12 months). Biliary and duodenal stenosis occurred in 89% of patients with cancer of the pancreatic head and 19%, respectively after a median delay following diagnosis of 0.1 months (0–24.1) and 2.1 months (0–37.9), respectively. Biliary endoprosthesis were successfully placed in 74 patients (90%); remaining patients underwent a percutaneous procedure. When a self-expandable metallic stent was first inserted (n = 59), a single stent was sufficient in 69% of cases (median duration of patency: 6.2 months [0.4–21.1]). Duodenal stenting was successful in 26 patients (96%) and 92% of them required only one stent (median duration of patency: 5.7 months [0.5–15.7]). Twenty one patients (15%) had either concomitant or metachronous development of biliary and duodenal obstruction. Combined stenting was successful in 89% of cases. No major complication or death occurred related to endoscopic treatment.

**Conclusions:** With improved survival of patients with unresectable pancreatic cancer, about 90% and 20% of patients presented biliary and duodenal obstruction, respectively. The feasibility of biliary and duodenal stenting was excellent. During follow-up, 69% and 90% of patients required a single self-expandable metallic biliary and duodenal stents, respectively.

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**Lymphotoxin-Alfa and Tumor Necrosis Factor Polymorphism in Pancreatic Cancer**

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Despite improving diagnostic methods, aggressive character and poor prognosis of this type of tumour, is the cause of its late detection in very advanced stages. The knowledge about the molecular biology and etiology of this carcinoma has increased during last years but is still not completely understood. Polymorphism within the tumour necrosis factor (TNF) and lymphotoxin-alfa (LT-alfa) genes has been associated with susceptibility, severity and mortality in a variety of autoimmune diseases e.g. Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA), some carcinomas e.g. gastric and lung cancer, and some infections. We investigated whether the TNF – 308 and LT-alfa – NcoI polymorphism does not contribute to the outcome of pancreatic cancer or survival rate among the patients studied. In the future we are going to investigate other polymorphic markers of the TNF or other genes coding proinflammatory cytokines, which may affect local responses to the growing tumour.

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**Molecular Panel of K-ras, c-erbB-2, p16, p53 and DPC4 Alterations in Pancreatic Adenocarcinoma – Clinical Implications**

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**Background:** The purpose of our study was to examine the prevalence of K-ras, c-erbB-2, p16, p53 and DPC4 alterations in pancreatic adenocarcinoma (PA) in order to evaluate their usefulness in clinical prognosis.

**Methods:** The study included 26 patients (aged 51–81 years) who underwent Whipple resection for PA. Normal pancreatic tissue was obtained from 6 patients with uninvolved pancreas tissue distal to Vater papilla carcinoma. DNA from pancreatic tissue was analysed for K-ras and p16 mutations with PCR amplifications. Paraffin-embedded tissue sections were immunostained with monoclonal antihuman p53 and c-erbB-2 antibody (Dako) and DPC4 antibody (Santa Cruz).

**Results:** The K-ras gene mutation has been shown in 20 (76.9%) and p16 mutations in 19 (73.1%) cases with PA. No K-ras or p16 mutations have been detected in normal pancreas. Positive ductal nuclear p53 immunostaining was demonstrated in 16 (61.5%) cases with PA and not in normal pancreatic tissue. Prevalence of c-erbB-2 expression in patients with PA was 15 (57.7%) and in normal tissue – 2 (33.3%). In PA loss of DPC4 expression have been seen in 14 (53.8%) of cases. Coexpression of all gene alterations was observed in 5 (19.2%) cases of PA. Overall median survival was 9.5 months with thirty-day surgical mortality 7.7%. Among examined mutation only loss of DPC4 expression was significantly associated with shorter survival time (p < 0.05). There was a correlation between K-ras mutation presence and lymph node metastasis (p < 0.05) and between p53 as well as K-ras alterations and well tumor differentiation (p < 0.01). No significant relationships were seen between the alteration of genes studied and patient’s age, sex and tumor localization.

**Conclusions:** The only significant factor influencing survival time was loss of DPC4 expression. Those results indicate that K-ras, c-erbB-2, p53, p16 and DPC4 play a role in pancreatic carcinogenesis, however only K-ras, p53 and DPC4 may be useful in clinical prognosis.

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Pancreatic Metastasis from Renal Cell Cancer
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Background: The pancreas is a rare target for metastasis from other cancers, but it plays a role in the diagnostic work-up in patients with pancreatic tumors, especially in patients with a history of renal cell carcinoma.

Patients and Methods: Between October 2001 and January 2004, 497 patients underwent pancreatic resection in our department. Data were analyzed for metastasis into the pancreas from renal cell carcinoma.

Results: Fourteen patients with metastasis to the pancreas from renal cell carcinoma were identified. In four patients the pancreas remains the only site of metastasis, whereas in 11 patients other organs, especially lung and thyroid gland, were also targets for metastasis. The interval between primary treatment for renal cell carcinoma and occurrence of pancreatic metastasis averaged 10.7 (4.2–21.5) years. Most patients showed only minor and unspecific symptoms such as moderate weight loss and were diagnosed during standard tumor follow-up. Metastases were multifocal in 36% (5/14) patients. We performed three pancreaticoduodenectomies, one segmental resection, seven left resections and three total pancreatectomies, in part in combination with other resections, such as hemicolectomy or liver segment resection. The mean operating time was 300 min with a mean blood loss of 550 ml. The median postoperative stay in hospital was 13 days. Until now, 13 patients remain alive with a median follow-up of 12 months.

Conclusions: Metastasis to the pancreas from renal cell carcinoma is rare, but can occur even more than twenty years after primary tumor manifestation. Our results show that pancreatic resections for metastasis can be performed safely. A pancreatic tumor in patients with a history of renal cell carcinoma should be also considered as a metastasis, even decades after nephrectomy.

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Pancreatic Resection – What is the Window of Opportunity to Avoid Biliary Drainage and its Complications?
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Introduction: Biliary drainage is often performed for pancreatic and peri-ampullary tumours and can have significant complications. The proportion of patients in whom these complications adversely influence patient management is unknown.

Aim: To assess the use and complications of preoperative biliary drainage procedures in patients with suspected pancreatic and peri-ampullary malignancy.

Methods: All suspected pancreatic and periampullary cancer cases in a large centre were prospectively identified from October 2000 to October 2003. Details of resectability on staging, jaundice, biliary drainage procedures and their complications and surgical procedures were recorded.

Results: Of 345 patients, 230 (66.7%) presented with jaundice. Resectable disease on staging was found in 191/345 (55%), of whom 146 (76%) underwent biliary drainage. In 68/146 (47%) more than one procedure was required to achieve adequate drainage. Complications of biliary drainage occurred in 20 (13.7%) patients with resectable disease. These included: acute pancreatitis–13; cholangitis–3; empyema–1; pneumonia–1; intra-abdominal collection–3; peritoneal seeding–1; death–2. Of 146 patients who had resectable disease on
staging 44 (30.1%) developed complications of biliary drainage or recurrent jaundice prior to surgery. A further 25 (17.1%) underwent multiple biliary drainage procedures because of previous failure. In 6 patients (4.1%), resection was abandoned or modified because of complications of biliary drainage: two deaths before surgery, one with peritoneal tumour seeding at a drain site and 3 requiring total pancreatoduodenectomy rather than pancreatoduodenectomy because of pancreatic necrosis secondary to ERCP induced pancreatitis.

Conclusions: Almost half of the patients undergoing biliary drainage with resectable pancreatic or peri-ampullary tumours experienced complications, recurrent jaundice or required multiple procedures. A lower threshold for operating on jaundiced patients, prompt referral and investigation and the minimisation of delays may lead to a reduction in these complications. Where drainage is unavoidable, surgery should subsequently be expedited prior to stent occlusion.

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Predictive Imaging Criteria of Malignancy for Intraductal Mucinous-Papillary Pancreatic Tumors: A Retrospective Study in 38 Patients
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Introduction: Intraductal mucinous-papillary pancreatic tumors (IMPT) are rather unfrequent tumors that can be degenerated in 15 to 45% of cases. Pancreatectomy is frequently indicated to prevent evolution into in situ or invasive adenocarcinoma. However the indication of pancreatectomy is sometimes difficult in aged and/or asymptomatic patients. The preoperative determination of malignancy is thus crucial to manage IMPT patients.

Patients And Methods: From 1988 to 2003, 38 successive IMPT patients (20 men, 18 women; mean age 65 ± 12 yrs) were investigated and treated in our institution. All patients were investigated with ultrasonography, CT scan, endoscopic ultrasonography (n = 35), ERCP (n = 19) and magnetic resonance cholangiopancreatography (MRL n = 12). The diagnosis was set up when one or more pancreatic cyst tumors communicated with the main pancreatic duct. Histological and/or cytological examination was obtained in 23 cases. The mean follow-up after diagnosis was 40 ± 28 months. ‘Symptoms, cyst tumor >30 mm in diameter, size of the main pancreatic duct >10 mm, presence of lymph nodes, presence of solid component within the cyst’ were retrospectively explored as potential malignant criteria for IMPT.

Results: Patients were divided into 2 groups: Group 1 including 18 patients (mean age 59 yrs) that underwent pancreatic surgery. Group 2 including 20 patients (mean age 70 yrs) including non operated patients because of: refusal of surgery or asymptomatic one single cyst lesion of branch pancreatic duct or asymptomatics >75 years patients. Pathological examination confirmed carcinomatous transformation in 6 cases of the Group 1. Five of these six patients died within 14 months after surgical resection. All patients of Group 2 and the other patients of Group 1 survived without relapse after a mean follow-up of 44 months. In our study, presence of measurable solid tissular component within the IMPT at CT scan and/or endoscopic ultrasound and/or MRI appeared as the unique malignant predictive criteria after an univariate analysis (Fischer indice p = 0.000).

Conclusions: The malignant transformation of IMPT had to be suspected when the presence of solid component is detected within the cystic lesion whatever the imaging technic. In these cases as well as in symptomatic IMPT patients we advocate systematic surgery. The management of asymptomatic small side branch duct cases of IMPT that display a better prognosis should be discussed case by case.

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Prognostic Value of p27kip1 Expression in Adenocarcinoma of the Pancreatic Head Region
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Background: p27kip1 (p27) is a tumour suppressor gene, functioning as cyclin-dependent kinase inhibitor. It has been characterised as an independent prognostic factor in breast, colon, and prostate adenocarcinomas, but conflicting data are reported for adenocarcinoma of the pancreas. Therefore, aim of the study was to establish the prognostic value of p27 expression in adenocarcinoma of the pancreatic head region.

Patients and Methods: Study included 45 patients (male/ female ratio – 2:1; mean age: 59, range: 38–82 years) with tumours of the pancreatic head region: 27 adenocarcinomas of the pancreatic head, 13 ampullary carcinomas, and five carcinomas of distal common bile duct. All patients underwent Kausch-Whipple pancreatoduodenectomy (n = 39), pylorus preserving pancreatoduodenectomy (n = 5), or nearly total pancreatectomy (n = 1). Eight patients received adjuvant chemotherapy postoperatively. Follow-up time ranged from 3 to 60 months. Tumours were staged as pancreatic or ampullary carcinomas according to the pTNM classification (UICC 1997). Immunohistochemistry was done on paraffin-embedded blocks from tumour sections using monoclonal antibody p27Kip1 (clone SX53G8, Dakocytomation). Quantitative determination of p27 expression was based on the proportion of p27-positive cells (p27-index). Cases with less than 10% positive cells were considered as negative. Pearson test, survival analysis using the Kaplan-Meier method and the log-rank test were used when appropriate.

Results: Positive p27 expression was detected in 21 tumours (45.7%), whereas 24 tumours (53.7%) were p27-negative. There was no significant correlations between p27-index and stage, lymph node involvement, or radicality of resection (R0 vs R1/R2). Median survival time in patients with p27-positive tumours was 25 months, whereas in patients with p27-negative tumours was 30 months (p = 0.73). Even when analysis was stratified according to ampullary and non-ampullary carcinomas, the survival comparison of patients with p27-positive and -negative tumours did not reveal significant differences.

Conclusion: p27kip1 expression has no prognostic value in patients following resection for adenocarcinoma of the pancreatic head region.
Protein Expression Profiling of Malignant Pancreatic Ductal Epithelium and its Surrounding Stroma
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Background: Pancreatic cancer is an extremely aggressive malignancy that is characterised by an intense desmoplastic response, the role of which remains unknown. Proteomic studies allow the analysis of the protein content of a cell or tissue and offer a more functional perspective than traditional gene based studies. Recent work has suggested that the surrounding stromal tissue actively participates in tumour progression and invasion and this may be detectable by critical changes in protein expression. We aim to characterize the protein expression in the pancreatic cancer microenvironment using a proteomic-based approach.

Methods: Pancreatectomy specimens obtained following surgery were sectioned, stained and specific cell populations obtained using laser capture microdissection (LCM). We obtained malignant ductal cells and stromal cells both immediately adjacent to tumour (juxtatumoral stroma) and distant to the tumour (panstroma). Proteins from these cell populations were subsequently separated using two dimensional gel electrophoresis (2DE) and visualized using silver staining. Spot profiles from each were compared to identify differentially expressed spots and mass spectrometry used to identify proteins from these spots of interest.

Results: Four groups of protein samples have been acquired, each gel displaying in excess of 700 spots. Comparison of the spot patterns revealed 11 consistent differences between ductal cells and stromal cells. Five of these differences were limited to the juxtatumoral stromal cells. Work is ongoing to identify these proteins and to validate the proteomic findings using independent methods.

Conclusions: We have shown that the combination of laser capture microdissection and two dimensional electrophoresis is capable of identifying proteins from complex tissues accurately and reproducibly. Our results confirm that there is a spatial difference in protein expression in the pancreatic cancer microenvironment. Identification of proteins involved in microenvironment biology will further our understanding of tumour development and may lead to the discovery of novel biomarkers or therapeutic targets.

Results of Pancreatic Resection due to Pancreatic and Periampullary Cancer
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Introduction: The aim of this study was to analyse the postoperative complications, perioperative mortality and survival rate after different types of pancreatic resections.

Methods and Materials: Between 1994 and 2003, 354 patients (age range 35–91 years; mean 58) were treated for pancreatic cancer (306) and periampullary cancer (48). Criteria of tumor resectability were: (1) no distant metastases, (2) no neoplastic infiltration into large vessels. In 183 patients resection was performed. In 138 cases pancreatectoduodenectomy (PD) was done: 109 Whipple’s and 29 Taverso-Longmire’s procedures. Pancreatogastrostomy was a standard method of pancreatic fistula prevention. 38 patients were treated with total pancreatectomy and 7 with distal pancreatectomy. In remaining 161 patients: 100 bypass procedures and 61 laparotomy were performed.
Results: Hospital mortality rate after resections was 4.2%. 8 patients died due to: circulatory and respiratory insufficiency (3), cerebral circulation insufficiency (1), intraoperative bleeding (1), mesenteric artery prothesis infection (1), myocardial infarction (1), pancreatic fistula and sepsis (1). The postoperative complications were noticed in 45 patients (25%). The most important of them were: upper GT bleeding (5 patients), intraabdominal bleeding (4), biliary fistula (4), acute pancreatitis (4). Pancreatic leakage was founded in 3 cases after PD (2%). In 31 patients (17%) developed delayed gastric empting. Wound infection appeared in 9% cases. Radical resection (R0) had undergone only in 39 patients (22%). The average survival rate after radical resection (R0) was 25.3 months and 14.7 months after palliative resection (R1). Long term survival after bypass procedure was 8.4 months and 3.2 months after laparotomy.

Conclusions: Pancreatoduodenectomy is a method of choice in the treatment of pancreatic and peripancreal cancer. Pancreatogastrostomy is a safe and relatively easy to perform method of panreatointestinal after pancreatoduodenectomy with low incidence of pancreatic cancer.

S100A6 is an Independent Prognostic Indicator in Patients with Pancreatic Cancer

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Introduction: Pancreatic cancer is a devastating disease, responsible for approximately 40,000 deaths in Europe each year. Using both RNA expression analyses and proteomics based studies we have previously demonstrated increased expression of the calcium binding protein S100A6 in malignant pancreatic ductal cells compared to normal ductal cells. The aim of this study was to determine the clinicopathological relevance of S100A6 expression.

Methods: Immunohistochemical analysis was performed on a tissue micro-array of sixty pancreatic cancer tissue specimens. The intensity of staining [negative (undetectable), weak, intermediate, strong] was scored along with the proportion of cells staining. For the purposes of analysis, immunoreactivity (expression) was categorised into two groups; undetectable or weak (‘low expression’) and intermediate or strong (‘high expression’). Univariate and multivariate survival analysis was then performed using S100A6 expression and other commonly used clinicopathological parameters.

Results: High S100A6 expression was observed in 40 (80%) of 60 patients. Kaplan-Meier analysis revealed a significantly decreased survival time in high S100A6 expressing cases (p = 0.02) when compared with low expression. Nodal metastases (p = 0.05) and resection margin involvement (p = 0.04) were also significantly associated with survival. Multivariate analysis with a Cox proportional hazards model using S100A6 expression, nodal status and resection margin status revealed S100A6 expression to be a significant independent prognostic indicator (p = 0.008).

Conclusions: Our findings suggest S100A6 may be at least as powerful a determinant of survival as existing prognostic indicators. The exact role that S100A6 plays in pancreatic adenocarcinoma is at present unknown. The findings of this study, and the implication that S100A6 may play a significant role in pancreatic tumour progression, clearly merit further investigation.

The Prognostic Factors of the Invasive Adenocarcinoma of the Head of the Pancreas

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Background: Various factors affected to the poor prognosis of the invasive adenocarcinoma of the head of the pancreas compositely. The aim of this study is to evaluate the prognostic factors of the invasive adenocarcinoma of the head of the pancreas from the viewpoint of clinicopathological factors.

Methods: We retrospectively investigated 346 patients who underwent pancreatectomy for the invasive adenocarcinoma of the head of the pancreas between 1978 and 2002. These patients could be followed up more than 3 years after surgery. According to the 2nd edition of JPS classification, age (<50/50–69/70), gender, portal/superior mesenteric vein resection, lymph node dissections (D1/D2), dissection of the extrapancreatic nerve plexus, blood loss (>1,000 g/1,000 g), blood transfusion (>1,000 g/1,000 g), operating time (>6 hr/6 hr), adjuvant therapy, pathology, lymphatic invasion (ly), venous invasion (v), intrapancreatic nerve invasion (ne), tumor size (TS), distal bile duct invasion (CH), duodenal invasion (DU), serosal invasion (S), retropancreatic tissue invasion (RP), portal venous system invasion (PV), extrapancreatic nerve plexus invasion (PL), invasion of other organs (OO), lymph node metastases (N), pancreatic cut-end margin (PCM), bile duct cut-end margin (BCM) and dissected peri-pancreatic tissue margin (DPM) were chosen as a prognostic factor in this study. These factors were evaluated by Cox proportion hazard model.

Results: Multivariate analysis detected each factor of v(+), TS1(>2 cm), PL(+), N(+) and DPM(+) as an independent prognostic factor.

Conclusion: Early diagnosis of less than 2 cm tumor and R0 resection for the patients with PL(–), N(–) are necessary to improve the prognosis of the pancreatic head carcinoma.
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The Use of Pre-Operative CA19-9 Can Reduce the Need for Laparoscopic Assessment in the Pre-Operative Staging of Pancreatic Adenocarcinoma

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Background: To determine if the use of pre-operative CA19-9 (cancer antigen) could reduce the need for laparoscopic assessment in the pre-operative staging of pancreatic ductal adenocarcinoma.

Methods: From a prospectively collected database (Nov-96-Dec2003) a retrospective analysis was performed. Inclusion criteria were histologically proven pancreatic ductal adenocarcinoma, computed tomography criteria of resectability, pre-operative CA19-9 and laparoscopic assessment. Patients were divided into 2 groups; CA19-9 ≤150 U/ml (Group 1) or >150 U/ml (Group 2). An adjusted CA19-9 in the jaundiced patient was calculated and compared. Medians (95% confidence intervals) are provided.

Results: 66 patients met the inclusion criteria. The median CA19-9 was 452 U/ml (-807-1711). 47/57 were jaundiced with a median bilirubin of 170 μmol/l (125–215). The median adjusted CA19-9 was 103 U/ml (-811-1074). The frequency or level of hyperbilirubinaemia was not different between the two groups (12/16, 150 mcg/l (63-363) Group 1 vs. 33/41, 237 mcg/l (102-371) Group 2, p = 0.35, p = 0.44). All Group 1 (n = 20) patients were deemed resectable by laparoscopic criteria compared to 35/46 of Group 2 patients (p = 0.03). The sensitivity, specificity, positive predictive and negative predictive values were 36%, 100%, 100%, 24% respectively. The respective values for the adjusted CA19-9 values were 63%, 55%, 88%, 22%.

Conclusions: In patients with radiologically resectable pancreatic cancer the use of pre-operative CA19-9 can reduce the need for laparoscopy in up to 30% of patients. Adjusting the CA19-9 for the presence of jaundice may not be be necessary.

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Total Lymphocytes Count Predicts Pancreatic Cancer Survival

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Background: An impaired host immunity might concur in determining the tremendous prognosis of pancreatic cancer (PC). Our aim was to ascertain whether the pattern of blood lymphocytes immunophenotype in PC correlates with: (1) tumor stage, (2) tumor grade, (3) the development of metastases after surgery, (4) survival.

Methods and Patients: We studied 46 PC patients. Staging was: stage I = 2.2%, stage II = 8.9%, stage III = 48.9%, stage IV = 40.0%. Grading was: G1 = 19.4%, G2 = 47.2%, G3 = 33.3%. Survival was available for 32 patients (minimum = 1, maximum = 24, median = 19 months). Metastases were found after surgery in 76% of these patients. Lymphocyte immunophenotype was determined by FACS analysis. The following were considered: CD16/CD56+ (natural killer), CD19+ (B lymphocytes), CD3+ (T lymphocytes), CD4+ (T helper), CD8+ (T cytotoxic).

Results: Tumor stage did not correlate with lymphocytes immunophenotype or total lymphocyte count. CD16/56+ were lower in patients with (14.7% +1.14, mean + SE) than in those without lymphnode metastases (25.4% + 4.9) (t = 9.83, p < 0.01). CD4+ were lower in patients with undifferentiated (47.6% + 2.1) than in those with well differentiated PC (55.6% + 2.1) (t = 2.65, p < 0.05). Tumor stage, not grade (χ² = 3.55, p:ns), correlated with the development of metastases after surgery (χ² = 12.75, p < 0.01). Patients who developed distant metastases after surgery had also significantly lower levels of total lymphocytes (Mann Whitney U = 31.5, p < 0.05). Total lymphocyte count discriminated patients who developed from patients who did not develop distant metastases after surgery with a sensitivity of 75% and a specificity of 64% (cutoff = 1.5 × 10⁹/L). The overall survival of patients correlated with tumor stage (Log rank = 12.4, p < 0.01), but also with total lymphocytes (Log rank = 16.5, p < 0.001). The association between survival and total lymphocyte count was confirmed when stage III (Log rank = 10.2, p < 0.005) or stage IV patients (Log rank = 3.0, p = 0.08) were considered singly.

Conclusions: The presence of lymphnode metastases at diagnosis or the development of metastases after surgery are significantly associated with a reduction of natural killer cells and total lymphocytes counts. A reduction in the latter at diagnosis could predict patients’ survival, indendently from tumor stage.

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Trends in Pancreatic Cancer Incidence and Survival with Deprivation – A Population Based Study

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Background: It is postulated that patients from affluent backgrounds with upper gastro-intestinal cancers survive better than their deprived counterparts, but robust demographic data on pancreatic cancer is lacking. We aimed to analyse the incidence, mortality and survival trends for pancreatic cancers in West-Midlands, England (population = 5.5 million) over fifteen years from 1985–2000 in terms of socio-economic deprivation.

Methods: Data was collated from West-Midlands cancer registry and geographical information system. The Townsend Score was employed as a well-validated indicator of social deprivation including 4 proxy indicators of socio-economic status collapsed into quintiles. Populations for Townsend bands were derived from Census data and individual cases allocated one of five deprivation categories using the postcode at diagnosis. Relative survival rates were calculated using
stratified actuarial life tables, regression trend analysis at one and five years performed and p value derived from t-test statistic.

**Results:** Total number of cancers = 8,820. Marginal overall decrease in incidence in men (12%) and women (4%) masked the preferential increase in the most affluent categories. Thus incidence in affluent men rose by 39% (age-standardised rate 8.9/100,000 to 12.4/100,000) and by 41% in affluent women (ASR 5.3/100,000 to 7.5/100,000). Incidence in most deprived women fell by 32% from 9.6/100,000 to 6.5/100,000. The affluent groups had a 3–5% survival advantage over the deprived but the differences were not significant at 95% CI level [p = 0.08 and 0.12 in men and p = 0.16 and 0.09 in women at 1 and 5 years]. 55 deaths would have been avoided between 1989–1993 if most deprived patients had survival rates similar to their affluent counterparts.

**Conclusions:** Modest overall decrease in incidence of pancreatic cancer over fifteen years masks the preferential increase noted in the affluent population and decrease in the deprived. The reasons for these temporal changes are unclear, could be lifestyle related, and warrant further stable longitudinal studies examining probable dietary factors along with other multi-factorial associations related to material deprivation and social affluence.

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**Clinical Acute Pancreatitis**

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**Endoscopic Transgastric Necrosectomy as Primary Therapy in the Management of Infected Pancreatic Necrosis**

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**Background:** Open pancreatic necrosectomy is the standard treatment for infected pancreatic necrosis but is associated with significant morbidity, mortality and prolonged hospital stay. Minimally invasive retroperitoneal necrosectomy (MIRPN) is not suitable for some patients with infected necrosis. We have used a novel technique, endoscopic transgastric necrosectomy (ETGN), to treat patients with infected necrosis.

**Patients and Methods:** Since May 2002 we have used ETGN as primary treatment for infected pancreatic necrosis in selected patients where the necrosis cavity was applied to the posterior wall of the stomach on CT scan. We have reviewed records of patients who had undergone ETGN and compared the outcomes with a historical group of open necrosectomy patients.

**Results:** Ten patients who underwent attempted ETGN for infected pancreatic necrosis were identified of whom 9 were successfully treated. Median age was 53 years (range: 30–64). Median (range) APACHE 2 score on presentation was 7 (0–18) and hospital stay was 21.5 days (13–98). Multiple ETGN procedures were performed in 7 patients. ETGN was abandoned in 1 patient and an open necrosectomy was performed. In addition to ETGN 1 patient each underwent MIRPN and laparoscopic drainage. Only 2 patients needed ITU care. One patient died of a chest complication unrelated to the procedure. All others recovered and had a follow-up of 11.5 months (1–20). In our previously reported group of 41 patients with infected necrosis treated with open necrosectomy we found a median APACHE 2 of 7 and hospital stay of 25 days with 12 (29%) deaths. However 26 patients needed ITU care with a median stay of 5.5 days.

**Conclusions:** ETGN is a feasible method of treatment of infected pancreatic necrosis which may reduce ITU stay. Careful selection of patients is essential. It should be combined with other modalities and routes of surgical intervention.
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Coagulation Factors and Adhesion Molecules of Endothelial Origin in the Course of Different Severity Forms of Acute Pancreatitis

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The endothelium is a central effector in the inflammatory response in acute pancreatitis (AP), therefore the aim of this study was to determine the blood concentrations of endothelial origin coagulation factors – von Willebrand factor (vWF:Ag), tissue factor (TF:Ag), tissue factor pathway inhibitor (TFPI:Ag) antigens and adhesion molecules – sE-selectin, sICAM-1 in AP.

Patients and methods: The study was performed in 97 patients, with mild AP (M-AP, n = 28), severe AP with sterile necrosis (SN-AP, n = 35), severe AP with infected necrosis (IN-AP, n = 34) and in 20 healthy controls.

Results: We have shown, for the first time, significant increase of TF:Ag concentration in IN-AP which was higher by 70.9%, 27.8% on the 1st day (at admission), by 94.7%, 38.1% on the 2nd day, by 92.7%, 40.7% on the 3rd day, by 99.3%, 19.9% on the 5th day and by 84.3%, 36.9% on the 10th day than in SN-AP and M-AP, respectively. The significant increase of TF:Ag in M-AP was found only on the 1st day, whereas in SN-AP – from the beginning until the 5th day, when compared to the controls. The significant decrease of TFPI:Ag concentration was only noted in IN-AP. The significant increase of vWF:Ag concentration was found in all patients with the highest values in IN-AP. It was paralleled by significant increase of sE-selectin and sICAM-1 concentrations. The significant correlation was found between TF:Ag, vWF:Ag and sE-selectin (Rs = 0.384, Rs = 0.478 p < 0.001), between TF:Ag, vWF:Ag and ICAM-1 (Rs = 0.437, Rs = 0.508, p < 0.001), respectively.

Conclusions: Early changes of coagulation factors and adhesion molecules concentrations of endothelial origin depend significantly on AP severity and pancreatic necrosis infection. The most pronounced changes found in IN-AP indicate massive activation of endothelial cells in severe AP with septic complications. TF:Ag and vWF:Ag may serve as an early predictors of multorgan and septic complications in AP.

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Detection and Identification of Bacterial DNA in Patients with Acute Pancreatitis

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Background: Infection of pancreatic and peripancreatic tissues complicates 30% of severe Acute Pancreatitis (AP), and implies a worse prognosis. The most frequent isolated micro-organisms are gram-negative enteric bacteria. Animal models, the increase of enteric colonization by these micro-organisms and the early increase of intestinal permeability on AP suggest a mechanism of bacterial translocation. More sensible test than cultures of blood specimens would be a progress in the study of bacterial translocation. Our aim was to investigate the presence of bacterial DNA in blood specimens of patients with AP, and to relate that to disease severity.

Patients and Methods: Blood samples were obtained on 3 consecutive days within the first 6 days after the diagnosis of AP, for standard culture and detection of bacterial DNA. Polymerase chain reaction technique was used to amplify the 16S ribosomal gene, which is universally widespread in all bacteria. Those positive samples were sequenced to determine bacterial species. Other infectious foci were investigated in those patients with septic state. Severity of AP was determined according to Atlanta criteria.

Results: Thirty three patients with AP were included, 9 with severe disease. Bacterial DNA was found in 8 samples from 6 patients, two of them with severe AP. The isolated micro-organism were C. freundii (5 samples) and P. aeruginosa (3 samples). One blood culture was positive for S. epidermidis, with negative DNA, which was attributed to skin contamination. Other infections were detected: a respiratory infection (DNA+), an infection of pancreatic necrosis by E. coli (DNA+), and an urinary tract infection by E. coli (DNA–).

Conclusions: The presence of DNA from enteric microorganisms in serum of patients with AP supports the translocation theory. In our study, this fact doesn’t seem to be related with severity.
223 patients (39%) and 99 of the controls (44.4%) were taking at least one potentially AP inductor (NS). The number of prescriptions was not different between cases (median of 1, range 1 to 5) and controls (median of 1, range 1 to 4). Analysis of individual drugs showed an inverse association of AP with aspirin intake (OR 0.57, 95% CI: 0.34–0.95; p = 0.04) and no significant association with any other drug. The number of medications was higher in the group of mild compared to severe AP (OR 0.43, 95% CI: 0.21–0.88; p = 0.02).

Conclusions: Our study does not support an association between potentially AP inductor drugs and increased risk or worse outcome of AP. Aspirin appears to protect against the development of AP, which should be confirmed in prospective studies.

**Imbalance of CXC Chemokine Expression in Human Pancreatic Cancer**


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Background: The CXC chemokine family is critical in the biology of many solid malignancies and is characterized by the presence or absence of the N-terminal motif Glu-Leu-Arg (ELR). These proteins have profound angiogenic (ELR+) and angiostatic (ELR−) properties and have been found to impact metastatic biology. The exact role of CXC chemokines in pancreatic cancer biology is unknown. To evaluate the expression of the ELR+ chemokines ENA-78/CXCL5 and IL-8/CXCL8, the ELR− chemokines IP-10/CXCL10 and MIG/CXCL9, and their receptors (CXCR2, CXCR3) in human pancreatic tissues.

Methods: Tissue samples from patients with pancreatic cancer (PaCa, n = 29) and without pancreatic disease (normal, n = 20) as a control group were evaluated. CXC chemokine and CXC chemokine receptor mRNA expression was determined by quantitative RT-PCR and by immunohistochemistry.

Results: Quantitative RT-PCR of CXCL5, CXCL8, CXCL9, and CXCL10 mRNA revealed expression in a distinct manner in human pancreatic tissues. ELR+ chemokines were strongly upregulated in pancreatic cancer compared to ELR− chemokines (mRNA copies normal vs. cancer: CXCL5: 30 + 12 vs. 5129 + 3157*, CXCL8: 283 + 116 vs. 2014 + 838*, CXCL9: 9 + 2 vs. 52 + 20, CXCL10: 78 + 24 vs. 862 + 213*, *p < 0.01). In addition, CXC receptor mRNA expression was increased in pancreatic cancer in comparison with normal controls.

Conclusions: Compared to normal tissues, there was an up-regulation of ELR+ chemokines in pancreatic cancer specimens, and to a lesser extent of ELR− chemokines. This imbalance of angiogenic and angiostatic chemokines suggests that angiogenesis may be activated in pancreatic cancer by CXC chemokine regulation.

**Positron Emission Tomography Does Not Add to Computed Tomography for the Diagnosis and Staging of Pancreatic Cancer**

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Introduction: The role of 18F-fluro-2-deoxy-D-glucose (FDG) positron emission tomography in both the pre-operative diagnosis and staging of pancreatic cancer remains controversial. The aim of this study was to report on the experience of FDG positron emission tomography in a tertiary pancreatic centre.

Methods: Between June 2000–August 2003 patients presenting with a mass in the head of the pancreas underwent both contrast enhanced computed tomography and FDG positron emission tomography prior to definitive treatment. A subgroup analysis was performed on those patients who underwent pre-operative laparoscopic assessment. Sensitivity, specificity, positive and negative predictive values were determined.

Results: 112 patients underwent assessment. Seventy-eight (70%) had malignant disease (72/78 histologically proven). The sensitivity, specificity, positive predictive value and negative predictive value of computed tomography were 89% (58/65), 65% (11/17), 90% (58/64), and 61% (11/18). The respective values for FDG positron emission tomography were 73% (51/70), 60% (18/30), 80% (51/63), and 49% (18/37). The sensitivity and specificity for detecting small volume metastatic disease as assessed by laparoscopy was 20% (2/10) and 94% (47/50) for computed tomography and 22% (2/9) and 91% (45/49) for FDG positron emission tomography.

Conclusions: FDG positron emission tomography does not add to either the preoperative diagnosis or staging of pancreatic cancer and cannot be recommended for routine use.

**Risk Factors of Mortality and Intra-Abdominal Morbidity after Pancreaticoduodenectomy – A Multivariate Analysis**

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Background: The aim of this study was to define, by multivariate analysis, the pre- and per-operative risk factors of mortality, intra-abdominal morbidity and pancreatic fistula after pancreaticoduodenectomy.

Patients: 300 patients (186 men and 114 women) with a mean age of 58 years (range: 22–81) were included in this study. The data
were collected prospectively from 3 French multicentre randomized studies concerning pancreatic surgery. The primary disease was a pancreatic cancer in 225 cases (74.6%) and a benign disease in 75 (25.4%). The pancreatoduodenal anastomosis was pancreatecojejunostomy in 170 cases (56.6%) and pancreaticogastrostomy in 130 (43.4%).

**Methods:** Eighteen factors were studied. Univariate analysis used the Chi square test and Student's t-test. The multivariate analysis used a logistic regression model with the maximum likelihood ratio (Odds ratio; OR).

**Results:** The mortality rate was 9% (28/300). Two risk factors were found: age > 70 years (OR = 3) and extended resection (OR = 5). The morbidity rate was 30.5% (90/300). Two risk factors were found: the size of main pancreatic duct inferior to 3 mm (OR = 2) and extended resection (OR = 5). The rate of pancreatic fistula was 16.6% (50/300). Only one risk factor was found: the size of the main pancreatic duct inferior to 3 mm (OR = 2.5).

**Conclusions:** This study demonstrated that age >70 years was an independent risk factor of mortality, and a main pancreatic duct inferior to 3 mm an independent risk factor of intra-abdominal morbidity and pancreatic fistula. Furthermore, an extended resection was a risk factor of mortality and intra-abdominal morbidity.

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**Clinical Value of Procalcitonin (PCT) in Predicting Infectious Complications and Overall Prognosis in Severe Acute Pancreatitis: A Prospective International Multicenter Study**


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Early and accurate severity stratification is of central importance in acute pancreatitis (AP). In this context, timely and reliable diagnosis of infectious complications is a major issue. PCT, the 116 amino-acid precursor of calcitonin is the first biochemical parameter for predicting bacterial infection and sepsis in various inflammatory diseases. However, in AP the clinical value of PCT determinations still remains controversial.

**Patients and Methods:** From December 1999 until September 2003 a total of 103 patients with severe AP were included in five European centers within 96 hours of disease onset. Ninety-three (90%) patients had CT-proven intra- and/or extrapancreatic necrosis of whom 16 (16%) developed infection (IN). Single organ failure was observed in 39 (38%) and multi organ dysfunction syndrome (MODS) in 29 (28%) patients, 7 patients (6.8%) died. CRP was determined routinely, PCT was assessed by a semi-automated chemoluminescent immunoassay (BRAHMS AG, Berlin, Germany) in a real-time fashion in each center over a maximum of 21 consecutive days.

**Results:** Median PCT concentrations revealed an early and significant increase in patients who developed infected necrosis which was not observed for CRP. If infected necrosis was associated with MODS or patients subsequently died median PCT values reached highest concentrations which already peaked at the third day after onset of symptoms, whereas CRP values did not differ. ROC analysis was based on the two highest PCT and CRP values reached during the total observation period with the following results:

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity, %</th>
</tr>
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<tbody>
<tr>
<td>Prediction of IN</td>
<td>PCT ≥ 4.0 ng/ml</td>
<td>63 90</td>
</tr>
<tr>
<td>Prediction of IN</td>
<td>CRP ≥ 350 mg/l</td>
<td>38 83</td>
</tr>
<tr>
<td>with MODS</td>
<td>CRP ≥ 300 mg/l</td>
<td>80 65</td>
</tr>
<tr>
<td>Prediction of death</td>
<td>PCT ≥ 3.4 ng/ml</td>
<td>100 90</td>
</tr>
<tr>
<td></td>
<td>CRP ≥ 315 mg/l</td>
<td>67 67</td>
</tr>
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</table>

Prediction of the above mentioned complications was already possible on the third day after onset of symptoms with favourable sensitivity and specificity.

**Conclusions:** Monitoring of PCT is a non-invasive and reliable method to predict IN and associated systemic complications as well as overall prognosis in SAP. This single test parameter significantly contributes to an improved stratification of patients at risk to develop major complications in AP and deserves routine clinical application.

This study was supported by BRAHMS AG, Berlin, Germany.

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**A Comparison of Infrared Spectra of Serum with Lipase and Amylase in the Prognostic Evaluation of Patients with Acute Pancreatitis**

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**Background:** Predicting the severity of acute pancreatitis early in the course of the disease is still difficult. The aim of the study was to compare the infrared spectra of serum with lipase and amylase in the assessment of severity of acute pancreatitis.

**Methods:** The analytes were measured on admission in 100 consecutive patients with acute pancreatitis. Twenty-four patients had severe and 76 mild acute pancreatitis. As reference methods we used serum amylase and C-reactive protein.

**Results:** The diagnostic accuracy of the markers was evaluated by receiver operating characteristic (ROC) analysis. On admission, infrared spectra of serum, lipase, and amylase differentiated patients with mild acute pancreatitis from severe acute pancreatitis with different accuracy and ROC analyses showed unsimilar areas under the ROC curves (AUC) for infrared spectra of serum (0.926), lipase (AUC 0.637), C-reactive protein (AUC 0.623), and amylase (AUC 0.549) (p < 0.05).
Conclusions: The infrared spectra of serum displayed the best accuracy for predicting a severe acute pancreatitis already at admission, which makes this test superior for clinical purposes. Thus, the serum makeup reflects the severity of acute pancreatitis. This is an intriguing finding, particularly as it is very unlikely that the infrared spectra are detecting any particular constituent that cannot be (and has not been) assayed previously. The strength of our approach therefore lies not in the ability of infrared spectroscopy to identify novel disease markers, but rather in the ability of multivariate pattern recognition and classification methods to perceive characteristic changes in the balance among the infrared detectable components.

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Bacteriological Study of Bile in Patients with Acute Biliary Pancreatitis
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Background: The aim of this study was to investigate several microbiological aspects of bile in human pancreatitis, and to compare it with bile from patients with symptomatic cholecystitis, but without pancreatitis.

Patients and Methods: A total of 192 patients admitted for acute biliary pancreatitis (ABP) was prospectively studied. On admission a series of clinical and biological data were recorded, and at the time of surgery bile was collected from the biliary tract for bacteriological examination.

Results: There were 68 men and 124 women and their age was 62.5 ± 1.2 years. On admission the serum amylase concentration was 1,420 ± 90 IU/L (N < 113) and the APACHE II score was 6.9 ± 0.3. The outcome according to the Atlanta classification was ‘mild’ in 160 cases and ‘severe’ in 32, including 3 deaths.

In 98 patients the bile cultures were sterile, whereas 94 patients had a positive bile culture. A total of 169 microorganisms were isolated. The most frequently cultured species was Escherichia coli (n = 33), followed by Enterococcus spp. (n = 30) and Streptococcus spp. (n = 27), while Clostridium perfringens was the predominant anaerobe (n = 8). There were 81 (48%) gram-positive bacteria, 72 (43%) gram-negatives and 16 (9%) obligate anaerobes. In the control group 150 different microorganisms were found. Compared with the ABP patients there were less gram-positive aerobes (29% versus (vs) 48%, p < 0.005), and more gram-negatives (61% vs 43%, p < 0.005). The anaerobes were equally represented in both groups (10% vs 9%, p = NS).

Conclusions: About half of the patients with ABP had positive bile cultures at the time of surgery. They had significantly more infections by gram-positive bacteria than patients with cholecystitis without pancreatitis. This distinctive bacteriological profile might have implications in both the pathophysiology and the treatment of acute pancreatitis of biliary origin.

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Compliance with Acute Pancreatitis Guidelines in Germany
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We asked 192 German gastroenterologists from non-university hospitals using written questionnaires (response rate 89%), whether and to what extent they complied with the three most recently published guidelines for acute pancreatitis. (see Table)

German hospital gastroenterologists are generally aware of treatment guidelines for acute pancreatitis. They prefer imaging
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techniques to multifactor scoring systems to determine severity and largely comply with novel treatment recommendations, such as enteral nutrition and antibiotics, if they are evidence based.

Does Post-Operative Pancreatitis Cause Pancreatic Fistula after Pancreatic Resection?

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Background: Pancreatic fistula is the most common complication after pancreatic resection. ‘Soft pancreas’ is more vulnerable to fistula development, but the pathophysiology of this complication is far from clear. The aim of this prospective study was to analyse pancreatic changes by CT scan and urine trypsinogen strip-test (U-TT) in the patients who undergo pancreatic resection.

Patients and Methods: 24 consecutive patients undergoing pancreatic resection (19 PPPD, 3 standard Whipple’s, 2 body/tail resections), were included – 13 males and 11 females, median age 62 (range 32–82) years: 15 periampullary cancer, 2 chronic pancreatitis, 2 pseudocyst, 1 mucinous tumour, 1 insulinoma, 1 carcinoid tumour, 1 mucinous ducectasia, 1 bile duct adenoma. Daily drain output and its amylase activity were measured on the fourth and sixth post-operative day – over 50ml fluid with amylase 3 times ONR in serum was defined as a fistula. CT scan was done on the second and sixth post-operative day for the diagnosis of post-operative pancreatitis. U-TT (Actim Pancreatitis®) was done daily during the first post-operative week.

Results: Seven patients developed pancreatitis as detected either by CT or by U-TT. All were detected by CT and 6/7 by U-TT. Six patients developed fistula, one requiring re-operation, but the others healed spontaneously. The patients with post-operative pancreatic fistula had pancreatitis changes more often in CT scan compared to the patients without a fistula (6/6 vs 1/18, p = 0.00001). Furthermore, the patients with a fistula had more often positive trypsinogen strip-test compared to the patients without a fistula (5/6 vs 1/18, p = 0.0001).

Conclusions: Post-operative pancreatitis is associated with post-operative pancreatic fistula after pancreatic resection. It is hypothesised that fistula develops due to post-operative pancreatitis.

Effect of Octreotide on Interleukin-8 Level in Acute Necrotizing Pancreatitis

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Background: Proinflammatory cytokines have been implicated in the pathogenesis of acute necrotizing pancreatitis (ANP) and mediates the systemic manifestation of the disease.

Patients and Methods: The aim of this study was to establish the influence of octreotide on serum levels of interleukin-8 (IL-8) in 43 patients with ANP (33 male, 10 female). The level of cytokine in the serum was determined by ELISA on the 1st and 10th day of treatment. The patients were divided into 4 groups: group 1 received the protease inhibitors (contrycal), group 2 – pentoxiphylline (100mg twice a day)+protease inhibitors, group 3 – octreotide (0.01mg subcutaneously 3 times a day during 5 days)+protease inhibitors, group 4 – the combination of octreotide, pentoxiphylline and protease inhibitors.

Results: The initial level of IL-8 did not differ in all groups. At the first 2 groups we observed a significant increase (p < 0.05) of
initial IL-8 level which occurred on the 1st and 10th day respectively at the 1st group 331.2 ± 36.67 and 492.4 ± 30.7 pg/ml, at the 2nd group 247.1 ± 31.47 and 353.4 ± 27.8 pg/ml. The application of octreotide prevented the increase of the serum level of IL-8 which occurred on the 1st day 331.9 ± 28.07 and on the 10th day 365.4 ± 14.6 pg/ml (p > 0.05). This is an evidence of the anti-cytokine properties of octreotide. The significant decrease of IL-8 by 10th day was noted in the 4th group from 370.0 ± 27.76 up to 278.3 ± 31.84 pg/ml. A decrease of IL-8 serum levels accompanied by a lower of frequency of pulmonary complications in the case of octreotide application at 2 persons (9.1%) as at the first 2 groups at 7 persons (33.3%).

**Conclusions:** Octreotide may decrease the serum level of IL-8. The combination of octreotide, pentoxiphylline gains ground the anti-inflammatory cytokine properties of both the medications. This anti-inflammatory effect may contribute to its complex effect in the treatment of ANP.

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**Endoscopic Sphincterotomy (ES) is Effective Treatment for Acute Gallstone Pancreatitis**


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Neoptolemos et al. published the first randomized controlled trial (RCT) to assess the effect of ES in gallstone associated AP and showed that early ES was beneficial with the effect being more pronounced in the severe group. A study from Hong Kong produced similar results. A multi-centre trial by Fölsch et al. showed no benefit from early ES and the trial was terminated due to high mortality in the ES group. We have studied the outcome of patients treated with ES for gallstone associated AP in our large university hospital.

Patients who, between October 1996 and May 2003 had ES following gallstone associated AP at the Royal Liverpool University Hospital, UK. Patients were identified from computerized hospital databases (all patients having had ES subsequent to a raised serum amylase) and then by case note analysis. Only those patients with proven gallstone associated AP were included.

There were 107 patients, median age 68 (range 20–98) years (78% female). 92 patients presented with their first episode of AP (92%) and 27 patients had previously known gallstones (25%). Based on the modified Glasgow score, 21 were predicted to have severe disease (20%). Indications for ES were: predicted severe AP 23%, cholangitis/suspicion of MBD stone 50%, unfit for or declined cholecystectomy 27%. 6 patients had local complications from ES (1 patient from the predicted severe group), 11 patients required pancreatic necrosectomy. The overall inpatient mortality was 3.7% (n = 4) with 1 patient dying in the predicted mild group (1%) and 3 patients dying from the predicted severe group (18%).

**Conclusion:** ES is an important treatment option in the management of gallstone associated AP with mortality rates comparable to previous studies and much lower than those in the Fölsch trial.

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**Haemostatic Abnormalities During Surgical Management of Severe Necrotizing Pancreatitis**

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**Background:** Clinical studies in severe necrotizing pancreatitis (SNP) suggest profound activation of coagulation as well as activation of the fibrinolytic system. Aim of this study was to evaluate the haemostatic derangements in patients who were managed for SNP.

**Patients and Methods:** Forty-one operated patients with SNP were analyzed regarding clinical outcome and activation of the coagulation systems. Serial measurement of coagulation, anticoagulation and fibrinolysis parameters; prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, antithrombin III (AT III), protein C, plasminogen activator inhibitor-1 (PAI-1), D-dimer, α2-antiplasmin and plasminogen were performed on days 1, 3, 5, 7, 10 and 14 after initial operation. According to treatment outcome at the end of study, groups of 26 survivors and 15 non-survivors were compared.

**Results:** Protein C levels were low on days 1, 3, 5, 7 and 14 in nonsurvivors and on day 1 in survivors. On the day 3 the difference between both groups was statistically significant. AT III levels were decreased on days 1 and 3 in survivors and on days 1, 3, 5, 7, 10 and 14 in nonsurvivors. On day 5 levels in survivors and nonsurvivors became significantly different. The PAI-1 levels were high in both groups on days 1, 3 and 5. On day 7 the difference between survivors and nonsurvivors reached statistical significance. The D-dimer levels were high in group D on days 1, 3, 5, 7, 10 and 14 and on days 1, 3 and 5 in survivors. Values on day 7 were significantly different between groups.

**Conclusions:** Cytokines released early in the course of SNP stimulate a disorder of haemostasis. In a later stage of disease, in patients with serious septic complications, consumption of clotting factors and inhibitors resulting in a procoagulant state. Low activity of protein C and AT III, and high concentrations of D-dimer and PAI-1 predict worse prognosis.

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**Hyperlipidaemia and Outcome in Acute Pancreatitis**


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**Background:** The association between hyperlipidaemia and clinical acute pancreatitis, in particular alcohol-induced acute pancreatitis is well recognised. In experimental models triglyceride infusion produces histologic changes of pancreatic oedema and haemorrhage
similar to those seen in acute pancreatitis. Further, hyperlipidaemia aggravates the course of experimental acute pancreatitis. Despite these associations suggesting a correlation between serum triglyceride level and the molecular mechanisms involved in the mediation of acinar cell injury, it remains unknown whether hypertriglyceridaemia influences the severity of the episode of acute pancreatitis and whether there is an association between hypertriglyceridaemia and peri-pancreatic complications.

**Aim:** To conduct a prospective study in acute pancreatitis to assess the relation between hyperlipidaemia and disease outcome using current disease descriptors.

**Methods:** Forty-three patients with acute pancreatitis admitted during the calendar year 2001 constitute the study population. There were 19 (44%) males. The median (range) age was 50 (21–86) years. Serum triglycerides, cholesterol and high-density lipids were measured on admission. Patients were followed until at least 6 months after discharge. Principal outcomes were: relation between hyperlipidaemia and peri-pancreatic complications and end-of-episode disease severity.

**Results:** Hypertriglyceridaemia was present in 14 patients (33%). There was a significant difference in mean (s.e.m.) serum triglyceride levels between patients with alcohol-induced pancreatitis compared to pancreatitis of other aetiologies [3.07 (1.0) mmol/L vs. 1.26 (0.11) mmol/L; P = 0.03, Fisher’s]. There was an inverse relationship between serum triglyceride and serum amylase (r² = 0.06). There was also an inverse relationship between serum triglyceride level and delay to sampling (r² = 0.07). There was no correlation between admission hypertriglyceridaemia and admission APACHE II score (r² = 0.0015). Similarly, there was no correlation between triglyceride level and either pancreatic inflammatory complications or final outcome.

**Conclusion:** There was no apparent correlation between hypertriglyceridaemia and either complications of disease or overall end-of-episode severity in this population of patients with acute pancreatitis.

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**83 Improvement in the Management of Patients Sustaining Severe Acute Pancreatitis**


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**Background:** As defined by Atlanta criteria, severe acute pancreatitis (SAP) is present in up to 25% of the patients with acute pancreatitis, with considerable mortality. The aim of this study is to compare the mortality and morbidity between patients managed by a prospective and specific protocol with others previously treated without this regime.

**Patients and Methods:** All patients with severe acute pancreatitis from 1999 to February 2004 admitted at Santa Casa School of Medicine in Sao Paulo, Brazil were included in this study. Atlanta criteria were used to assess the pancreatitis severity. We compared the results of a retrospective review from 1999 to 2002 (Group A) with a prospective protocol, started in August 2003 (Group B). APACHE II > 8 was the indication for computed tomography. Antibiotics were administered if any local complication or organ failure were present. Chi square, Fisher and Student’s t-tests were used for statistical analysis, considering p < 0.05 as significant.

**Results:** In group A, 395 patients had acute pancreatitis and 24 (6%) sustained SAP, which was observed in 16 out of 47 patients (36%) in group B (p < 0.001). Considering only the patients with SAP the mean age was 49 ± 17 y.o. in group A and 60.1 ± 15.8 y.o. in group B (p = 0.044). The mean APACHE II in groups A and B were, respectively, 10.7 ± 3.5 and 10 ± 4.6 (p = 0.59). Mortality in the first group reached 45.8%, whereas only 12.5% of the patients in the second group died (p = 0.04).

**Conclusions:** A prospective and specific protocol improved the care of patients sustaining severe acute pancreatitis. Due to a more careful evaluation, patients sustaining severe pancreatitis could be identified earlier, allowing the specific care to be applied in time.

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**84 Influence of Octreotide on Lipid Peroxidation in Acute Necrotizing Pancreatitis**

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**Background:** Oxidative stress has been involved in acute necrotizing pancreatitis (ANP). The present study was performed to investigate the effect of octreotide on lipid peroxidation (LP).

**Patients and Methods:** The indices of lipid peroxidation were determined at hemolysates of patients with ANP on the 1st and 10th day of admission: malondialdehyde (MDA) – according to thioharburic acid reactive substances content; superoxide dismutase (SOD) activity and catalase – by spectrophotometry. 53 patients with ANP (male – 33, female – 20) were divided into 2 groups: at the 1st group (35 patients) we used protease inhibitors (contrycal), at the 2nd group (18 patients) – protease inhibitors + octreotide (0.01 mg subcutaneously 3 times a day during 5 days). The indices obtained (MDA, SOD, catalase) were compared with those of healthy control.

**Results:** On the 10th day at the 1st group we observed a significant increase of MDA concentration up 1.30 ± 0.060 as in comparison with an initial level 1.08 ± 0.031 and in compared to the control level 1.01 ± 0.031 μmol/mgHb. The catalase activity was significantly reduced on the 10th day from 71.5 ± 2.41 up 63.8 ± 1.09 μmol/mgHb*ml and was lower (p < 0.05) than during the control 73.8 ± 2.0. We did not observe the change of SOD activity neither at the observation’s dynamic (4.9 ± 0.20 and 5.0 ± 0.25 U/mgHb on the 1st and 10th day respectively), nor compared to the control 4.4 ± 0.020. The octreotide application was accompanied by MDA reduction from 1.97 ± 0.242 on the 1st day up to 1.11 ± 0.055 μmol/mgHb on the 10th day. At the same time the antioxidative enzyme activity is increased – SOD from 5.0 ± 0.28 up 5.7 ± 0.33 U/mgHb and catalase from 65.0 ± 2.52 up 77.7 ± 3.59 U/mgHb*ml (on the 1st and 10th day respectively), their value exceeded the control index by the 10th day.

**Conclusion:** The therapeutic effects of octreotide are partly caused by a reduction of lipid peroxidation and enhancement of the activity of antioxidative enzymes in ANP.
Interleukin 18 and Soluble ICAM-1 in Clinical Course of Severe Pancreatitis

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Migration leukocytes to the site of inflammation one of the initial steps in the development of acute pancreatitis. Frequent observations have confirmed that cytokines are markers of severity and major mediators involved in the pathogenesis of acute pancreatitis. One of the most important target of cytokine action are the blood vessels, which undergo some structural and functional changes that results in activation of endothelium. Activated endothelium expresses several adhesion receptors, which control the leukocyte recruitment at the inflamed zone. The goal of this study was to examine the relationship between IL-18 and soluble ICAM-1 (sICAM-1) in acute pancreatitis.

Applying the ELISA technique, plasma levels of IL-18 and sICAM-1 were studied in 27 patients with acute pancreatitis. According the Atlanta criterion the mild pancreatitis was established in 13 patients and severe – in 14 patients. All patients have the alcoholic pancreatitis and admitted in clinic not later 48 hours after disease onset.

The highest levels of IL-18 and sICAM-1 were observed in patients with necrotising pancreatitis. During first week the levels of IL-18 gradually increased in patients with severe pancreatitis, while in patients with the edematous pancreatitis it's levels decreased starting from the third day. sICAM-1 levels gradually increased during first three day with the following decrease after this term. The clear correlation between IL-18 and sICAM-1 was noted in both groups of patients. Besides that, the clear strong correlation was observed between IL-18 and quantity of circulating granulocytes in patients with necrotising pancreatitis.

Our study confirms the importance of activation of endothelium as a part of the systemic inflammatory response in patients with acute pancreatitis.

Is there an Association Between Cholecystokinin and Recurrent Acute Alcohol Induced Pancreatitis?

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Background: After the first acute alcohol induced pancreatitis (AAIP) episode less than half of the patients will have a recurrent attack even though two thirds continue alcohol consumption. The pathophysiology of these recurrent attacks is unclear. Hyperstimulation of the pancreas with cholecystokinin (CCK) induces acute pancreatitis in rodents. In post-ERCP-pancreatitis patient's plasma (P-) CCK concentration has been shown to be increased. Alcohol induces temporary stimulation of the pancreas and CCK could be a possible mediator. CCK secretion is regulated by the releasing peptides e.g. diazepam binding protein (DBI), which is secreted from the proximal small intestine. The aim of the study was to investigate whether there are changes in CCK plasma levels and DBI expression in the duodenum in patients with recurrence of the AAIP.

Patients and Methods: Study population: 43 patients (female 7, male 36); mean age 42 yrs. Study groups: A: Patients with one AAIP (n = 10), B: Patients with >3 AAIP (n = 11), C: Patients with heavy alcohol consumption, with no AAIP (n = 11) and D: Healthy controls (n = 11). Median time since last alcohol intake was 7 days. Diagnostic criteria for pancreatitis were epigastric pain, P-amylase 3 × upper normal limit and possible pancreatitis changes in imaging. Alcohol consumption was considered heavy when exceeded 20 g/day in females and 40 g/day in males of pure alcohol. CCK levels were measured by RIA. Biopsies during upper endoscopy were taken from duodenal epithelium to extract and analyze DBI mRNA by real-time PCR. Helicobacter Pylori (Hp) infection was also analysed by histology.

Results: In 40 of 43 biopsy samples DBI mRNA could be detected. There was no significant difference in CCK levels or DBI expression between the different groups. Eight patients had Hp infection. In the infected Hp patients DBI expression was reduced (p = 0.013) and alcohol consumption heavy more often (p = 0.011). Logistic regression analysis of age, amount of alcohol consumed, DBI, CCK and Hp-status with AAIP or its recurrence revealed that only the Hp-status had a tendency to be an independent factor (p = 0.079).

Conclusions: DBI expression and CCK plasma concentrations are not associated with recurrent AAIP. Hp infection was more common with heavy alcohol consumers than with the AAIP patients.

Nutritional Status and Complications of Acute Pancreatitis

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Background: In patients with severe acute pancreatitis two separate types of complication are recognized, a systemic illness characterized by varying degrees of organ failure and local pancreatic complications. The aims of the recent study were to determine the correlation between the body mass index (BMI) body fat distribution, nutritional status and the different complications in the course of acute pancreatitis.

Patients and Methods: 88 patients with acute pancreatitis were consecutively examined in a clinical study. The possible prognostic factors were evaluated for each patient (age, etiology, Ranson’s criteria etc). Laboratory examinations, ultrasonography, and computer tomography assessed the clinical progression. Severe acute pancreatitis was defined by the development of organ failure or local complications. The nutritional status was evaluated by measurement of BMI, laboratory parameters, the percentage of body fat by impedance method and by the body fat distribution measured as waist/hip ratio.
Results: The obese patients (BMI > 30 kg/m² or body fat percentage >40%) had a higher incidence of developing local complications than the non obese ones (p < 0.001). There was also statistical difference between the patients with higher waist/hip ratio, (>0.88) without extreme BMI (p < 0.001) in the incidence of local complications. There were no statistical differences in the incidence of systemic complications (p = 0.31) between the over or ideal nutritioned patients.

Conclusions: Results suggest a very strong correlation between the late severe complications of pancreatitis and BMI over 30 kg/m². The android type obesity is an independent risk factor for local complications of acute pancreatitis. However obesity did not carry an increased risk of organ failure.

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Abstracts

88 Percutaneous Drainage for Acute Fluid Collection and Pancreas Abscess in Severe Acute Necrotizing Pancreatitis

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Introduction: Percutaneous drainage is a possible therapy for the treatment of peripancreatic fluid collection and pancreatic abscess in severe acute necrotizing pancreatitis.

Patients and Methods: Authors treated 67 patients (46 male, 21 female) with severe necrotizing pancreatitis. The most common cause of the pancreatitis were alcohol abuse and gallstone disease (67.2% and 22.4%). Beside Imipenem-Cilastatin® antibiotic profilaxis, early naso-jejunal feeding was executed and a delayed operation for at least 10 days after the admission were preferred. Percutaneous drainage was used for those patients, with 2–3 cm or wider peripancreatic fluid collection (17 patients) or pancreatic abscess (6 patients).

Results: No complication were found related to this intervention. Seven patients (30.4%) were recovered without surgery after an 26.8 days average time of drainage. The remaining 16 patients underwent a late (>10 days of admission) operation. The overall mortality rate of the 67 patients was 16.4%.

Conclusions: Authors suggest the percutaneous peripancreatic drainage as a first intervention for acute fluid collection or pancreatic abscess in patients with severe pancreas necrosis. If septic symptoms or MOF develop inspite of drainage operation is mandatory. In these cases the advantage of the drainage is to avoid the early operation.

89 Polymorphic Genes of TNF-alpha, Hsp70-2 and CD14 in Patients with Acute Pancreatitis

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Aims: Pro-inflammatory cytokines and heat shock proteins play a fundamental role in the pathogenesis of acute pancreatitis. Since TNF production and the expression of heat shock protein Hsp70 in individuals has been shown to be genetically influenced, we hypothesized that the polymorphism of the TNF gene locus and of Hsp70 may influence disease susceptibility and course in acute pancreatitis. Moreover, the role of genetic polymorphism of the LPS receptor CD14 could not be excluded.

Patients and Methods: DNA from 77 patients with acute pancreatitis was used for genetic experiments. The patients were grouped according to the disease severity on the basis of the Ranson scores. The –308 polymorphism of TNF-alpha gene was determined by NcoI RFLP, the polymorphism for Hsp70-2 gene by Pst I RFLP, and the CD14-159 polymorphism by melting point analysis. DNA isolated from healthy blood donors (n= 71) served as controls.

Results: No significant difference was noted in the frequency of the TNF 2 allele, or between the groups stratified according the disease severity. The frequency of CD14-159 TT genotype only moderately was higher in patients with severe pancreatitis (p = 0.045). However, there was a significant difference in the frequency of Hsp70-2 G allele between the groups of patients with severe acute pancreatitis (Ransone score 6.2) and the normal controls (51/96 versus 48/142, p = 0.002) and especially between groups of patients with mild and severe pancreatitis with a Ranson score of 2.8 and 6.2 respectively, (11/58 versus 51/96, p < 0.001).

Conclusions: The high frequency of Hsp70-2 G allele -with a concomitant lower Hsp70 production in severe acute pancreatitis provides further evidence that genetic determination of the defence mechanisms in acute pancreatitis is rather dependent on the polymorphism of the heat shock genes than on that of the TNF-alpha or CD14 genes.

90 Relationship of Pancreatic Necrosis to Organ Failure in Patients with Severe Necrotizing Pancreatitis

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Background: Development of pancreatic necrosis and organ failure (OF) are the most important complications in the course of
severe acute pancreatitis (SAP). The relationship between the necrosis and organ failure and multiple organ failure were analyzed.

**Patients and Methods:** This prospective study included 71 patients suffered for severe necrotizing pancreatitis (SNP). The diagnosis of SAP was done by clinical and laboratory findings, contrast enhanced CT and/or intraoperative findings. Percutaneous and/or intraoperative assessed microbiological status of necrosis. The occurrence of OF was defined according to the Atlanta classification system. Multiple organ failure (MOF) was defined by the simultaneous occurrence of 3 organ failures. Surgical treatment was performed in patients with infected necrosis, whereas sterile necrosis was treated conservatively (except 6 patients with deterioration despite ICU treatment at least 3 days).

**Results:** Twenty-nine (41%) patients had infected necrosis, whereas 42 (59%) had sterile necrosis. Incidence of OF was greater in patients with infected necrosis (79% vs. 55%; p = 0.033). Distinct differences were found in prevalence of MOF in patients with infected necrosis than in those with sterile necrosis (38% vs. 17%; p = 0.043).

**Conclusions:** Bacterial infection of pancreatic necrosis has a strong impact on the occurrence of OF and MOF in patients with SNP. Organ failures increase the severity of illness and have strong impact to outcome of patients with SAP. Prevention of pancreatic infection is the major goal in the treatment of patients with necrotizing pancreatitis.

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**91 Role of Endoscopy in the Treatment of Acute Biliary Pancreatitis**

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**Introduction:** Acute biliary pancreatitis (ABP) is one of the most severe complications of the biliary stone disease. Its contemporary management is early endoscopic papillotomy followed by stone extraction if necessary, and the best conservative treatment of pancreatitis.

**Patients:** During the last five years we have treated forty-two patients (mean age: 64.5/21–96/years) with ABP. Thirty-seven patients had stones in the gallbladder, four had previous cholecystectomy and in one patient the gallbladder was stonefree. The severity of the pancreatitis was assessed by a modified Ranson score, and the majority of pts (26/42, 61.9%) had severe disease.

**Methods:** In case of suspected biliary origin of the pancreatitis we have performed duodenoscopy and if it was possible, endoscopic retrograde cholangiography and endoscopic papillotomy followed by stone extraction was done. At the end of the procedure we inserted feeding tube into the second loop of the jejunum. At the first day we have administered saline, on the second day diluted nutrient and from the third day to definite healing we applied 1 ml/kcal nutrient as continuous infusion into the jejunum.

**Results:** We performed 35 papillotomy, followed by stone extraction in 17 patients, and jejunal feeding was applied in 36 patients. The median time from the start of the symptoms to the intervention was 1 (1–21) day. The hospital stay was 11 (2–81) days in average. Two old patients died of pneumonia with healed pancreatitis and five died of complicated pancreatitis, all of them suffered from the severe form of the disease (mortality rate: 16.7%).

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**92 Role of Interleukin-18 in Metabolic Disorders in Acute Pancreatitis**

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The metabolic disorders often accompany the clinical course of acute pancreatitis. While the role of proinflammatory cytokines, such as TNF-alpha, IL-1beta, IL-6, and IL-8, in acute pancreatitis is well established, the role of IL-18 is still unclear.

Plasma levels of IL-18 were measured in 27 patients with acute pancreatitis. According the Atlanta criterion severe course was established in 14 patients and the mild pancreatitis – in 13 patients. In this study enrolled patients who admitted in clinic not later than 48 hours after disease onset. Among all patients the biliary pancreatitis was in 6 of them and the rest had an alcoholic pancreatitis.

Increased levels of IL-18 were observed in all patients already after admission. Besides that, the highest levels were noted in patients with prognostically severe acute pancreatitis (APACHE II score more than 15). Among the patients with necrotizing pancreatitis increased glucose plasma levels were noted in 7 (50%) of them. The clear correlation between IL-18 and glucose plasma levels was documented.

Increased glucose blood levels in patients with acute pancreatitis can be caused by the damage of pancreatic islet cells or by the disorders of metabolism of main energetic substrates in the liver due to its toxic or hypoxic damage. Besides that, the interferon-γ plays an important role in the death of pancreatic islet cells and IL-18 is a potent inductor of interferon-gamma expression.

Thus, the IL-18 may play an important role in the development of metabolic disturbances in acute pancreatitis.

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**93 TAP and Acute Pancreatic Damage – A Pilot Study Using a New Technique for TAP Determination**

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**Aim:** To evaluate the clinical value of a new immunoassay for serum and urine TAP determination in assessing the diagnosis and the severity of acute pancreatitis.
**Patients:** Thirty-four patients with acute pancreatitis (AP) (22 mild pancreatitis and 12 severe disease); 12 patients with non-pancreatic acute abdomen (AA), 11 healthy subjects (HS) and 16 patients who underwent therapeutic ERCP (ERCP).

**Methods:** Serum TAP (optical density, OD), amylase (reference range 64–92 IU/L) and lipase (reference range 46–67 IU/L) were determined in AP, AA, and HS at their initial observation; AP patients were also studied for six consecutive days from admission. In ERCP patients, serum TAP, amylase and lipase, as well as urine TAP and amylase (upper reference limit 460 IU/L), were determined before and 6 hours after ERCP.

**Results:** Mean ± SD serum TAP levels on admission were 0.35 ± 1.60 OD in AP patients, 0.005 ± 0.001 OD in AA patients, while HSs had no detectable serum TAP levels. ERCP patients had no detectable serum TAP levels before and 6 hours after the execution of ERCP, whereas urine TAP concentrations before the execution of the endoscopy were 1.72 ± 3.43 OD (mean ± SD) and decreased 6 hours after ERCP (mean ± SD: 0.75 ± 1.49 OD) (P = 0.249). Using a cut off range of 0.013–0.020 OD for TAP, 138–142 IU/L for amylase, 67–98 IU/L for lipase, the sensitivity and specificity of the three markers in assessing the diagnosis of AP were 23.5% and 91.7%, 94.1% and 100%, 97.1% and 100% respectively. Using a cut off range of 0.005–0.008 OD for TAP, 409–448 IU/L, for amylase, 375–406 IU/L for lipase, the sensitivity and specificity in assessing the severity of AP were 29.9% and 73.5% for TAP, 38.8% and 81.2% for amylase, 28.4% and 83.6% for lipase.

**Conclusion:** TAP is of limited value in assessing the diagnosis and the severity of acute pancreatic damage.

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**The Incidence and Effect of Complications on Outcome Following Pancreatic Necrosis**

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**Background:** Surgery for pancreatic necrosis is associated with a high morbidity and mortality. The aim of this study was to review the incidence of early and long term complications following pancreatic necrosectomy and their effect on outcome in a large contemporary series of patients.

**Method:** A retrospective case note review of all cases undergoing pancreatic necrosectomy between August 1997–December 2003 was performed. Early and long term complications were recorded. Medians (95% Confidence Intervals) are provided for continuous data. Chi squared and Fisher exact tests were performed for nominal data.

**Results:** Eighty-eight patients underwent pancreatic necrosectomy, 47 by a minimally invasive technique (MIRP). 25 (28%) died (9/47 MIRP vs. 16/41 open necrosectomy). The median age was 55.5 yrs (52–59). 81 (93%) had at least one complication. 44 (50%) patients developed had a degree of organ dysfunction. 11 (13%) patients developed gastrointestinal or pancreatic fistulae, 9 secondary haemorrhage, 11 portal or splenic vein thrombosis. Of the 63 patients who survived 19 (30%) developed new onset diabetes, 14 (22%) exocrine insufficiency, pancreatic insufficiency. 11 (17%) required readmission for further intervention. Post operative complications associated with an increased mortality included secondary fungal infection (p = 0.005), Organ dysfunction (p = 0.004) and post-operative bleeding (p = 0.01).

**Conclusions:** Almost all patients undergoing pancreatic necrosectomy develop significant early and/or late complications. Post-operative bleeding and secondary fungal infection are associated with an adverse outcome and should be treated aggressively. Long term follow up is important as 17% will require further interventions for delayed complications.

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**The Influence of Octreotide on Interleukin-1α Production in vitro**

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**Background:** Proinflammatory cytokines play an important role in progression of acute necrotizing pancreatitis (ANP). So blockade of its production can improve the course of the disease. The aim of this study was to determine if octreotide, pentoxiphylline and its combination can inhibits the production of interleukin-1α (IL-1α).

**Patients and Methods:** The blood of the patients suffering with ANP was taken before the treatment and was incubated during 48 hours at complete cultural medium RPMI-1640. Depending on chosen dose, IV series of experiment in vitro were made. The doses of the medications used at I–IV series for octreotide 20; 10; 5; 2.5 ng, for pentoxiphylline 20; 10; 5; 2.5 μg; in case of a combination of drugs we used the following combinations of octreotide and pentoxiphylline doses 20ng + 20μg; 10ng + 10μg; 5ng + 5μg; 2.5ng + 2.5μg. We took as a control the spontaneous production of IL-1α. The level of IL-1α in supernatants was determined by ELISA.

**Results:** During the application of all drugs we noted the dose-dependning reduction of IL-1α concentration at supernatants, this effect was noted at the I–III series, for pentoxiphylline at I–IV series. The concentration of IL-1α made for octreotide respectively 1,210 ± 351.7; 2,768 ± 497.3; 3,164 ± 467.5; 5,018 ± 328.8 pg/ml, for pentoxiphylline – 1,416 ± 361; 2,109 ± 220.5; 3,600 ± 643.2; 3,732 ± 664.7 pg/ml. The lowest IL-1α level was observed in the case of combination of drugs and made at I–IV series of experiment respectively 12.8 ± 0.49; 1,148 ± 143.9; 2,142 ± 320; 2,236 ± 316.2 pg/ml. The IL-10 values were significantly lower not only of its spontaneous production (5,614 ± 447.2 pg/ml) but also in comparison with isolated application of drugs at I and II series of experiment.

**Conclusions:** Octreotide seems to have a dose-dependent anti-cytokine effect with regard for IL-1α, more significant in the case of its combination with pentoxiphylline.
Inhibition of Tyrosine-Kinase Mediated Cellular Signaling by Tyrphostins AG126 and AG556 Modulates Murine Experimental Acute Pancreatitis

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Background: Exaggerated systemic inflammatory response is a hallmark of severe acute pancreatitis (AP) with elevated circulating and tissue levels of proinflammatory cytokines and chemokines. Tyrosine kinases (PTKs) play a key role in cellular signaling during the inflammatory cascade. PTK inhibitors Tyrphostin AG126 and Tyrphostin AG556 act by competitive inhibition of substrate binding to PTKs and modulate the inflammatory response in experimental inflammatory disease models. This study tests the hypothesis that tyrosine kinase inhibitors modulate the course of experimental acute pancreatitis.

Methods: In a murine model of cerulein-induced pancreatitis, tissue levels of malondialdehyde and myeloperoxidase activity, expression of the adhesion molecule ICAM-1, inducible nitric oxide synthase (iNOS), serum amylase and lipase levels and histologic evidence of pancreatic injury were measured in six groups: Sham Group (vehicle, Tyrphostin AG126 and Tyrphostin AG556), Cerulein Group. (Vehicle, Tyrphostin AG126 and Tyrphostin AG556), Cerulein Group. (Vehicle, Tyrphostin AG126 and Tyrphostin AG556) given 30 minutes before induction of pancreatitis for ‘pretreatment’ and 20 minutes after the induction for ‘posttreatment’.

Results: Intraperitoneal injection of cerulein in mice resulted in severe, acute pancreatitis characterised by oedema, tissue haemorrhage, necrosis and elevation of serum amylase and lipase along with infiltration of pancreas with neutrophils (measured as increase in malondialdehyde activity) and enhanced lipid peroxidation (increased tissue levels of malondialdehyde). Tyrphostins AG126 and AG556 ameliorated these changes in both pre-treatment and post-treatment groups. Immunohistochemical examination demonstrated a marked increase in immunoreactivity for, iNOS and upregulation of ICAM-1 expression in the pancreas of cerulein-treated mice and this was markedly reduced by Tyrphostin AG126 and Tyrphostin AG556.

Conclusion: Inhibition of tyrosine-kinase mediated intercellular signaling pathways by Tyrphostins AG126 and AG556, suppresses inflammatory response and disease severity in cerulein-induced murine experimental acute pancreatitis providing evidence for specific targeting of non-constitutively expressed inflammatory pathways.
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**Pioglitazone, a Specific Ligand of Peroxisome Proliferator-Activated Receptor-γ, Protects Pancreas Against Acute Cerulein-Induced Pancreatitis**


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Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of the nuclear hormone receptor superfamily whose activation has been linked to the antiinflammatory effects by inhibiting the induction of inflammatory response genes and NF-κB-dependent transcription. The aim of the present study was to determine the effect of pioglitazone, a specific PPARγ-ligand, on the development of acute pancreatitis and on the expression of heat shock protein-70 (HSP-70) in the pancreas.

**Methods:** Acute pancreatitis was induced in rats by cerulein infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion. Pioglitazone was administered at the dose 50 μg/kg i.g. 30 min before cerulein. Immediately after cassation of saline or infusion.

**Results:** Treatment with pioglitazone markedly attenuated the pancreatic tissue damage in cerulein induced pancreatitis as demonstrated by the improvement of pancreas histology, the reduction in plasma lipase activity (288.3 ± 18 vs. 400.3 ± 10.6) and plasma concentration of pro-inflammatory IL-1β (131.3 ± 7.2 vs. 212 ± 8.6 pg/ml). Moreover, administration of pioglitazone reduced the gene expression of IL-1β in the pancreas and attenuated the pancreatitis-evoked fall in pancreatic blood flow. Cerulein-induced pancreatitis increased pancreatic HSP-70 mRNA and protein expression in the pancreas and this effect was enhanced by pioglitazone treatment.

**Conclusions:** Pioglitazone attenuates the cerulein-induced pancreatitis. The beneficial effect of this pioglitazone is multifactorial and is due, at least in part, to its anti-inflammatory activities, to the suppression of IL-1β and finally to the overexpression of HSP-70. PPARγ-ligands could represent a new therapeutic option in the treatment of acute pancreatitis.

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**Targeting Neutrophil-Elastase in Severe Experimental Acute Pancreatitis: A Potential Therapeutic Approach**


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Activated neutrophils are important mediators of local and systemic complications in acute pancreatitis (AP) by releasing various deleterious proteases. In this context, neutrophil-elastase, a serine protease, is an established biochemical marker of severity in AP. However, little is known about the pathophysiological role of this protease within the course of the disease.

**Materials and Methods:** Severe acute pancreatitis (SAP) was induced by retrograde infusion of 4% sodium taurocholate in male Wistar rats. A new synthetic inhibitor of neutrophil elastase (NE) was intravenously (i.v.) administered at the time of SAP induction and continued in 12 hourly intervals (SAP-NE, n = 42), nontreated control rats (SAP-S, n = 55) received saline. After observation periods of 6 h, 12 h, 24 h and 3 days the following parameters were assessed: intrapancreatic extent of necrosis, leukocyte infiltration and myeloperoxidase (MPO) activity, systemic hemococoncentration, differential blood count and survival.

**Results:** Treatment with NE inhibitor significantly decreased the progression of intrapancreatic necrosis and inflammatory infiltrate (p < 0.05). A marked reduction of pancreatic and pulmonary MPO-activity was observed in NE inhibitor treated animals compared with nontreated controls (p < 0.05). Moreover, following NE inhibitor administration both ascites formation and hemococoncentration were less expressed (p < 0.02). Mortality rate was significantly improved from 59.1% (13/22) in the SAP-S to 8.3% (1/12) in the SAP-NE group (p < 0.009).

**Conclusions:** Neutrophil-elastase is an important mediator of local and systemic complications as well as mortality in AP. Our results suggest that targeting this protease could serve as a potential therapeutic approach in SAP.

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**Inhibition of both TNF-α Receptor 1 (TNF-R1) Ectodomain Shedding and Nuclear Factor kappa B (NF-κB) Activation Unveils the Pro-Apoptotic Effect of TNF-α in Pancreatic Stellate Cells**

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Tumor necrosis factor α (TNF-α) can activate both survival and death signaling pathways. Proteolytic cleavage of the extracellular region of TNF-R1 (ectodomain shedding) and activation of NF-κB regulate TNF-α responses. In pancreatic stellate cells (PSC) TNF-α is a pro-survival mediator. Since PSC apoptosis is essential for effective pancreatic repair, our aim was to investigate putative apoptotic TNF-α dependent pathways in PSC.

**Methods:** Rat PSC were incubated for 24 h either with TNF-R1 shedding inhibitors (1,10-phenanthroline, N-acetyl cysteine – NAC) or NF-κB inhibitors (MG-132, Bay 11-7082, and gliotoxin). Apoptosis was determined by measuring caspase-3 and -8 activities by fluorogenic assay and DNA fragmentation by ELISA. TNF-R1 shedding was assessed by measuring soluble TNF-R1 in cell supernatants by ELISA and cell surface TNF-R1 expression by flow cytometry.
Results: Exposure of cells to both 1,10-phenanthroline and NAC effectively down-regulated TNF-R1 shedding, as indicated by a significant reduction of TNF-R1 on supernatants (0.4 ± 0.06 and 0.4 ± 0.07 fold vs. control, respectively) and increased TNF-R1 cell expression (1.6 ± 0.2 and 1.6 ± 0.2 fold increase vs. control, respectively). Such effect correlated with a substantial caspase-3 activity increase (2.9 ± 0.7 and 2.7 ± 0.3 fold vs. control, respectively) and DNA fragmentation (7.2 ± 1.6 and 2.7 ± 0.1 vs. control, respectively). Neutralizing anti-TNF-α antibody partially reverted caspase-3 activation and DNA fragmentation induced by 1,10-phenanthroline, what links TNF-R1 shedding to the apoptotic action of TNF-α. NF-κB inhibitors increased more than 10 times both caspase-3 activity and DNA fragmentation. No apoptosis occurred after recombinant TNF-α exposure, but, upon pretreatment with NF-κB inhibitors, TNF-α potently enhanced (more than 20 times) caspase-3 and -8 activities as well as DNA fragmentation, suggesting that NF-κB might preclude pro-apoptotic TNF-α signaling pathways in PSC.

Conclusion: TNF-α dependent apoptosis in PSC can be unmasked by inhibiting TNF-R1 ectodomain shedding and NF-κB activation.

Model of Chronic Alcoholic Pancreatitis

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Background and Aims: Although alcohol abuse is the major cause of chronic pancreatitis, the pathogenesis of this disorder remains elusive. A critical obstacle to understanding of alcoholic pancreatitis is lack of animal models. We sought to develop one such model.

Methods: Rats were pair-fed for 8 weeks control or Lieber-DeCarli ethanol diet. For the last 2 weeks, they received cyclosporin A (CsA) or vehicle. 1 week after start of CsA, rats were subjected to 1 episode of acute cerulein pancreatitis. Animals were killed 3h or 2, 4 (CsA) or vehicle. 1 week after start of CsA, rats were subjected to an episode of acute cerulein pancreatitis. Animals were killed 3h or 2, 4, and 7 days after cerulein treatment.

Results: Neither ethanol alone nor treatments of control-fed rats produced post-acute pancreatic injury. However, the combination of ethanol diet plus cerulein and CsA treatments resulted in severe pancreatic injury characterized by loss of parenchyma, widespread fibrosis, and massive inflammatory infiltration. Thus, ethanol sensitized pancreas to responses of chronic pancreatitis. Time-course analysis showed that ethanol-induced changes developed post-acute. Inflammatory infiltration (with prominent macrophage presence) persisted in ethanol-fed rats while subsiding within 7 days after cerulein pancreatitis in control-fed rats. Pancreatic NF-κB activation was similarly sustained. We found a marked increase in total collagen, fibronectin and TGF-b1, as well as proliferation and activation of pancreatic stellate cells. Microarray analysis on pancreatic RNA, using genes thought to play a protective role, but it did not by itself induce gene expression changes characteristic of pancreatitis. However, in rats treated with cerulein plus CsA ethanol induced dramatic changes in pancreatic gene expression.

Conclusions: We have developed a model of alcohol mediated post-acute pancreatitis induced by synergistic effect of ethanol diet, acute (cerulein-induced) injury, and CsA treatment. The model displays all 3 responses characteristic of the human disease, thus allowing investigations

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A Novel Cell-Permeable Peptide Attenuates the Severity of Cholecystokinin-Induced Acute Pancreatitis

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Background: A previous finding demonstrated that a cell-permeable peptide containing the nuclear localization sequence of the p50-NF-κB subunit of NF-κB inhibits the nuclear import of NF-κB and suppresses systemic inflammatory responses in vivo. To enhance the efficacy of the substance, we replaced the cell permeable motif with the more efficient cell transporter penetratin. In vitro Luciferase reporter gene assay with pNF-κB-Luc (in LPS stimulated RAW 264.7 macrophages and TNF stimulated L929 fibroblasts) revealed that our newly designed peptide named PN50 could suppress transcription activity of NF-κB.

Aim: To assess the effect of the NF-κB inhibitory peptide PN50 in an experimental model of acute pancreatitis (AP).

Methods: Pancreatitis was induced in male Wistar rats by administering 2 x 100 μg/kg of cholecystokinin-octapeptide (CCK) intraperitoneally at an interval of 1 h. The animals were sacrificed 4h after the first injection of CCK.

Results: Both pre- and after treatment with PN50 (200 μg/animal ip 30 min before or after the first dose of CCK) ameliorated many of the examined laboratory (the pancreatic weight/body weight ratio, the serum amylase activity, the pancreatic levels of TNF-α, and IL-6, the degree of lipid peroxidation, the pancreatic and lung myeloperoxidase activity) and morphological parameters of the disease. According to the histologic findings, treatment with PN50 protected the animals against AP by favoring induction of apoptotic, as opposed to the necrotic acinar cell death associated with severe AP.

Conclusion: Our study implies that reversible inhibitors of the nuclear import of stress-responsive transcription factors like NF-κB might be clinically useful for the suppression of severity of AP.
Alcohol Sensitizes the Rat Pancreas to Endotoxin and Exacerbates Pancreatic Injury in a Dose-Dependent Fashion

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Introduction: Chronic alcohol consumption increases susceptibility to development of acute and chronic pancreatitis, and also alters gut permeability, resulting in variable levels of endotoxia. We hypothesize that alcohol increases pancreatic susceptibility to endotoxin-related immune-mediated pancreatic damage in a dose-dependent manner. The aims was to determine if increasing LPS concentrations exacerbate pancreatic injury in alcohol-fed rats as compared to control rats, and whether further injury is associated with apoptosis or necrosis.

Methods: Rats were fed a liquid diet (Lieber-DeCarli) containing either ethanol or maltose-dextrin in isocaloric amounts for 14 weeks and killed 24 h after endotoxin (LPS) injection (0.2, 0.8, 1.5 and 3 mg/kg bw). Pancreatic injury was assessed after tissue H&E staining by scoring parenchymal edema, acini necrosis, vacuolization, and inflammatory cell infiltration. Activities of caspase-3 (apoptosis executor), caspase-8 (receptor initiator) and caspase-9 (mitochondrial initiator) were investigated.

Results: Alcohol alone had minimal effect on pancreatic histology. Pancreatic injury increased after LPS injection in control and alcohol-fed rats in a dose-dependent fashion. The injury in alcohol-fed rats was two-fold greater than in controls. LPS induced more severe necrosis and vacuolization in pancreatic acini in alcohol-fed as compared to pair-fed control at the highest LPS dose. Alcohol alone reduced caspase-3 (~2.9-fold) and caspase-9 (~6.7-fold) activities, while caspase-8 activity was not altered, suggesting an inappropriate deactivation of the mitochondrial apoptotic pathway by alcohol. In control rats 0.8 mg/kg LPS injection markedly increased caspase-3 (+14-fold), caspase-8 (+10-fold) and caspase-9 (+31-fold) activities, with no further increase at 1.5 or 3 mg/kg LPS. Alcohol plus 0.8 to 3 mg/kg LPS resulted in a reduction of all three caspase activities.

Conclusions: LPS increases pancreatic injury in a dose-dependent fashion. Alcohol inhibits the mitochondrial-associated apoptosis pathway at caspase-9 and the down-stream executor caspase-3, while the receptor-mediated apoptotic pathway at caspase-8 was not affected. LPS causes dose-dependent pancreatic injury that is potentiated in alcohol-fed rats. In the presence of alcohol, the induction of LPS-induced apoptosis is blunted, suggesting that the alcoholic pancreas cannot eliminate injured acinar cells and thus may sustain greater injury.

Detailed Characterization of Complement Activation in Experimental Edematous and Necrotizing Pancreatitis: Implications for the Clinical Setting

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Background: In acute pancreatitis, local as well as systemic organ complications are mediated by the activation of various inflammatory cascades. The aim of this study was to characterize the activation of complement components in the course of acute pancreatitis.

Methods: Edematous pancreatitis was induced in male Wistar rats by intravenous infusion of cerulein (5 μL/kg/h). Necrotizing pancreatitis was induced by the combination of intravenous cerulein and retrograde infusion of glycodeloxycholic acid (GDOC 10 mM/L, volume-, time-, and pressure-controlled) into the biliopancreatic duct. Ringer-solution was infused into controls. C3a levels in plasma collected at baseline, 0.5, 1, 2, 3, and 6 hours after start of infusions were measured by ELISA (BMA Biomedical, Switzerland). Likewise, hemolytic activity of plasma (CH50) was measured by complement-dependent lysis of sheep erythrocytes. The severity of acute pancreatitis was determined by wet-to-dry weight ratio, myeloperoxidase activity, and histology of the pancreas.

Results: Only minor complement activation was found in edematous pancreatitis, with no significant difference compared to control animals infused with saline only. In necrotizing pancreatitis, C3a levels were significantly increased whereas CH50 levels were decreased, reflecting major complement activation and consumption, respectively. Complement activation occurred as early as 30 minutes after induction of necrotizing pancreatitis. Cerulein-pancreatitis was characterized by significant edema and moderate leukocyte infiltration in the pancreas, whereas GDOC-pancreatitis showed moderate edema, major leukocyte infiltration, and severe necrosis. Regular morphology of the pancreas was found in control animals.

Conclusions: Complement activation occurs very early in the course of necrotizing pancreatitis and correlates with the severity of the disease. However, because of its activation right after induction of pancreatitis, complement inhibition may not be a valuable option for delayed treatment of severe pancreatitis in the clinical setting.
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Development of a Novel Anti-TAP Cross-Species Reactive Immunoassay for the Detection of Acute Pancreatitis

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The pathological activation of trypsin activating peptide (TAP) within the pancreas constitutes a critical step in the initiation of acute pancreatitis. We describe the generation of a new anti-TAP antibody which is reactive across animal species (rat, mouse, dog, monkey, man) and has a very high affinity for its antigen. The 8-aminoo-acids TAP antigen FPLEDSDK was synthesized and used for covalent coupling to keyhole limpet hemocyanin (KLH) as a carrier protein via an added C-terminal cysteine residue appended after a small glycine spacer (FPLEDSDKGC). The molecular weight of the derived immunogen is equal to 1,195 Da. Three rabbits were immunized following the procedure for the generation of anti-idiotypic antibodies to enhance immunogenicity of the peptide. The rabbits generated a potent humoral response which proved specific to the peptide in a quantitative ELISA. The developed antibodies were purified using an affinity column where the TAP peptide had been immobilized. Yields of the purified antibodies varied between 23 to 88% of bound and recoverable antibodies. The antibodies were further characterized for their potency, specificity and functional dynamic range of TAP detection using diverse immunoassays such as Western or Dot-blotting and direct or competitive ELISAs. When comparing the performance of our best antibody (563) to a commercially available one (Biotrin) in a competitive immunoassay format, we found that our assay provides a similar dynamic range but better sensitivity at lower TAP concentrations. Developing reverse phase protein arrays from the plasma of treated animals, we show that the assay can be easily miniaturized and requires as little as few microlitres of samples. The antibody can be efficiently used to identify and quantify the levels of TAP in body fluids, both in experimental animals as well as in clinical samples from human donors.

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Development of a Transgenic Model of Pancreatitis

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Pancreatitis remains a common condition with mortality of 6–10%. Understanding of the molecular events underlying pancreatitis is still poor and there have been no significant breakthroughs in management. Nevertheless, new insights into the pathogenesis of pancreatitis have followed the discovery of mutations in the cationic trypsinogen gene PRSS1, associated with Hereditary Pancreatitis (HP). HP is characterised by recurrent attacks of acute pancreatitis, leading to chronic pancreatitis and with a 40-fold increased risk of pancreatic cancer. Therefore HP can be used as a model to study both pancreatitis and pancreatic cancer.

The two most common mutations of PRSS1 are R122H and N29I. These are thought to result in trypsin resistant to inactivation, but the mechanisms leading to pancreatic autodigestion and pancreatitis are not known and there are conflicting theories. As pancreatitis arises in the intact pancreas it cannot be studied effectively without a relevant animal model.

To generate a tissue specific, inducible transgenic model for pancreatitis we have chosen the Tet-On system. Wild-type PRSS1 cDNA was generated from normal pancreatic RNA and the mutants R122H and N29I were created by PCR mutagenesis. These have been cloned into a vector with a bi-directional tet-responsive promoter enabling monitoring of tet induced transgene expression with a Lac Z reporter.

In addition we have also cloned wt and mutant PRSS1 into a CMV driven vector to study the enzyme activity and stability encoded by these forms of the gene, using fluorescent substrates in transiently transfected rodent acinar cell lines.

By combining cellular and molecular studies of the intrinsic properties of PRSS1 and its mutants with the more sophisticated transgenic animals we are currently creating, we will gain a better understanding of the molecular events involved in pancreatitis, hopefully resulting in improvements in the management of this disease.

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Does Endothelin-1 Receptors Antagonists Affect the Trypsinogen Activation, Monocyte Chemotactic Protein-1 and Interleukin-6 Level in Early Caerulein Acute Pancreatitis in Rats?

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Some beneficial or undesired effects of endothelin-1 receptors (ET R) antagonists were found in experimental acute pancreatitis (AP). The role of monocyte chemotactic protein-1 (MCP-1) in AP remains unclear.

Aim: The aim of the study was to assess the effect of LU-302146 (selective ET R1) and LU-302872 (non-selective ET R1,3) antagonist on trypsinogen activation, MCP-1 and IL-6 levels in early caerulein-induced AP in rats.

Methods: In 39 male Wistar rats, AP was induced by two injections of caerulein (40 μg/kg i.p.) in 1 h interval; 29 were treated with each antagonist, 10 or 20 mg/kg i.p. Six healthy rats receiving only vehicle were control (C). Three hours later, pancreases were excised and homogenised. In 12,000 X G supernatants, free active trypsin (FAT), total potential trypsin after activation with enterokinase (TPT) and lipase were assayed. %FAT/TPT was an index of trypsin activation, MCP-1 and IL-6 levels in early caerulein-induced AP in rats.

Results: FAT increased to 1.253 ± 0.112 μg/mg protein and %FAT/TPT to 22.4 ± 5.0 in untreated AP vs 0.366 ± 0.083 (p < 0.001) and 3.0 ± 0.6 (p < 0.01) in C. Lipase increased to 6.67 ± 0.59 U/mg protein (p < 0.05) and α-amylase to 28.2 ± 4.3 U/mL (p < 0.001) in the intact pancreas it cannot be studied effectively without a relevant animal model.

To generate a tissue specific, inducible transgenic model for pancreatitis we have chosen the Tet-On system. Wild-type PRSS1 cDNA was generated from normal pancreatic RNA and the mutants R122H and N29I were created by PCR mutagenesis. These have been cloned into a vector with a bi-directional tet-responsive promoter enabling monitoring of tet induced transgene expression with a Lac Z reporter.

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By combining cellular and molecular studies of the intrinsic properties of PRSS1 and its mutants with the more sophisticated transgenic animals we are currently creating, we will gain a better understanding of the molecular events involved in pancreatitis, hopefully resulting in improvements in the management of this disease.

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Effects of Antioxidants and ICI 192605 (Thromboxane A2 Receptor Antagonist) on Pancreatitis-Induced Renal Dysfunction  

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Impairment of renal function may occur during acute pancreatitis and may herald the onset of multiorgan failure. No specific therapy has been shown to prevent renal dysfunction other than hemodynamic assistance. Acute pancreatitis induces oxidative stress that generates bioactive molecules that may induce renal vasoconstriction acting through thromboxane-like receptors.

**Aim:** To evaluate the effects of antioxidants and thromboxane A2 receptor antagonists on pancreatitis-induced renal dysfunction.

**Methods:** Edematous pancreatitis (EP) was induced in rats by cerulein hyperstimulation (40 μg/kg i.p. × 4) and necrotizing pancreatitis (NP) by intraductal injection of taurocholate (3.5%, 0.4ml). Renal function (creatinine clearance and fractional sodium excretion) was evaluated at 36h (EP) or at 72h (NP) from pancreatitis induction. Separate groups of rats with either condition (EP or NP) received either daily combined doses of antioxidants (N-acetylcysteine 200mg/kg/d + lipoic acid 10mg/kg/d + trolox 2.5mg/kg/d, i.p.) from 72h before induction of pancreatitis, or the thromboxane A2 receptor antagonist ICI 192605 (4mg/kg/12h, i.p.). Blood and 24h urine was collected before sacrifice.

**Results:** Plasma TBARS raised after pancreatitis induction (EP: 55 ± 25μM; NP: 64 ± 22; controls: 5 ± 2μM) whereas creatinine clearance (EP: 0.39 ± 0.1; NP: 0.18 ± 0.1 ml/min) and fractional sodium excretion (EP: 0.3 ± 0.1; NP: 0.19 ± 0.03%) decreased in comparison with controls (0.7 ± 0.1 and 0.8 ± 0.1; p < 0.01). Administration of antioxidants significantly reduced TBARS and improved creatinine clearance (EP: 0.8 ± 0.1; NP: 0.5 ± 0.1) and fractional sodium excretion (EP: 0.6 ± 0.1; NP: 0.42 ± 0.05; p < 0.01). The thromboxane A2 antagonist ICI 192605 had no effect on plasma TBARS, it improved creatinine clearance (EP: 0.52 ± 0.06; NP: 0.52 ± 0.06; p < 0.01) but it failed to ameliorate fractional sodium excretion (EP: 0.12 ± 0.01 and NP: 0.22 ± 0.05 respectively).

**Conclusion:** Antioxidants significantly improve pancreatitis-induced renal dysfunction.

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Endoscopic versus Surgical Therapy of Chronic Pancreatitis Long-Term Prospective and Randomize Study  
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**Aim of the Study:** Chronic pancreatitis is a disease with negative impact on the live, it is a risk factor for the pancreatic cancer and disease with irreversible damage of pancreatic tissue.

The aim of our study was to compare the long-term therapeutic effect of endoscopic and surgical therapy.

**Materials and Methods:** Totally 140 pts were treated, 64 endoscopically and 76 surgically, randomization was performed by protocol in 35 pts treated endoscopically and in 36 pts treated surgically. All procedures were performed by 2 endoscopists and 1 surgeon respectively. Inclusion and exclusion criteria were used. Patients were controlled by 1, 3 and 5 years after the therapy and changes of body weight and pain relief were evaluated.

**Results:** 48.3% pts were treated by endoscopic papillosphincterotomy, 39.2% by papillosphincterotomy and pancreatic duct drainage and in 12.5% pts stents to both ducts were inserted after papilotomy. Surgeon performed in 43.4% duodenum preserving pancreatic head resection, hemipancreato-duodenectomy in 30.3%, distal pancreatectomy in 6.6% and Partington-Rochelle anastomosis in 19.7%. Total complications rate was in both methods similar 7.0% and 10.5% respectively. Surgical complications were more severe. In randomized groups 5 years follow-up results: complet pain absence and increase of body weight were more significant in the group treated surgically (p = 0.002).

**Conclusions:** Endoscopic therapy of painful chronic pancreatitis is effective and safe method, but in our study long term results were better after surgical procedure. Complications in both group were similar, surgical complications were more severe.
(pancreatico-bile duct). This study aimed to examine the surface disturbances of pancreatic duct cells under conditions of oxidative stress induced acute pancreatitis (AP). We have chosen an electron spin resonance – using specific labels to analyse this surface structure disturbances under hydroperoxide – generated oxidative stress and influence of oxidants on those processes.

**Methods:** Oxidative stress was induced by retrograde injections of 150 micromoles of tert-butyl hydroperoxide (ButOOH) into PBD. Experiments were performed on male Wistar rats under anaesthesia with Ketamin and Xylosin. The duodenal loop was identified and the catheter (venocath 17) was inserted into the common PBD. The stibeline derivatives (resveratrol and diethylstibostrol) were dissolved in 200 microl of ethanol/0.9% NaCl mixture and administered intraperitoneally in the dose 2 mg per animal. Physiological saline – 500 microl during 15 minutes were administered by infusion pump into CPBD. 15 minutes after ButOOH (or 0.9% NaCl solution) administration CPBD was washed with physiological saline and then 300 microl of 1 mM 5-doxyl-stearate solution was given during next 5 minutes.

**Results:** Three ESR parameters namely S (order parameter) and rotational correlation time TauB and TauC were estimated. S parameter has been found to decrease from 0.6633 to 0.6392 after ButOOH infusion TauB has been found to decrease dramatically from initial 4.4 × 10⁻⁹ s as a result of ButOOH triggered oxidative stress. Antioxidants prevented charges in order parameters as well as mobility of spin probe in pancreatic duct cell membrane.

**Conclusion:** Our results strongly confirmed the crucial role of free radical reaction in initial phase of acute pancreatitis.

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**Influence of Ischemic Preconditioning on Pancreatic Regeneration and Pancreatic Presence of FGF-2, PDGF-A and VEGF in the Course of Ischemia/Reperfusion-Induced Pancreatitis**


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In various organs preconditioning by brief exposure to ischemia protects the organ against damage evoked by subsequent severe ischemia. The aim of our study was to evaluate the influence of ischemic preconditioning (IP) on the development of ischemia/reperfusion-induced pancreatitis (I/R) and pancreatic regeneration, as well as on the pancreatic presence of FGF-2, PDGF-A and VEGF.

**Methods:** In male Wistar rats, IP of the pancreas was performed by short-term clamping of celiac artery (2 × 5 min). I/R was induced by clamping of inferior splenic artery for 30 min followed by reperfusion. Rats were sacrificed 1, 5, 12h or 1, 2, 3, 5, 7, 9 and 21 days after the start of reperfusion. Biochemical and morphological evaluation of severity of pancreatitis and immunostaining for growth factors were determined.

**Results:** Exposure to regular 30-min pancreatic ischemia followed by reperfusion led to the development of acute necrotizing pancreatitis. In I/R-induced pancreatitis, the pancreatic damage reached the maximal value between the first and second day of reperfusion with subsequent regeneration. IP applied prior to I/R caused the reduction in pancreatic damage and accelerated pancreatic regeneration. It was manifested by the reduction in plasma lipase, plasma IL-1ß and histological signs of pancreatic damage, as well as attenuation of I/R-induced reduction in pancreatic blood flow and pancreatic DNA synthesis. Exposure to IP before I/R caused an increased the lobular presence of FGF-2 from 5–12 h and 5–7 day of reperfusion, PDGF-A from 3–5 day of reperfusion and VEGF from 3–7 day of reperfusion.

**Conclusions:** IP reduces pancreatic damage and accelerates pancreatic healing in the course of I/R-induced pancreatitis. This effect is accompanied by an increase in the pancreatic lobular presence of FGF-2, VEGF and PDGF-A in early stage of pancreatic regeneration suggesting the involvement of these factors in healing-promoting effects of IP.

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**Inhibition of Nitric Oxide Synthesis Enhances Oxidatively-Modified Proteins in Rat Pancreatic Lysosomes during Experimental Acute Pancreatitis Development**

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Oxidative damage to proteins has been described as an early event in the development of experimental acute pancreatitis (AP). In order to identify targets of protein oxidation, the purpose of our study was to analyse the pattern of oxidatively-modified proteins (OMP) in pancreatic lysosomes during the development of AP and to investigate the effect of nitric oxide (NO) synthesis inhibition on such proteins. Wistar rats were treated with two or four hourly subcutaneous injections of caerulein (20 mg/body weight, pancreatic rats), equivalent volumes of saline (controls), the NO synthase inhibitor L-NAME + caerulein (L-NAME rats) or L-NAME + saline. 2, 5 or 9 hours after the first injection, animals were killed, the pancreas removed and a lysosomal mitochondrial (L + M) fraction obtained by subcellular fractionation. Protein oxidation patterns were visualised by blotting, film images analysed and data referred to the whole pancreas. Percentages of total OMP located in L + M fraction (considering the total amount of OMP in the pancreas as 100%) increased after longer periods of induction of AP (i.e. they change from 5% in control to 10% in pancreatic rats, after 9h). Nevertheless, OMP formation had already started at 2 h of first injection of caerulein, because the amount of OMP in L + M fraction at this time was increased by 1.8 times over control. L-NAME administration produced an increase in OMP (2.2 times over control, at 9h) by itself and potentiated the effect of caerulein on this parameter (3.4 times over control in...
L-NAME rats). Certain protein bands in blotts were also studied. Our data indicate that during acute pancreatitis, an accumulation of OMP in lysosomes takes place, which is greater when nitric oxide synthesis is inhibited. Changes observed in OMP suggest variations in formation and degradation of oxidized proteins during AP development and a modulatory effect of NO on such processes.

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Introduction: Hydroperoxides (ROOH) precursors of free radicals display transient increase during cerulein induced acute pancreatitis. ROOH have been found to induce ATP depletion in isolated normoxic cells initiating apoptotic or necrotic way of their elimination. We synthesized a new kind of aminoxy sensing ATP binding site in AMP-deaminase. The aim of present study is to prove if new aromatic aminoxy can prevent both lipid peroxidation and ATP pool depleting activity of AMP-deaminase.

Methods: AMP-deaminase from rat pancreas was purified by affinity chromatography using cellulose phosphate column. AMP-deaminase activity was measured by the phenol-hyposulfite method of Chaney and Marbach. Pancreatic endoplasmatic reticulum membranes were obtained after 1 hour centrifugation of postmitochondrial supernatant of pancreatic tissue homogenate at 100,000 × G.

Results: Carboxy QAL analog: 1,2 dihydro-2,2-diphenyl-4-carboxy-quinoline-1-oxyl (C-QAL) was found to inhibit AMP-deaminase activity in ATP countervactive way. Similar inhibitory effect was displayed by arachidonic acid creating close to ring shaped molecule while arachidonic acid methyl ester was noninhibitory in respect to AMP-deaminase activity. Close analog of C-QAL, having o-methyl group instead of carboxyl one at position 4, loose ability to inhibit the enzyme. Lipid peroxidation in pancreatic endoplasmatic reticulum membrane was strongly inhibited by C-QAL.

Conclusion: Our results provide opportunity to protect inflamed pancrea by preventing both ATP depletion and ROOH accumulation.

Pancreatitis Associated Protein (PAP) Acts as an Anti-inflammatory Protein through the Induction of SOCS-3

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Background: Pancreatitis Associated Protein (PAP) is a secreted protein expressed in pancreatic acinar cells during acute pancreatitis. We have previously reported a potential anti-inflammatory

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function for this protein. It seems to play a similar role to other anti-inflammatory cytokines (IL-6 or IL-10). These cytokines induces the expression of mediators, as Suppressor Of Cytokine Signaling (SOCS-3), that acts blocking the proinflammatory downstream signaling. Here, we have evaluated the possible involvement of SOCS-3 in the anti-inflammatory effects of PAP and the relation between PAP and the cytokine IL-10.

**Methods:** Rat pancreatic cell line AR42J was incubated with PAP (500ng/ml) or IL-10 (20ng/ml). Total RNA was obtained 15 min, 1h, 2h and 4h after treatment and the expression of PAP and SOCS-3 was examined by RT-PCR. In addition, NFκB activation was measured by immunofluorescence using specific antibodies against p65 in cells activated with TNFα.

**Results:** PAP expression was induced by IL-10 and also by PAP itself in a dose- and time-dependent manner. Maximal induction was observed 1h after treatment and maintained until 4h. In addition, TNF-induced translocation of NFκB was prevented by PAP administration. The expression of SOCS-3 was transiently induced by PAP. It achieved maximal expression 1h and then returned to the basal levels. This profile is close similar to that observed for IL-10 treatment.

**Conclusions:** PAP exerts an anti-inflammatory role in pancreatic acinar cells similar to IL-10. The induction of a common intracellular mediator (SOCS-3) could explain the similarities between PAP and IL-10.

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**116 Platelet Activation Contributes to Microcirculatory Disturbances in Acute Experimental Pancreatitis**

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**Background:** Acute pancreatitis (AP) is characterized by a disturbance of pancreatic microcirculation leading to ischemia and consequently necrosis of pancreatic tissue. Leukocyte-endothelium-interaction and erythrocyte flow pattern have been well investigated during these pathophysiological processes. In contrast, it remains unclear how platelets contribute to these perfusion disturbances.

Aim of our study was the investigation of platelet activation and function in experimental models of AP.

**Methods:** AP of graded severity was induced in rats. (1) control animals (n = 6) Ringer’s solution i.v.; (2) mild AP (n = 12) cerulein i.v.; (3) severe AP (n = 12) glycodeoxycholic acid 2.5mM intraduc-
al + cerulein i.v. 12h after induction of AP intravital microscopy was performed in 6 animals of each group after separation and staining of platelets (1 ml blood, rhodamin 6G). Platelet velocity as well as temporary and firm platelet adhesion to the endothelium was investigated in capillary fields and postcapillary venules. In addition serum thrombox-
ane B2 levels were measured. In the remaining 6 animals per group, histological damage was evaluated 24h after induction of AP.

**Results:** Histology after 24h showed a mild AP in the animals that had received cerulein. In contrast, in animals with GDOC application, inflammation and necrosis were significantly more evident (inflammation 1.3 ± 0.18 vs. 1.9 ± 0.21, necrosis 1.20 ± 0.11 vs. 1.80 ± 0.28). Intravital microscopy showed significantly more platelet-endothelium interaction in animals with AP compared to control animals (roller: control 3.2 ± 0.9, mild AP 9.2 ± 1.7*, severe AP 11.0 ± 1.8**, sticker: control 0.9 ± 0.5, mild AP 1.1 ± 0.4, severe AP 3.5 ± 0.6*). In addition, TBX-levels were significantly higher in AP animals (control 15.3 ± 10.3, mild AP 47.8 ± 12.1*, severe AP 61.9 ± 15.8* [pg/50μl]). *p < 0.05 vs. control.

**Conclusions:** Platelet activation plays an important role in the pathophysiology of acute, especially necrotizing pancreatitis. During mild AP mainly temporary platelet-endothelium interaction is observed, while severe AP is characterized by firm platelet adhesion with consecutive coagulatory activation and perfusion failure.

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**117 Protective Effect of Ischemic Preconditioning in Edematous Cerulein-Induced Pancreatitis. Involvement of Cyclooxygenases and Heat Shock Protein 70**


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Previous our study has shown that ischemic preconditioning (IP) inhibits the development of ischemia/reperfusion-induced necrotizing pancreatitis. The aim of present study was to determine whether IP affects the development of edematous cerulein-induced pancreatitis (CIP) and to determine the role of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and heat shock protein 70 (HSP-70) on this effect.

**Methods:** IP of the pancreas was performed by clamping of celiac artery (2 × 5 min with 5 min interval). Thirty minute after IP or sham-operation, acute pancreatitis was induced by caerulein. Activity of COX-1 and COX-2 were inhibited by resveratrol and rofecoxib, respectively (10mg/kg). The pancreatic blood flow (PBF), serum lipase and poly-C RNAase activity, serum interleukin-1beta; (IL-
beta) and interleukin-10 (IL-10) concentration, cell proliferation, and morphological signs of pancreatitis were examined. Production of HSP-70 was detected by Western blot.

**Results:** IP significantly reduced pancreatic damage in CIP as demonstrated by the improvement of pancreas histology, reduction in serum lipase and poly-C RNAase activity, and serum concentration of pro-inflammatory IL-1beta. Also, IP attenuated the pancreatitis-evoked fall in pancreatic blood flow and pancreatic DNA synthesis. IP did not affect serum level of anti-inflammatory IL-10. CIP and IP increased the HSP-70 content in the pancreas and maximal increase in HSP-70 was observed, when IP was combined with CIP. Resveratrol and rofecoxib given alone tended to reduce pancreatic damage in CIP, however, inhibition of cyclooxygenases, especially COX-2, in combination with IP, reduced protective effect of IP in CIP.

**Conclusions:** Ischemic preconditioning reduces pancreatic damage in cerulein-induced pancreatitis. This effect, at least in part, depends on cyclooxygenases activity and production HSP-70.
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Protective Effects of Calcitonin Gene Related Peptide (CGRP) on Acute Experimental Pancreatitis

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Introduction: Vasoactive mediators play an important role in the initiation and progression of acute pancreatitis. CGRP is stored in pancreatic perivascular nerves and is known to act as an intestinal vasodilator. In addition, it attenuates cytokine release in several inflammatory models. The role in acute pancreatitis has not been investigated. The present study evaluates the influence of CGRP on acute pancreatitis in rats.

Materials: Severe necrotizing pancreatitis was induced using the GDOC-model in rats. CGRP or saline solution (control) was administrated over 6hrs starting at onset of the disease. Microcirculation of the pancreas (blood flow, leukocyte adhesion (LEI)) as evaluated by intravital microscopy, histological damage (edema, inflammation, necrosis), and immunohistochemistry (ICAM-1 expression) were investigated.

Results: Microcirculation was significantly disturbed (increased capillary blood flow, decreased LEI) in control animals after induction of acute pancreatitis. CGRP-administration normalized both, capillary blood flow and leukocyte adhesions to baseline values (p < 0.001). Histological damage was significantly improved by CGRP compared to control animals (p < 0.05). At the same time ICAM-1 expression was diminished after CGRP-application.

Conclusions: CGRP plays a pivotal role in the regulation of pancreatic microcirculation and attenuates pancreatic damage in experimental necrotizing pancreatitis. CGRP seems to play a key role in the pathophysiology of acute necrotizing pancreatitis. The relevance of this endogenous neuropeptide in human disease has to be evaluated in further investigations.

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Severe Acute Pancreatitis Does not Enhance the Liver Production of Potent Neutrophil Activators, in Response to a Toll-Like Receptor-4-Targeted ‘Second Hit’

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Background: A ‘second hit’ in severe acute pancreatitis (AP) may exacerbate the systemic inflammatory response. We have shown previously that AP does not enhance rat liver de novo pro-inflammatory cytokine production in response to a ‘second hit’ of the toll-like receptor-4 (TLR-4) agonist lipopolysaccharide (LPS). Other mechanisms, such as activated neutrophils, are implicated in organ damage during AP. We therefore sought to evaluate liver production of potent neutrophil activators, in response to a TLR-4-targeted ‘second hit’, in AP.

Methods: 24 rats were randomised into three groups: AP (n = 10), sham laparotomy (n = 8) and controls (n = 6). Severe AP was induced by intraductal infusion of glycodeoxycholic acid, followed by intravenous caerulein infusion for 6hr. Sham-operated animals underwent laparotomy and saline infusion. Control rats had no first intervention. 18hr after this ‘first hit’, all animals underwent isolated liver perfusion with oxygenated Krebs-Hensleit buffer. A bolus of LPS (10ng/ml) was delivered into the portal vein from 0–10 min, to simulate a ‘second hit’. Perfusate was collected at 30–40 min and 90–100 min. The ability of this perfusate to activate freshly-isolated rat neutrophils was measured using the fluorogenic probe dihydro-rhodamine-123.

Results: Liver perfusate from all groups activated the neutrophil respiratory burst at both time points, compared to unstimulated neutrophils (P < 0.001, one-way ANOVA). There was no difference in the extent of neutrophil activation between AP, sham and control groups at either time point (P = 0.901, ANOVA, post-hoc Student-Neuman-Keuls). Fluorescence counts in each group were: Unstimulated cells: 30.6 (30.5–30.7) kU/well; At 30 min: AP 44.2 (41.9–46.8) kU/well; sham 44.2 (41.7–46.4) kU/well; control 44.9 (42.9–46.5) kU/well. At 90 min: AP 43.3 (40.7–45.7) kU/well; sham 42.7 (40.7–45.0) kU/well; control 43.4 (41.0–45.5) kU/well.

Conclusion: AP does not appear to enhance liver production of potent neutrophil activators in response to a TLR-4-targeted ‘second hit’.

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The Effect of a Free-Radical Scavenger on the Severity of L-Arginine-Induced Experimental Acute Pancreatitis in Rats

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Free radicals and lipid peroxidation have an important role in the pathogenesis of the local and systemic inflammation during acute pancreatitis. The aim of this study was to determine the effect of the free-radical scavenger melatonin on the early phase of acute necrotizing pancreatitis.

Methods: Male Wistar rats were divided into five groups. Rats in group A received 3.2 g/kg body weight L-arginine (L-Arg) i.p. twice at an interval of 1h. In group MA, rats were treated with 50 mg/kg body weight melatonin i.p. 30 min prior to L-Arg administration. In group AM, the rats received the same dose of melatonin 1h after the induction of pancreatitis with L-Arg. In group M, a single dose of melatonin was administered as described previously. Group C served as control group and received physiological saline
injections i.p. Rats were exsanguinated 24h after the last L-Arg injection.

Results: In groups A, AM and MA L-Arg caused a severe necro-
tizing pancreatitis confirmed by the significant elevation of serum amylose levels, pancreatic weight/body weight ratio (pw/bw), and pancreatic myeloperoxidase activity vs. groups C. Elevation of the serum amylose level was significantly reduced in group AM vs. group C. The activity of pancreatic antioxidant enzymes (Cu/Zn-
superoxide dismutase (SOD), Mn-SOD and catalase (CAT)) was sig-
ificantly increased in the liver, whereas melatonin pretreatment abolished these
tathion peroxidase and Cu/Zn-SOD activity were significantly
increased in the liver, whereas melatonin pretreatment abolished these changes and decreased the activity of liver CAT vs. group C.

Conclusion: In severe acute pancreatitis a moderate protective effect of melatonin was observed 24h following pancreatitis induc-
demonstrated by the decrease of serum amylose activity, and lipid peroxidation.

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The Effect of Endothelin-1 Receptors (ET R) Antagonists on Histological Changes and Ultrastructure of Acinar Cells in Early Caerulein Acute Pancreatitis (AP) in Rats
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The effect of both, non-selective and selective ET R antagonists in AP remains controversial. Morphological, especially ultrastructural aspects of this problem have not been adequately investigated.

Aim: The aim of the study was to assess the effect of non-selective ET R (ET R) and selective ET R (ET R) antagonists on pancreatic histology and acinar cells ultrastructure in caerulein AP.

Methods: Male Wistar rats with caerulein AP lasting 4hrs were treated i.p. injection of 10 and 20 mg/kg b.w. of each antagonist at the start of AP. The specimens of pancreases were taken for histo-
tic peroxidase and Cu/Zn-SOD activity were significantly increased in the liver, whereas melatonin pretreatment abolished these changes and decreased the activity of liver CAT vs. group C.

Conclusion: In severe acute pancreatitis a moderate protective effect of melatonin was observed 24h following pancreatitis induc-
demonstrated by the decrease of serum amylose activity, and lipid peroxidation.

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The Influence of Obesity on Acute Experimental Pancreatitis is Unrelated to Systemic Inflammation, Cyclooxygenase-2 or 5-Lipoxygenase Expression
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Obesity is for unknown reasons associated with increased severity of human acute pancreatitis and acute experimental pancreatitis (AEP). This study investigated whether obesity is associated with an altered inflammatory response, cyclooxygenase-2 (COX-2) or 5-lipoxygenase (5-LOX) expression in AEP.

Methods: AEP was induced by retrograde bile duct infusion of 3% sodium taurocholate in obese (OAP) and lean Zucker rats (LAP). Untreated rats served as controls (LC and OC). Eight hours after induction pancreatic histology, amylase, and myeloperoxidase (MPO) expression in pancreas and lung were determined. COX-2 and 5-LOX gene- and protein expression were evaluated by real-time qRT-PCR, Western blot (WB), and immunohistochemistry (IHC).

Results: Pancreatic MPO levels were significantly greater in OAP compared to LAP (P < 0.05). No differences in amylase, pancreatic necrosis, pulmonary MPO or mortality (OAP 44%; LAP 20%) were seen between OAP and LAP. In controls, COX-2 IHC showed staining of islets. In AEP, positive cytoplasmic COX-2 staining was observed in acinar cells and infiltrating leukocytes. WB confirmed increased COX-2 protein expression in pancreas and lung. Controls showed 5-LOX staining of perivascular leukocytes. In AEP, strong cytoplasmic staining of acini and infiltrating leukocytes was seen. WB did not detect 79kD 5-LOX, but a marked increase of a low mol-
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Cytokines are released during acute pancreatitis (AP) from different cell sources. Cytokine expression may be regulated by oxidant-sensitive transcription factors.

**Aims:** To study the time-course of TNF-α production in acinar cells during AP induced in rats by bile-pancreatic duct obstruction (BPDO) and to analyse the effect of N-acetyl cysteine (NAC).

**Methods:** NAC (50 mg/kg, ip) was administered 1 h before and 1 h after BPDO. At different BPDO times (3 h, 6 h, 12 h, 24 h and 48 h) flowcytometric analysis of oxygen free radicals (OFR) and TNF-α were carried out in isolated acinar cells using dihidrorhodamine and monoclonal antibody against TNF-α labelled with phycoerithrin, respectively. Activation of NF-κB was evaluated using NF-κB p65 transcription factor kit (Active Motif). Cytoplasmic IκB degradation was measured by Western blot. The effect of NAC on malondialdehyde (MDA) levels, histological damage and neutrophil infiltration were also evaluated in pancreatic tissue.

**Results:** TNF-α was found significantly increased in acinar cells from 6 h after BPDO onwards as a result of NF-κB activation. NAC treatment effectively prevented the OFR generation and TNF-α production in acinar cells. NAC prevents acinar cell TNF-α production during AP induced by BPDO by blocking the activation of NF-κB at stages in which acinar cells display maximal oxidative stress, thus palliating the severity of AP.

**Conclusion:** Treatment with CP-96345 protects mice against acute pancreatitis and associated lung injury. NK1 receptor antagonists may potentially be of use for the treatment of acute pancreatitis and its systemic complications.

**Up-Regulation of Uncoupling Protein 2 in Isolated Pancreatic Islets may Contribute to Beta-Cell Impairment in Acute Experimental Pancreatitis**

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Impairment of pancreatic endocrine function is believed to play an important role in the development of glucose intolerance in acute pancreatitis. The mechanisms behind this are not elucidated. Uncoupling protein 2 is a mitochondrial protein that increases the e-entry of protons into the mitochondrial matrix, thereby providing protection against reactive oxygen species. However, as a consequence of up-regulation, heat instead of ATP is produced, and cellular energy metabolites are depleted. UCP2 is expressed in the beta-cells of pancreatic islets and evidence show that an increased UCP2 expression results in suppression of glucose stimulated insulin secretion. The aim of this study was to evaluate whether UCP2 transcripts would increase in the pancreas and/or isolated islets from mice with cerulein-induced acute experimental pancreatitis (AEP).

**Methods:** AEP was induced by 12 hourly injections of cerulein (AEP) or saline (controls). Pancreas was removed 12 and 24 hours after the 1st injection. In another group of mice islets were isolated 24 hours after 1st injection. Total RNA was extracted and UCP2 gene expression was determined by real-time quantitative RT-PCR.

**Results:** AEP was confirmed biochemically, histologically, and by increased myeloperoxidase (MPO) activity. UCP2 mRNA was not significantly different from saline injected control values at 12 hours but was nearly 12-fold greater than control levels after 24 hours (P < 0.001). Only a weak correlation was seen between MPO activity and UCP2 gene expression suggesting a contribution from other cell types. In isolated islets UCP2 transcript was more than 2-fold increased (P < 0.01) in AEP compared to controls.
Conclusions: UCP2 mRNA was increased in the pancreas and in isolated islets in acute experimental pancreatitis. As UCP2 represents a novel negative modulator of glucose stimulated insulin secretion, we hypothesize that the up-regulation of UCP2 in pancreatic islets may contribute to the impairment of pancreatic endocrine function in acute pancreatitis.

Background: Vagal nerves are implicated in the regulation of pancreatic secretions, but the role of these nerves in pathogenesis of acute pancreatitis is unknown.

Aim: To evaluate the effect of vagal stimulation on the course of acute caerulein-induced pancreatitis (CIP) in the rat.

Methods: The study was performed on Wistar rats. Animals were surgically prepared with subdiaphragmatic microchip (MP) with two electrodes. Left vagal nerve was continuously stimulated by a constant stimulus (amplitude 100 mV, frequency 1 Hz). Sham-operation was performed in the control group of rats. A week after surgery, CIP was induced by subcutaneous infusion of caerulein (5 \( \mu \)g/kg-h, 5h) in CIP rats (without MP) pancreatic edema, rises of plasma amylase and lipase (by 500% and 800%, respectively), and usual morphological signs of CIP were observed. Stimulation of vagal plasma amylase and lipase (by 500% and 800%, respectively), and pancreatic secretions, but the role of these nerves in pathogenesis of acute caerulein-induced pancreatitis (CIP) in the rat.

Conclusions: Stimulation of vagal nerve aggravates pancreatic acute inflammation though activation of vagal inflammatory reflex.
diagnosis of chronic pancreatitis by EUS were evaluated. Data are shown as median (95% CI) and compared by the U-Mann-Whitney test for unpaired data.

**Results:** 19 patients (51.3%) suffered from EPI. Patients with EPI showed a higher number of parenchymal (p < 0.01), ductal (p < 0.001) and total EUS criteria (p < 0.001). The probability of EPI in a logistic model is at best defined by the number of ductal EUS criteria (OR = 5.6, 95%: 2.0–16.1). The model does not improve by the addition of the number of parenchymal criteria or of any single EUS criteria. The probability of suffering from EPI is 0% in patients with 2 or less EUS ductal criteria but as high as 80.1% if 4 or 5 criteria are present. Analyzing individual EUS criteria as independent variables, only the presence of ductal dilatation is significantly associated to EPI (OR = 28.3, 95%: 3.16–261.9). The addition of parenchymal calcifications (OR = 5.6, 95%: 0.9–30.6) tends to improve the model (p = 0.06). The probability of having EPI is as high as 70.9% in patients with ductal dilatation and as low as 29.1% if ductal dilatation is absent.

**Conclusions:** The number of EUS ductal criteria and the presence of ductal dilatation allows to predict the probability of EPI in patients with severe CP. This may have clinical relevance if functional tests are not readily available.

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**Prevalence of CFTR Gene Mutations in Patients with Idiopathic Chronic Pancreatitis**

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**Background:** Cystic Fibrosis (CF) is the most common recessive disease in Caucasians. Using screening panels containing the most common CFTR mutations their prevalence has been found to be increased in patients with idiopathic chronic pancreatitis. We have investigated the frequency of CFTR mutations in patients with sporadic chronic pancreatitis by sequencing the entire CFTR coding region.

**Patients and Methods:** 66 patients with idiopathic pancreatitis as determined by unequivocal CT and/or ERCP criteria were included in the study. The classification as ‘idiopathic’ was based on exclusion known nutritive, biliary, metabolic or endocrine risk factors of the disease. Patients with trypsinogen gene mutations as well as patients with a history or symptoms of CF were excluded. CFTR mutations were identified by sequencing of the entire coding region of the gene (24 exons).

**Results:** A total of 12 patients (18.2%) carried at least one abnormal CFTR allele. Four patients (6.1%) were compound heterozygous for two CFTR mutations. Two new, not previously reported mutations in the CFTR gene (M348V and A1087P) were identified. The 5T allele was present in 8 of the 66 patients (12%). Patients, who were compound heterozygous were significantly younger (29 ± 9 years) at disease onset than patients carrying only one abnormal CFTR allele (46 ± 3 years).

**Conclusions:** In 66 German patients with unequivocal idiopathic chronic pancreatitis the prevalence of abnormal CFTR alleles was significantly higher than in the control population. Compound heterozygous CFTR mutation carrier patients had a significantly earlier disease onset. However, in contrast to prior assumptions our data clearly show that heterozygous CFTR mutation carrier status represents an independent risk factor for developing chronic pancreatitis.

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**Analysis of TGF-beta Gene Polymorphism in Pancreatic Adenocarcinoma (PA) and Chronic Pancreatitis (CP)**


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**Background:** Transforming growth factor beta (TGF-beta) is a regulator factor for cell growth and differentiation and is often disrupted during pancreatic tumorigenesis. We investigated the clinical significance of C509T TGF-beta gene polymorphism in pancreatic adenocarcinoma (PA) and chronic pancreatitis (CP).

**Methods:** The study included 49 patients (aged 47–81 years) who underwent Whipple resection or distal pancreatectomy for pancreatic adenocarcinoma (26 subjects, 10 men and 16 women) or chronic pancreatitis (23 subjects, 14 men and 9 women). Normal pancreatic tissue was obtained from 6 patients with uninvolved pancreas tissue distal to Vater papilla carcinoma. C509T TGF-beta polymorphism was studied in genomic DNA isolated from pancreatic tissue after operation. Survival probabilities in patients with PA were computed using univariate Kaplan-Meier analysis.

**Results:** The heterozygous C509T TGF-beta genotype was found in 11 (42.3%) cases with pancreatic adenocarcinoma, 15 (65.2%) with chronic pancreatitis and in 5 (83.3%) cases of normal pancreatic tissue. We found an increased frequency of the homozygous 509C/C TGF-beta genotype in patients with PA (14 cases − 53.8%) compared with CP (3 case − 21.7%) (p < 0.05). The homozygous 509T/T GGF-beta genotype was observed only in 1 (3.4%) cases with PA and 3 (13%) cases with CP. In normal pancreatic tissue only one TT homozygote and no CC homozygote were found.

Overall median survival in patients with PA was 9.5 months. There was no significant association between survival time and TGF-beta gene polymorphism. The correlation between presence of homozygous 509C/C TGF-beta genotype and tumor size > 3 cm has been observed (p < 0.05). No statistically significant relationship between TGF-beta gene polymorphism and sex or patient age, tumor differentiation or lymph nodes metastases has been shown.

**Conclusion:** Those results indicate an association between TGF-beta polymorphism and tumorigenesis and they suggest that the homozygous 509C/C TGF-beta genotype as a high risk factor for the PA development and prognosis.

This work was supported by State Committee for Scientific Research, Grant # 3 P05B 121 23.
Alcohol is a main contributory factor to the development of chronic pancreatitis (CP), and pain is the weightiest consequence of the disease. To investigate the impact of increasing alcohol consumption on disease manifestations and to elucidate factors associated with increasing pain, we analysed 113 patients with CP.

**Method:** The patients fulfilled a questionnaire comprising data on alcohol consumption and pain (VAS). Furthermore disease duration, exocrine and endocrine function, tobacco use and educational level were recorded. They were divided into 4 groups according to alcohol use: (a) No alcohol use (n = 30), (b) alcohol use but not >60 gram/day for a year (n = 19), (c) alcohol use >60 gram/day for >one year, but no current use (n = 39), (d) alcohol use >60 gram/day for >one year and continued use (n = 47).

None had undergone surgical resection of the pancreas. Age: 23–83 years. Disease duration: 0.5–18 years. Exocrine function (Lundh-meal test): normal, reduced, abolished. Endocrine function: normal glucose tolerance (GT), impaired GT, NIDDM, IDDM.

Statistical analysis: Spearman’s correlation analysis and multiple regression analysis.

**Results:** Increasing alcohol use was associated with decreasing exocrine function (p < 0.008) and endocrine function (p < 0.02) pancreatic function, but not with pain score, or educational level.

High VAS score was negatively correlated with age (p < 0.0001) and exocrine function (p < 0.03), but not with disease duration, or any other factor. Reduced exocrine function was not correlated with long disease duration but with high tobacco use (p < 0.01). Endocrine function decreased with long disease duration (p < 0.02).

**Conclusion:** High alcohol use increases the risk of exocrine and endocrine pancreatic insufficiency, but is not associated with increased pain. Younger patients with CP experience more pain than the elder and pain is reduced with declining exocrine pancreatic function. Surprisingly, the duration of the disease has no effect on the exocrine function.
Endoscopic Evaluation and Management of Hereditary Pancreatitis Patients


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Background: Most patients with hereditary pancreatitis develop progressive chronic pancreatitis with associated, disease specific complications. We have investigated impact of diagnostic and interventional ERCP on the clinical course of hereditary pancreatitis patients.

Patients and Methods: From a cohort of 40 kindreds with hereditary pancreatitis (R122H-, R122C-, N29I-mutations), 30 patients underwent ERCP including 16 interventions.

Results: According to the Cambridge Classification 10 patients had grade I, 3 grade II and 17 grade III pancreatitis. Two third of the patients had high grade ductal changes and 50% pancreatic duct strictures that may have disguised intraductal neoplastic lesions. Although 88% of patients who underwent repeat ERCP studies demonstrated disease progression, none had developed pancreatic cancer during a 4 year period, even though 4 were found to have common bile duct obstruction from hereditary pancreatitis. Both patients who received pancreatic duct stents to treat strictures developed post-ERCP pancreatic ductal stenosis. Neither of the 2 patients who underwent repeated ESWL for pancreatic stone fragmentation and removal.

Conclusion: The spectrum of therapeutic interventions by ERCP in hereditary pancreatitis is similar to that in patients with other causes of pancreatitis, but pancreatic duct stent insertion (unlike ESWL) may be accompanied with a higher complication rate. Because of rapidly evolving and highly variable ductal changes in hereditary pancreatitis ERCP is not likely to be a high-sensitivity procedure for the detection of early – and thus curable – intraductal neoplasia.

New-Onset Symptom-Free Diabetes Mellitus in Chronic Pancreatitis – A Sign of Progressive Endocrine Pancreas Damage?

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Diabetes mellitus is a common complication of chronic pancreatitis (CP), and it has been suggested to be a marker of pancreatic parenchyma damage in the course of this disease.

The aim of the study was to assess b-cell function as a measure of CP severity in CP patients with newly diagnosed diabetes mellitus. Three groups of subjects were studied: Group 1 – patients with CP with newly diagnosed symptom-free diabetes mellitus (n = 10, mean age 44.0 ± 9.3 years); Group 2 – CP patients with normal glucose tolerance (n = 10, mean age 48.0 ± 9.3 years); and Group 3 – healthy age-matched controls (n = 10). Clinical characteristics of chronic pancreatitis as well as plasma glucose, insulin (RIA), C-peptide (RIA) and amylin (ELISA) concentrations were assessed during the oral glucose tolerance test (OGTT) performed according to the WHO protocol. Area under the curves of plasma insulin (IAUC) and amylin (AAUC) were calculated.

Mean plasma insulin in OGTT was similar in Group 1 and Group 2, but significantly lower than in Group 3 (p < 0.001): 0 min = 5.7 ± 2.3 and 6.0 ± 3.2 vs 19.0 ± 7.6; 60 min = 28.7 ± 11.7 and 26.6 ± 7.8 vs 136.4 ± 45.7; 120 min = 24.4 ± 9.7 and 17.7 ± 6.7 vs 41.9 ± 34.0 mIU/ml, respectively. Mean IAUC in Group 3 was four-fold higher than in Group 1 and 2: 166.9 ± 53.4 vs 43.8 ± 16.8 and 38.8 ± 11.3 mIU/ml/h, respectively. A similar trend has been observed in plasma C-peptide. There were no significant differences in mean plasma amylin between Group 1 and Group 2 subjects: 0 min = 6.9 ± 3.9 and 4.9 ± 2.6; 60 min = 8.9 ± 4.8 and 8.3 ± 3.2; 120 min = 12.4 ± 17.8 and 8.6 ± 4.5 PMI or in AAUC: 18.5 ± 14.5 and 25.8 ± 27.1 PMI, respectively (p > 0.05). There were no differences between groups 1 and 2 according to Cambridge criteria. However, CP patients with diabetes were diagnosed for CP almost three times earlier than non-diabetes patients: 9.4 ± 5.3 vs 3.5 ± 1.4 years (p < 0.05).

In conclusion, new-onset symptom-free diabetes does not seem to be the result of progressive endocrine pancreas damage, and its pathogenesis is likely to involve other mechanisms, in addition to apparent insulin deficiency. The duration of chronic pancreatitis is a significant predictor of pancreatic diabetes development.

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An Endoscopic Pancreatic Function Test (EPFT) can Decrease Frequency of Negative EUS in Evaluation of Patients with Chronic Abdominal Pain (CAP)


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An extensive medical evaluation is often performed on patients with chronic abdominal pain (CAP) of suspected pancreatic origin, including referral to tertiary care centers for EUS. Endosonography criteria for chronic pancreatitis (CP) have been established. We have developed an endoscopic pancreatic function test (ePFT) that can be performed by the practicing gastroenterologist during standard upper endoscopy (Gastrointest Endosc 2003;57:37).

Purpose: Determine if ePFT is an effective screening test for patients with CAP.

Methods: A total of 45 patients with CAP (21M/24F; mean age 44 yr) had both ePFT and EUS. Endoscopic Pancreatic Function Test (ePFT) protocol: (1) upper endoscopy (Olympus GIF-XP 160) with or without MS, (2) IV synthetic porcine secretin (0.2 mg/kg, ChiRhoClin, Inc) after test dose, (3) duodenal fluid aspirated for...
60 minutes and autoanalyzed for \([\text{HCO}_3^-]\). EUS protocol: (1) EUS images obtained from gastric and duodenal stations, (2) scoring based on 9 standard parenchymal and ductal criteria.

**Results:** There was a reciprocal correlation between EUS score and \([\text{HCO}_3^-]\); EUS score decreased as duodenal \([\text{HCO}_3^-]\) increased (-0.517, \(p < 0.01\), Spearman’s correlation coefficient). No patient with a peak \([\text{HCO}_3^-]\) greater than 84 mEq/L had an EUS score greater than 5.

**Conclusions:** (1) the endoscopic pancreatic function test (ePFT) is an effective screening test for CAP patients: If peak \([\text{HCO}_3^-]\) is greater than 64 mEq/L, EUS will not be diagnostic for CP and is unnecessary. A peak \([\text{HCO}_3^-]\) less than 84 mEq/L does not diagnose CP, but only indicates the need for further testing.

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**A Prospective, Randomized Trial Comparing the Effects of Moderate Sedation (MS) on Exocrine Pancreas Function**


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Secretin enhanced pancreatic function testing with measurement of peak \([\text{HCO}_3^-]\) or total \([\text{HCO}_3^-]\) output is the gold standard in evaluating chronic abdominal pain (CAP) that may be pancreatic in origin. Traditional gastroduodenal collection methods are rarely performed, as they are cumbersome and technically difficult. We have reported that a timed endoscopic aspiration (ePFT) at 30 minutes can screen CAP patients for chronic pancreatitis (Gastrointest Endosc 2003;57:37–40). While our ePFT is simpler and less cumbersome, it requires MS that may alter pancreatic secretion.

**Purpose:** Determine effects of MS on pancreatic \([\text{HCO}_3^-]\) secretion in a prospective trial.

**Methods:** Healthy subjects were randomized by computer to ePFT with or without MS, then crossed-over after a 7-day washout. MS was accomplished with meperidine/midazolam dosed from a published nomogram (Am J Gastroenterol 2000;95:2242). Endoscopic Pancreatic Function Test (ePFT) protocol: (1) upper endoscopy (Olympus GIF-XP 160) with or without MS, (2) IV synthetic porcine secretin (0.2 mg/kg, ChiRhoClin, Inc) after test dose, (3) duodenal fluid aspirated for 60 min and autoanalyzed for \([\text{HCO}_3^-]\). Samples were grouped in four 15-minute aliquots for statistical analysis. Mean of maximum \([\text{HCO}_3^-]\) values of timed intervals reported.

**Results:** 14 subjects (7M/7F) were randomized. Median meperidine/midazolam dose: 62.5 mg/2.5 mg. MS had no effect on the overall peak \([\text{HCO}_3^-]\), estimated \([\text{HCO}_3^-]\) output (area under curve, AUC), and maximum \([\text{HCO}_3^-]\) secretion during the 0–15 min and 15–30 min collection time intervals but remained well above the historical cut-point of 80 mEq/L.

**Conclusions:** (1) MS does not affect the pancreatic secretory physiologic parameters (peak \([\text{HCO}_3^-]\) or total \([\text{HCO}_3^-]\) output) utilized to diagnose chronic pancreatitis. (2) A decrease in \([\text{HCO}_3^-]\) is observed in the later phases of secretion, but this appears to have no diagnostic significance. **Clinical Implication:** A secretin stimulated endoscopic pancreatic function test using MS is feasible for the practicing gastroenterologist.

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**Absence of an Association between Genetic Polymorphisms of UDP-Glucuronosyltransferase 1A7 and Pancreatic Diseases**


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Although the pathogenesis of pancreatic diseases remains largely unknown, it is likely that genetic factors modify the susceptibility to pancreatic cancer and pancreatitis. Xenobiotic-mediated cellular injury is thought to play a major role in the pathogenesis of pancreatic diseases. Genetic variations reducing the expression or activity of detoxifying phase II biotransformation enzymes such as the UDP-glucuronosyltransferases might be important in this respect. Recently, a UGT1A7 low detoxification activity allele, UGT1A7*3, has been linked to pancreatic cancer and alcoholic chronic pancreatitis. Genetic polymorphisms in the UGT1A7 gene were assessed in a large cohort of patients with different types of pancreatitis and pancreatic cancer originating from the Czech Republic (n = 93), Germany (n = 699), the Netherlands (n = 136), and Switzerland (n = 106), as well as in healthy (n = 1,409) and alcoholic control (n = 123) subjects from these European countries. Polymorphisms were determined by melting curve analysis using fluorescence resonance energy transfer probes. Additionally, 229 Dutch subjects were analysed by restriction fragment length polymorphism.

The frequencies of UGT1A7 genotypes did not differ between patients with acute or chronic pancreatitis as well as pancreatic adenocarcinoma and alcoholic and healthy control individuals. We found evidence that genotype errors probably account for the detected UGT1A7 differences in a recent other study.

**Conclusion:** Our data suggest in contrast to earlier studies that UGT1A7 polymorphisms do not predispose patients to the development of pancreatic cancer and pancreatitis. Moreover, the previously reported association is most probably based on genotyping errors.
Assessment of Health-Related Quality of Life (HRQL) in Patients with Chronic Pancreatitis

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**Background:** Measurement of HRQL is becoming an increasingly important endpoint to researchers and clinicians in management decisions in patients with chronic pancreatitis (CP) – progressive and debilitating disease, that causes not only pain and endocrine–exocrine insufficiency of pancreas, but also in correlation with alcohol etiology and loss of work it leads to social consequences.

**Aim:** The aim of the study was to assess HRQL in patients with CP in correlation with the environmental or social factors, which can influence well-being of these patients.

**Materials and Methods:** The study group comprised 43 patients with CP (M/F 37/6; mean age 47.9 ± 8.6 yr). Different degrees of CP activity were defined using Cambridge classification; pain intensity and frequency were assessed using pain index. Control group consisted of 40 healthy volunteers of comparable demographic data. HRQL was assessed using Short Form-36 questionnaire, which was validated for Poland.

**Results:** Mean HRQL scores in CP were lower compared to control group in all SF-36 domains. Statistical differences were observed in general health perception, physical functioning, role-physical (p < 0.001) and vitality (p < 0.05).

We observed negative correlation of HRQL results and pain index in all domains; and number of relapses in 5 from 8 domains and positive correlation between HRQL and BMI in 5 from 8 domains (p < 0.001; p < 0.05). The worst HRQL scores were obtained in retired/disabled patients, as well as in unemployed persons in almost all SF-36 domains (p < 0.001).

There was no statistical correlation between mean HRQL scores and gender, age, marital status, education as well as with etiology of disease (alcohol vs idiopathic), smoking habits, duration or activity of CP, history of surgical or endoscopic treatment and diabetes mellitus coincidence.

**Conclusion:** Many of social factors have a significant negative impact on CP patients’ HRQL parameters, so the measurement of HRQL in CP is very important.

Clinico-Immunogenetic Parallels in Chronic Alcoholic Pancreatitis (CAP)

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CAP pathogenesis still remains unclear, although alcohol is the main etiologic factor. Probably there is a possibility of immunogenetic predisposition for CAP development.

Aim: To study immunogenetic characteristics of CAP patients and relations between peculiarities of the disease clinical picture and patient’s phenotype.

Materials and Methods: We examined 92 CAP patients and 450 healthy persons (inhabitants of the same region). We studied incidence of erythrocyte’s antigens of Rhesus-factor and ABO system and leukocyte antigens – HLA system, A, B, C, DR locus using hem agglutination method, microlymphocytotoxicity test and polymerase chain reaction. On the basis of these results we calculated development risk of the disease (R).

Results: CAP risk is increased in case of patient blood group A(II) (R = 2.2), combination of Rhesus-factor antigens CDe (R = 2.0), presence in phenotype HLA antigens A1 (R = 3.1), B13 (R = 2.8), B18 (R = 3.1), B27 (R = 3.0), Bw40 (R = 4.8), DRB1*11 (R = 2.6), DRB1*13 (R = 2.2), haplotype A10-Cw6 (R = 3.9), Antigen Cw4 acts as an antigen-protector (R = 0.23). In presence of blood group A(II) there was increased risk of progressive pancreatic excretory insufficiency (R = 3.2). Haplotype A10-Cw6 was associated with expressed pain syndrome (R = 2.1), significant hyperferremenia (R of elevated blood level of immunoactive trypsin = 3.4). HLA antigen Bw40 was associated with evident reduction of lipase debit-part (R = 3.7) and with increase of index L of pancreas ultrasound histogram (R = 3.7). HLA antigen B13 was associated with pancreas calcification (R = 4.0).

Conclusion: CAP develops under influence of immunogenetic predisposition and some peculiarities of the disease clinical course are associated with the presence of erythrocyte and leukocyte antigens in patient’s phenotype.

Comparison of the Results of Pancreatic Secretin Stimulation and Indirect Pancreatic Exocrine Function Tests in Patients after Partial Pancreatectomy

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Aim: In order to clarify if the pancreatic enzymes activity secreted into the stomach and bicarbonate level are comparable to results of indirect pancreatic exocrine function test in patients after partial pancreatectomy with pancreatogastrostomy.

Methods: 17 patients (2001–2003 year) underwent partial pancreatectomy for malignant or benign pancreatic tumor. 16 pancreatoduodenectomies with pancreatogastrostomy (12 Whipple, 4 pylorus-preserving-PPPD) and 1 distal pancreatectomy were performed.

Pancreatic exocrine output was measured 2 months after operation. Pancreolauryl test (PLT), Elastase 1(E1) stool test and secretin stimulation were used estimate pancreatic remnant exocrine function.

Results: The preoperative level of Pancreolauryl test results and E1 stool level in the all patients significantly decreased after 2 months. About 90% of patients had severe pancreatic exocrine insufficiency. Only 9% (PLT) and 17% (E1) of patients had mild pancreatic exocrine insufficiency. Only 2 patients after partial
pancreatectomy had normal total bicarbonate output during secretin stimulation (1 Whipple procedure and 1 distal pancreatectomy). For 9 patients level of bicarbonate to be indefinite. For most of the patients the pH of gastric juice was in range between 4 to 8. 2 PPPD-patients exhibited had pH of gastric juice below 3. Increased amylase and lipase activity were observed during secretin stimulation. No significant difference amylase and lipase activity between Whipple procedure and PPPD during secretin stimulation. Dependence between pancreatic juice pH, amylase activity and bicarbonate concentration were found. Dependence between E1, PLT and bicarbonate output during secretin stimulation not found.

Conclusions: No correlation between results of indirect tests and results secretin pancreatic remnant stimulation can be partially explained by bicarbonate neutralization by low pH of gastric acid. Additionally these two types of assessment pancreatic exocrine function are not fully comparable because pancreatic secretin stimulation was performed in fasting patients while the indirect tests required intake of standard meal by patients.

Results: The Cambridge, ABC, Heidelberg and Manchester systems (see graph) all demonstrated a significant progression towards end-stage over 10 years (P < 0.01 Kruskal-Wallis with post-correction).

Conclusions: This is the first study to compare multiple classification systems for CP evaluating change in disease category over a prolonged observation period. The results confirm that for clinical categorisation, the Heidelberg, ABC and Manchester scoring systems are valid and practical. These systems now require prospective validation.

Design and Evaluation of a Clinically-Based Classification System for Chronic Pancreatitis: Assessment in Comparison to the Marseilles, Cambridge, Heidelberg and ABC Systems Over a Ten-Year Study Period

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Introduction: Classifications of chronic pancreatitis (CP) based on either histologic (Marseilles) or endoscopic (Cambridge) criteria are not widely used. The aim of this study is to design a clinically-based classification (CBC) for CP and to evaluate this in comparison to other systems over a prolonged period.

Methods: Patients with a diagnosis of CP(577.1:ICD-9) for 1993 were identified from the records of the Hepatobiliary Service of a University Hospital. Endoscopic (ERCP) or CT evidence of CP were mandatory for inclusion. Forty-one new patients met criteria and were allocated a category according to the Marseilles, Cambridge, Heidelberg and ABC systems and also according to the Manchester clinically-based 3-stage system:

Mild CP: Abdominal pain I either ERCP or CT evidence of CP I obligatory: no regular (weekly) opiate, preserved endocrine, exocrine (ex/end) function and no peri-pancreatic complications (PPC).

Moderate CP: Abdominal pain I at least 1 of: regular opiates, impaired ex/end function, no PPC.

End-stage CP: ERCP or CT I at least one obligatory: biliary stricture, portal hypertension, duodenal stenosis 6 one of: pain, diabetes, steatorrhea.

Charts were reviewed for the subsequent 10-years with annual categorical allocation. Principal outcomes were death and progression to end-stage CP.

Diagnosis of Chronic Pancreatitis (CP) by Endoscopic Ultrasound (EUS): An UP-to-Date of the Cambridge Classification is Needed

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According to the Cambridge classification of CP, patients are included in one out of four different groups based on the presence of minimal, mild, moderate or severe morphological changes on imaging methods. The advent of EUS, which provides highly accurate images of both pancreatic ductal system and parenchyma, may increase the morphological information obtained in these patients. We hypothesize that each one of the Cambridge groups includes a wide range of USE changes and that, therefore, the Cambridge classification should be brought up to date. To support that, this study aimed at analyzing the different USE patterns observed in patients with severe CP based on ERCP or magnetic resonance cholangiopancreatography (MRCP).

Methods: 52 consecutive patients (mean age was 46 years, range 27–86 years, 42 male and 10 female) with severe CP according to ERCP and/or MRCP findings were prospectively included. In all cases, EUS was performed under conscious sedation by the lineal scanning Pentax FG-38UX echoendoscope, by a single operator who was aware of the clinical history but blinded to ERCP and MRCP findings. The different ten criteria accepted for the diagnosis of chronic pancreatitis
by EUS were evaluated: parenchymal changes included hyperechoic foci, hyperechoic strands, lobularity, cysts and calcifications; ductal changes included dilation, irregularity, hyperechoic duct margins, visible side-branches and ductal calcifications. The number and the different EUS criteria accomplished were analyzed.

Results: 12 patients (23.1%) fulfilled all 10 USE criteria for chronic pancreatitis, 7 patients (13.5%) fulfilled 9 criteria, 10 (19.2%) 8 criteria, 9 (17.3%) 7 criteria, 7 (13.5%) 6 criteria and 7 (13.5%) 5 criteria. The most common parenchymal criteria were hyperechoic foci and pancreas lobularity (100% of patients) and hyperechoic strands (94.2% of patients). Parenchymal calcification was present in 31 patients (59.6%) and pseudocyst in 15 patients (28.8%). The most common ductal changes were hyperechoic duct margins (100% of patients) and irregularity (94.2%). Pancreatic duct dilation and visible side branches were present in 39 patients (75%) and ductal calcifications in 21 patients (40.3%).

Conclusion: The group of advanced CP according to the Cambridge classification includes a heterogeneous group of patients based on USE findings. A more specific classification of CP should be thus defined taken into account recent advances in imaging diagnosis.

143 Diagnosis of Pancreatic Cystic Lesions with Combined Endoscopic Ultrasonography and Aspiration Fluid Analysis
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Background: From data that are currently available, it appears that endoscopic ultrasonography (EUS) can help to reliably distinguish between the majority of benign and neoplastic cystic lesions. In cases where high suspicion for malignancy exists the use of EUS-guided fine needle aspiration (EUS-FNA) for obtaining cytology and cystic fluid for analysis of various tumor markers improves the diagnostic yield. The aim of study was to assess the performance of EUS-guided fine needle aspiration (EUS-FNA) in the management of pancreatic cystic lesions using additional cystic content analysis (cytology, amylase content, CEA, CA19-9).

Methods: In total 26 patients (9 women, 15 men) in an age from 34–81 (mean age 59 years) with one or more pancreatic cystic lesions detected by US, EUS, MRI, or CT were involved. All pancreatic cystic lesions were considered for single time EUS-FNA.

Results: EUS detected pancreas parenchymal (structure, echodensity) changes (n = 19; 73%), cystic lesions with septae, calcifications, solid structures (n = 11; 42.3%), pancreatic duct size, structure changes (n = 8; 50%). Lesions bigger than 20 mm were 80.7% (n = 19). Surgery treatment n = 8 (43.75%). Cytology study of the cyst content; malignant cells (n = 5; 19%), high grade adenocarcinoma (n = 3), low grade adenocarcinoma (n = 1), Non-Hodgkin lymphoma (n = 1).

Conclusions: EUS-FNA helps in decision making for medical or surgical approach. EUS guided puncture with single time drainage of pancreatic cystic lesions is simple, safe procedure (success rate up to 87.5%). EUS-FNA success is depending of he cystic content, size, localization and age. Laboratory examination (L, Er, protein, amylase) of pancreas cyst fluid showed low diagnostic sensitivity. Separate CA19-9 and CEA values showed less diagnostic specificity. Combined CEA + CA19-9 and cytology studies enhanced diagnostic sensitivity up to 100%.

144 Direct Measurement of Pancreatic Elastase 1 (PE-1) in Duodenal Aspirates from Healthy Subjects (HS) and Patients with Chronic Pancreatitis (CP)

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Fecal PE-1 is an indirect pancreatic function test (PFT) with good sensitivity and specificity for moderate/severe pancreatic insufficiency (Gut 1996;39:580). Direct PFT is superior to indirect PFT for evaluation of early CP. The clinical utility of direct PFT with measurement of PE-1 levels in duodenal fluid has not been determined.

Purpose: (1) To compare duodenal fluid [PE-1] in HS and patients with CP. (2) Evaluate the difference in PE-1 secretion with CCK (Bracco Diagnostics, Princeton, NJ) vs. Secretin (ChiRhoClin, Burtonsville, MD) stimulation.

Methods: Duodenal fluid was obtained by our previously described Secretin or CCK stimulated endoscopic PFT (s-PFT; c-PFT), and sent for analysis of [PE-1], bicarbonate (s-PFT), and lipase (c-PFT). [PE-1] was measured using an ELISA assay (Genova Diagnostics, Asheville, NC).

Results: 10 HS and 10 CP pts were studied (8 M/12 F; mean age 42.3 y). C-PFT: Median peak lipase for HS and CP was 1605IU/L and 87IU/L, respectively (p = 0.001). S-PFT: Median peak bicarb for HS and CP was 102mEq/L and 40mEq/L, respectively (p = 0.001). Median [PE-1] for HS and CP were 317 and 17IU/ml (p = 0.02) respectively after CCK stimulation, and 87 and 17IU/ml (p = 0.09) after Secretin stimulation.

Conclusions: (1) [PE = 1] can be measured directly from duodenal fluid obtained by endoscopic aspiration. (2) Duodenal aspirate [PE-1] are decreased in CP compared to HS. (3) CCK is the best secretagogue for PE-1 analysis.

145 Duodenopancreatectomy Versus Duodenum Preserving Pancreatic Head Excision for Chronic Pancreatitis

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Background: Aim of this study was to compare two surgical procedures in the treatment for chronic pancreatitis (CP). Pancreato-duodenectomy resection (classical Whipple – PD and pylorus
preserving – PPPD) with the duodenum-preserving pancreatic head excision (DPPHE) to define the advantages of each procedure with regard to postoperative complications, pain relief and the quality of life.

**Materials and Methods:** One hundred and four consecutive patients were included in this study. The duodenopancrectectomy procedure 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 two times (before and in the follow up period (median months after operation) with the European Organization for Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire (QLQ-C30). Test was reevaluated for patients suffering from CP. Pain intensity was quantified using a specially designed pain score. Early postoperative morbidity and mortality were observed and evaluated in the 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 global quality of life improved by 30.4% in the PJA – DPPHE group and by 23.2% in the PD, PPPD group. 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 a quality-of-life assessment in patients with chronic pancreatitis.

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**Electrogastrography in Pancreas Diabetes**


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Background: Timing and criteria of testing for gastric dysmotility and the management of diabetic patients with pancreatic insufficiency is not well established. The aim of the present study is to investigate the pattern of gastric myoelectrical activity (MA) and autonomic neuropathy (AN) in patients with pancreas diabetes mellitus (PDM), and to clarify the relationship between AN, alcohol consumption, glucose homeostasis, diabetes duration, and gastric myoelectrical abnormalities.

**Patients and Methods:** 30 patients with PDM were enrolled into the study. Mean duration of diabetes mellitus was 11 (0–25) years, mean blood glucose levels: 8.13 (±2.7)mmol/l, HbA1c 8.3 (±2.96)%. 25/30 patients were treated with insulin, the others were on rigorous diet, all of them received high dose pancreatin substitution treatment. Ten matched controls without diabetes and pancreatic insufficiency were also examined. AN was evaluated by the cardiovascular reflex tests according to the Ewing’s criteria (Diab Care 1985;8(5):491–497), MA was monitored for 30–30 min in both fasting and in postparandial states, using a Digitrapper EGG (Synectic Med., Stockholm). EGG rhythm disturbances (bradygastria: 0–2 cpm, tachygastria: 4–10 cpm) and meal evoked EGG signal amplitude (power) changes were determined.

**Results:** 9/30 pts had mild to moderate parasympathetic AN, 1/30 pts had sympathetic AN, 5/30 pts had both parasympathetic and sympathetic AN; 17/30 pts demonstrated myoelectric abnormalities: 5/30 pts had bradygastria, tachygastria or mixed dysrhythmia, and in 9/30 pts an absence of increase in the postprandial signal amplitude was found, 3/30 pts had both alteration. Overall, 7/30 pts with abnormal EGG did not demonstrated AN. Abnormal dominant frequency and MA showed no correlation with actual blood sugar values or HbA1c, but it was associated with diabetes duration more than 10 years.

**Conclusion:** Our results suggest, that beside neuropathy other factors, such as alcohol toxicity, sympathetic and parasympathetic imbalance or inappropriate glucose control may be involved in the gastric myoelectric abnormalities provoked by pancreas diabetes.

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**Electrolyte Composition of Endoscopically Collected Duodenal Fluid After Secretin Stimulation in Healthy Normal Volunteers**


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Hormonal stimulated pancreatic function tests (PFT) are the non-histologic gold standard for diagnosis of chronic pancreatitis. Traditional (gastro-duodenal) collection tubes are cumbersome, and are no longer manufactured. We have developed a purely endoscopic collection method for PFT (Gastrointest Endosc 2003;57:37–40). Criticisms of the endoscopic method include; (1) uncertainty of the effects of sedation on pancreatic secretion, (2) Scarcity of data in healthy normal patients, and (3) the test remains time-consuming.

**Purpose:** To collect and analyze the duodenal fluid electrolyte composition of healthy subjects after secretin stimulation using sedationless endoscopy.

**Methods:** Healthy subjects underwent our previously described ePFT, without the use of sedation. After synthetic porcine secretin stimulation (time ‘0’), an ‘Ultrathin’ Olympus GIP-XP160 endoscope was used to obtain timed duodenal aspirates every 5 minutes for 1 hour. Fluid was sent on ice for measurement of [Na⁺], [K⁺], [Cl⁻], and [HCO₃⁻].

**Results:** Seventeen healthy subjects were enrolled. Sixteen subjects (8 M/8 F) tolerated the sedationless ePFT. The mean age was 35.6 years (range 19–51 years). Mean electrolyte concentrations were plotted for each time point (Figure 1). The concentrations of sodium and potassium remained relatively constant, similar to normal plasma concentration (mean [Na⁺] = 145 mEq/L, mean [K⁺] = 4.8 mEq/L). [HCO₃⁻] and [Cl⁻] exhibited an inverse and reciprocal relationship. The median peak [HCO₃⁻] was 108 mEq/L (IQR 99–110 mEq/L). 88–100% of subjects had a bicarbonate concentration >80 mEq/L at 20–60 minutes.

**Conclusions:** (1) Endoscopic collection of pancreatic juice reproduces the anion-cation pancreatic secretory curve seen in previous studies of pancreatic physiology. (2) The pancreatic fluid electrolyte composition seen with endoscopic collection is identical to the traditional (gastro-duodenal tube) collection method. (3) Analysis of the pancreatic fluid secretory curve makes a single, timed aspiration (i.e. shortened ePFT) for screening for pancreatic insufficiency feasible.
Endoscopic Sphincterotomy in Patients with Stenosis of Ampulla of Vater: Three Year Follow up of Exocrine Pancreatic Function and Clinical Symptoms


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In stenosis of ampulla of Vater endoscopic sphincterotomy is the treatment of choice. In this study the effect of endoscopic sphincterotomy (ES) on exocrine pancreatic function and clinical symptoms was investigated retrospectively.

Materials and Methods: Sixty patients of our department, who all had undergone previously (mean 37.7 months) diagnostic endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sphincterotomy because of stenosis of ampulla Vater, were examined. Indications for diagnostic ERCP were cholestasis as well as serum lipase and amylase were measured.

Results: 80% of patients reported an improvement in their general condition after ES. The concentration of fecal elastase of all patients attained with this operation in a 4-year period.

Operative endocrine function of 81 patients, but 5 patients with latent diabetes mellitus (DM) progressed to IDDM.

Conclusion: This 4 years of experience clearly reveals that this organ-preserving pancreatic head resection is a safe and effective procedure for definitive control of the complications following the inflammatory alterations of CP.

Four Years of Experience with Organ-Preserving Resection of the Pancreatic Head in Patients with Chronic Pancreatitis

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Background: In chronic pancreatitis (CP), enlargement of the head of the pancreas develops in consequence of inflammatory alterations. A safe procedure has been developed for duodenum-preserving pancreatic head resection, and this report is concerned with the results attained with this operation in a 4-year period.

Patients and Methods: In 100 patients a new surgical management was introduced. The preoperative morbidity comprised frequent abdominal pain, a weight loss in all patients, jaundice in 8 patients, and latent and insulin-dependent diabetes mellitus (IDDM) in 10 and 9 patients, respectively. The surgical procedure consists in a wide excision of the inflammatory tumour in the region of the pancreatic head, without division and cutting of the pancreas over the portal vein. Reconstruction, with drainage of the secretion from the remaining pancreas into the intestinal tract, takes place through a jejunal Roux-en-Y loop. In 8 icteric cases and in 12 patients with stenosis of the common bile duct, preapillary bile duct anastomosis was also performed with the jejunal loop.

Results: Only one reoperation was required, in consequence of anastomosis bleeding, but no mortality was noted in the postoperative period. The duration of hospitalization ranged between 7 and 12 days. In the mean follow-up period of 2.35 years (range 0.5–4.0), 92 patients became complaint-free, 8 patients displayed moderate symptoms and the weight increased by a median of 10 kg (range 4–25). One or 2 years postoperatively bilio-digestive anastomosis was performed in 2 patients in consequence of bile duct stenosis. No change was noted in the preoperative endocrine function of 81 patients, but 5 patients with latent diabetes mellitus (DM) progressed to IDDM.

Conclusion: Endoscopic sphincterotomy of the ampulla of Vater should be considered as an effective therapy in patients with chronic pancreatitis.

Functional Polymorphisms of UDP-Glucuronosyltransferases 1A1, 1A6 and 1A8 are not Involved in Chronic Pancreatitis

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Background/Aim: Chronic pancreatitis (CP) is associated with alcohol abuse, smoking and other dietary or environmental factors. UDP-glucuronosyltransferases (UGTs) are phase II detoxifying enzymes responsible for glucuronidation of various exogenous and endogenous compounds. Genetic variations, resulting in variable rates of glucuronidation, are of toxicological and physiological importance and are frequently associated with diseases. Recently, a genetic polymorphism in UGT1A7 was associated with an increased risk for chronic pancreatitis. We investigated whether polymorphisms in the genes for UGT1A1, UGT1A6 and UGT1A8 modified the risk for chronic pancreatitis.

Methods: DNA samples were obtained from 258 adult CP patients with alcoholic (n = 153), hereditary (n = 25) or idiopathic (n = 80) origin. DNA from 140 healthy controls was analysed for comparison. Patients and controls were all of Caucasian origin. Genetic polymorphisms in UGTs were determined by PCR, eventually followed by restriction-fragment-length-polymorphism analyses in all subjects.

Results: The distribution of the various alleles of UGT1A1, UGT1A6 and UGT1A8 did not differ between CP patients and healthy controls.
**Groove Pancreatitis Due to Heterotopic Pancreas in the Minor Duodenal Papilla – Two Case Reports**


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**Introduction:** Groove pancreatitis (GP) is a rare form of segmental chronic pancreatitis which involves the anatomic space between the dorso-cranial part of the head of the pancreas, the duodenum and the common bile duct. We report two new cases of GP particular by the presence of pancreatic heterotopia in the minor papilla. The role of heterotopic pancreas in the minor papilla, as in our two cases, is probably underestimated.

**Case Reports:** Patient n°1 was a 44 year-old woman and patient n°2 a 47 year-old man. Both had a past history of alcohol consumption. They presented with abdominal pain, vomiting and weight loss due to duodenal stenosis. Liver function tests, serum pancreatic enzymes and tumor markers were normal. Abdominal computed tomography revealed thickening of the duodenal wall, enlargement of the pancreatic head in patient n°1 and an hypodense mass of the pancreatic head with calcification, confirmed by ultrasound endoscopy in patient n°2. In patient 1, ultrasound endoscopy showed a dilated duct in the head of the pancreas. On microscopic examination, biopsies of the lesions revealed fibro-inflammatory changes of the pancreatic parenchyma. Pancreaticoduodenectomy was performed to rule out pancreatic adenocarcinoma. In both cases, gross and microscopic examinations showed fibrosis of the duodenal wall with Brunner's gland hyperplasia and chronic pancreatitis of the groove area. Santorini's duct was dilated and contained protein plugs in patient n°1. There were protein plugs and calcification in Santorini's duct with abscesses in patient n°2. In both cases there were microscopic foci of heterotopic pancreas with mild fibrosis but without cystic dystrophy in the wall of the minor papilla. Both patients had a favourable postoperative course.

**Discussions:** Groove pancreatitis is often diagnosed in 40 to 50 year-old alcoholic men presenting with clinical symptoms due to duodenal stenosis. The distinction between groove pancreatitis and pancreatic head adenocarcinoma is often difficult on imaging. The pathogenesis of this rare entity is still unclear but could be due to disturbance in the pancreatic secretion via the minor papilla. The role of heterotopic pancreas in the minor papilla, as in our two cases, is probably underestimated.

**Conclusion:** These data suggest that genetic polymorphisms in UGT1A1, UGT1A6 and in UGT1A8 do not predispose to the development of CP in Caucasians.
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Interleukin-18 Promoter Polymorphisms and Interleukin-18 Serum Levels are not Correlated in Patients with Alcoholic Chronic Pancreatitis

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Background: Interleukin-18 (IL-18) is a cytokine with a prominent immune-modulatory function by inducing Th1 cytokine production and stimulating natural killer cells. Recent data demonstrated a correlation between IL-18 serum levels and severity of acute pancreatitis. Our previous studies have shown that IL-18 expression is upregulated in patients with alcoholic chronic pancreatitis (ACP). Several polymorphisms in the IL-18 promoter region have been identified comprising a total of three distinct haplotypes. In vitro studies proved an association of these alleles with differential IL-18 transcription activity and corresponding IL-18 protein levels. We investigated the relation between IL-18 serum levels and promoter polymorphisms in patients with ACP.

Patients and Methods: After obtaining informed consent, whole blood was drawn from 90 patients with ACP (63 male, 27 female, median age 55 (29–81) years. After DNA isolation, genotyping was performed for the −607 and −137 promoter polymorphisms using allele-specific polymerase chain reaction. IL-18 levels in serum were quantitated by a specific ELISA in 81 patients. Differences of IL-18 levels between groups were analyzed by means of analysis of variance.

Results: The frequencies for the resulting haplotypes A1, A2 and A3 were 0.59, 0.27 and 0.14, respectively. No significant differences between the different resulting genotypes and the respective serum levels of IL-18 were found.

Conclusions: In our study, serum levels of IL-18 in patients with ACP were not correlated with promoter polymorphisms in the IL-18 gene. Additional factors may potently influence IL-18 expression in vivo, thus overriding transcriptional regulation. The role of IL-18 promoter polymorphisms in ACP may be of minor importance.

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Low Fecal Elastase-1 Values do not Reliably Indicate Exocrine Pancreatic Insufficiency in Type 1 Diabetes Mellitus

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Background: Using fecal elastase-1 (FE-1) estimation up to 40% of type 1 diabetes mellitus patients have exocrine pancreatic insufficiency and expensive pancreatic enzyme substitution seems to be necessary (Z Gastroenterol 2001;39:823, Pancreatology 2003;3:395). This study aim was to compare the results of FE-1 estimation in diabetic patients (type 1) with the secretin caerulein test (SCT), the ‘gold standard’ for measuring exocrine pancreatic function.

Patients and Methods: A SCT and two FE-1 estimations (Schebo-Tech, D-Wettenberg; Bioserv, D-Rostock; abnormal <200 µg pancreatic elastase-1/g stool) were performed in 32 consecutive patients with type 1 diabetes.

Results: The SCT was abnormal in 12 (37.5%) patients, fecal elastase-1 estimation was abnormal in 15 (47.6%); ScheboTech) and 9 (28.1%; Bioserv) patients, respectively. Fecal elastase-1 estimations (Schebo-Tech; Bioserv) were falsely abnormal in 40% and 25% of patients with normal SCT and falsely normal in 57% and 67% of patients with exocrine pancreatic insufficiency, respectively. When the test results were evaluated in comparison (Schebo-Tech; Bioserv) the following values were reached: Sensitivity 64%; 33%; specificity 60%; 75%; positive predictive value 47%; 44%; negative predictive value 75%; 65%.

Conclusion: Fecal elastase-1 estimations are unreliable for detecting exocrine pancreatic insufficiency in type 1 diabetes mellitus and low values should not lead to pancreatic enzyme substitution.

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One-Step Drainage of Pancreatic Pseudocyst with Multiple Stents Using a Large Channel Therapeutic Echoendoscope

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Background: Until recently, EUS-guided approaches to endoscopic cyst-gastrostomy have utilised single, 7 Fr or 8.5 Fr stents, limiting use to carefully selected patients. We report our experience with the placement of multiple stents using a one-step EUS guided approach in 11 patients with pancreatic pseudocysts.

Patients and Methods: All patients had persistent, symptomatic cysts. Aetiology was acute pancreatitis (9 cases) or chronic pancreatitis (2 cases). 2 cysts were infected. ERCP was either unsuccessful (2 cases) or demonstrated no communication (5 cases). In 2 cases ERCP was not attempted. In the remaining 2 cases, cysts persisted despite pancreatic duct stenting. Cyst drainage was carried out under EUS control using a therapeutic echoendoscope with a 3.7 mm working channel and bridge. 2 or more pigtail stents were inserted after dilatation of the cystgastrostomy to 10 mm.

Results: There were no procedure-related complications. Cysts resolved in 8 cases. One patient died following surgery for a persistent, infected cyst. A second death occurred due to liver failure following successful cyst drainage.

Conclusion: One step, EUS guided endoscopic cyst-gastrostomy with multiple stents is feasible using the new generation of therapeutic echoendoscopes. The use of EUS facilitates selection of a safe puncture site and allows cyst drainage in cases with no visible endoscopic ‘bulge’.
Pancreatic Exocrine and Endocrine Function after Pancreatectomy

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Aim: To evaluate long term detailed pancreatic endocrine and exocrine function after pancreatectomy.

Methods: Between 2001–2003, 23 patients underwent partial pancreatectomy, 16 for malignant and 7 for benign disease. 20 pancreateoduodenectomies with pancreatogastrostomy (15 Whipple and 5 Pylorus-preserving – PPPD) and 3 distal pancreatectomies were performed. Pancreatic exocrine and endocrine function were assessed before operation, in both short (2 months) and long follow up (6 months). The Pancreolauryl test and Elastase 1 stool test were used to assess exocrine pancreatic function (PF). The level of fasting plasma glucose (FPG), oral glucose tolerance test (OGTT) and plasma insulin response were used to determine endocrine PF.

Results: Comparing the preoperative level of endocrine PF for all patients significantly decreased in short term follow. No significant difference in the postoperative exocrine PF was observed while comparing Whipple procedure vs PPPD. All patients with benign disease and ~90% of patients with malignant disease had severe exocrine pancreatic insufficiency (PI) 2 months after operation. In a long term follow up in both groups only ~10% of patients had mild exocrine PI. No significant difference between results of the preoperative FPG level and OGTT results for patients with benign vs patients with malignant disease was observed. The preoperative percentage of glucose intolerance patients in malignant group significantly decreased in a short term follow up while the percentage of normal glucose curves patients increase. There was no difference in the number of diabetics in a benign group in a short term follow up while the percentage of normal glucose curves patients increase. There was no difference in the number of diabetics in a benign group in a short term follow up while the percentage of normal glucose curves patients increase.

Conclusion: Operative considerations for the treatment of pancreatic disease should include strategies to minimize the exocrine and endocrine impairment of pancreatic resection.

Pancreatic Fistula (PF) in the Course of Chronic Pancreatitis: Non-Invasive Diagnostic Methods and Therapeutic Strategies

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The diagnosis of PF is facilitated by new non-invasive imaging techniques (thin-slice helical CT-scan and MR-pancreatography [MRP]). Results of conservative treatment (enteral nutrition and somatostatin analogues) with or without eventual pancreatic endoprosthesis remain largely unknown.

Aims: Evaluate (a) performance of helical CT and MRP in the diagnosis of PF; (b) the number of patients requiring surgery following conservative treatment failure.

Patients and Methods: PF was defined as a tubular tract communicating with the pancreas and the pleural or peritoneal cavity. 16 consecutive pts (13 M; median age 45 (14–54) yrs) with chronic pancreatitis (alcoholic 15, hereditary 1) and a PF were included between March-01 to September-03; the median follow-up since the first sign of CP was 12.5 (3–33) months. All serious effusions (ascites, n = 9; pleural effusion, n = 6) contained high amylase (median: 8,154 (323–46,000) U/ml); 1 pt had a Weber-Christian syndrome. Pts with communicating pancreatic pseudocysts were not included. An acute episode of pancreatitis preceded the diagnosis of PF in 10 pts (63%).

Results: The diagnosis of PF and its site were determined in 12/16 pts by CT and 14/15 pts by MRP (site of rupture: head: n = 5; isthmus: n = 6; body-tail: n = 5) and confirmed by ERCP or surgery in 10 pts. Localised atrophy of pancreatic parenchyma adjacent to pancreatic duct rupture was observed in 13 pts (81%). Concerning treatment of PF, immediate surgery was required in 3 pts (2 of whom had infection of serous fluid at initial aspiration analysis). In 6 of the remaining 13 pts, simple conservative treatment (enteral nutrition combined with somatostatin analogues) was efficacious within 2–12 weeks. A trans-papillary stent was successfully inserted at ERP in 6 of 7 other pts (the 7th was operated-on following ascites superinfection after failed stent insertion). Pancreatic endoprosthesis allowed PF closure in 3 pts, but secondary complications with infection occurred in 3 requiring surgery. Overall, 7/16 pts (44%) underwent surgery (pancreatocoduodenectomy: n = 1; distal pancreatectomy: n = 2; wirsungo-digestive bypass: n = 4), 4 of which following ERCP.

Conclusions: (1) In addition to correctly identifying the site of pancreatic duct rupture and direction of the fistula, helical CT and MRP also help in guiding endoscopic and surgical procedures. (2) The high frequency of infections in serous effusions due to PF (either spontaneous or secondary to endoscopic intervention) ensures the need for surgery in almost half. (3) Endoscopic treatment of PF should be undertaken prudently and followed by careful monitoring.
Transabdominal and endoscopic ultrasound, CT scan, BMR were used to determine the number, size and location of PPCs. Symptomatic and/or bigger than 6 cm PPCs with close opposition to gastric or duodenal wall were drained by endoscopic route after diathermy puncture, filling the cyst with contrast agent to evaluate its possible communication with the pancreatic duct. Pseudocystogastrostomy or pseudocystoduodenostomy was performed by increasing an opening to 0.5–6 cm in 11 cases, transmural drainage with 3–6 cm 7 Fr double pigtail stent in 11 cases and cystonasaal drain in 4 cases. One PPC was treated by pancreatic duct sphincterotomy.

**Results:** In 18 cases PPC resolved. There were 6 bleeding episodes during endoscopic drainage. Endoscopic haemostasis was achieved in 5 cases and one patient underwent emergency operation. There were 3 emergency operations because of gastric wall perforation. Because of insufficient endoscopic drainage 1 patient recovered only after surgical treatment (pancreatoduodenal resection).

**Conclusions:** (1) Endoscopic drainage is an effective alternative to surgical treatment of PPCs. (2) Bleeding is the most frequent complication of endoscopic transmural drainage of PPCs and it is successfully managed by endoscopic haemostasis. (3) Contraindications for this procedure there are PPCs without close opposition to gastrointestinal wall, pancreatic head enlargement and/or pancreatic duct cut off or dilation (>6 mm) when pancreatic resection and/or duct drainage operations are indicated.

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**Percutaneous Drainage of Pancreatic Pseudocyst into the Stomach – Our Experience**  
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**Background:** The therapeutic options for treatment of pancreatic pseudocyst are numerous, ranging from endoscopic drainage, percutaneous drainage, to various surgical procedures. Selection of the appropriate method of drainage is controversial and influenced by several factors, including pseudocyst size, age, location, number, etiology and the presence of symptoms or complications.

A method was developed using ultrasonographically guided percutaneous intervention to ensure a prolonged internal cystogastric drainage until the pseudocyst collapses and seals off.

**Patients and Methods:** In the period from 1995 to 2003 percutaneous drainage of pancreatic pseudocysts with the double pig-tail catheter was performed in 23 patients under local anesthesia. The procedure failed in 2 patients. The needle insertion through both gastric walls, as well as the final position of the proximal curve of the catheter, were monitored with a gastroscope, whereas the position of the distal curve of the catheter was checked by ultrasound. The patients were followed up monthly by clinical and ultrasound examination.

**Results:** On the first follow-up examination 1 month after the intervention, none of the patients showed evidence of a pseudocyst on ultrasound. The catheter was removed endoscopically 6 to 9 month after intervention. In two cases pseudocyst showed up again 3 month after catheter removal. In both cases the catheter was introduced again in the same way.

**Conclusions:** The method is minimally invasive and possible also in high-risk surgical patients. Selection of patients is made mainly on the basis of ultrasound examination. The procedure is feasible in institution where a team consisting of an interventional radiologist, ultrasound expert and endoscopist can be set up.

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**Prevalence and Determinants of Exocrine Pancreatic Insufficiency Among Older Adults: Results of a Population Based Study**  
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**Background:** Data describing the burden of exocrine pancreatic insufficiency in the general population are sparse. We investigated the prevalence and main determinants of exocrine pancreatic insufficiency in a large population based sample of older adults by measuring fecal pancreatic elastase-1 concentrations (FEC).

**Patients and Methods:** This study was carried out in the context of the baseline examination of a large scale population-based cohort study which was conducted 2000–2002 in the State of Saarland, Germany. 9,961 participants aged 50 to 75 years were recruited by their general practitioner in the context of a general health examination. Patients and physicians were asked to fill out a standardized questionnaire. Stool samples were mailed to the study centre for laboratory analyses. FEC were analysed with a commercially available ELISA (ScheBo® Tech, Giessen, Germany) in a random sample of 914 participants.

**Results:** 525 women and 389 men, mean age 61.9 years (50–75), were included. 105 subjects (11.5%) showed signs of exocrine pancreatic insufficiency (EPI, FEC ≤ 200 μg/g), 47 (5.1%) had severe exocrine pancreatic insufficiency (SEPI, FEC < 100 μg/g stool). The prevalence was higher in males compared to females (EPI 13.6% vs. 9.9%, p = 0.08), EPI increased with age from 6.0% (50–54 years) up to 15.5% (65–69 years) and 13.4% (70–75 years) (p = 0.005 after adjustment for gender). Smoking was associated with a higher prevalence of EPI, while there was no correlation to alcohol consumption, gallstone disease or diabetes mellitus. Patients on ACE-inhibitors had a lower prevalence of EPI.

**Conclusions:** The prevalence of EPI may be much higher in the general population than previously estimated. The prevalence increases with age and seems to be tentatively higher in men compared to women. Smoking seems to be an independent risk factor and ACE-inhibitor intake might be protective. The findings may point to new avenues for concepts of chronic pancreatitis.
Quality of Life and Nutritional State Measurements Following Endoscopic and ESWL Therapy in Patients with Chronic Pancreatitis

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Aim of Study: Most evaluations in chronic pancreatitis aim at measuring pain, morbidity and mortality. In our study the QOL and NS changes were the center of attention following ESWL and endoscopic stenting in chronic pancreatitis in order to compare the effectiveness of these therapeutic modalities.

Methods: QOL was tested using EORTC QLQ C30 and PAN 26 questionnaires. These were developed originally for pancreatic cancer but in several clinical studies their suitability was evidenced for chronic pancreatitis. The questions reflect on clinical symptoms, general physical and psychological state. NS evaluation was done by using standard parameters like bodyweight, height, upper-arm circumference, waist-, hip circumference and triceps skin fold.

Patients and Results: 82 patients were measured altogether, 29 following ESWL and 53 after endoscopic stenting. Average age was 51.2 years (39–61). Follow up time was 1.2 years (1 month–2.5 years). Increase in body weight was 1.2kg, return to working activity was 82%. There was significant improvement in the pain-, general physical state-, daily activity-, digestive functions-, and fatigue-scores.

Conclusions: NS and QOL measurements are important tools in the evaluation of the effectivity and comparison of the different treatment modalities. There was significant improvement in QOL and NS after both ESWL and Endoscopic stenting, but in comparison of the two groups during the same follow up period no significant differences were found.

Reliability and Validity of the QLQ-C30 and PAN26 as Quality of Life Instruments in Patients with Chronic Pancreatitis

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Aim: To evaluate the validity and reliability of quality of life questionnaires QLQ-C30 and PAN26 in patients with chronic pancreatitis.

Patients and Methods: Between Jan 2002 and Dec 2003 health related quality of life (HRQoL) in patients with chronic pancreatitis was assessed by EORTC Quality of life-questionnaires C-30 and PAN26. At all, 301 patients completed the questionnaires (252 male, 83.7%). All patients had chronic pancreatitis due to alcohol abuse.

Internal consistency of the component scales of the questionnaires was assessed using Cronbach’s alpha. Validity of the questionnaires was assessed using a modified multitrait-multimethod-matrix.

Results: Apart from the jaundice scale, all component scales met accepted standards for internal consistency of EORTC modules, with a Cronbach’s Alpha coefficient of >0.60. Several scales of the QLQ-C30/PAN26 showed moderate to high correlation (0.6–0.8) with other related scales, whereas conceptually distinct scales exhibited low correlation of <0.4. The convergent validity of the two pain scales from the C30 (PA) and PAN26 (PP) is high (0.79), however this is not 1, suggesting that these scales are measuring some different aspects of pain and pain perception.

Summary: Reliability and validity of QLQ-C30/PAN26 was demonstrated. Both instruments may serve as standards for the assessment of HRQoL in patients with chronic pancreatitis.

Risk Factors of Intra-Abdominal Morbidity after Distal Pancreatectomy: A Multicenter Study

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Objective: To analyse short-term outcome after distal pancreatectomy and to determine risk factors of intra-abdominal complications.

Methods: This prospective multicentric study included 82 patients undergoing distal pancreatectomy with splenic preservation in 6 cases (7%). The diagnoses included pancreatic adenocarcinoma (n = 11), neuroendocrine tumors (n = 19), benign neoplasm (n = 27), pseudocyst (n = 5), chronic pancreatitis (n = 2), gastric cancer (n = 5), others diagnoses (n = 13). Analysed factors were age, gender, body mass index (BMI), diabetes, type of pathology, site of the initial lesion, texture of the pancreatic remnant parenchyma, the main pancreatic duct diameter, extension of the resection to nearby organs, splenic preservation, the technique of closure of the pancreatic stump, reinforcement of the closure or ductal occlusion with fibrin glue, postoperative octreotid, drainage.

Results: The median duration of the postoperative hospital stay was 11 days (range 5 to 155 days). Five patients (6%) died postoperatively. 15 patients (18%) had one or more intraabdominal complication with a reoperation in 5 cases (6%): 8 pancreatic fistula (10%), 14 intraabdominal collections (17%), 2 postoperative hemorrhages (2%). Multivariate analysis showed that a BMI > 25 kg/m² was the only independent risk factor for intraabdominal complication (OR = 8.1; p = 0.009; IC: 1.6–39.4).

Conclusions: Distal pancreatectomy is associated with an intra-abdominal morbidity rate of 18% which is significantly elevated for a BMI > 25 kg/m². Morbidity is unrelated with the texture and the technique of closure of the pancreatic remnant.
Serum Interleukin-4 and TNF-Alpha at the Patients with Chronic Pancreatitis
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Introduction: Proinflammmatory and antinflammmatory cytokines are involed in the pathogenesis of chronic pancreatitis.

Aims: To measure the serial levels of TNF-alpha and IL-4 at the patients with alcoholic and relapse forms chronic pancreatitis of depending on severity of an aggravation.

Methods: 112 consecutive patients with chronic pancreatitis admitted to the Emergency Hospital (32 patients with alcoholic pancreatitis and 80 – with relapse chronic pancreatitis). Severity of aggravation was determined according to the Atlanta criteria.

Serum levels of TNF-alpha and IL-4 by immunoenzyme multiplied were determined.

Results: In group of the patients with chronic alcoholic pancreatitis with severe aggravation an the high levels of TNF-alpha (638.64 ± 126.55 pg/ml) and IL-4 (57.18 ± 28.9 pg/ml) were marked with the subsequent consecutive decrease of with mild aggravation (TNF-alpha – 173.54 ± 40.15 pg/ml, IL-4 – 2.42 ± 14.84 pg/ml) and with easy aggravation (TNF-alpha – 80 pg/ml, IL-4 – 13.9 pg/ml).

At the patients with relapse chronic pancreatitis were marked the other parameters levels of cytokines: with severe aggravation (TNF-alpha – 529.63 ± 88.19 pg/ml, IL-4 – 268.49 ± 81.06 pg/ml) with the mild aggravation (TNF-alpha – 184.97 ± 22.30 pg/ml, IL-4 – 22.38 ± 5.22 pg/ml), and decrease at the easy aggravation (TNF-alpha – 74.33 ± 12.01 pg/ml, IL-4 – 42.84 ± 12.98 pg/ml).

Discussion: The received results have allowed to establish significant amplification production of TNF-alpha proportionally the severity of an aggravation and have shown, that it can is a reliable marker of a degree of severity of an aggravation.

The parameters of IL-4 also can be important in definition of a degree of severity, and its parameters authentically are higher at the patients with chronic alcoholic pancreatitis.

Surgical Treatment in Pancreatic Ascites without a Direct Approach to the Duct Disruption
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Introduction: Pancreatic ascites is an uncommon but well-recognized complication of chronic pancreatitis. Surgical treatment (either resection or permanent internal drainage) addresses the duct disruption, or the ruptured pseudocyst.

Case Report: A 51 years old male patient with chronic alcoholic pancreatitis was admitted with well-documented pancreatic ascites. After 3 weeks of unsuccessful conservative therapy, a severe duct disruption in the pancreatic neck area was shown by endoscopic retrograde colangiopancreatography (ERCP). Endoscopy failed to place a transpapillary stent across the leakage site (the guide-wire went out through the duct disruption and failed to cannulate the pancreatic duct to the tail). Post-ERCP-pancreatitis develops, ascitic fluid became bloody and surgery was needed. At laparotomy, a 4 cm acute pseudo-cyst in the hilus of the spleen was found, with little, but continuous hemorrhage, which imposed a distal pancreactectomy with splenectomy. Because we could not find the leakage site, situated on the posterior wall of the pancreatic neck, we used a 6 F tube (with multiples holes and guide-wire) to cannulate the 2 mm main pancreatic duct across the leakage site to the duodenum. The tube was brought out to the abdominal wall through the Roux-en-Y loop of a pancreatico-jejunosotomy and was maintained for 2 weeks. Postoperative pancreatectomy showed no leakage of the pancreatic duct. The patient recovered uneventfully, without recurrence of ascites (3 years follow-up).

Conclusion: Our procedure implies a minimal resection of the tail (with preservation of the spleen if possible) and a pancreatico-jejunosotomy with antegrade pancreatic duct stenting. Patients with pancreatic ascites, no dilated pancreatic ducts and a duct disruption in the head, neck, or body area which can not be localized, will benefit from our procedure, in order to avoid an extensive resection (usually followed by pancreatic insufficiency) or an external drainage which will transform pancreatic ascites into a pancreatic fistula.

The 282 C>T Polymorphism of the N-Acetyltransferase-2 (NAT2) Gene is Associated with Alcoholic Chronic Pancreatitis
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Background: The polymorphic NAT2 is responsible for slow or rapid acetylation of arylamine and hydrazine drugs and able to bioactivate several known carcinogens. The slow acetylator phenotype and genotype has been associated with a higher risk for several malignancies. Recently, an association of slow acetylator genotypes with acute alcoholic pancreatitis has been reported. The aim of our study was to investigate the relation between polymorphisms of the NAT2 gene and alcoholic chronic pancreatitis (ACP).

Patients and Methods: After obtaining informed consent, whole blood was obtained from 90 patients with ACP (63 male, 27 female, median age 55 (29–81) years). After DNA isolation, genotyping was performed by means of restriction fragment length polymorphism (RFLP) analysis for the polymorphisms 282 C>T (FokI), 481 C>T (Kpnl), 590 G>A (TaqI), and 803 A>G (DdeI). The 341 T>C polymorphism was analyzed using allele-specific polymerase chain reaction techniques. Results were compared with data from 95 healthy blood donors. Association analysis was performed using contingency table analysis and the chi-square test for allele frequencies of the individual polymorphisms.
Results: A significant difference was found in the frequency of the 282 C > T polymorphism between ACP patients and controls. The relative frequency of the mutant allele was 39.6% in ACP patients vs. 24.1% in controls (p < 0.01). The differences between the other NAT polymorphisms did not reach statistical significance.

Conclusions: We observed an association between the NAT2 282 C > T polymorphism and ACP. An increased number of slow acetylator alleles among ACP patients may represent an additional genetic risk factor for the development of ACP.

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The Assessment of Chronic Pancreatitis in Children – Own Experiences
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Chronic pancreatitis in children is rather rare condition, but if it’ll happen may be difficult for treatment. In childhood it’s characterized by many attacks of acute episodes, therefore could become life threatening states.

The aim of the study was the analysis of pancreatitis etiology, clinical course, treatment and complications in children.

15 children (aged 3–17) hospitalized because symptoms of acute episode of pancreatitis were analyzed. They were assessed by ultrasound and CT examination. After the acute symptoms were withdrawn endoscopic retrograde cholangiopancreatography (ERCP) was performed in all patients.

The analysis of the children suffered from chronic pancreatitis showed the following results: hypercalcemia (one case), hyperlipidemia (one case), CF (two cases), hereditary pancreatitis (4 cases), deposits in the Vater’s papilla (2 cases), congenital anomalies (4 cases) and idiopathic (one case) as a etiological factors were detected. The clinical course of the disease was rather moderate except the sometimes dramatic acute attacks. ERCP was failed in two children who underwent surgery. In the rest of children a lot of pathology like intraductal sludge, calcified deposits, cysts, congenital anomalies were showed. The endoscopic (ERCP) treatment was successful in most of the children at least immediately. After the ERCP procedures some complications like liver abscess, cholangitis and secondary acute pancreatitis occurred in three cases. In some children ERCP prevented the subsequent attacks of acute episodes in the following observations.

Conclusions: Etiological agents of chronic pancreatitis in children are very diversified. ERCP is a very useful technique for diagnosis and treatment of chronic pancreatitis in children.

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The Clinical Course of Chronic Pancreatitis Associated with Anatomic Anomalies in Children
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Background: The etiology of chronic pancreatitis (CP) in children is varied and includes gene mutations, anatomic anomalies, metabolic disorders and others. The reported pediatric experience with CP is small and the role of anatomic anomalies is not well elucidated. The aim of this study was to evaluate the role of anatomic anomalies of pancreatic duct (PD) as a cause of chronic pancreatitis in children.

Methods: 90 children with CP, hospitalized since 1995 to 2002, were enrolled into the study. The medical records of these patients were reviewed for data on the presentation, diagnostic findings and endoscopic treatment.

Results: Anatomic anomalies were found in 14 patients (15.5%) (5 girls and 9 boys; mean age 9.3 years, range: 3.6–14.2 years). We detected pancreas divisum in 11 patients, ansa pancreatica in 2 patients, one patient had two pancreatic ducts. In 5 patients with pancreas divisum we found gene mutations (CFTR-deltaF508/-, PRSS1-R122H/-, R122C/- and SPINK1-N34S/N34S); hyperlipidemia was found in two other patients. There was no difference in age of the disease onset between anatomic anomalies (AA group) and non-anatomic anomalies group (NAA group) (8.2 years vs. 8.7 years, NS). In all children ERCP had evidence of CP (5 moderate and 9 severe- according to the Cambridge Classification System, mean 2.50 Cambridge grade vs. 1.90 in NAA group, p < 0.05). Therapeutic intervention, including both surgical and endoscopic intervention, was more frequent in the AA group (78.5% vs. 54%; p < 0.05). Pancreatic duct stenting was done in 9 children (64.3% vs. 30% in NAA group; p < 0.05).

Conclusions: (1) CP associated with anatomic anomalies in children has worse clinical course than CP in NAA group. (2) In the CP group pancreas divisum is 2 times more frequent than in normal population. (3) We should be aware of coexisting anatomic anomalies of PD and other factors causing CP, as gene mutations.

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Treatment of Pancreaticopleural Fistulas – The Role of Nasojejunal Nutrition
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Background: Pancreaticopleural fistula is a rare but serious complication of chronic or recurrent inflammatory pancreatic disease. Increasing number of data have been published recently in the literature regarding the good results of nasojejunal feeding in the treatment...
of acute pancreatitis for reducing septic complications. The aim of this retrospective study was to evaluate the role of enteral nutrition in therapeutic strategies of internal pancreatic fistulas.

**Patients, Materials and Methods:** In the past decade, authors treated 10 patients with pancreaticopleural fistulas. Seven of the patients had a previous history of inflammatory pancreatic disease. Diagnosis was made by finding a markedly elevated amylase level in the pleural fluid. Pancreaticopleural fistula was successfully demonstrated by ERCP in six patients. Initial treatment was non-operative in each case. In the first part of the period (6 pts) conservative treatment contained total parenteral nutrition (TPN). Whereas later on four patients received nasojejunal nutrition (NJJ). Anti-secretary octreotide therapy and multiple thoracocentesis or thoracic drainage were used in all patients.

**Results:** Conservative treatment was successful in three patients (3/10). One in the TPN group and two in the NJN group. Septic complication occurred only in the TPN group, in one case. Unsuccessful medical therapy or septic complication recommended surgical intervention. Decompression procedure (3 pts) or distal resection (4 pts) were performed based on the location of the fistula. Surgery was successful in all seven patients. No patients were lost related to pancreaticopleural fistulas, and none of them required subsequent surgical treatment.

**Conclusions:** Authors suggest nasojejunal nutrition in the conservative treatment of pancreaticopleural fistulas. In case of septic complication or persistent fistula despite of non-operative therapy after two weeks surgical procedure is highly recommended.

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**Tumor Necrosis Factor Alpha (TNF-α) and its Receptors: TNF-αRI and TNF-αRII Plasma Concentrations are Elevated in Patients with Chronic Pancreatitis**

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The exact immunologic mechanisms underlying chronic pancreatitis (CP) are not clearly identified yet. The role of TNF-α in CP has been evaluated only rarely. The possible pathogenetic role of the cytotoxic TNF-α signal transduction pathway in the clinical course of CP has been recently suggested.

The aim of the study was to assess the plasma concentration of TNF-α as well as its receptors: TNF-αRI and TNF-αRII in patients with CP of different clinical stage.

TNF-α, TNF-αRI and TNF-αRII plasma concentrations have been measured with enzyme-linked immunosorbent assay (ELISA) in 39 patients with CP as well as in age-matched healthy volunteers. The percentage of smokers was not different in CP and the control group. The CP stage has been classified according to Cambridge scoring system. The correlation between above mentioned parameters and clinical data, as: CP etiology, disease duration and comitant diabetes has been assessed.

The mean TNF-α concentration in patient with CP was 24.6 pg/ml ± 15.07 which was significantly higher (p < 0.01) than in control group – 11.3 pg/ml ± 2.01. The mean values of TNF-α receptors concentrations: TNF-αRI and TNF-αRII have also been significantly higher (p < 0.01) in patients with CP, compared to controls. No significant differences in TNF-α, TNF-αRI, TNF-αRII values dependently on patient alcohol consumption, diabetes and the disease duration have been found. However, the significantly higher TNF-α and TNF-αRII concentration (p < 0.01) has been observed in patients with Cambridge IV stage compared to patients of all other stages. We conclude that TNF-α signal transduction pathway may play a significant role in chronic pancreatitis, particularly of advanced clinical stage.

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**Miscellaneous**

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**A Novel Basolateral HCO3⁻ Transport Mechanism in Human Pancreatic Duct Cells**

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**Background:** Pancreatic duct cells secrete the HCO3⁻ found in pancreatic juice. This HCO3⁻ secretion occurs in two stages. First, HCO3⁻ ions are accumulated across the basolateral membrane of pancreatic duct cells then exit the cell to the ductal lumen. Previously, basolateral HCO3⁻ transport has been studied mainly in rat and guinea pig duct cells, only few data are available for the human pancreas. The aim of this study was to characterize basolateral HCO3⁻ transport mechanisms in CFPAC-1 human pancreatic duct cells (derived from a patient stably expressing the AF508 cystic fibrosis transmembrane conductance regulator mutation).

**Methods:** To prepare polarised monolayers, CFPAC-1 cells were seeded at high density onto permeable supports and grown to confluence. The cells were loaded with the pH sensitive fluorescent dye BCECF, and mounted in a special perfusion chamber that allowed the simultaneous perfusion of different solutions to the basolateral and apical membranes. Basolateral HCO3⁻ transport activity was measured from intracellular pH changes as described earlier (Hegyi et al., Am J Physiol 2003;285:C268–76).

**Results:** Besides the Na⁺/H⁺ exchanger, we have identified a novel Na⁺/Cl⁻ independent electrogenic HCO3⁻ influx mechanism on the basolateral side of CFPAC-1 cells. Basolateral HCO3⁻ flux was partially blocked by the carbonic anhydrase inhibitor acetazolamide, the Cl⁻ channel inhibitor NPPB and was also sensitive to alterations in extracellular K⁺ concentration. The transporter was insensitive to temperature (22 vs 37°C), to inhibitors of the vesicular type H⁺-ATPase (concanamycin), anion transport (H⁺-DIDS), Cl⁻ channels [glybenclamide (p = 0.06),...
Conclusion: The novel transporter has characteristics of an anion channel and indicates that current models of human HCO$_3^-$ transport need to be modified.

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Cholecystokinin Receptor-1 and -2: Two Pancreatic Receptors which can Display Modified Behaviour when Co-Expressed
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In the human pancreas, two cholecystokinin receptors coupled to G-proteins bind cholecystokinin (CCK): the CCK1-R and CCK2-R. CCK2-R binds gastrin too. In human pancreatic adenocarcinoma, these receptors are co-expressed whereas only CCK2-R is present in healthy exocrine pancreas. Moreover, in ElasCCK2 transgenic mice where CCK2-R is co-expressed with endogenous CCK1-R in pancreas, 15% of specimens develop tumours. This work aims at studying if CCK-receptors co-expression could induce a new signal. COS-7 cells were used for analysing PLC and ERK1/2 activation in a context of co-expression of the receptors. Binding experiments enabled us to estimate the CCK1-R:CCK2-R proportion, and so, to obtain a ratio close to that observed in ElasCCK2 mice pancreas (5:1). In order to investigate potential receptor interaction, activity of CCK1-R was modulated by adding its specific antagonist (SR27897), or by using an inactive CCK1-R obtained by mutation in the NPXXY motif.

Whereas SR27897 antagonist did not change CCK2-R activity, its use in the co-expression context reduced potency of CCK to activate PLC through the CCK2-R without disturbing its efficacy. On the contrary, gastrin potency on the CCK2-R was not affected but its efficacy was decreased by 60%. Similar results were observed by co-expressing the inactive CCK1-R with the inactive CCK2-R. Moreover, reduction of the efficacy of CCK was noticed. In contrast to PLC activation, ERK1/2 activation profiles were not affected in the co-expression context.

This suggests that the inactive CCK1-R could act like a dominant negative receptor sequestrating the G-proteins, as we previously demonstrated for inactive CCK2-R (JBC Gales et al. 2000); that when CCK1-R and CCK2-R are co-expressed, they directly interact. In light to recent reports, interaction might involve heterodimerization of the two receptors. Blocking the CCK1-R by a specific antagonist may affect biological function mediated by the CCK2-R.

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Expression of Trypsinogen Isoforms in the Stimulated Mouse Pancreas
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Trypsin is thought to play a critical role in the initial phase of acute pancreatitis. Various genes have been reported to encode human and murine trypsingens and three human isoforms are expressed at the protein level (PRSS1, PRSS2, mesotrypsinogen). While the mouse is frequently used to investigate pancreatitis in experimental models, the murine trypsinogen isoenzyme pattern is unknown. Using a proteomic approach, we studied trypsinogen isofrom expression patterns in mouse pancreas from controls and after induction of experimental pancreatitis.

Pancreas of NMRI mice was obtained from unstimulated (saline injections) and caerulein-treated (7 hourly i.p. injections of 50 μg caerulein/kg bw), and the secretory granule fraction was enriched by differential centrifugation. 2D-PAGE was performed on these protein extracts. After silver staining protein spots were excised, proteolytically cleaved and the resulting peptides analysed by MALDI-TOF mass spectrometry. Identification of proteins was performed using NCBI Protein databases. Trypsin activity was determined spectrophotometrically using the colorimetric substrate Z-GPR-pNA.

We detected several protein spots that corresponded to trypsinogen isoforms. According to molecular mass, pl and peptide fingerprinting these proteins match trypsinogen 4/5, 7, 8, and 20. Trypsinogens 20 (PRSS2) and 8 were found as multiple spots of comparable masses, but of different pl. Trypsinogen 4/5 is practically absent from the pancreas in control animals, but is strongly expressed after supramaximal caerulein stimulation and reaches ~10–20% of the total trypsinogen content. Titration of trypsin activity in enterokinase-activated homogenates with soybean trypsin inhibitor showed that supramaximal caerulein stimulation induced a trypsin activity with lowered inhibitor sensitivity.

Our results demonstrate that the mouse also expresses several trypsinogen isoforms in the pancreas, and an additional cationic form that is different from the human. Hormonal stimulation induces a trypsinogen isoform, which has decreased inhibitor sensitivity, a property also observed with human mesotrypsin or rat trypsin 4.
Fluid Secretion Driven by Cl⁻ and HCO₃⁻ Transport in Ducts Isolated from the Pancreas of CFTR Null Mice

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Genetically modified mouse models of cystic fibrosis (CF) do not show the severe pancreatic alterations that characterize CF in humans. In spite of the complete loss of CFTR function, the pancreatic ducts of CF mice still secrete fluid, albeit at a much reduced rate. Our aim was to analyse whether this residual fluid secretion was driven by Cl⁻ and/or by HCO₃⁻ transport and to investigate its regulation by cAMP and Ca²⁺-mediated agonists. Fluid secretion was studied in isolated pancreatic duct fragments from control or CFTR null mice by measuring duct swelling using digital videomicroscopy. In an incubation medium containing both HCO₃⁻ and Cl⁻, CF ducts secreted fluid in response to forskolin, attaining a secretory rate of 91 ± 5 pl mm⁻² min⁻¹ under the same conditions. Carbachol also elicited a significant secretory response but, unlike the response to forskolin, this was transient in spite of the continuous presence of the agonist. CF ducts secreted 150 ± 18 pl mm⁻² min⁻¹ immediately after carbachol stimulation compared to 125 ± 48 pl mm⁻² min⁻¹ in control ducts. The secretory responses to forskolin and carbachol in ducts from CF mice were only partially inhibited by bumetanide, thus demonstrating the likely contribution of a HCO₃⁻-driven component of fluid secretion. To analyse the Cl⁻-driven component, experiments were repeated in a HCO₃⁻-free medium. Under these conditions, both forskolin and carbachol significantly stimulated secretion in ducts from CF mice (85 ± 7 and 213 ± 26 pl mm⁻² min⁻¹ respectively). We conclude that murine pancreatic ducts can secrete fluid in response to forskolin and to carbachol by a mechanism that is independent of CFTR function. This secretory response is driven by both HCO₃⁻ and Cl⁻ transport.

Endotherapy of Pancreatic Fistulas

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The pancreatico-cutaneous fistulas are rare but challenging complications of surgery or interventional therapy. In the last 5 years we have treated 24 patients (4 females, 20 males, mean age 54.9 yrs, range 33–81 yrs) in our institutions. Before endoscopic therapy a mean of 1.9 (0–4) pancreatic operations were performed: 11 necrectomies (2 repeated cases), 2 distal resections, 7 splenectomies, percutaneous drainage and/or lavage of fluid collections and abscesses (11 cases), cholecystectomy (4 cases), choledochoduodenostomy (1 case), excision of the fistula (1 case), choledocho-duodenostomy (1 case). The complex endoscopic therapy of the fistula consisted of jejunal feeding (24 pts), pancreatic (12) and/or biliary papillotomy (9) combined with endopancreatic (11) + endobiliary (4 pts) and/or nasopancreatic (10) + nasobiliary (2) drainages followed by Sandostatin treatment (7) and/or endoscopic cystogastrostomy (2), cystoduodenostomy (2) with nasocystic drainage (3) and/or extracorporeal shockwave lithotripsy (1). A mean of 2.33 (1–8) endotherapies were performed in the different cases. Sixteen patients (66.7%) healed, 8 patients were operated on again. In 2 cases distal resection with splenectomy became necessary because of a large disruption of the pancreatic duct. In an other patient with biliary pancreatitis repeated necrectomies with splenectomy and Hartmann operation resulted in a pancreatico-cutaneous fistula with periopancreatic fluid collection, a sigmoidostomy and retained gall-bladder with stones all indicating further surgery. Jejunal feeding with biliary and pancreatic papillotomy and temporary endopancreatic drainage as well as Sandostatin treatment significantly diminished the output of the fistula but it has not closed totally. After a 2-month recovery period he underwent reconstructive surgery. Another patient needed cholecystectomy with pseudosyst decompression. Four cases underwent operation because of sepsis.

Conclusion: Endotherapy of pancreatico-cutaneous fistulas is feasible but a combination of difficult techniques may be necessary for healing of pancreatic leakage. In several cases further surgery is inevitable later on but in a better condition of the patient.

A Membrane Permeable Protein Kinase C Inhibitor Reverses the Inhibitory Effects of Substance P and Phorbol Ester on Pancreatic Ductal Bicarbonate Secretion in the Guinea Pig

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The regulatory pathways that stimulate pancreatic ducal HCO₃⁻ secretion are well described, however, less is known about inhibitory pathways. Inhibitory pathways may be important in terms of limiting the hydrostatic pressure within the ducts (so preventing leakage of enzymes into the parenchyma of the gland), and in terms of switching off pancreatic secretion after a meal. Substance P (SP) inhibits secretin-stimulated HCO₃⁻ secretion by inhibiting an apical Cl⁻/HCO₃⁻ exchanger in the duct cell. The aim of this study was to investigate a role of protein kinase C (PKC) in the action of SP.

Methods: Small intra/interlobular pancreatic ducts were isolated from guinea pigs. The rate of HCO₃⁻ secretion (base efflux -J(B-)) was determined from the buffering capacity of the cells and the initial rate of intracellular acidification (1) after sudden blockage of basolateral base loaders with diisothiocyanatostilbene-disulfonic acid (100 µM) and amiloride (200 µM), and (2) after alkali loading the ducts by exposure to NH₄Cl. All the experiments were performed in HCO₃⁻ buffered Ringer at 37°C (n = 6 ducts for each experimental condition).
Results: Secretin (10nM) elevated the basal J(B-) about 3-fold and this effect was totally inhibited by SP (20nM). Phorbol 12, 13-dibutyrate (PDBu, 100nM), an activator of PKC, reduced the basal J(B-) by 38%, and completely blocked secretin-stimulated J(B-). Bisindolylmaleimide, a membrane permeable PKC inhibitor (BIS, 1μM) blocked the inhibitory effect of SP and PDBu. In addition, BIS (1, 30, 300, 1000nM) caused a dose-dependent block of SP's inhibitory effect.

Conclusions: We clearly demonstrate that the PKC inhibitor (BIS) reverses the inhibitory effects of SP and PDBu on pancreatic ductal bicarbonate secretion. Therefore, we conclude that SP exerts its inhibitory effect on pancreatic duct cells by activating PKC.

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Basolateral NBC1 and NHE1 Regulate Intracellular pH in Capan-1 Cells
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Aim: Pancreatic ductal cells secrete a bicarbonate-rich fluid, which is important for duodenal neutralisation of gastric acid. The present study sought to identify the transporters that maintain intracellular pH (pHi) during bicarbonate secretion.

Methods: The expression of Na⁺-HCO₃⁻ cotransporters (NBC1 and 4a), Na⁺-H⁺ exchangers (NHE1 and 4) and CFTR was compared in normal human pancreas and in cultured Capan-1 human pancreatic ductal cells. Polarised monolayers of Capan-1 cells were grown on Transwell-COL PTFE membranes. The transporters responsible for maintaining pHi were investigated using the NH₄Cl pulse technique to acid load the cells. Intracellular pH was monitored by microfluorometry using BCECF, a pH-sensitive fluorochrome. The activity of the transporters was calculated from the initial rate of recovery of pHi in the presence and absence of selective blockers: DIDS for inhibition of the Na⁺-HCO₃⁻ cotransporter (NBC) and amiloride for inhibition of the Na⁺-H⁺ exchanger (NHE).

Results: By RT-PCR we found that NBC1, NBC4a, NHE1 and CFTR were expressed both in normal human pancreas and in Capan-1 cells. In functional studies the initial rate of recovery of pHi (0.0013 ± 0.0004dpH/min) following the NH₄Cl pulse was almost completely inhibited when amiloride (0.3mM) and DIDS (0.1mM) were applied to the basolateral side of the cells (0.0002 ± 0.0001dpH/min, p < 0.01). Separate application of the inhibitors resulted in a weaker inhibition of the recovery rate. When DIDS and amiloride were added to the luminal side, no inhibition was observed.

Conclusions: According to our results the NBC and NHE transporters are present in both normal human pancreas and in Capan-1 cells. In Capan-1 cells they are located exclusively at the basolateral membrane and are therefore likely to contribute to the supply of bicarbonate ions during secretion.

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Biodegradable Biliary Stent for the Hepaticeo-Jejunal Anastomosis: A Long-Term Follow-up Study in a Big Animal Model
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Introduction: In Whipple operations with non-dilated bile ducts (BD), e.g. with small non-ampullary tumours such as endocrine and uncinate pancreatic and duodenal tumours, there is an increased risk for HJ complications. A biodegradable biliary stent (BDBS) could be one solution, of which previously early results have been encouraging. The aim of this study was to investigate the use of BDBS with longer follow-up.

Materials: The braided, self-reinforced, gamma-sterilised, polylactide-barium sulphate BDBSs were used in this study with 50 Yorkshire pigs (weight 54.0 ± 1.3 kg). Cholecystectomy plus Roux-Y hepaticeo-jejunosotomy were performed with (Group 1) or without (Group 2) the BDBS in the anastomosis. The pigs were followed by repeated 99mTc dynamic biligraphy (DBG), abdominal X-ray and blood and serum determinations at 2, 6, 12, 26, 52 and 78 weeks, and sacrificed at 6, 12, 26, 52 and 78 weeks (5 animals in each time group) when the anastomosis was measured and histological analysis performed.

Results: During the follow-up time the pigs did not differ in the weight gaining, blood haemoglobin or leukocyte counts, or serum liver function tests. One control animal died because of HJ anastomosis leakage (NS difference). In the X-ray the BDBS was seen open and in situ immediately p.o. in all, at 2 weeks in 25/25 and at and after 6 weeks in 0/25 of the BDBS pigs. In DBG the liver clearance (LC) 15, 30, 45 and 60min after the 99mTc injection was significantly reduced in Group 2 at 12, 26, 52 and 78 weeks compared to the preoperative clearance (p < 0.05), whereas in Group 1 (BDBS pigs) the LC did not differ significantly from the preoperative LC during the follow-up time. The width of the HJ anastomosis increased from 3.2 ± 0.2 mm and 3.5 ± 0.3 mm at 6 weeks to 7.4 ± 0.5 and 6.3 ± 0.9 mm at 78 weeks in Group 1 (p < 0.05) and Group 2 (p < 0.05), respectively (mean ± SEM), without significant difference between the groups. Histological determinations did not differ between the groups.

Conclusions: This novel BDBS improves biliary drainage, manifest as improved liver clearance, during the 78-week follow-up and thus seems promising for HJ performed in non-dilated BDs, e.g. after Whipple for small non-ampullary tumours such as endocrine and uncinate pancreatic and duodenal tumours.
Characterization of the Cholecystokinin CCK1 Receptor Agonist SR146131 in the Rat Pancreas in vivo

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Hyperstimulation of cholecystokinin CCK1 receptors by the CCK analogue cerulein (CRL) is the standard rat model for acute edematous pancreatitis. However, we have reported previously that CRL increases pancreatic blood flow via CCK2 receptors, which precludes conclusions about inflammation-related changes in tissue perfusion. SR146131, 2-[4-(4-chloro-2,5-dimethoxyphenyl)-5-(2-cyclohexyl-ethyl)-thiazol-2-ylcarbamoyl]-5,7-dimethyl-indol-1-yl-1-acetic acid ethanalamidic salt is a selective CCK1 receptor agonist (Bignon et al: J Pharmacol Exp Ther 1999;289:742–751, 752–761) and unlike previous compounds was believed to activate both high and low affinity CCK1 receptors. Therefore, we have investigated whether SR146131 could be used as a new experimental model to investigate acute pancreatitis without direct effects on tissue blood flow. Bolus i.p. injections of SR146131 (18–1,800 nmol/kg) in anesthetized Sprague-Dawley rats stimulated pancreatic enzyme secretion into the biliopancreatic duct in a dose-dependent manner. Effective doses were about 10-fold higher than those of CRL. Enzyme secretion was blocked by the CCK1 antagonist dextoxiglumide, but not by the CCK2 blocker iriglumide. However, in contrast to CRL, the effects of SR146131 did not decline at supramaximal levels but remained at maximum values even at i.p. doses up to 1,800 nmol/kg. While CRL induced pancreatitis at i.p. doses of 2.5 nmol/kg (2 × at a 1 h interval) and above, SR146131 was devoid of such actions since it did not elicit an inflammatory edema or increases in amylase activity in the blood serum or in the pancreatic tissue even at maximum doses of 2 × 1,800 nmol/kg. In conclusion, SR146131 does not induce pancreatitis even at supramaximal dose levels and thus apparently activates CCK1 receptors in vivo only in the high affinity state but not in the low affinity state.

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Chemical Designing of New Indolicin Aminoxyls Displaying Different Ability to Prevent Lipid and Protein Peroxidation in Pancreatic Subcellular Membranes

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Introduction: Reactive oxygen species – free radicals seems to play an important role in the development of acute pancreatitis (AP). Participation of lipid or protein peroxides in progression of AP is still unsolved problem. The aim of present study is to design and evaluate new aromatic aminoxyls having ability to be insert inside biological membrane and to inhibit selectively lipid or protein peroxidation.

Methods: IA-C2 namely 1,2-dihydro-2-ethyl-2-phenyl-3N-indole-3-phenylimino-1-oxyl and IA-C18, 1,2-dihydro-2-octadecyl-2-phenyl-3N-indole-3-phenylimino-1-oxyl were synthesised at University of Ancona Italy. Endoplasmatic reticulum plasma membrane were isolated from pancreatic postmitochondrial fraction of pancreatic homogenate.

Results: IA-C2 while added to pancreatic endoplasmatic reticulum membrane inhibited almost completely lipid peroxidation. Under the same conditions IA-C18 inhibited but slightly lipid peroxidation. In these conditions the degree of protein protection against oxidative stress showed a pattern opposite to that observed in lipid peroxidation system. ESR spectrum of IA-C2 and IA-C18 in organic solvent display well resolved hyperfine spectra. The presence of biological membrane changes the characteristics of respective aminoxyl spectra pointing to efficient membrane insertion of IA-C2, whereas almost complete broadening of IA-C18 indicated of strong immobilization and spin-spin interaction of aminoxyl in localized possibly membrane protein domains.

Conclusion: IA-C2 seems to be good candidate for substitute of vitamin E in biological membranes while IA-C18 will be a prototype of selective protein protecting molecule. Application of these aminoxyls in biological systems will allow to demonstrate the particular impact of lipid or protein peroxidation in acute pancreatitis.

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**Effect of Apoptosis-Inducing Agents on Caspase-3 Activity in Isolated Pancreatic Acinar Cells**

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**Background:** Mild acute pancreatitis has earlier been shown to be associated with extensive apoptotic acinar cell death while severe acute pancreatitis involves extensive acinar cell necrosis but very little acinar cell apoptosis. Our earlier work has shown that induction of the pancreatic acinar cell apoptosis in vivo by crambene (1-cyano-2-hydroxy-3-butene – CHB), a plant nitrile, has protective effect against pancreatic acinar cell apoptosis in vivo by crambene (1-cyano-2-hydroxy-3-butene – CHB), a plant nitrile, has protective effect against pancreatic acinar cell apoptosis in vivo. Crambene is a cytotoxic aspartate that has been implicated in the effector phase of apoptosis and is part of both intrinsic and extrinsic pathways of apoptosis. In the current study, we report the effect of three different inductors of apoptosis – crambene, caerulein, and menadione on caspase 3 activity in isolated pancreatic acini.

**Methods:** Mouse pancreatic acini were prepared by collagenase digestion. Acini were incubated with different concentrations of crambene, caerulein, and menadione for 0–6 h and cell lysate assayed for caspase 3 activity using the fluorophore Ac-DEVD-AFC as the substrate (Ext. 394 nm/Emm. 535 nm).

**Results:** Caspase 3 activity in acini treated with crambene, caerulein, and menadione was significantly higher than in untreated acini. Stimulation of caspase 3 activity in pancreatic acini was observed after 1 h incubation with menadione. Crambene and caerulein were able to stimulate caspase 3 activity only after a 3 h incubation.

**Conclusions:** Treatment of isolated pancreatic acini with crambene, caerulein, and menadione causes a stimulation of caspase 3 activity. The present study shows evidence of induction of apoptosis by crambene in vitro which would facilitate the study of the mechanism of pancreatic acinar cell apoptosis.

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**Effects of Caerulein and Melatonin on Heat Shock Protein 60 mRNA Signal in AR42J Cells**

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**Introduction:** Heat shock proteins (HSP) are involved in the regulation of inflammatory process and protection of the pancreas from the acute damage produced by caerulein overstimulation. However the effect of caerulein and melatonin on mRNA signal for HSP 60 in the pancreatic acinar cells is yet unknown.

**Aims:** (1) To investigate the gene expression for HSP 60 in the pancreatic AR42J cells stimulated by melatonin, caerulein and their combination. (2) To compare above changes with mRNA signals of HSP 60 in pancreatic AR42J cells subjected to heat shock.

**Methods:** AR42J cells were incubated in standart medium at 37°C for: 0, 1, 3, 5, 12 and 24h, under basal conditions, or in presence of caerulein (10⁻¹⁰⁻¹⁻⁰⁷M), melatonin (10⁻⁸−10⁻⁶M), or combination of above. AR42J cells were subjected to heat shock (48°C) for 0, 1 and 2 h. Gene expression for HSP 60 was determined by RT-PCR.

**Results:** The signal for HSP 60 has been observed in AR42J pancreatic cells under basal conditions. This signal was markedly and dose-dependently increased in AR42J caerulein and melatonin stimulation. The strongest signal was observed in the cells incubated with combination of caerulein (10⁻¹⁰⁻¹⁻⁰⁷M) and melatonin (10⁻⁸−10⁻⁶M). The signal for HSP 60 was markedly and time-dependently increased in AR42J cells subjected to heat shock.

**Conclusions:** (1) Gene expression for HSP 60 was detected in pancreatic AR42J cells under basal conditions. (2) Exposure of these cells to heat shock, caerulein or melatonin significantly increased this signal.

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**Experimental Obstructive Jaundice Causes an Early Upregulation of Uncoupling Protein 2 and Decreased ATP Content in Liver**

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**Background and Aims:** Obstructive jaundice impairs liver function with effects on liver energy metabolism. We have previously shown that uncoupling protein 2 (UCP2) in the liver is upregulated in experimental obstructive jaundice. Upregulation of UCP2 has been proposed to impair energy metabolism and thus increase liver vulnerability. The aim of the present study was to study the time course of...
liver UCP2 expression and contents of energy metabolites in experimental obstructive jaundice. 

**Methods:** There were three groups of animals. (1) Rats operated with bile duct ligation (BDL). (2) Rats shamoperated and then paired to the BDL rats (PF). (3) Rats shamoperated and then given food ad libitum (AL). Animals were sacrificed 2, 4, and 8 days postoperatively. UCP2 mRNA levels were determined in liver tissue with Northern blot. LiverATP content and plasma levels of glucose, insulin, and free fatty acids (FFA) were determined.

In BDL rats there was a two-fold increase of liver UCP2 mRNA levels 4 days postoperatively compared to both PF and AL rats. This difference increased progressively over time. At four days there was a significant decrease in Liver ATP levels in BDL rats compared to controls (3.31 ± 0.49 vs. 5.06 ± 0.53 mM/kg dw, P < 0.05). In BDL rats FFA increased already at two days. Plasma glucose levels were significantly decreased in the BDL group compared to PF and AL rats at four days (7.88 ± 0.64 vs. 11.84 ± 0.59 and 11.56 ± 0.41, P < 0.05). Plasma insulin concentrations were similar across groups. PF rats lost weight in the same magnitude as the BDL rats during the first four days but then gained weight at the end of the experimental period.

**Conclusions:** In experimental obstructive jaundice there is a marked and progressive upregulation of liver UCP2 and depletion of energy metabolites. 

This alteration is independent of reduced food intake, but may enhance lipid utilization in the catabolic state associated with obstructive jaundice.

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**Functional Comparison of Human Pancreatic Elastase 2 Isoenzymes**

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**Background:** Human pancreatic elastase 2 is the only protease with reported elastolytic activity in the pancreatic juice. According to available cDNA and genomic sequences, pro-elastase 2 has two distinct isoenzymes termed 2A and 2B. The two genes are located next to each other on chromosome 1, and expression of both isoforms at the mRNA level has been verified by cDNA cloning and numerous EST-sequences. To date, only elastase 2A has been purified from pancreatic juice. Pro-elastase 2A and 2B exhibit nearly 90% sequence identity, however, potentially important amino-acid differences are apparent in the substrate binding pocket.

**Objectives:** Our goal was to compare the two human elastase 2 isoforms with respect to activation, catalytic activity and substrate specificity.

**Methods:** Recombinant human pancreatic pro-elastase 2A and B were expressed in _Escherichia coli_, purified with affinity-chromatography, activated with trypsin and assayed with the fluorescent DQ-elastin substrate (Molecular Probes).

**Results:** Recombinant human pro-elastase 2A was readily activated by human trypsins and to a lesser degree by cathepsin B. Activated elastase 2A exhibited elastolytic activity on DQ-elastin. Surprisingly, despite the high degree of homology between the two isoforms, elastase 2B was devoid of detectable proteolytic activity. To identify the evolutionary mutations that inactivated elastase 2B, we have created several elastase 2B-specific amino-acid changes in elastase 2A by site-directed mutagenesis. We found three mutations, which independently or in concert inactivated elastase 2A.

**Conclusions:** The elastolytic activity of human pancreatic elastase 2B has been abolished by several evolutionary mutations. The observations raise the question whether elastase 2B is an expressed nonprocessed pseudogene product with no biological function or a novel digestive enzyme with yet unknown substrate specificity.

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**Hypoxia Induced Cytosolic Calcium Increase in Perfused Exocrine Rat Pancreas**

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**Aim:** Changes of intracellular calcium concentration [Ca]i in the exocrine pancreas can cause uncontrolled premature protease activation, which may play a major role in the pathogenesis of ischemia/reperfusion-injury (Lerch, M.M. and F.S. Gorelick: Med Clin North Am 2000;84(3):549–63). In previous fluorescence microscopic investigations performed at an isolated perfused rat pancreatic preparation we observed that the basic [Ca]i increase during hypoxia.

**Methods:** We applied Thapsigargin (2.5 μM), the mitochondrial protonophore CCCP (10 μM) or calcium-free perfusion medium 45 min prior and during hypoxia to check possible involvement of the ER, mitochondria or extracellular calcium, respectively.

**Results:** (1) Application of Thapsigargin and/or CCCP caused a transient increase in [Ca]i. (2) Under the prolonged application of Thapsigargin, CCCP and calcium-free solution there was no significant change in the hypoxia induced increase of [Ca]i.

**Discussion/Conclusion:** The results obtained from an isolated perfused rat pancreatic preparation did not provide evidence of an involvement of ER, mitochondria or extracellular calcium in hypoxia induced cytosolic calcium increase. Comparable experiments on isolated acini remain to be performed for further investigation of related mechanisms.

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**New Technical Method of Proximal Stump Closure After Distal Pancreatectomy**

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**Background:** The optimal management of the stump of the pancreas after distal pancreatectomy remains unresolved. The overall incidence of pancreatic fistula can reach even 20–30%. Neither the
Insulin Suppresses Somatostatin Expression in RIN-14B Cells through MAP Kinase Activation

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**Background:** In streptozotocin-diabetic rats, significant increases in somatostatin (SS) mRNA in SS islets contents were observed. These alterations were completely reversed upon insulin treatment with new increases after cessation of insulin. This study was undertaken to determine if the negative control of insulin on SS mRNA expression was direct, concentration and time dependent and influenced by glucose.

**Methods:** RIN-14B cells grown in 1640 medium were used throughout at around 70–80% confluency. The specificity of these cells for SS was established by confocal microscopy and SS mRNA estimated by RT-PCR.

**Results:** As indicated by confocal microscopy and specific antibodies, the RIN-14B cells are free of insulin and glucagon and loaded with SS. In these cells, SS mRNA expression is upregulated by increasing concentrations of glucose. Glucose at 30 mM is 92% more efficient than those at 2.8 and 5.6 mM. In 30 mM glucose, increasing concentrations of insulin gradually reduced SS mRNA expression within 4 h by 16% at 1 nM, up to 70% at 1 μM. This inhibitory effect is glucose independent as it also occurred at 2.8 mM glucose. Insulin’s effects are also time-dependent reaching maximal inhibition after 12 h of incubation, and glucose independent. Preincubation of the cells for 30 min with 20 μM LY294002 or 10 μM U0126, specific inhibitors of PI3-kinase and MEK, respectively, and then incubation with insulin 100 nM for 4 h in 30 mM glucose, indicates that the negative control of SS mRNA expression by insulin operates via the MEK pathway with only U0126 preventing inhibition by insulin.

**Conclusions:** The inhibitory effects of insulin on SS mRNA expression are concentration and time dependent, glucose concentration independent. Insulin seems to operate via the MEK pathway.

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Involvement of Sensory Nerves and Growth Hormone in the Pancreatic Protection Afforded by Ghrelin

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**Background:** Ghrelin, an orexogenic peptide isolated from the stomach, has been identified in gastrointestinal tissues and in the brain. Ghrelin protects the gastric mucosa from acute damage, stimulates growth hormone (GH) release and inhibits pancreatic exocrine and endocrine secretions. Ghrelin receptors has been detected in the pancreas but the role of ghrelin in the pancreatic protection has not been investigated.

**Aim:** To determine the effects of central and peripheral application of ghrelin on the course of caerulein-induced pancreatitis (CIP) and to investigate the involvement of sensory nerves (SN) and GH in above effects.

**Method:** The study was carried out on the rats with intact SN or capsaicin-deactivated SN. To deactivate SN capsaicin was given to one group of rats at total dose of 100 mg/kg 10 days before the tests. CIP was induced by subcutaneous caerulein infusion (25 μg/kg) to the conscious rats. Ghrelin was given to the rats intraperitoneally (i.p.) at doses of 10, 5, or 50 μg/kg, or into the right cerebral ventricle (i.c.v.) at doses of 1, 2.5 or 5 μg/kg, 30 min prior to the start of CIP. Plasma levels of ghrelin and GH were measured by RIA.

**Results:** CIP was confirmed by histological assessment and characterized by usual edema and rise of plasma amylase and lipase (by 500% and 800%, respectively). Ghrelin given i.p. at dose of 50 μg/kg increased plasma levels of GH and ghrelin and attenuated CIP. These effects were reversed in the rats with capsaicin-deactivated SN. Central application of ghrelin prevented from the CIP development and resulted in the dose-dependent rise of plasma GH, but not ghrelin. Deactivation of SN by capsaicin completely reversed above effects of icv ghrelin on the pancreas.

**Conclusions:** Ghrelin prevents from the development of acute pancreatitis though activation of central mechanisms including the GH release and sensory nerves activation.
Leptin Inhibits Pancreatic Exocrine Secretion in Anaesthetized Rats through Indirect Mechanisms

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Recent study have demonstrated that leptin may inhibit the CCK1-vagal afferent-dependent mechanism controlling the secretion of pancreatic juice in rats, the direct effect on pancreatic acini was doubtful (Matyjek et al. Regul Pept 2003;114:15–20). The aim of present study was to further investigate the mechanisms by which leptin may control the secretion of pancreatic juice. In the first study we investigated the influence of leptin on the insulo-acinar axis which is known to enhance pancreatic secretion. Stimulation was done with 2-deoxy-glucose (2DG, 0.25g/kg b. wt. per h) in continuous i.v. infusion. In the second study, we applied surgical fundectomy to remove major portion of gastric leptin thus allowing, after the recovery, to study pancreatic secretion in terms of reduction in gastric leptin pool. Anaesthetized Wistar male rats, 2DG infused (300 ± 27 g) or fundectomized (340 ± 18 g), were fitted with silicone catheters in the right external jugular vein and polyethylene tubing in the common pancreatic-biliary duct. Pancreatic-biliary juice (PBJ) was not introduced into the duodenum. Leptin was administered in i.v. boluses of 0, 0.1, 1 and 10 μg/kg b wt. Intravenous infusion of 2DG significantly increased the PBJ protein output but not PBJ volume. Intravenous leptin boluses reduced the elevation in protein output produced by 2DG in a dose-related manner. In fundectomized rats leptin inhibited PBJ protein and trypsin outputs in a dose-related manner. Moreover, the inhibition was stronger in comparison with control rats, and it occurred with a 15 min delay, whereas in control rats the effect occurred immediately. In conclusion, present data suggest that leptin may depress the activity of the insulo-acinar axis, possibly the effect is due to reducing insulin release. It is also speculated that partial removal of gastric leptin may up regulate leptin receptors thereby increasing the sensitivity to circulating leptin.

Leptin is Produced by the Pancreas

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Background: Leptin produced by adipocytes could be also released from the stomach and involved in the regulation of pancreatic enzyme secretion. However the ability of the pancreas to produce endogenous leptin is unknown.

Aim: To investigate the ability of the pancreas to produce leptin and to determine the effect of caerulein on pancreatic leptin concentration.

Methods: The study was performed on Wistar rats. Caerulein was given at doses of 1, 10 or 25 μg/kg intraperitoneally (i.p.) to the separate groups of animals. The blood samples and fragments of pancreatic tissue were taken at 20, 40, 60 and 90 min after caerulein administration to measure leptin plasma level and leptin concentration in the pancreas by RIA. Leptin gene expression was determined in the pancreas by RT-PCR.

Results: Leptin mRNA was present in the intact pancreas and this signal has been dose-dependently increased by caerulein. Under basal conditions leptin was detected in the plasma and in the pancreatic tissue (0.3 ± 0.05 ng/ml and 1.2 ± 0.1 ng/g of tissue, respectively). Following caerulein administration plasma leptin level significantly increased to 1.5, 5.0 and 7.0 ng/g, respectively at 90 min after caerulein administration.

Conclusion: Pancreas is able to produce leptin. Caerulein increased pancreatic leptin content.
Melatonin or its Precursor L-Tryptophan Stimulates Pancreatic Enzyme Secretion via Activation of Duodeno-Pancreatic Reflex


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Background: Melatonin, a pineal product, inhibits insulin secretion, but the role of melatonin in the physiological modulation of pancreatic enzyme secretion is yet unknown.

Aim: To evaluate the secretory effects of melatonin or its precursor L-tryptophan given intraduodenally (i.d.) to the conscious rat with intact or capsaicin deactivated sensory nerves, and to investigate the involvement of CCK in the secretory effects of tested substances.

Materials and Methods: In order to deactivate sensory nerves capsaicin was given to part of the rats at a total dose of 100 mg/kg 10 days before surgery. Melatonin (1, 5 or 25 mg/kg) or L-tryptophan (10, 50, or 250 mg/kg) were administered i.d. Samples of pancreatic juice were collected in 15 min aliquots. Tarazepide, a CCK-1 receptor antagonist (2.5 mg/kg i.d.), was given to the rats 15 min prior to the administration of tested substances. The volume of pancreatic juice, protein and amylase content of each sample was measured. CCK plasma level was determined by RIA.

Results: Intaduodenal application of melatonin or L-tryptophan significantly and dose-dependently increased CCK plasma level, pancreatic juice volume, protein and amylase outputs. Above secretory effects were totally abolished by capsaicin deactivation of sensory nerves or pretreatment of the rats with Tarazepide.

Conclusion: Melatonin or its precursor L-tryptophan stimulates pancreatic enzyme secretion via activation of duodeno-pancreatic reflex and stimulation of CCK release.

Pentaghrelin Inhibits Pancreatic Secretion through a Vagal-Dependent Mechanism in Anaesthetized Rats

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Background: Ghrelin, a novel 28 amino acid peptide, is widely distributed in the gastrointestinal tract mucosa as well as pancreas, pituitary and hypothalamus. Studies in rodents revealed that besides other functions, exogenous ghrelin-28 may control gastrointestinal motility and secretion. Only the first 5 amino acid residues are necessary to maintain the activity of ghrelin, but a large hydrophobic group on the Ser³ side chain is still required for activity. Recently, ghrelin-28 was found to inhibit pancreatic enzyme output in rats, though the effect of pentaghrelin was not studied so far. The aim of present study was to determine the influence of pentaghrelin on the secretion of pancreatic juice in anaesthetized rats.

Methods: In anaesthetized male Wistar rats (200 ± 15 g body weight, b. wt.) the external jugular vein was catheterized and the common biliary-pancreatic duct was cannulated with a polyethylene tubing for collection of pancreatic-biliary juice (PBJ). Pentaghrelin boluses (1.2, 12 and 500 nmol kg⁻¹ b. wt.) were injected intravenously every 30 min with or without CCK-8 background infusion (i.v. 12 pmol kg⁻¹ b. wt.). This dose of CCK-8 is known to stimulate pancreatic enzyme output via a duodenal mucosal CCK-vagal mechanism. Pentaghrelin injections were done also in vagotomized (subdiaphragmatic vagotomy) and capsaicin pre-treated rats.

Results: In examined rats, pentaghrelin boluses decreased PBJ volume both in the basal and CCK-8-stimulated conditions. The effect was dose-dependent. Pentaghrelin inhibited the CCK-8-stimulated protein and trypsin outputs stronger than the unstimulated PBJ secretion. Vagotomy and capsaicinization significantly decreased the PBJ volume and abolished the effects of pentaghrelin.

Conclusion: The present study demonstrates ghrelin pentapeptide is effective in reducing the secretion of the exocrine pancreatic, it also confirms previous studies that the mechanism of ghrelin action on the exocrine pancreas is related to CCK and vagal afferent pathways.

Protective Effect of Lauryl Gallate on Lipid Bilayer Integrity of Pancreatic Duct Cells Exposed on Oxidative Stress

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Introduction: This study aimed to inspect lipid bilayer integrity of pancreatic duct cell plasma membrane under condition of oxidative stress induced by intraduodenal infusion of prooxidant tert-butyl hydroperoxide (ButOOH). Lauryl gallate has been used as antioxidant food additive registered in European Union as E-312.

Methods: Gallic acid esters were synthesized in Department of Medical Chemistry, Medical University of Gdańsk, Poland. Oxidative stress was induced by retrograde infusion of 150 micromoles of ButOOH into common pancreatic-biliary duct (CPBD) of rats. 200 microl of 2.5 mM of lauryl gallate was administered intraperitoneally before induction of oxidative stress. 15 minutes after 0.9% NaCl solution (control rats) or ButOOH solution (stressed rats) administration, CPBD was washed with physiological saline and then 300 microl of 1 mM 5-doxyl-stearate solution was given during the next 5 minutes. Then the duct was washed again, excised and placed in ESR Spectrometer Varian E4 (USA).
Results: ESR spectrum from surface of epithelial duct cells were gained electronically, saved digitally and calculated using EPRIK programme. Three ESR parameters namely order parameter S and two rotational correlation time TauB and TauC were evaluated. S parameter has been found to decrease from 0.6633 to 0.6392 after oxidative stress induction. TauB time has been found to decrease distinctly from initial $4.4 \times 10^{-9}$ to $1.5 \times 10^{-9}$s as a result of But OOH triggered oxidative stress. Lauryl gallate has been found to improve significantly both order parameter S - 0.644 and TauB - $4.1 \times 10^{-9}$s

Conclusion: Our results suggest potential usefulness of gallic acid ester in prevention of oxidative stress during acute pancreatitis.

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196 Stellate Cells are Present in Freshly Isolated Pancreatic Acini and Interact with Acinar Cells after Stimulation with Cholecystokinin or Epidermal Growth Factor

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In rat pancreatic acini, three separate mitogen-activated protein kinase (MAPK) cascades: extracellular signal-regulated kinase (ERKs), p38MAPK and stress-activated protein kinases/c-Jun NH2-terminal kinases (SAPK/JNKs) have been studied so far and all can be activated by cholecystokinin (CCK). CCK and epidermal growth factor (EGF) activate the ERK cascade in rat acini by distinct mechanisms.

Methods and Results: In the present work, we compared the effects of 1nM CCK and 100nM EGF on activation of ERKs, p38MAPK and SAPK/JNK, measured by Western blotting and immunocytochemistry with the use of antibodies recognizing active, dually phosphorylated kinases in isolated rat pancreatic acini. Both stimuli, applied separately, caused rapid activation of ERKs. CCK markedly activated p38MAPK while EGF had almost no effect on this kinase activation. CCK caused substantial activation of SAPK/JNK and the effect of EGF on this kinase was much weaker. Stimulation of isolated pancreatic acini with the mixture of 1nM CCK and 100nM EGF had diverse effects on MAPK family members. In the case of ERKs, it resulted in amplification of this kinase activity, it had no any additional effect in the case of p38MAPK, while in the case of SAPK/JNK – strong inhibition took place. With the use of immuno- cytochemical methods and anti-pTy-ERK antibody, we showed that CCK and EGF activate ERKs in two different cell types present in isolated pancreatic acini. CCK activated ERKs predominantly in pancreatic acinar cells, while EGF caused activation of ERKs in pancreatic peri-acinar cells. These peri-acinar cells stained with anti-desmin antibody, which is characteristic for stellate cells.

Conclusions: We have made the novel observation that isolated pancreatic acini contain pancreatic stellate cells. These cells respond to EGF by activation of the ERK cascade. We suggest that stimulation of isolated pancreatic acini with CCK may induce cross-talk between acinar and stellate cells.

197 The Effect of Pentaghrelin on Amylase Release from the Dispersed Rat Acinar Cells

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Ghrelin, a 28 amino acids polypeptide was recognized as an endogenous ligand for the growth hormone secretagogue receptor. It turned out that the entire sequence of ghrelin is not necessary for performing the above-mentioned functions. It was suggested that 5 residues (Gly-Ser-Ser(n-octanoyl)-Phe, pentaghrelin) constituted functionally active part of the full-length peptide. Ghrelin-28 was found to inhibit pancreatic enzyme output in rats, though the effect of pentaghrelin was not studied so far. The aim of present study was to investigate the effect of pentaghrelin on amylase release from rat pancreatic acinar cells in vitro. Dispersed pancreatic acinar cells were obtained from Wistar rats by collagenase digestion. Amylase secretion was inhibited in the concentration range between $10^{-11}$ and $10^{-8}$M with the maximum inhibition in $10^{-9}$M of pentaghrelin. In pentaghrelin concentration higher than $10^{-8}$M (i.e., $10^{-7}$ and $10^{-6}$M) the inhibition was no longer observed. A different pattern of pentaghrelin action was observed in a study with stimulation with 75mM KCl – the increasing doses from $10^{-7}$ to $10^{-2}$M of pentaghrelin strongly inhibited amylase release. Moreover, amylase release pretreated with cholecystokinin-octapeptide (CCK-8) in doses between $10^{-12}$ and $10^{-8}$M showed that pentaghrelin in low dose ($10^{-10}$M) increased amylase release although in high dose ($10^{-8}$M) did not have a significant effect. Present data suggest that pentaghrelin may reduce amylase release by a direct influence on acinar cells as well as through a neural pathway. A week stimulatory effect in concentrations higher than $10^{-8}$M, however, needs further elucidation.

198 The Src-Kinase Lyn is present in Rat Pancreatic Acini and is Activated by Diverse Stimuli

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Background: Src Family Kinases (SFK) play a central role in directing intracellular signals in many cells from growth factors (GF), cytokines, G protein-coupled receptors (GPCR) and other stimuli. Three Src-family-kinases (i.e., Src, Fyn, Yes) have been shown to be activated by CCK or GFs in pancreatic acini. The SFK, Lyn, is present mostly in hematopoetic tissues and little is known of its function in other tissues such as the pancreas, although it is reported to be present in other tissues.
Aim: To establish if Lyn is present in pancreatic acini and if so, its activation, regulation of its action, and its ability to activate other cellular tyrosine kinases.

Methods: Dispersed rat pancreatic acini were prepared. Lyn was detected by immunoprecipitation (IP) using Lyn-specific antibodies and its activation assessed by Western blotting (WB) with a phospho-specific pY418 Src antibody.

Results: Lyn was expressed in rat pancreatic acini by WB. The addition of a specific Lyn-blocking peptide abolished the WB signal. Ca²⁺ mobilizing agents [CCK, carb, bombesin, A23187, thapsigargin (TG)], cyclic AMP agents (VIP, secretin, 8-Br cAMP) and GFs (HGF, EGF, PDGF, IGF) all activated Lyn by stimulating tyrosine phosphorylation (TyrP) of the active Y418 site. The action of CCK was investigated in detail. CCK (1 nM) caused rapid TyrP of Lyn, reaching a maximum by 0.5 min followed by a rapid decrease. The addition of the specific SFK inhibitor, PP2, decreased Lyn-TyrP; however, the inactive analogue, PP3, had no effect. Inhibition of changes in [Ca²⁺]i by TG (1 µM) in a Ca²⁺-free medium inhibited the CCK-stimulation of Lyn-TyrP by 55%; GFX (5 µM), a PKC inhibitor, inhibited it by 36%; and the combination inhibited by 95%. CCK stimulated an association of Lyn with PKC-delta, Shc, p125FAK and PYK2, as well as with their autophosphorylated form (i.e., p397FAK, p402 PYK2).

Conclusions: Lyn is expressed in rat pancreatic acini and is activated by a wide range of pancreatic GPCRs and GFs. CCK activation of Lyn requires CCK activation of both.