ABSTRACTS

1 Early Administration of Natural Somatostatin (S) Increases the Efficacy of Sclerotherapy (ES) in Acute Bleeding Oesophageal Variceal Episodes: The European ABOVE Study

The additional efficacy of S to emergency ES in cirrhotics with an acute upper gastrointestinal bleeding episode was assessed. Immediately after admission, 205 subjects were randomly assigned to receive continuous IV infusion of S (6 mg/24 hr for 120 hr), or placebo (Pi) in a double blind way. Additionally, iv boluses S (250 μg) or Pi were injected: a) after start of infusion, b) before endoscopy for c) if active bleeding occurred. ES was performed between 1 to 8 hr after initiation of treatment. The study drug was continued in 151 pts (74%) in which oesophageal varices were the origin of bleeding (S = 75, Pi = 76). Overall failure during the 5 day study period was defined as: death or clinical signs of rebleeding or the requirement of an excess of blood products or rescue therapy. Both groups S/Pi were comparable with regards to age (mean ± sd = 59.5 yrs ± 12.1/8.9 yrs ± 3.9), gender (M: 52/52; F: 23/24), Child’s class (A = 10/11; B = 39/39; C = 26/25) and alcoholic origin (4/46%). Overall failure was observed in 32 subjects (S) and 31 (Pi) with p = 0.003. During the infusion 2 patients (2.7%) died in S and 7 (9.2%) in Pi (p = 0.17); units of blood products transfused: 2.6 ± 0.35 (S) vs 3.6 ± 0.35 (Pi) (i.e., p = 0.05). In conclusion the results of this study suggest that the early administration of natural S increases the efficacy of S in control of acute bleeding from oesophageal varices in cirrhotics.

3 The Actin Bundling Protein Fascin, Is Ungрегulated by Transforming Growth Factor-α
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Fascin is a highly conserved, ubiquitously expressed actin-bundling protein, involved in the assembly and reorganisation of actin bundles and networks necessary for cell motility and attachment to intercellular contacts and the substratum. A recent report has suggested interaction of fascin with the cadherin-catenin complex at adherens junctions.

Alain: 1. To examine the expression of fascin in gastric and colonic carcinoma cell lines and its response to stimulation of the epidermal growth factor receptor (EGFR) by TGF-α. 2. To examine the interaction of fascin with the E-cadherin-catenin complex in these cell lines.

Methods: Fascin expression was examined in gastric (MKN45 & HSC39) and colonic cell lines (HT29, HCT116, & LS174T) by immunocytochemistry and Western blotting. Intensity of expression was assessed on days 1-4 following seeding and in response to stimulation of the EGFR by TGF-α (10 ng/ml for 24 hrs). Immunoprecipitation of fascin-associated proteins followed by Western blotting was performed.

Results: Fascin co-localised with E-cadherin at the cell membrane. A 55 kDa band consistent with fascin was demonstrated in all cell lines. Band intensity was inversely proportional to monolayer confluence. Stimulation by TGF-α was associated with increased fascin expression in MKN45 & HT29. Immunoprecipitation experiments confirmed fascin interaction with E-cadherin, β and γ-catenin.

Summary and conclusions: TGF-α stimulation is associated with upregulation of fascin expression. The interactions between the cadherin-catenin complex, fascin and the EGFR may play a role in modulating cell-cell adhesion and cytoskeletal interaction during cell migration in epithelial repair, and invasion by neoplastic cells.

4 Preoperative Radiotherapy in Rectal Cancer: Influence of the Interval between Radiotherapy and Surgery: A Multicenter Randomised Trial from Lyon
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Preoperative radiotherapy has been shown to decrease local recurrence after surgery in rectal carcinoma. However the best moment for surgery after completion of radiotherapy is not known. Therefore the aim of this prospective multicenter randomised trial was to determine the influence of the interval between radiotherapy and surgery on sphincter conservation, complications of surgery and local recurrences.

Methods: Operable patients with rectal carcinoma reachable at digital examination, staged T2–T3, Nx, MO were included. Preoperative radiotherapy delivered 39 Gy in 13 fractions within 17 days. Randomisation was performed before radiotherapy in 2 arms: in the "short" arm, patients were operated on within 2 weeks after completion of radiotherapy, while in the "long" arm, surgery was performed 6 to 8 weeks after radiotherapy.

Results: 210 patients were randomised between 1992 and 1995. 177 patients had for the moment complete: a 6 month follow-up, 90 in the short arm, 87 in the long arm. There were 113 male and 64 female, mean age 63 yrs (range 35-82). There was no statistical difference between the 2 groups for sex, age, and the mean distance between the lower part of the tumor and the anal verge as determined by rigid endoscopy and endosonography (respectively 5.8 cm and 6.1 cm). 21 patients (12%) could not have curative resection (liver, peritoneum metastasis, parietal invasion). Sphincter preservation was possible in 68% (61/90), short arm and 75% (66/87) (long arm (p = 0.25). After radiotherapy, decrease in tumoral size (> 50%) measured by digital examination was observed more in the long arm, 69% vs 55% (p < 0.05). Operative specimen showed no or only a few tumoral cells in 11/86 vs 25/83 (p = 0.008). Median operative stay, peroperative mortality, anastomotic complications and reinterventions were similar in the 2 groups. With a median follow-up of 20 months, actuarial survival was at 1, 2 and 3 yrs, 89, 83, 78% in the short arm, 91, 77 and 64% in the long arm (NS). Local recurrence was observed in 6 patients in the short arm and 5 in the long arm. All these patients had sphincter saving surgery.

Conclusion: In this large series of rectal carcinoma with preoperative radiotherapy, sphincter conservation was possible in 72% of patients. Patients of the long arm has a more important tumor regression than in the short arm. However there was not statistical difference in the percentage of sphincter saving intervention or operative complications. A longer follow-up will confirm the lack of difference in survival and local recurrence.

5 Effects of Smoking on the Clinical Course of Crohn’s Disease
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Purpose A previous study by our group showed no relationship among 1) years of smoking 2) the cumulative estimate of the total amount of cigarette smoked and the clinical course and attack of Crohn’s disease. This study was performed with the hypothesis that the effect of smoking is not long-lasting. In order to test the hypothesis a short-term effect of smoking during long-term follow up a phone interview based on a standardized questionnaire about smoking habits was performed. For methods and Ethics Methods Of 203 patients (127 M; 76 F) with a follow-up of more than 10 years duration, 73 (36%) stopped smoking after the diagnosis of CD and were excluded from the study. The median follow-up period for the remaining 130 patients was 16 years (10-33). At the time of diagnosis the disease involved ileum 60 (46%), ileum-colon 29 (22%) and colon 41 (32%). The effect of daily cigarette smoking irrespective of life time exposure was evaluated by dividing patients in 64 smokers (49%) and 66 non-smokers (51%).

Results: The comparison of non-smokers versus smokers shows: mean age 49 and 46 (P = 0.17), mean age at diagnosis 33 and 28 (P = 0.11), months between first symptom of CD and diagnosis 38 and 22 (P = 0.09), pain at diagnosis 45 and 55 (P = 0.09), weight loss at diagnosis 39 and 61% (P = 0.04), mean number of hospital admission 2.9 and 2.6 (P = 0.06), steroid months 18 and 26 (P = 0.06), azathioprine months 7 and 18 (P = 0.12). Disease localization, number of major surgical operations, indications for surgery, number of postoperative recurrences, and mortality are not statistically associated with smoking. There is no difference in the timing of the first, second, third and further operations estimated by life table analysis.

Discussion This study confirm the absence of both long-standing and cumulative effects of smoking on Crohn’s disease. Our data suggests that smoking could have a short-term effect on some symptoms, signs and disease severity as indicated by the statistical association or consistent trend with age at diagnosis, latency between symptoms and diagnosis, hospital admission and therapeutic needs as suggested recently by Cosnes et al.

6 Hepatitis G Virus Infection in Patients with Hepatocellular Carcinoma

Purpose of the study: Hepatitis G virus (HGV) is a RNA virus and has been
implicated as a causative agent in acute and chronic hepatitis. We investigated the prevalence of HGV in patients with hepatocellular carcinoma (HCC).

Methods: Serum of 76 patients (53 m, 23 f, 61 ± 11 yrs) with HCC was studied for the presence of HGV-RNA by reverse-transcription polymerase-chain reaction (RT-PCR).

Results: 74% of 76 patients with HCC were infected with HGV. In 6 patients (8%) HGV was the only hepatotropic virus found. In 9 patients (12%) coinfection with other hepatitis viruses was present. 4 patients (26%) were HBsAg+, 5 others (33%) were HCV-RNA+, in one of the patients triple infection of HBV, HCV and HGV was found. Of 67 patients with HCC 30 patients (39%) had no evidence of infection with hepatotropic viruses, 46 patients (61%) had markers virus infection: of those 12 patients (27%) were HBV infected, 9 patients (20%) were positive for HCV-RNA, and 6 patients (14%) were HGV-RNA+. In 61 control patients with chronic hepatitis B (without HCC) 10 patients (16%) were HGV-RNA+, whereas 4 of 12 HBsAg+ patients with HCC (30%) were infected with HGV. 18 of 88 patients (21%) with chronic hepatitis B (without HCC) and 5 of 26 of HCV-RNA+ patients with HCC (20%) were positive for HGV. The prevalence of HGV in patients with HCC (20%) was significantly higher than that found in healthy controls (3%; p = 0.003).

Conclusion: The prevalence of HGV is significantly increased in patients with HCC as compared to the healthy population. HGV could be a risk factor for HCC.

Circulation of Tumorous Cells and Intravenous Spreading of Hepatic Cells in Patients with Liver Cancer (LC)

Background: Pro-inflammatory cytokines (IL-1α, IL-6, IL-10) and neutrophils were increased in intestinal infection in IBD. Monocytes as well as epithelial cells have been discussed as a source. However, predominant cells in inflammatory infiltrates are granulocytes (PMN). The Aim: of this study was to evaluate the cytokine profile of PMN to secrete pro-inflammatory cytokines as well as the regulatory capacities of IL-4 and IL-10 in PMN. Methods: PMN from 35 patients with ulcerative colitis (UC), 28 patients with Crohn's disease (CD) and 25 normal volunteer controls (NC) were obtained from peripheral blood by dextran sedimentation and density centrifugation. Release of pro-inflammatory cytokines (ELISA) into culture supernatants as well as mRNA (semiquantitative RT-PCR) were assessed. Results: Only low levels of pro-inflammatory cytokines were secreted by PMN after stimulation with LPS. PMN from patients with active UC or CD secreted significantly more IL-1α, TNF-α and IL-10 than NC (Figure). Pro-inflammatory cytokine secretion was related to disease activity. IL-4 as well as IL-10 down-regulated pro-inflammatory cytokine secretion as well as mRNA levels in a dose dependent manner without differences between IB and NC PMN. Elements of IL-4 receptor signal transduction (STAT-6) were induced by stimulation of PMN with IL-4. Conclusions: PMN could be an important contributor to enhanced concentrations of pro-inflammatory cytokines in intestinal mucosa of IBD patients. PMN appear to be fully capable to partake in mucosal immunoregulation by anti-inflammatory cytokines. Elements of IL-4 signal transduction (STAT-6) are fully conserved in PMN. Future therapeutic developments need to consider PMN as potent pro-inflammatory cells capable of participating in mucosal immunoregulation.

11 Self-Reported Ulcer Incidence and Changes in Levels of Serum IgG Antibodies to H. Pylori: A Prospective Cohort Study Comprising 2,404 Unselected Danes

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Aim: To examine the relationship between changes in levels of IgG antibodies to H. pylori and the incidence of self-reported peptic ulcers (PUD) in an 11-year period. Methods: A random sample of 3,589 Danes aged 30-60 years entered a population-based prospective cohort study in 1983. After 11 years, 2,656 participants attended a follow-up examination. Blood samples were drawn at both attendances (mean 10 ± 4.04). IgG antibodies against H. pylori were measured in an in-house ELISA assay. Antibody levels were categorized as sero-negative, border-line, or sero-positive. People who sero-converted in IgG antibodies to H. pylori were regarded as having acquired H. pylori infection within the study period. Participants with no history of peptic ulcer disease at study entry reported that they had had an ulcer diagnosed at follow-up. Information on lifestyle practices, socio-economic factors, and medical history was obtained from a questionnaire. Results: Cumulated 11-year incidence of PUD was 28.3 [21.7–34.9] per 1,000 persons at risk. Ulcers were more often reported in those who were seropositive at both attendances (OR 2.18 [1.13–4.21], in people with an increase in IgG antibodies from border-line to seropositive levels (OR 4.89 [1.13–4.31]), in heavy tobacco smokers (OR 6.24 [2.45–15.91]), and in people categorized as psychosocially vulnerable at study entry (OR 2.86 [1.45–5.56]). Age, sex, family history of PUD, sero-conversion in IgG antibodies, and alcohol consumption did not act as risk factors to PUD in this study. Conclusions: Long-term H. pylori infection, tobacco smoking, and psychic vulnerability are independent risk factors to PUD. An increase in IgG antibody levels may be useful as a marker for ulcer formation. Recent infection with H. pylori (< 11 yrs.) may not be a risk factor to PUD.
Conclusion: FDG-PET, which provides biochemical information, is accurate in identifying pancreatic cancer, and has significantly higher sensitivity and specificity than CT and US.

13 Human Papillomavirus (HPV) Infection and p53 Overexpression as Prognostic Factors in Patients with Esophageal Squamous Cell Carcinoma

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Infection with high-risk human papillomavirus (HPV) has been detected in high percentages of patients with several types of cancer such as in the uterine cervix, anus, skin and esophagus. On the other hand, p53 protein mutation and overexpression in the multistep process of esophageal carcinogenesis is still under debate. The present study was undertaken in order to investigate the possible involvement of HPV in the esophageal squamous cell carcinoma (ESC) and the relation between p53 overexpression and prognosis of patients in ESC.

Material and Methods. One hundred twenty three samples, formalin fixed and paraffin embedded, were examined for this study. The detection of HPV was performed with dot blot hybridization (DBH), polymerase chain reaction (PCR) and in situ hybridization (ISH) methods. p53 protein was assessed by means of an antibody against the monoovalent form of the p53 protein and in both the ISH method, p53 overexpression and prognosis of patients with ESC were analyzed by multivariate survival analysis. Results. The detection rate of HPV were 21% by DBH and PCR, respectively. In ISH method, HPV types 16, 18 and 33 were detected in 30% (37/123). In addition, 43 of 123 samples (35%), nuclear immunohistochemical reactivity for p53 protein overexpression was detected. The survival rate (Kaplan-Meier method) of the group was significantly worse than negative group and in p53 protein overexpression positive group was significantly worse (p < 0.05) than negative group.

Conclusion. These results suggest that the HPV infection and p53 protein overexpression were detected in a high percentage of ESC. The presence of HPV and p53 protein overexpression have strong impact on the prognosis of the patients with ESC. However, additional studies on a large series of patients with ESC will be necessary for verification of these results.

14 Esophageal Squamous Cell Papilloma and Human Papillomavirus (HPV) Infection

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Human papillomavirus (HPV) is involved in the development of cervix, lar-ynx, lung and anus papillomas as well as the progression of the papilloma into cancer. Esophageal squamous cell carcinoma (ESCC) is a rare benign condition, considered as precarcinomatous, but its etiopathogenesis remain controversial. The prevailing etiological considerations are chronic chemical irritation from the reflux and infection. In addition HPV and p53 protein type 16 or 18, is detected in a percentage of squamous epimalignancies. The aim of our study was to investigate the possible relationship between HPV and ESCC in an area of low prevalence of squamous esophageal cancer. Patients and Methods: 14 ESCCs (5-15 mm of diameter) from 12 patients (29–72 years old) were analysed for HPV using in situ hybridization (ISH) from paraffin-embedded tissue (ENZO PathoGene®). The HPV types tested according to the DNA probe reagents provided were 6/11, 16/18, 13/33/51. The method was validated as the manufacturer suggests, using 3 control slides inoculated with 6 and 16 HPV types. Geneticogastroscopy reflux was diagnosed in 5 patients, one patient had gastric ulcer while the rest of the patients (6) had a fibrosarcoma for chronic dyspeptic syndrome. One patient had a stenosis but distinct a monolayer epithelium and while the other patient had a recurrence at the same place of mid-esophageal, one year after ablation. Results: No evidence for HPV DNA was detected in any of the examined specimen. Conclusions: 1) We were unable to detect HPV DNA using ISH in all ESCC samples as we tested 21 Other authors using more sensitive methods, like PCR, rarely report HPV DNA in ESCCs. This supports our findings and suggest that other than HPV infection, pathogenetic mechanisms are more important for ESCC's etiology at least in our area.

15 Clinicopathological Study of Esophageal Squamous Papilloma-including Immunohistochemical Staining

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Clinicopathological study for 100 cases of esophageal squamous papilloma were performed. Patients were frequently found in 6th and 7th decades. The majority of patients had no remarkable complication and the esophageal squamous papillomas were accidentally found at the investigation of gastric disease by X-ray and endoscopy. The white colored elevated lesions located frequently in lower 1/3 (60%) and middle 1/3 (30%) of esophagus showing lobulated or vilious appearances. The white colored elevated lesions observed were classified into 3 grades: 1) polypoid type, 2) papillomatoid type, and 3) flat type.

Hemorrhagic and sessile polypoid lesions (94%) were frequently found compared with the pedunculated ones. Single lesion was found in 90% of the cases, and 93% of the lesions were less than 5 mm in diameter. Histologically, squamous epithelial thickening and papillomatous growth without atypia were observed. Although several factors like as regurgitation of gastric juice associated with gastroesophageal reflux, gastric nasal reflux, gastroesophageal reflux in pregnancy, etc., and HPV (human papilloma virus) infection have been reported in relation to the histogenesis of esophageal squamous papilloma, genuine mechanism has been unknown.

According to the immunohistochemical staining, positive rate was 21.2%, 5.0%, 8.4%, 15.4%, 23.1%, 9.0% and 7.6% for p53 (proliferating cell nuclear antigen), p16, o-erbB-2, EGF, EGFR, K-ras and HPV, respectively. These positive rates were extremely low in comparison to those of esophageal carcinoma. It seemed likely that esophageal squamous papilloma is not precancerous.

16 A Retrospective Review of a Consecutive Series of 90 Oesophageal Resections

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To assess the perioperative mortality and morbidity of oesophageal surgery, we present a retrospective series of 90 patients who underwent oesophageal resection at our institution from 1.1.1986 through 31.12.1995.

There were 73 males and 17 females, mean age was 64.2 years (range: 21–78 years). Indications for resection were esophageal cancer in 64 patients, cardial adenocarcinoma in 20, and benign lesions in six. Twenty-five patients received preoperative radiotherapy. In 78 patients surgery was performed in a curative intent and was palliative in twelve. There were thirty-seven total esophagectomies, nineteen thoracic esophagectomies, and thirty-four partial oesophagogastrectomies. The detection of HPV and p53 protein overexpression were detected in a high percentage of ESC. The presence of HPV and p53 protein overexpression have strong impact on the prognosis of the patients with ESC. However, additional studies on a large series of patients with ESC will be necessary for verification of these results.

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17 An Original Evaluation of Gastric and Colonic Transplants after Oesophageal Resection

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We present a clinical and radiological evaluation of gastric and colonic grafts used to restore digestive continuity after oesophageal resections.

Out of 90 patients who underwent an oesophageal resection at our institution, from 1.1.1986 through 31.12.1995, we evaluated the alimentary comfort and the quality of life with a standard questionnaire, and performed a dynamic contrast-swallowed radiography (radiocinema) in 35 patients who were alive more than one year after oesophageal resection.

There were 23 males and 12 females; mean age was 64 years. In 30 patients endoscopic resection was performed for benign lesions in five. 28 patients had a gastric pull-up and in 7 patients a colonic segment of the stomach was used to restore digestive continuity. Major long-term complaints were sensation of early fullness during eating in 8 patients, nocturnal cough in seven, postprandial sweating in five, dysphagia in four, and diarrhoea in three. Most patients considered the side effects of the operation was mild to moderate and mean rating of alimentary comfort was 8.2/10. Twenty-four patients qualified their quality of life as good, eight as satisfactory, and four as poor. Thirty patients did not experience any complications. Postoperative complications were colon transplants were essentially non contractile and emptied by gravity; in gastric grafts, the contrast progressed passively in the antral segment of the gastric pull-up, and subsequently by antral contractions. In 21 patients the contrast progressed passively in the antral segment of the gastric pull-up, and subsequently by antral contractions. In 21 patients the contrast progressed passively in the antral segment of the gastric pull-up, and subsequently by antral contractions.
colonic graft, particularly when the proximal portion of the oesophagus triggers the progression.

18 Five-Year Survival Following Resection for Oesophageal Cancer in 733 Patients

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The aim of this retrospective study was to determine survival and prognosis factors of a group of 733 patients undergoing surgery for oesophageal cancer between 1981 and 1991. Cancers of the pharyngoeosophageal and oesophageal gastric junction were excluded from this study.

Patients and methods: The group comprised 696 males and 37 females with a mean age of 59 years. 526 resections were performed using various surgical procedures: Sweet (5%), Ivor-Lewis (30%), Akiyama (18%), Thorek (6%), MacKeown (18%), transhiatal oesophagectomy (23%). The tumor was located at the upper, middle and lower third of the oesophagus in 14%, 65%, and 21% of patients respectively. 95% of tumors were squamous cell carcinomas.

Results: The resectability rate was 72% and 76% of resections were considered as being curative. Thirty-day operative mortality was 9%. Excluding operative mortality, 5-year actuarial survival following curative resection was 25.7% versus 3.4% following palliative resections (p < 0.0001). When TNM staging was considered, 5-year actuarial survival was 87% for stage 0 lesions, 51% for stage I lesions, 25% for stage IIA and 13.6% for stage IIB lesions, 6% for stage III lesions and 0% for stage IV lesions (p < 0.0001). 5-year survival showed no relationship to patient gender or histology.

Conclusions: There exists a statistically significant relationship between TNM tumor staging for oesophageal cancer and 5-year survival.

19 Results of the Operative Treatment for Esophageal Squamous-Cell Carcinoma

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From 1988 to 1995 one hundred and fifty eight patients with squamous-cell esophageal carcinoma located in its thoracic part were treated in our Department. According to the preoperative staging (TNM) 84% of patients were classified as stage II/III.

The most common and most important presenting symptom was dysphagia, which occurred in 96% of patients (mean 4.6 months).

Patients qualified to the operative resectional procedures included those in good general condition, in whom the weight loss have not exceed 20% of body weight.

Resectional operations were carried out in 64 patients (42%). The operation of choice was the resection of thoracic esophagus with tumor as well as the lesser curvature of the stomach. Two-field lymphadenectomy (mediastinal and upper abdominal lymph nodes) was also performed. The continuity of the alimentary tract was achieved by stomach graft placed in the anterior mediastinum, with cervical esophagogastic anastomosis. In only 17% of patients the proximal part was used for reconstruction.

Pulmonary (14 patients – 21.9%) and cardiovascular (6 patients – 9.4%) complications were significant source of morbidity following esophagectomy. Anastomotic leak occurred in 5 patients (7.8%).

Early mortality (<30 days) was of 19% (12 patients).

One-year survival equalled 45% (29 patients). Six patients (9%) survived 3 years and 2 (3%) - 5 years.

Conclusions: Better results of treatment of esophageal squamous-cell carcinoma depend on early diagnosis and treatment, aggressive surgical procedures and appropriately chosen adjuvant therapy (chemo- and/or radiotherapy).

20 Esophageal Functional Evaluation of Laryngeectomy Rehabilitated with Esophageal Voice

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Aim: laryngeectomies who had laryngeal Ca and rehabilitated with esophageal voice were studied to evaluate the incidence of alterations regarding the esophageal motility and the presence of gastroesophageal reflux disease (GERD). We studied 75 patients (males, age range: 44–71 years, mean age 54 ± 9 years) with laryngeal Ca and who were to undergo surgical total laryngectomy and subsequent phonic rehabilitation with "esophageal voice". GERD symptoms were absent in these patients. All subjects underwent pre and post operative swallow protocol (generally after six months) including: anamnesis, video-taped oesophagus-stomach Rx and manometric and 24 hours pH monitoring evaluation. No patient showed GERD symptoms or pathologies. The protocol was repeated after the logopedic treatment, generally one year after the laryngectomy. Of the patients investigated GERD symptoms, 12 (16%) dysphagia, 2 (2.6%) presented hialt, 17 (22.7%) abnormal sliding of abdominal oesophagus, 22 (29.3%) acid pathologic GER at the pHmetry, 37 (49.3%) alterations of esophageal motility. All patients were considered "good speakers" referring to the quality of the esophageal voice. The patients were given Omeprazole 20 mg and Cisapride 10 mg 4 times a day for an average period of 8 weeks. GERD symptoms were significantly decreased by this therapy in 24 pts. The result of our study showed a certain incidence of GERD and alterations of esophageal motility following the speech therapy and suggested that a therapy with a double localization in order to avoid esophageal diseases.

21 Comparison of Double Neoplasms of Oesophagus and Head and Neck: Metachronous Versus Synchronous

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Head and neck neoplasms and oesophageal neoplasms have the same risk factors. They can occur in any segment of the oesophagus (n = 19) and H and N (n = 6). In the group I, the first localization found was oesophageal cancer (n = 19) and H and N (n = 6). In the group II, the first localization was H and N (n = 21), oesophageal cancer (n = 16), was principally middle third (n = 14) for the group I and upper third (n = 11) for the group II. The H and N localization was principally oropharyngeal (n = 14) in group I and oropharyngeal or hypopharyngeal (n = 14) in group II. The treatment in the group I was radiotherapy: 28 (14%) with 14 complete responses. Radiotherapy and surgery (n = 11) for head and neck cancers and radio-chemotherapy (n = 15) for oesophageal neoplasms were the principal strategy of treatment in the group II with only 9 complete responses. The major problem in cases of resection was the control group (n = 20): 16.5 months in group I and 18 months after the second neoplasms in the group II.

Conclusion: Oesophageal is the first site found in case of synchronous neoplasm, and head and neck cancers in case of metachronous neoplasm. Main difficulties were the field of radiotherapy in the group I and few possibility of surgery in the group II.

24 Perioperative Immunotherapy with Recombinant Granulocyte-Colony Stimulating Factor (rG-CSF) in Patients Undergoing Surgery for Esophageal Cancer

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Neutrophil granulocytes play an important role in host defense mechanisms, including those of microbial, inflammation and wound healing. We investigated the effects of r-G-CSF (Filgrastim) on the function of neutrophils and the high infection rate in patients undergoing surgery of esophageal cancer. Here we present our results of the first study phase (stage I) in 22 patients (16 male and 6 female). The mean age was 60 years (range 49–75). All patients received 300 µg (≤ 75 kg) or 480 µg (> 75 kg) of Filgrastim s.c., starting on day –2 before surgery until day 7 after surgery. Application stopped when leucocytes increased above 50 000/µl. The treatment was well tolerated. One patient was found with low grade wound healing disorder; no other complications or infections were observed. To evaluate the effect of G-CSF on neutrophil function, we measured the percent of neutrophils with phagocytosis and the oxidative burst on day –2, 2 and 10. 10 patients undergoing major surgery served as controls. Phagocytosis increased in the study group (day 2: IgG-beads: +30% ± 29%, Albumin-beads: +22% ± 47%; day 10: IgG-beads: +9% ± 47%, Albumin-beads: +28% ± 92%) in contrast to the control group (day 2: IgG-beads: –1 ± 35%, Albumin-beads: –1 ± 19%, day 10: IgG-beads: –11 ± 18%, Albumin-beads: –18% ± 27%). The microbial activity as measured by the oxidative burst was substantially higher in the G-CSF-treated study group (day 2: +219% ± 167%, day 10: –37% ± 77%) as compared to the control group (day 2: –11% ± 55, day 10: –46% ± 46%). In conclusion, perioperative immunotherapy using G-CSF to stimulate neutrophil function in patients with esophageal cancer might be effective to prevent infection after surgery. Enrollment is continuing.

25 Retrospective Analysis of Treatment of 640 Patients with Oesophageal Squamous-Cell Cancer

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The aim was to study the immediate results of surgical and combined treatment in esophageal cancer. In 1980–1994 640 patients (523 males and 117 females) aged 46–70 years (1/3 being of 60 years and older) were treated in the centre. Superior esophageal cancer was in 33 patients, meso-esophageal in 406, inferior-in 201 patients. There were cases of stage I, 220-of stage II, 305-of stage III, 164-of stage IV. We have performed esophagectomy
The diagnostic incidence age The model For echoendoscopy the 18 been undertaken Staging was prognosis H. ratio i by FranciscO Gentil, F. Gut Among these research patients in 1989 to 1994. Staging was based upon the histopathological analysis of the resected tumors (when resection had been possible), thoracic and abdominal CT scan, endoscopy and/or bronchoscopy. Blood samples were collected in the time of diagnosis. TPS was determined by an "in vitro" monoclonal radioimmunoassay; the value of 70 U/L was considered the upper limit of normal. For statistical analysis of data, ANOVA and the proportional hazards survival model were used. Results: Seventy three patients (89.0%) were male; mean age was 61.6 ± 10.3 years (40-88). In only 24 patients (29.3%) had it been possible to undertake surgical resection. Forty four patients (53.7%) were dead, 28 (34.1%) alive and 10 (12.2%) were lost after a median follow-up of 4.5 months (range: 0-41); 22 (26.8%) were stage I, 36 (43.9%) stage III and 24 (29.3%) stage IV. TPS values were above normal in 34 patients (41.5%). TPS correlated positively with stage (p < 0.001) and inversely with survival (p < 0.002), the hazard ratio for patients with a TPS value above 70 U/L being 2.7 (95% CI: 1.4-5.1). After controlling for surgical resectability, TPS kept its independent prognostic value. Conclusion: 1) TPS has little diagnostic accuracy in SCE. 2) TPS values correlated with stage and showed promising prognostic meaning in SCE. This research was funded in part by Biki Diagnostics AB, Bromma, Sweden.

Multiple Primary Malignancies and Oesophageal Cancer
H. Lombaviana, A. Correia, R. Silva, L. Dias, R. Lombaviana. Dept. of Gastroenterology, Oncology Portuguese Institute, Oporto, Portugal

The association between multiple primary malignancies, synchronous (s) or metachronous (m) is an interesting subject in Oncology. With the aim of detecting any association between the oesophageal cancer and other malignancies, the authors made a retrospective study in 407 patients, in which oesophageal cancer was diagnosed between July 1974 and December 1999.

Among these 407 patients, 20 (4.9%) had at least one other cancer associated. Sixteen patients (80%) presented metachronous malignancies and four (20%) synchronous ones. Fourteen patients (70%) were male, with an average age of 66.1 years and 17 (85%) were female with an average age of 69.6 years old. The oesophageal cancer was associated to another malignancy in 18 cases and with two or other cancers in 2 cases. The time interval between the diagnostic of the metachronous primary malignancies ranged from 6 months to 27 years, with an average of 5.6 years. The follow up after the diagnostic of the oesophageal cancer ranged from 1 to 84 months, with an average of 15.7 months. The mortality at the end of the first year was 60% (12 patients) and at the end of the third year was 95% (19 patients).

Conclusion: The coincidence of multiple primary malignancies in patients with oesophageal cancer was 4.9%; 2 – high incidence in male; 3 – higher incidence of metachronous malignancies than synchronous ones; 4 – a more acute association between the oesophageal cancer and the others of the head and neck (50%); 5 – reduced time of follow up (15.7 months) of the patients with oesophageal cancer; 6 – very high mortality (95%).
Conservative Treatment of Oesophageal Perforations after Endoscopic Palliation in Advanced Oesophageal Cancer

T. Bisgaard, M. Vejdemann, H. Heinidorn, L.B. Svendsen. Dept. of Surgical Gastroentrology, Rigshospitalet, Copenhagen, Denmark

Purpose: The aim of the study was to evaluate the effect of conservative treatment of oesophageal perforations due to endoscopic palliation in patients with unresectable oesophageal or cardiac cancer.

Methods: From January 1993 to January 1996, 148 consecutive patients with advanced oesophageal or cardiac cancer were subjected to a total of 686 palliation procedures (argon plasma electrocoagulation, Nd:YAG laser photoagulation, dilatation, intubation and stenting procedures). When palliation-related perforations were diagnosed, patients received conservative treatment: Broad spectrum antibiotics, fasting and nasogastric suction. Pneumothorax or pleural effusions were drained. Stenting or intubation was also regarded as conservative treatment.

Results: Perforations were seen in 9 patients (6%) corresponding to 1% of palliation procedures. The mean time lap between perforation and treatment was 30 hours (1–56 hours). Conservative treatment succeeded in 6/8 patients (75%) and the perforation-related mortality after conservative treatment was 1/8 (13%). The patient died without further possibility of general condition prior to palliation. Two patients did not respond to the conservative treatment (1 patient developed a tracheoesophageal fistula and 1 patient developed pleural empyema, and decortication with drainage was performed).

Conclusions: Conservative treatment of oesophageal perforations seems sufficient in selected patients with advanced oesophageal or cardiac cancer.

Preoperative Nutritional Status in Oesophageal and Gastric Cardia Cancer

Z. Jankovic, Z. Gerzic, P. Pesko, T. Randjelovic, J. Knezevic, M. Popovic. Institute of digestive disease, Clinical center of Serbia, Belgrade, Yugoslavia

Using several clinical and anthropometric parameters in pts who underwent different oesophageal and gastric cardia cancer: transorhaphic oesophagectomy (TT), transthoracic oesophagectomy (TH), coloplasty in benign oesophageal disease (C), total gastrectomy (G), we wanted to assess preoperative nutritional status and its correlation with postoperative pulmonary complications. On table 1 we can see anthropometric and nutritional parameters in four groups of pts:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TT (45 pts)</th>
<th>TH (41 pts)</th>
<th>C (42 pts)</th>
<th>G (82 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>61.0 ± 10.5</td>
<td>65.6 ± 12.4</td>
<td>56.1 ± 13.0</td>
<td>66.3 ± 13.2</td>
</tr>
<tr>
<td>Height</td>
<td>169.8 ± 8.1</td>
<td>165.6 ± 9.1</td>
<td>164.0 ± 10.1</td>
<td>170.0 ± 8.7</td>
</tr>
<tr>
<td>Weight loss</td>
<td>9.9 ± 3.3</td>
<td>11.7 ± 11.2</td>
<td>10.0 ± 9.1</td>
<td>11.8 ± 9.8</td>
</tr>
<tr>
<td>% weight loss</td>
<td>14.7</td>
<td>19.5</td>
<td>18.9</td>
<td>17.9</td>
</tr>
<tr>
<td>MAC</td>
<td>25.6 ± 3.6</td>
<td>23.8 ± 3.4</td>
<td>24.5 ± 2.9</td>
<td>26.5 ± 3.2</td>
</tr>
<tr>
<td>TSF</td>
<td>7.1 ± 2.7</td>
<td>8.9 ± 3.9</td>
<td>8.4 ± 4.4</td>
<td>10.8 ± 4.8</td>
</tr>
</tbody>
</table>

According to Blockbum classification and MAC and TSF values pts from all groups were severely malnourished. There were no significant difference between groups in any parameter measured (ANOVA, p > 0.05), although pts from C group had benign oesophageal narrowing. The incidence of pulmonary complications was 37.7%, 53.6%, 21.4% and 23.1% respectively. Lack of the influence of parameters measuring nutritional state on the incidence of pulmonary complications is the consequence of multifactorial influence on their occurrence.

Preoperative Chemotherapy and Concurrent Irradiation for Localized Esophageal Carcinoma: Results of a Phase II Study

M. Vychov, P. Senesse, C. Debrigode, P. Rouzet, F. Quenet, S. Salas, B. Saint Aubert, C. Astre, J.B. Dubois. C.R.L.C Val d’Aurelle, Montpellier, France

Purpose: A prospective phase II study to determine the outcome of patients (pts) with oesophageal cancer who receive preoperative chemoradiotherapy. Patients and Methods: Between January 1992 and October 1994, 46 pts with localized esophageal carcinoma were treated with chemotherapy and concurrent external beam irradiation followed by esophagectomy. Each patient received two courses of chemoradiotherapy on day 1 to 5 and on day 21 to 25. Chemotherapy consisted of leucovorin 200 mg/m²/d followed by 5 FU 400 mg/m² id in one hour infusion and Cisplatinum 70 mg/m² on day 2. Irradiation was delivered one hour after 5 FU, at 3 Gy per fraction for a total dose of 30 Gy. Twenty-two pts had lesions measuring 5 cm or less, 20 had lesions measuring more than 5 cm and data was unknown in 3 cases. Thirty two pts had squamous cell carcinoma and 14 had adenocarcinoma.

Clinical, Endoscopic & Pathologic Features of 416 Pts with Esophageal Cancer from Iran

H. Frount Pishskari, R. Rabiee, G.A. Amirian Mojardar. Endoscopy Unit, Imam Khomeini Hospital Tehran, Iran

We reviewed the clinical, endoscopic & pathologic findings of 416 pts with esophageal CA (285 Pts from Tehran & 131 Pts from Sistan Province, south east IRAN). There were 232 (56%) Males & 183 (44%) Female Pts, with a mean age of 57.2 Yrs. (Range 28-81 Yrs), 67% of Pts were in their 5th & 7th decades. Clinically, the most common presenting feature was dysphagia observed in 93% of Pts. with a mean duration of 4 Mths. Anemia (HB < 11 g/dl) was present only in 20% of Pts. Endoscopically 8.3% of tumors were on upper third, 47.5% on middle third & 44.1% were in lower third of esophagus. Pathologically 84.4% of tumors were adenocarcinoma & 15.6% were S.C.C. 94% of tumors in upper third, 91% in middle third & 75.5% of lower third of esophagus were adenocarcinomas with the remainder being S.C.C. 81% of adenocarcinoma Pts were Males & 39% Females with a mean age of 56 yrs. 55% of SCC Pts were Males & 45% Females with a mean age of 56.8 Yrs. The adenocarcinoma Pts in Sistan province (Southeast IRAN) Were on average 5 yrs younger than Pts in Tehran. The SCC Pts. were also 2 Yrs younger than Tehran Pts.

Histological Features of Chronic Hepatitis C and HCV Genotype: Correlation with Bioclinical Data and Knodell Index


Purpose: to study the relation of characteristic histological features of chronic hepatitis C, with HCV genotypes, their eventual correlation with ALT and GGT values, and Knodell Index. Methods: the study includes a group of 40 patients (24 M, 16 F; mean age: 44 ± 15.5 years) with the diagnosis of chronic hepatitis C confirmed by serological tests (RBAlBl-Chiron), presence of RNA-VHC.
Hepatitis C and Rheumatic Diseases


Hepatitis C infection may induce several immune alterations including rheumatoid factor and type II cryoglobulinemia. The purpose of the study is to find the prevalence of HCV in a miscellaneous of rheumatic diseases and to determine a pattern to suspect the virus presence in this population. We performed a longitudinal study in 279 patients, aged 53.7 ± 15.3 years followed at a rheumatology outpatient clinic, regarding ALST, AS, Alkaline Phosphatase, γ-GT, leucocyte count, anti-nuclear antibody (ANA), rheumatoid factor (RF), HCV determination by ELISA (3rd gen) and RNA by PCR. An hepatic biopsy was performed in patients RNA+. We considered 5 major groups of rheumatic diseases: osteoarthritis (n = 43), fibrositis (n = 30), Rheumatoid arthritis (n = 61), Sjögren’s Syndrome (n = 27), miscellaneous (misc) not belonging to any of the previous groups (n = 118). Results:

<table>
<thead>
<tr>
<th>OA (43)</th>
<th>Fibrom (30)</th>
<th>pSS (27)</th>
<th>RL (61)</th>
<th>Misc (118)</th>
<th>Total (279)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV (Elisa) 2.3%</td>
<td>3.3% (1)</td>
<td>11.1% (3)</td>
<td>9.8% (6)</td>
<td>16.1% (19)</td>
<td>9.7% (27)</td>
</tr>
<tr>
<td>RNA (PCR) 2.3%</td>
<td>3.3% (0)</td>
<td>0%</td>
<td>8.2% (5)</td>
<td>8.5% (10)</td>
<td>6.5% (18)</td>
</tr>
</tbody>
</table>

Results: We found a higher prevalence of HCV in a heterogeneous group of 118 patients (16.1%), associated frequently with immunological alterations: Mixed Cryoglobulinemia (3), Cutaneous vasculitis (3), Weber-Christian disease (1), Polymyalgia rheumatica (1). Sera were collected from 84% of the patients (n = 234) and RNA by PCR. An hepatic biopsy was performed in patients RNA+. We considered 5 major groups of rheumatic diseases: osteoarthritis (n = 43), fibrositis (n = 30), Rheumatoid arthritis (n = 61), Sjögren’s Syndrome (n = 27), miscellaneous (misc) not belonging to any of the previous groups (n = 118). Results:

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</table>

Conclusion: A positive pathergy test is more frequently seen in patients with chronic HCV infection compared to normal and disease controls and may represent an extraneous phenomenon associated with HCV infection. Further studies are ongoing to determine the immunohistological characteristics of the pathergy reaction in this group of patients.

50 Infants and Neonates Screening for Hepatitis C Virus Infection

Abou El Magd Enas, El Rashidi Zeinab, Saad Eldin Koula, Kholal Ahmed, Nabawy Zekry Abdel Rahman. Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt

The aim of this study was to screen infants born to anti-HCV seropositive and seronegative mothers for evidence of HCV markers. Symptoms free 61 month infants pairs were classified into 4 groups according to infant’s age. Blood samples were taken from all mothers and infants. Saliva samples was collected from all infants.

Collective and specific (core, NS3, NS4a and NS5a) IgM anti-HCV antibodies, IgG and IgM anti-HCV antibodies by ELISA test, HCV RNA by RT, PCR and HCV antigen in lymphocyte Lysate by Dot ELISA test were done.

Eight out of 61 (13.1%) of mothers were seropositive for collective IgM anti-HCV antibodies, 5 of them were seropositive for IgG anti-HCV and NS3, 4 of them were seropositive for IgG anti-NS4 and only one was seropositive for IgG anti NS5. 5 mothers were seropositive for IgG anti-core, NS3 and HCV RNA. Only 4 infants had who were seropositive for IgM anti HCV core and NS3 but only 3 were seropositive for IgM anti-HCV NS5a and only 2 for IgM anti-HCV NS5b (N 4). HCV RNA were detected in 2 out of 4 IgM anti HCV seropositive infants. The same 2 infants were also positive for salivary IgM anti-HC. All seropositive mothers and infants for IgM anti-HCV were also negative for HCV RNA and other markers tested.

In conclusion mother to infants transmission of HCV does exist.

51 Hepatitis C Virus Infection among Medical Personnel

El Sherif Ahmas, El Sherif Assem, Saad Eldin Koula, Aal Maged Abdel, El Shinnawy Gamal. Faculty of Medicine, Al Azhar University, Cairo, Egypt

The aim of the present work was to study the prevalence of HCV seromarkers indicative of past or current infection of medical personal as a high risk group. This study was carried out on 202 volunteer medical and paramedical staff members working in different hospitals and medical institutes. Sera were collected and tested serologically for HCV-antibodies using 3rd generation ELISA Technique and the positive cases were subjected to PCR test, liver function tests, HBsAg and abdominal ultrasonography. Saliva samples was also obtained and examined for anti-HCV.

The results revealed that anti-HCV was detected in 13.86% of the medical personnel while only 4.95% of them showed positive PCR reaction indicative of viraemia. A significant increase in the frequency of HCV was noticed from persons aged 40-49 years old and duration of employment. A positive correlation was also found between the serum level of anti-HCV (optical Density Level) and the presence of anti-HCV antibodies in saliva.

From this study, it is obvious that frequent contact with blood, blood products and body fluids during health care work practice carries a definite risk of HCV transmission as high frequency of HCV seropositivity was found among laboratory personnel.

52 Hepatitis C Genotypes in French Haemophilics: Kinetic and Reappearance of Mixed Infections


Aims: The aim of this study was to investigate the distribution and kinetic HCV genotypes and prevalence of mixed genotypes of hepatitis C in haemophiliacs repeatedly exposed to non-virus inactivated clotting factor.

Patients and Methods: We analysed 45 patients with A or B haemophilia or Von Willebrand's disease (37, 7, respectively) and 2 mixed infections. We analysed 36 haemophiliacs who were genotyped twice with a mean of 96.3 months follow up (min = 56-max = 171). We analysed the HCV genotype in the core and 5' untranslated regions by means of modified core and non-LIPA methods, respectively. Sensitivity for detection tests was evaluated by the detection of type specific NS4 antibodies was performed for 43 patients.

Results: 1) HCV genotype prevalence: We revealed genotypes 1 (n = 23: 51%), 1a (n = 10: 22%), 1b (n = 13: 28%), 2 (n = 10: 22%), 2a (n = 3: 6%), 3a (n = 4: 8%) and 3b (n = 1: 2%). Since the circulation in the group of genotype 3 was performed in SUTR revealed 2/8 mixed infections (1a + 1b and 2b + 3a). Our core modified method showed 8/45 mixed infections: 6/8 a + 1b and 23/8 + 2. By designing new primers more specific to genotype 3 we could confirm such a + 1b mixed infection in only 1/5 cases. This result was also confirmed by direct sequencing. 2) Evolution upon time: Only 5/33 haemophiliacs showed a change of genotype during follow-up: 2 from 1a to 1b, 1 from 1b to 1a,
A8

1 from 1a to 2a and 1 from 1b to 3a. 4) Serotyping: Seventeen of 21 anti-HIV patients showed concordance with 5’UTR genotype; only 6/19 anti-HIV patients showed detectable serological reactivity. 3 serum samples showed reactivity toward 2 HCV types, whereas genotyping assays only revealed 1 type in these three cases. Conclusions: We have 1) observed a similar HCV genotypes distribution between French haemophiliacs and non-haemophiliac HCV+ patients; 2) demonstrated the difficulties to assess with the available genotyping and serotyping assays the real prevalence of mixed infections in polytransfused subjects.

Analysis of the Core Region of HCV Genome Isolated from Patients with Chronic Hepatitis C during Intervals of Normal ALT Concentration
First Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan.

There are several reports on hepatitis C virus-specific cytotoxic T lymphocytes (CTLs) recognizing an epitope in the core region. In this study, we determined core region nucleotide sequences of specimens from patients with chronic hepatitis C during intervals of normal ALT concentration without treatment.

Materials and Methods: Six patients analyzed in this study had chronic hepatitis C and their ALT concentrations had remained normal for more than one year without treatment. In one patient, we were able to compare the amino acid sequence during normal ALT concentration with that during elevated ALT. The core region of the HCV genome was amplified by the PCR. PCR products were then cloned. At least 5 independent clones were sequenced.

Results: In 2 of the 6 patients, some clones that could be sequenced showed deletions in more than 2, in the 6 patients, most of the isolated clones that could be sequenced had mutations at specific amino acids. When the ALT concentration was normal, we could sequence 10 independent clones. However, when the ALT concentration in the same patient was elevated, six of ten clones that could be sequenced were equivalent to one clone that was obtained during normal ALT concentration.

Conclusion: These results suggest that expression of the wild-type HCV core region genome as well as diversity of the HCV core region genome was associated with liver cell damage.

Relationship between Hepatitis C Virus (HCV) Genotypes and Sources of Infection in a Sample of Patients from Northern Portugal
T. Vasques 1, J.A. Sarmiento 2, F. Carneiro 1, G. Macedo 2, A.M. Vale 2, J.J. Riezu 1, T. Ribeiro 1, J. Pieto 1, Institute of Molecular Pathology and Immunology of University of Porto (IPATIMUP), Medical Faculty, Porto, Portugal; 3 Gastroenterology Unit of H. S. J. Porto, Porto, Portugal; 3 Internal Medicine, University of Navarra, Pamplona, Spain.

Aims: To study the relationship between HCV genotypes and gender, age, and putative source of HCV infection, in a sample of patients from northern Portugal.

Patients and methods: The present study included 143 patients anti-HCV- positive (ELISA) (94 men, mean age 43 ± 15 years) referred to H.S.J. between 1989/95. Patients were divided according to age (<40 years and >40 years) and source of infection (past history of blood transfusions (BT), intravenous drug users (IVDU), and unknown cause (UCI)). HCV-RNA was detected in serum by nested PCR with primers directed to the 5’UTR. Genotyping was performed by means of a hybridization procedure using specific probes for HCV genotypes 1a, 1b, 2a, 2b, 3a, and 4, according to Simmonds et al, and the amplified nested PCR product of the HCV Core region. The Qui-square method was used to statistical analysis.

Results: HCV-RNA was detected in 119/143 patients (83.2%) and genotyping was done in 107 patients from the latter group: 1a (71.8%), 1b (53.3%); 2a (2.9%); 3a (19.6%); 4 (2.8%), 1b + 2b (1.9%), unclassifiable (1.9%)

The statistical analysis was performed after excluding double infections and unclassifiable genotypes:

<table>
<thead>
<tr>
<th>n</th>
<th>Genotypes (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>36.6</td>
<td>9.0</td>
</tr>
<tr>
<td>1b</td>
<td>78.9</td>
<td>9.1</td>
</tr>
<tr>
<td>2a</td>
<td>1.9</td>
<td>7.7</td>
</tr>
<tr>
<td>3a</td>
<td>19.6</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Year of birth before 1945: 69% 50% 8% 67% after 1945: 31% 50% 92% 100% 33% 100%

Infection period before 1970: 63% 75% 95% 100% 70-1970: 13% 31% 50% 100% after 1980: 10% 12% 69% 50% – 100%

Conclusions: Genotype 1b was more frequent in more aged patients with suggestions of old infection by parenthorical inapparent routes. Genotype 2c was distributed in the old ages and was related mostly to iatrogenic factors. Genotypes 3a and 1a were more frequent in young males and related to drug addiction. These data are consistent with the hypothesis of secular changes in HCV genotype spread.

Soluble IL2 Receptor Levels in Peripheral Blood of Patients with Chronic Hepatitis and Asymptomatic Hepatitis Virus Carriers — In Special Comparison with B Type and C Type
Hiroaki Ishimaru. 1st Dept of Intern Med, St. Marianna Univ School of Med, Kwasaki, Japan.
It is known that soluble IL2R (sIL2R) levels in peripheral blood can be used...
as index of activated T cells. In present study, IL2R levels were measured in peripheral blood of patients with chronic hepatitis positive for HBV or HCV and asymptomatic carriers, using EIA kits for sIL2R measurement.

(Subjective Method) Cases with chronic hepatitis (CH) and asymptomatic carriers (ASCs) were positive for HBV or HCV. Classification (CH, CAH Ila, CAH IIb) of CH was done due to basis on histological findings, and ASC was diagnosed due to normal level of serum transaminase for two years, none chronic phenomena and normal finding of liver and spleen by abdominal US. In methods, sIL2R in peripheral blood was measured, using cell-free IL2R measurement kit of EIA (T cell diagnostics company).

(Results) In HBV carriers, serum sIL2R revealed normal to high levels in order of ASCs and CH. In HCV carriers, sIL2R revealed the increased levels in each group, which were higher in later in order of ASC, CH and CAH. The level of sIL2R was higher in C-ASC than in B-ASC. In fatty liver with increased level of serum transaminase, it was within normal range. Furthermore there was the significantly positive relationship between the levels of serum sIL2R and transaminase. There was not the significant change of serum transaminase levels at an interval of several months. sIL2R showed the significant high level one week after start of IFN+ therapy, compared with one before it. The change was similar to one of ratios of T cell subsets positive for IL2R on the membrane. The levels of sIL2R and transaminase were lower two months after start of SNM (glicynrhimcin) – intravenous injection, compared with ones before it. Next capacity of IL2 production in peripheral blood mononuclear cells (PBMN) increased in B-CH and B-ASC and in C-CAH IIb. Capacity of IL2R response in PBMN was not significantly different between each group.

(Conclusion) Circulating sIL2R levels either in HBV or HCV carrier were higher than CH than in, and increased in progression of CH. In comparison with HBV and HCV carriers, it was suggested that the activation of T cells might be accelerated in HCV carrier rather than in HBV carrier.

[58] Cirrhosis, HCV Infection and Diabetes Mellitus

G. Macedo, A. Correia, N. Fernandes, H. Queiroz, J. Pires, T. Pinto, J.A. Sarmento, A.M. Vale, T. Ribeiro. Gastro Unit and Faculty of Medicine, Porto, Portugal

Introduction and aim: It is known for a long time the association between cirrhosis, glucose intolerance and Diabetes Mellitus (DM), and several contributive factors have been proposed: hyperinsulinemia, insulin resistance, reduced glucose uptake by cirrhotic livers. In cases with pancreatic disease associated (hemochromatosis, alcoholism) or with underlying autoimmune diseases, DM is a common observation. As HCV infection is associated with autoimmune phenomena, and being clinically observed a high prevalence of DM in those patients, we compared the prevalence of DM in cirrhotics from different etiologies.

Material and methods: In a retrospective study of 96 cirrhotic patients, several parameters were registered: age, sex, etiology, HCV, DM, steroids use, B blockers use. DM diagnosis was based upon the need for oral hypoglycemic agent or insulin, or glucose > 12 mmol.

Results: The distribution of etiology and DM prevalence was:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cirrhos</th>
<th>No. of DM patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>28</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>HBV</td>
<td>13</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>PSC</td>
<td>5</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hemoc.</td>
<td>5</td>
<td>480%</td>
</tr>
<tr>
<td>Crypto.</td>
<td>4</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>PSC</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

No patient had been on B blockers before DM diagnosis, and one had been treated with steroids for over.

Conclusion: DM prevalence is significantly higher in HCV cirrhotic patients than in other common etiologies (HBV, alcohol).

This retrospective study supports a genuine link between HCV cirrhosis and DM.

[59] Only Some Autoimmunity Markers Are Associated with Hepatitis C Virus

J. Graus, R. Bárcena, V. Ureña, A. Moreno. Hospital Ramón y Cajal de Madrid, Spain

Purpose: To investigate if the Hepatitis C Virus (HCV) induce autoantibody production in Chronic Hepatitis C patients.

Methods: We analyzed antinuclear (ANA), antimitochondrial (AMA), antistriatified (SMA), antiliver and kidney microsomes antibodies (LKM) in 153 patients HCV+ (91 males); antismooth muscle (SMA), antimitochondrial (AMA), antiliver kidney microsomes (LKM-1), thyroid microsomal, thyroid globulin, gastric parietal antibodies and rheumatoid factor.

Results: The autoantibodies levels were higher in CHC patients than in asymptomatic. The question of if and how HCV is associated with autoimmune disease remains unknown. If the association exists, it may at least in part be genetic, and HLA type may provide an answer. Larger numbers are also required before genotypic influence can be excluded.

[60] Thyroid Dysfunction in Chronic Active Hepatitis (C)

H. Risk, 1, N. Gharb, 2, 1 Department of Internal and Tropical Medicine, Mansoura University, Egypt; 2 Department of Clinical Pathology, Mansoura University, Egypt

Thyroid disorders are now considered as an extrapathetic manifestation of chronic hepatitis C. The aim of this work is to study changes in thyroid function tests and thyroid autoantibodies in chronic active hepatitis C and also to find if there are correlations between the degree of hepatic damage and these changes. In this study, 20 patients with chronic active hepatitis C (12 males & 8 females) age ranging from 28 to 60 years were included. Statistically significant differences in the level of TBG and gammaglobulin were found between the chronic hepatitis C patients and control group concerning the antithyroglobulin titre (51.97 ± 64.501 Versus 7.41 ± 5.371: P < 0.05). And no significant difference was observed concerning the antithyroidperoxidase titre (55.84 ± 26.711 Versus 41.689 ± 17.915: P > 0.05). 8 patients revealed border line elevation in anti TPO (30%) and only one female patient showed significantly high anti TPO level > 125 μIU/mL (5%). On the other hand only one female (5%) revealed border line increase in anti TG levels. Also, there were statistically significant positive correlation between (the level of albumin and Free T4, Free T3, and TBG levels), (ALT level and TGB level), and (prothrombin activity and Free T4, and Free T3 levels), and there were statistically significant negative correlation between (Total bilirubin and Free T4, and Free T3 levels) and (Alkaline phosphatase and Free T3 levels). From this study it can be concluded that, in addition to the characteristic low T3 syndrome and low T3y-T4o syndrome which can occur with any non thyroidal illness. Chronic HCV infection is commonly associated with presence of thyroid autoantibodies indicating that immune stimulation and direct effect of HCV on the thyroid gland may play a role in thyroid disturbance in chronically infected HCV patients.

[61] Lack of Autoimmune Disease in Irish Hepatitis C Patients

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Purpose: To determine the prevalence of autoimmune disease and/or autoantibody positivity in Irish Hepatitis C patients. Methods: 90 Irish Hepatitis C patients (55 Anti-D recipients, 35 intra-Venous Drug Abusers and 10 Transfusion recipients) were surveyed clinically and by autoantibody serology to anti-nuclear (ANA), anti-smooth muscle (SMA), anti-mitochondrial (AMA), liver-kidney microsomes (LKM-1), thyroid microsomal, thyroid globulin, gastric parietal antibodies and rheumatoid factor.

Results: Anti- D group (all female); 2 of the Anti-D group and 1 of the transfusion group complained of generalised musculoskeletal symptoms but without clinical signs. In 5/5 (10.9%) thyroid microsomal antibodies were detected (2/5, thyroid globulin antibodies also positive). In 5/5 (9.1%), ANA titres were weakly positive and in 5/5 (9.1%), gastric parietal antibodies were positive. 47/55 were genotype 1 and 9/55 were genotype 3. IVDA group (8 females, 17 males); No autoantibodies were detected. Of 725 genotypes tested, 3 were genotype 1 and 5 were genotype 2. No genotypes 5, 6, 7, 8, 9, or 10 were detected. 5 were from 5 females, 5 males); No autoantibodies were detected. 5 were of genotype 3 and the other 5 were of genotype 1. Conclusions: These findings suggest that in Irish Hepatitis C patients, neither genotype nor source (and dose) of infection determines susceptibility to autoantibody development. The question of if and how HCV is associated with autoimmune disease remains unknown. If the association exists, it may at least in part be genetic, and HLA type may provide an answer. Larger numbers are also required before genotypic influence can be excluded.

[62] Jaundice at Onset Signifies a Good Prognosis in Anti-D Associated HCV Infection

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Introduction: Acute hepatitis presenting with jaundice occurs in less than a quarter of patients infected with the hepatitis C virus. These patients may be associated with a more benign clinical course than those who are asymptomatic. Purpose: To compare and contrast the PCR and RIBA status, serum ALT levels and histological scores in age, disease duration and viral load matched HCV anti-D recipients with and without a history of jaundice. Methods: Healthy controls and HCV+ patients showed no significant differences in ANA, AMA, AT, AD, ICA, Ap, AI and TSAb titres. HCV+ males showed a significantly higher SMA prevalence if compared to controls (p < 0.05). HCV+ females also showed higher LKM prevalence compared to controls (p < 0.1).

Conclusions: 1. RF titres are higher in CHC patients than in controls, their values being directly related to portal necrosis.

2. SMA male prevalence and LKM females prevalence are significantly higher in HCV+ patients than in controls.

3. Other antibodies’ prevalence was not significantly higher in HCV+ patients compared to controls.
HCV status was confirmed by detecting HCV-RNA by PCR and antibodies to HCV using ELISA and RIBA-3. Serum ALT levels were measured in all patients and a liver biopsy was performed in 2934 patients. All patients were genotyped. Results: 14/17 jaundiced patients were PCR positive and negative for HCV. Also 4/17 had RIBA scores greater than nine and all non jaundiced patients were PCR positive and all seventeen had RIBA scores greater than 9. 13/17 jaundiced had normal ALT values; 9/17; mildly elevated (41–100) and 1/17 > 100. 6/17 non-jaundiced had normal ALT levels, 9/17 (41–100) and 2/17 (> 100). 7/9 jaundiced had mild histological scores, 9/9 moderate and 2/9 severe. 5/17 non jaundiced had mild, 9/17 moderate and 3/17 severe. All 54 patients were of the presence of the antibodies. Patients with jaundiced associated with lower antibody scores, increased PCR negativity, normal serum ALT levels and low-normal histological scores. Jaundice at onset was an indicator of good prognosis.

63 Chronic Cryptogenic Hepatopathy: A Still Puzzling Entity In 1995

Even now, chronic elevation of transaminases without evidence of viral, toxic, alcoholic, genetic, autoimmune or vascular liver disease (defined as chronic cryptogenic hepatitis (CCH)) is considered puzzling. There are 257 immunocompetent patients with grade I–III of 1095 to liver biopsy (percutaneous: 138, laparoscopic: 2, transvenous: 117) because of chronic elevation of transaminases were reviewed retrospectively. Thirty patients (12%) had to the criterion of CCH. Blood HCV-RNA measured in 25 of them was always negative. These 30 patients with CCH (group I) were matched according to their sex and age to 30 HCV + patients (group II) and clinical, biochemical and histological features were compared between the two groups by univariate analysis (Kaplan–Meier survival). Patients with CCH were significantly older (p < 0.02) in group I. Histological analysis revealed greater inflammatory activity (using Metavir classification), higher number of lympho-plasmacytic aggregates, greater portal inflammation and greater sinusoidal activation in group II. Final histological diagnosis in group I patients was steatosis/liver cirrhosis in 18, chronic hepatitis in 5, normal liver in 4 and cirrhosis in 1. CCH represents 13% of chronic parenchymal liver disease and is characterized by abnormal lipid profile and histologically by steatosis/liver cirrhosis in most cases with minor inflammatory activity. This entity could represent a new metabolic dysregulation or a new non A non B non C viral hepatitis.

66 Prevalence of Anti-HCV Antibodies in Healthy Individuals, Alcoholics and Immigrant Population from Albania in Northern Greece
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Purpose: The aim of the present study was an attempt to the better approach of the prevalence of anti-HCV antibodies in the Greek population. Subjects and Methods: The prevalence of anti-HCV antibodies was investigated in 6742 healthy (5.815 male and 1.457 female, mean age: 38.5 years, range: 0–66 years), 684 refugees from Albania (473 male and 211 female, mean age: 39.2 years, range: 0–70 years) and 146 alcoholics (95 male and 51 female, mean age: 46.5 years, range: 30–80 years). This latter group consisted from 83 alcoholics with LD and 68 without LD. 5835 subjects from healthy were blood donors who were accounted for only once during the study while more than 50% of them were first time donors. Third generation anti-HEV immunoglobulin and anti-HEV IgG antibodies were used. Results: We found that: (a) 0.83% of healthy (50/67742) were reactive with EIA 3.0 but 21.4% (12/56) of them were positive by RIBA-II, (b) 1.75% (2/128) of refugees were positive both with EIA 3.0 and RIBA-II and (c) 1.32% (2/151) of alcoholics (1/93 with LD and 2/158 without LD) were also positive both with EIA 3.0 and RIBA-III. The seropositivity for anti-HCV antibodies was significantly more frequent among refugees than healthy (p < 0.05) but not for alcoholics and the two groups (comparison between male only). In addition, the presence of anti-HCV antibodies in healthy controls was significantly (p < 0.01) more frequent in older ages (more than 30 years). The latter finding, however, was not confirmed for the refugees. Conclusions: (1) The prevalence of anti-HCV antibodies in our region is approximately similar with that reported in most European countries. (2) The refugees from Albania had significantly higher prevalence of these antibodies than the healthy controls. (3) By contrast, the present study did not confirm the presence of high prevalence of anti-HCV antibodies among alcoholics which has been reported by others.

65 The Significance of Hepatitis B Markers in Chronic Hepatitis C Egyptian Patients
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Purpose: It is well known that both HCV and HBV share several routes of transmission. It is our aim to assess the prevalence and clinical significance of hepatitis B markers in chronic hepatitis C patients.

Methods: A total of 372 chronic hepatitis C (CHC) patients with elevated ALT were studied; 306 males and 66 females with a mean age of 42 years. All cases were ELISA-2, repeatedly positive, HCV-RNA-PCR was performed as a pre-abstinence before antiviral therapy. They were tested for hepatitis B markers (HBSAg, HBeAg/Ab HBcAb IgG, whereas cases positive for HBSAg were screened for HBV-DNA-PCR). Liver biopsy was performed in 242 patients.

Results: HBV seromolecular data are demonstrated in the following table:

<table>
<thead>
<tr>
<th>No.</th>
<th>% anti-</th>
<th>HCV-RNA</th>
<th>HBSAg</th>
<th>HBeAg</th>
<th>anti-HBc</th>
<th>HBV-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: HCV + HBV</td>
<td>dual infection</td>
<td>19</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>G2: HCV + HBeAg</td>
<td>44</td>
<td>12</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/-</td>
</tr>
<tr>
<td>G3: hepatitis C</td>
<td>207</td>
<td>56</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/+</td>
</tr>
<tr>
<td>G4: patients CHC</td>
<td>102</td>
<td>27</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/+</td>
</tr>
</tbody>
</table>

Histological diagnosis in dual infection versus (vs) isolated HCV infection revealed CPH in 16% vs 19.6%. CAH in 21% vs 58.8%, cirrhosis in 63% vs 21.5% respectively.

Conclusion: The results show a high prevalence of markers of past HBV infection among HCV viremic patients. Results also might suggest suppression of HBV replication by coexisting HCV infection. We also concluded that dual HBV & HCV infection hasten the progress & severity of liver disease.

65 Lack of Evidence of IgA Deficiency in Hepatitis C Virus Infection
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A previous study (Ilan et al. Arch. Int. Med. 1993; 3: 1588–1592) has suggested that IgA deficiency predisposes towards chronic Hepatitis C Virus (HCV) infection in some patients. It has also been suggested that IgA deficiency may occur as a secondary event as a result of the viral infection. Aims: To determine the prevalence of both selective IgA deficiency (IgA < 0.05 g/L), and partial deficiency of IgA (IgA < 0.82 g/L) in a group of patients with chronic HCV infection attending our clinics. Methods: Immunoglobulin levels including IgG, IgA and IgM were determined in 67 HCV infected patients using a nephelometric technique. These patients had been infected with HCV contaminated blood, or anti-D immunoglobulin between 1977 and 1990. Mean immunoglobulin concentration (g/L) was compared with 100 controls without HCV infection. Results: 59 women and 8 men were examined with HCV infection, mean age 45.3 years (range 27–72 years). No patient was found to be either selectively or partially deficient in IgA. Mean IgA 2.59 g/L versus 2.81 g/L in the control group (2 tailed t = 0.890). No patient had no deficiency of serum IgA or IgM was found in either patients or controls. Conclusion: No evidence of IgA or other immunoglobulin deficiency states exist among this group of patients with chronic HCV infection. While it is possible that IgA levels may decrease transiently following infection, no evidence exists that this phenomenon persists in the long term (mean duration of infection > 15.01 years). Differences between this study population and the previous report may relate to ethnic factors alone.

67 Quality of Life of Italian Patients with Chronic Hepatitis C
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After the diagnosis, patients (pts) suffering from chronic hepatitis C undergo to several changes of their life style, because of treatments, fear to infect other people, progression of the disease etc. Measurement of health related quality of life (QoL) is an important index to evaluate outcomes induced by therapies and the use of QoL questionnaires is the most suitable way to collect these informations. A multicentre survey to measure the QoL of Italian chronic hepatitis C pts started during the spring of 1996. As in Italy no specific questionnaire for CHC pts is available, the protocol proposed for the use of Italian version of the general questionnaire SF36. It was administered to anti-HCV positive males and females with compensated CHC
and serum ALT values at least 1.5 times the upper limit of normal, who never underwent a hepatic biopsy, were not previously treated with interferon or antiviral drugs and/or corticosteroids, who were free from relevant psychiatric or neurological diseases, and were HIV-1 negative. SF36 was administered to over 500 patients by 50 Investigators throughout Italy.

Analysis of the first 62 pts (mean age 46 ± 6, 65% males) showed that scores in some domains of SF36 were lower than the norm: role physical = −2.0, general health = −0.7, vitality = −1.4, social function = −0.9, mental health = −3.1. Other domains scored greater than the norm: physical functioning = +3.8, bodily pain = +1.1, vitality = +1.1. These data suggest that CHC pts perceive their disease as a serious risk to their health with alterations of social functions in spite of having a good enough physical functioning and lack of symptoms. It is notable that psychological aspects of pts are lower than normal however pts were not treated with any drugs and this does not support hypotheses on psychotropic effects of specific drug for CHC.

**68 Hepatic Glutathione Deficiency in Chronic Hepatitis C:**

G. Barbaro, G. Di Lorenzo, M. Soldini, G. Bellomo 1, G. Belloni 2

**Methods:**

A total of 125 patients with serologically and histologically documented CHC (65 HIV-positive and 60 HIV-negative); 61 healthy individuals served as a control group for P-GSH and L-GSH concentrations. H-GSH concentration was determined by High Performance Liquid Chromatography on liver specimens obtained by ultrasound-guided biopsies according to the method described by Reed et al. The concentrations of P-GSH and L-GSH were determined according to the method described by Suarez et al. The detection of HCV-RNA strands in PBMC was performed according to the method described by Qian et al. 1

**Results:** H-GSH, P-GSH and L-GSH were significantly reduced in patients affected by CHC compared with healthy controls (p < 0.001). H-GSH was significantly correlated with both P-GSH (r = 0.99; p < 0.001) and L-GSH (r = 0.65; p < 0.001). The reduction of H-GSH, P-GSH and L-GSH was significantly correlated with the replication activity of HCV in PBMC (r = 0.75 vs H-GSH; p = 0.001); r = 0.92 vs P-GSH (p < 0.001); r = 0.97 vs L-GSH (p < 0.001) and to the grade of activity of the liver disease assessed by the values of ALT (r = 0.74 vs H-GSH; p < 0.001); r = 0.83 vs P-GSH (p < 0.001); r = 0.64 vs L-GSH (p < 0.001) and by histopathological score of CHC (r = 0.75 vs H-GSH; p < 0.001). H-GSH and, particularly, L-GSH were more significantly reduced in HIV-positive patients compared with HIV-negative ones (p < 0.001), without significant correlation with the values of T cells subsets CD4+ (r = 0.068; p > 0.507 vs H-GSH and r = 0.063 vs L-GSH; p = 0.343). In both groups of CHC patients L-GSH was more significantly reduced in drug addicts compared with non drug addicts patients (p < 0.001).

**Conclusions:** In patients with CHC and, particularly, in HIV-positive patients, a systematic depletion of GSH is present. This depletion may be a factor underlying the resistance to interferon therapy and, in HIV-positive patients, to antiretroviral drugs, fostering HCV and/or HIV replication. This may represent the biological basis for GSH replacement therapy with GSH-prodrugs.

**69 HCV Heterogeneity and Response to Alpha IFN Therapy in Five Patients:**

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**Objectives:** It has been suggested that Hepatitis C virus (HCV) genome heterogeneity might be a predictive virologic parameter for responsiveness to IFN treatment. We have investigated this issue in a series of French patients treated by IFN for chronic active hepatitis B and C. Nineteen 19/95 cirrhosis) before IFN treatment were classified into three groups: long term responders (LTR) (n = 20), relapers (n = 31) and non-responders (n = 40). In these patients, HCV cytotype was determined by restriction fragment length polymorphism analysis (RFLP), 1b (n = 45), 1a (n = 11), 2a (n = 4), 4a (n = 2) and 3a (n = 29) and HCV RNQ quantitation was analyzed by the Branched DNA assay (Quantiplex 2.0). The quasispecies complex of HCV HVR 1 was analyzed by PCR-mediated single-strand conformation polymorphism (SSCP) and classified as low (SSCP band ≤ 3) or high complexity patterns (SSCP band > 3). Univariate and multivariate analyses were performed (BMDP Statistical Software Inc., Los Angeles, CA, USA). Results: In univariate analysis high complexity pattern showed the best response to IFN therapy measured by age, HCV genotypes and viral load (p < 0.0001). A low complexity pattern was associated with long term response for genotype 3a (10/14). Multivariate analyses showed that the best predictor of response to IFN therapy in HCV types associated to both low and success rates was important to embedded within a more specific study.
The Genotype 1b Replicates More Actively and It Is More Resistant to the IFN in the Patients with Chronic Hepatitis C

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Objective: To study the influence of viral genotype and viremia on the response to IFN alpha treatment in chronic hepatitis C patients.

Patients and Methods: 50 hepatitis C patients, diagnosed by liver biopsy and the presence of anti-HCV in serum (ELISA and RIBA-II), were treated with IFN alpha (Wellferon, Wellcome) for one year: 3 MU daily for 10 weeks followed by 3 MU thrice to week. Viremia was determined immediately before and after treatment by quantitative PCR (Monitor, Roche). HCV genotype was determined by the treatment by INNO-400. To normalize ALT level following treatment was considered as response to IFN.

Results: Of the 50 patients treated, 34 (68%) showed no response and 16 (32%) responded, 8 of whom (16%) maintained the response after one year of follow-up. HCV RNA level was 100 000 copies/ml for the responders and 600 000 copies/ml for non responders (P < 0.0032). All but one of those who didn't respond were RNA HCV positive at the end of treatment but to much reduced level: 150 000 copies/ml (P < 0.005). They were accomplished genopatizations with the basal serum anti C at all patients. 15 of 16 responders were genotyped (94%) and 22 of 34 non responders (64%). The most frequent genotype was 1b (20/23) (70%), 19 of 22 non responders genotyped were 1b (90%). Responders genotyped were 1b (p = 0.0130). It has been studied the patients that were presenting the genotype 1b as compared to those which were presenting other genotypes in relation to the viremia. The viremia mean of the patients with genotype 1b was 5.7 times; 10^6 copies/ml (9.7 times; 10^6) and that of the others genotypes (non-1b) was 9.8 ± 10^6 (standard desviation: 1.37 times; 10^6) (p < 0.0019).

Conclusion: The replication of HCV is greater in the patients infected with the type 1b and the response to IFN treatment in these patients is lower. Furthermore the genotype 1b is the most frequent in the population.

Chronic Hepatitis C and Severe Autoimmune Diseases

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Objectives – To test the effectiveness and tolerance of IFN interferon combined with low-doses of CSA and steroids for the treatment of patients with chronic hepatitis C virus (HCV) and exacerbation of severe autoimmune diseases.

Methods – 20 Patients (12 females and 8 males) ages ranging from 25 to 42 years were included in the study. Each patient were affected with chronic hepatitis C virus and lymphomatous crisis (new ARA classification criteria) 10 patients, and/or L.E.S. (new ARA classification criteria) 6 patients, vasculitis (histological or arteriographic evidence) 4 patients. Inclusion criteria for patients: a therapy refractory condition after an at least 3-weeks treatment with prednisone at a dose of 1 mg/kg body weight, which usually corresponded to 40–70 mg, IFN interferon (Fronese-Serona) was administered at a dose of 5 million U/week on 5 days for 6 months. Cyclosporin A (CsA) was administered initial daily dose of 5 mg/kg body weight (ideal weight in the case of overweighted objects). Blood levels of the drug were between 100 and 200 mcg/ml by the third day of treatment. All the patients also received Flucorcolone at a dose to control disease activity, that is, 80–70 mg weekly on 5 days depending on the case, and then tapered in relation to the course down to a maintenance dose of 15–20 mg/week administered on 3 days.

Results – All Patients presented a significant improvement of the clinical picture. 2 months after onset of treatment the AST had normalized and there was a statistically through Anti-D were evaluated. Mean age: 42.5 (range 33–60) yrs. 83 were PCR+ve; 71 type 1b, 2 type 2b, 10 type 3. At mean follow-up of 18 years there was no clinical evidence of glycurboglobinemia. However 1PCR+ve case with small joint arthropathy had a cryicit of 2%. There was 1 case of mild sepsisive RA and 1 of palatineic RA. 4 had small hand joint arthropathy while 1 R.J. Farrell, D. McGonagle, R. Pilkington, E.B. Casey, D. Kelleher. Departments of Hepatology and Rheumatology, St James’s Hospital, Dublin 8, Ireland

Introduction: Hepatitis C Virus (HCV) infection is associated with several connective tissue diseases. The most well recognized is Sjogren’s disease (SD), an asymptomatic and non-specific arthritis. In 1977–78 Anti-D Immunoglobulin batches infected with HCV were inadvertently administered as prophylaxis against Rhesus blood group incompatibility. Virtually all patients exposed to HCV whether symptomatic or not have been evaluated thus allowing the accurate determination of the true prevalence of rheumatic conditions at a mean time of 18 years since exposure. Methods: HCV was diagnosed using Riba testing and confirmed with PCR. Groups where HCV was acquired by other means (VUD, blood transfusion, sex, coinfection and transplant and sporadic) were eliminated. Patients with suggestive symptoms were carefully re-evaluated with full autoantibody screen, cryoglobulins and Schirmer test. Results: 136 patients who acquired HCV were evaluated. The other 18 patients who had other methods of infection were included for comparison. HCV-RNA was present in the sera of all but one of the anti-HCV+ve patients. It was concluded that chronic HCV infection is more common in the patients with NHL, in comparison to general population. There is no correlation between the history of transfusion and anti-HCV positivity in lymphoma.

Abdominal Lymphadenopathy and Histological Pictures in Chronic Hepatitis C

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Aim: To evaluate the relationship between ultrasoundography (US) findings of upper abdominal lymphadenopathy and histological activity index (HAI) and serum liver function tests (LFTs) in subjects with chronic HCV infection.

Methods: 58 subjects (41 M, 17 F) (mean age 46.4±12.4 years), with chronic hepatitis C were studied. None were HBSAg+ve, alcoholics or had other known causes of liver disease or neoplasia. US scans was performed using a real time equipment (Toshiba SSA 360) with a 3.5–7.5 MHz convex transducer. Liver biopsies were performed with needles 16 G (Surecut, Hospital Service, Rome). HAI was evaluated with Knodell’s score (K), which expresses necrosis-inflammation and fibrosis, and Desmet’s score which separately determines gradient (G) necrosis-inflammation and staging (S) expressing fibrosis. The common LFTs (AST, ALT, ALP, GGT, total bilirubin, prothrombin activity, serum albumin, y-globulin) were assayed. Statistical analysis was performed using Student’s t test, Pearson’s and Spearman’s correlation coefficient r.

Results: 36 subjects did not present lymph nodes (LN) on US (LN–), while 22 did (LN+). Mean values of the K and G scores were significantly higher in the LN+ vs. LN– subjects.

Hepatitis C Infection in Non-Hodgkin Lymphoma

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Chronic Hepatitis C virus (HCV) infection was proposed as a predisposing factor in the development of non-hodgkin lymphoma. Despite its oncogenic mechanism is far from clear; HCV infection is a factor in B and T lymphoproliferation and its long-term presence in immune system may promote the infiltration of bone marrow with expanded clones of Ig-secreting lymphocytes. We examined the serum samples from 30 patients (17 male, 13 female, median age 52, range 16–84 y) with non-Hodgkin lymphoma (NHL) and the lymphomas and its clinical manifestations in 34 patients (10 male, 8 female, median age 26, range 17–61 y) with Hodgkin lymphoma (HL) for anti-HCV antibodies by ELISA II (Abott, HCV EIA) Anti-HCV positive sera was tested for HCV RNA by nested PCR as well. The serological results from sera of 948 healthy blood donors (age range 16-65 y) were used as controls. 4/30 (13%) of patients with NHL and 74/4948 (0.8%) of blood donors were anti-HCV-positive (p = 0.007). None of the 18 patients with HL was +ve for anti-HCV. There were positive correlation with: age of the patient and anti-HCV positivity (24/4 of anti-HCV+ve vs 13/26 of anti-HCV in NHL and 5/18 in HL) Serum ALT levels of 24% of anti-HCV positive were elevated. Liver biopsy showed chronic hepatitis in these patients. Biopsy was not performed for all the patients due to other complications. HCV-RNA was present in the sera of all but one of the anti-HCV+ve patients.
was had family. had days. hepatitis-A. increase of restaurant patients of and the questionnaire among 41% of are representing the the host immune response have been suggested to play a role in liver injury occurring in patients with chronic hepatitis C. In order to explore the relationship between the relative proportions of intrahepatic and peripheral blood lymphocytes (ILL, PBL), the levels of viremia, and the histological hepatitis activity score, three-colour fluorescence-activated cytometric analysis was performed for 36 patients with chronic hepatitis C before interferon therapy and 6 control subjects without chronic hepatitis.

Each liver biopsy was divided into two parts: one for histological examination and one for immunological analysis. Tri-color CD45 was used to improve "lymphogating". Fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies with specificity for CD3, CD4, CD8, and CD20 (lymphocyte subpopulations), for CD69 (activated lymphocytes), and for CD16/56 (natural killer cells) were used. Levels of viremia were determined by PCR (Monitor, Roche Diagnostic Systems).

The proportion of IHL CD4+ was significantly increased in patients with chronic hepatitis C, resulting in an IHL CD4+/CD8+ ratio significantly higher than in control subjects (0.55 ± 0.21 vs 0.32 ± 0.12, p = 0.046), while the IHL CD8+ T lymphocytes (40.7 ± 13.9% vs 26.0 ± 6.6%, p = 0.0001) and a smaller percentage increase was observed in both, which was interpreted to be due to local immune responses. We report about 201, which exhibited marked expression of CD69, compared to peripheral blood. No statistical correlation was found between the intrapancreatic CD4+/CD8+ ratio and the level of viremia, Knodell's score, or transaminase activities.

Our findings indicate that a cellular immune response does take place in HCV-infected livers, and could contribute to the pathogenesis of HCV-induced liver damage.

78 High Prevalence of Antibodies to Hepatitis A Virus (HAV) Infection in Healthcare Workers

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Objective: To determine the occupational risk of contracting HAV infection among healthcare workers in a large general adult hospital which should represent the relative risk among healthcare workers in other general hospitals. Methods: 625 healthcare workers were recruited of whom 264 were student nurses, 98 medical students, 87 staff nurses, 65 administrative staff, 60 physicians, 31 laboratory staff and 30 physiotherapists. Each participant completed a questionnaire by interview and 10 ml of venous blood was withdrawn which was tested for total anti HAV immunoglobulin (primarily IgG) using an ELISA competitive assay.

Results: 17% of student nurses were HAV antibody positive, 18% of medical students, 48% of staff nurses, 41.5% of administrative staff, 40% of physicians, 41% of laboratory staff and 23% of physiotherapists were also HAV antibody positive. Most of them had received vaccination, specifically physicians and administrative staff are at significant risk of HAV infection whereas physiotherapists, medical students and student nurses were not.

Conclusion: These findings suggest that patient exposure does not alone account for increased susceptibility. This suggests that healthcare workers may be at increased risk of HAV infection and would benefit from a HAV vaccination programme.

81 Nosocomial Transmission of HBV

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Purpose: The study's purpose is the determination of the dispersion of HBV in chronic patients and the investigation of the possible significance of hospitalization as a risk factor for HBV infection.

Patients and Methods: 657 chronic patients, who were hospitalized in our hospital between Sept. 91 and Dec. 94, were classified in two groups. Group A consisted of 248 transfused patients, 157 (63.3%) males and 91 (36.7%) females, with a mean age 65.2 years (23-92), a mean number of hospitalizations 15.8 (2-60), a mean number of transfusions 5.5 units (1-25), a mean number of transaminases 5.3 units (0.1-90), and a mean number of transfusions 5.5 units (1-25). Group B consisted of 409 non transfused patients, 262 (64%) males and 147 (36%) females, with a mean age 67.3 years (22-92), a mean number of hospitalizations 15.9 (2-60), and a mean number of transaminases 5.3 units (0.3-20). Patients with other risk factors for hepatitis B were excluded from the control group. As a control group, fully comparable for age and sex, we used 480 healthy controls with a history of chronic disease, hospitalizations or transfusions. High risk individuals for hepatitis B were excluded from the control group as well. The determination of the dispersion of HBV was made through the search of anti-HBc and HBsAg using the ELISA method. The statistical analysis was made on PC (D Base IV, SPSS).

Results: In group A the prevalence of anti-HBcAg was 60.3% and of HBsAg 8.9%, in group B 54.6% and 7.85%, whereas in the control group 34.5 and 4.5% respectively. The statistical analysis showed important difference regarding the presence of HBsAg and anti-HBcAg between groups A, B and the control group. A positive correlation between serum positivity and number of transfusions in group A and number and duration of hospitalizations in group B was confirmed.

Conclusions: The results show a high dispersion of HBV in chronic patients and demonstrate that besides transfusion hospitalization consists a significant risk factor for HBV infection as well.

B2 Prediction of Severity in Chronic Active Hepatitis B (CAHB) by PCR-RFLP Method

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We have reported that amino acid substitutions Ile-141 to Leu (L) at codon 87 and Ser to Gly (G) at codon 97 due to DNA mutation in core region were noted in patients with CAHB, whose serum GPT (sgGTP) were below 100 IU/l (published in 4th UEGW). However, it is time consuming to detect mutations by DNA sequencing. We studied whether these mutations could be detected or not by Restriction Fragment Length Polymorphism (RFLP) method.

Twenty-six patients with CAHB were studied. CAHB were characterized of two groups according to SGTP value. In group 1 (10 patients) and group 2...
(16 patients), SCPT were over 100 IU/l and below 100 IU/l, respectively. HBV DNA was extracted from patient's sera and amplified by PCR with core region specific primer. Digestion study at codon 87 and 97 for PCR products was performed with AluI and DdeI, respectively. To prevent incomplete digestion, PCR products were incubated with restriction enzyme at 37°C over night and analysed for argarose gel-electrophoresis. In group 1, DNA fragments ladder was the same pattern as wild type allele. The other hand, different ladder pattern (mutant type) were found in group 2. In digestion with AluI, DNA fragment of 88 bp was detected in group 1, but not in group 2. And also, in digestion with DdeI, DNA fragment of 410 bp was detected in group 1, but not in group 2. Further ladder pattern that mentioned above in wild and mutant type, mixed type, was frequently found in group 2. Therefore, in group 2, mutant or mixed type were detected by RFLP. These results showed that PCR-RFLP at codon 87 and 97 is available for prediction of severity in chronic active hepatitis B without DNA sequencing.

83 Is There a Real Nuclear Localization of HBV Core Protein? A Detailed Subcellular Localization Study

H. Sima, O. Rosmorduc, D. Kremsdorfer, C. Bréchot. INSERM U370, Paris, France

The subcellular distribution of HBV capsid protein (C) and its implications for the viral life cycle is still a matter of debate. In this study, we examined the localization of C in different cell lines as well as in the livers of humans and replicative transgenic mice (TG) in dependence of cell cycle and viral replication status. Methods: In vitro: HuH7, stably and transiently transfected with constructs containing HBV, HBVΔ5 (generated from singly spliced 2.2 kb HBV RNA) or C-ORF alone. HuH7 cells were synchronized using TGF-β, double thymidine block and nocodazole (N). The subcellular localization of C was studied by indirect immunofluorescence (IF) and biochemical fractionation of synchronized cells. In vivo: Localization of C in the livers of highly replicating TG (kindly provided by F. Chisari) and humans from both high and low HBV replicators was studied by immunocytochemistry (IC) and CM-permitting 3D reconstructions (3D). Results: In vitro: There was no evidence for a cell cycle related nuclear translocation of C, contrasting with recently reported data. C was in fact exclusively cytoplasmic characterized by diffuse granular staining with perinuclear attenuation. Transfection of COS cells stably expressing large T-Antigen (LTA) revealed C is not a nucleoprotein like LTA. Double labelling with antibodies against nuclear pore complex (NPC) and lamin showed further an association of C with nuclear membrane (NM). Biochemical fractionation of synchronized cells confirmed further the in situ localization data. In addition, N-arresting cells in mitosis led to a diminution of C as revealed by immunoblotting. In vivo: TG showed cytoplasmic C staining in centriobular hepatocytes whereas nuclear C was parallelly revealed by IF as recently reported (Guidotti et al.). The human livers from high replicators showed in virtually all hepatocytes cytoplasmic C in addition to the nuclear staining. In low replicators C was restricted only to the nuclei. A detailed CM analysis of nuclear C distribution in TG and human hepatocytes with a 3D proofed expression is used to localize C of only 40 nm. Our results show that C is restricted exclusively to the cytoplasm. However, we provide evidence for an association of C with the NM and mitotic disassembly of NM destabilize C. 2) In vivo, cytosolic C is an indicator of high viral replication status as observed in fresh frozen samples and data are consistent: low viral replication is accompanied by low C expression. The reported nuclear localization signal of C leads to its binding to the NM but is not sufficient for a translocation through the NM. Rough mutants and stabilization of C at the NM is disturbed with each mitotic NM disassembly.

84 Randomised Controlled Trial of Interferon α2b in Prolonged Hepatitis B

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Objective: With the hypothesis that interferon is effective and well tolerated in prolonged hepatitis B (> 10 weeks), we designed this randomised controlled trial.

Setting: Tertiary referral centre.

Participants: Patients with virus B hepatitis of > 10 weeks and positive of anti-HBc IgM, HBeAg with elevated alanine aminotransferase (1.5 x N) were recruited.

Intervention: a-interferon (INTRON A) 3 MU thrice weekly (group A) for 16 weeks or conventional treatment (group B) was given.

Study variables: Clinical status (weekly), biochemical and haematological parameters (every 2 weeks), ALT and HBeAg. Subjects were followed up for 6 months after the trial. Compliance to therapy and side effects were monitored.

Outcome: Clinical improvement, clearance of HBeAg and HBsAg.

Results: Of 20 patients enrolled, 9 received interferon and 11 conventional treatment. Age ranged from 18 to 42 years and baseline characteristics were similar in both groups. Compliance therapy was 100% and side effects such as malaise, arthralgia were observed in no patient. HBeAg became negative in two patients each in group A & B, HBeAg was negative in 3/9 (group A) and 2/11 (group B). One patient in group B progressed to subacute hepatitis failure and expired.

Conclusion: Interferon treatment is beneficial in prolonged B hepatitis and is a promising modality of treatment. Further long term trials are needed in larger samples.

* Study funded by Fulford India Limited (Schering Plough, USA).

85 Pre-Core Stop Codon Mutations among Patients with Chronic HBV Infection in Turkey

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Background: Pre-core stop codon mutation, G to A substitution at nucleotide 1896 that preclude the production of HBsAg develops around the time of HBs seroconversion. Pre-core stop codon mutation (A1896) is not found in patients (pts) infected with HBV genotypes that have a C at nucleotide 1858 due to a blocking in the stem-loop structure of the pregenome signal. Thus the prevalence of the pre-core stop codon mutant (A1896) among HBeAg+ pts varies in different geographical areas depending on the predominant HBV genotype.

Aim: To determine the prevalence of pre-core stop codon mutation (A1896) in Turkish pts with HBeAg+ and HBeAg− chronic hepatitis B.

Patients and Methods: Sera from 14 HBeAg+ and 18 HBeAg− pts were analysed by direct sequencing of the pre-core region PCR amplified DNA.

Results: All the patients studied had T at nucleotide 1858. Three of 14 HBeAg+ pts had mixture of wild type and stop codon mutant A1896, the other 11 had wild type sequence only. Fifteen of 18 (83%) HBeAg− pts had the stop codon mutation. The other three had another common substitution involving mutation of G to A change at nucleotide 1896. Mutations involved in the initiation codon was not observed in any pts studied.

Conclusion: Most HBeAg+ Turkish pts with chronic hepatitis B have the pre-core stop codon mutation (A1896) that is related to the fact that the predominant HBV genotype in Turkey has a T at nucleotide 1858 which permits G to A mutation at nucleotide 1896.

86 Hepatitis B X-Protein is Exclusively Localized in the Cytoplasm and Colocalizes with Proteasomes

H. Sima, O. Rosmorduc, D. Kremsdorfer, C. Bréchot. INSERM U370, Paris, France

In the view of potential implication of HBV X-Protein (X) in hepatocarcinogenesis, we have studied its cellular localization in the following reasons: 1) The subcellular localization of X is still a matter of debate. II. X may exert, depending on its sublocalization, different effects and interact with proteins which are temporary expressed and compartmentalized in function of the cell cycle. Methods: 1) 1) HuH7 cells were stably transfected with HBV and HBVΔ5 (generated from singly spliced 2.2 RNA) containing constructs. 2) Expression of X was studied by immunoblotting. 3) Cell synchronization was achieved by TGF-β (G1), double Thymidine block (aT) and Nocodazole (N). 4) X was additionally labeled by BrdU pulse. Cellular localization of X was studied by immunofluorescence (IF). 5) To analyze the behaviour of X in living cells we have used the green fluorescent protein (GFP). GFP was used as a C-terminal tag to create X-GFP chimeras and was expressed in cells by transient transfection. Subsequently, the distribution of GFP and X-GFP was analysed in living cells by fluorescence microscopy. Using fixed cells, we have further performed double-labelled using 7-Aminomycin D and antibodies against Lamin and Proteasomes. The biological activity of the chimera protein was tested using the stimulation of NF-κB dependent transcription by X. Results: 1) The cells could be synchronized to high degree (> 80%). X was distributed irregularly as granules embedded in diffuse cytoplasmic staining with perinuclear dominated corona. 2) GFP alone was distributed locally in the nucleus and the cytoplasm. In contrast, X-GFP was located only in the cytoplasm as granulo-glubular structures and juxtanuclear caps. Using double labelling in fixed cells, X confirmed the exclusively cytoplasmic location of X as already seen in living cells. IF and CA studies further demonstrated a colocalization of the cytoplasmic proteasomes at sites of X. X-GFP chimera activate NF-κB dependent transcription consistently with recently reported data. Thus, addition of GFP to X does not alter one of its well characterized biological activity. Conclusions: 1) X is located exclusively in the cytoplasm. There is no cell cycle dependent translocation into the nucleus. These data support a model of X acting indirectly on transcriptional process. 2) The colocalization of X with proteasomes suggest that X may interfere with cellular protein degradation leading to modulation of the half-life and activity of transcriptional and other regulatory factors; this would provide an unifying explanation for the markedly pleiotropic effects of X.
88 Prevalence of Hepatitis E Virus Antibodies in Tunisia

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Hepatitis E virus (HEV) infection is common in Asia and Africa, but its prevalence is unknown in our country. Furthermore, although HEV represents the main etiological agent of enterically transmitted non-A, non-B (NANB) hepatitis, the risk factors associated with anti-HEV positivity are not well known yet.

Aim: This study investigates the prevalence of HEV antibodies in different populations and etiological hepatitis A and E antibodies prevalence.

Method: We studied the prevalence of anti-HEV IgG in 205 asymptomatic male volunteer blood donors (AVBD), 87 health related staff (HRS), 49 hemodialysis patients and 21 cirrhotic patients. All samples were tested with HEV A/B/C (LABNIA, France). ELISA technique was used to detect anti-HAV IgG and anti-HEV antibodies.

Results: (see table)

<table>
<thead>
<tr>
<th>n</th>
<th>age (years)</th>
<th>anti HAV</th>
<th>anti HAV</th>
<th>anti HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVBD</td>
<td>205</td>
<td>24</td>
<td>4.9%</td>
<td>100%</td>
</tr>
<tr>
<td>HRS</td>
<td>87</td>
<td>32</td>
<td>8.9%</td>
<td>98%</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>47</td>
<td>41</td>
<td>14.9%</td>
<td>68%</td>
</tr>
<tr>
<td>Cirrhotic patients</td>
<td>21</td>
<td>60</td>
<td>19%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*p < 0.05 referring to AVBD

89 Detection of Hepatitis G/G/C-Viral RNA but not HCV RNA in the Different Semen Fractions of Infected Patients

T. Persico,1,2, V. Thiers,1 R. Tuveri1, M. Di Fins1, A.E. Semprini2, C. Brochet1,3, 1 INSERM-U572, 2 teacher and CBMS Pasteur Institut Paris, France, 3 Dept. Obstetrics and Gynecology, ISEM San Paolo, Univ. of Milan, Italy; 4 C. M. S. Patrinieri, Rimini, Italy

The issue of sexual transmission of HCV is still debated. A new RNA virus, HGV/G/C, related to HCV, has been recently identified. There is no information on the risk of its sexual transmission. Objectives: Investigate the presence of HCV and HGV RNA in the different fractions of semen: seminal plasma (SP), spermatozoa (SPz), round cells (RC, leucocytes and spermatogenesys cells), swim-up (SU) by PCR. Methods: 90 anti-HCV (+) Italian previously drug addicted males, were included (27 HIV-). The different fractions of the semen samples were obtained after discontinuing Percoll Percoll. HGV RNA was tested in sera and different semen fractions by PCR (5'NCR). HGV RNA was tested in sera and in some selected semen samples by PCR (NS5 NCR).

5 negative results were only interpreted in semen fractions in the absence of PCR inhibition (previously reported in such samples). Results: Serum: HGV RNA was detected in 55/50. 26/60 samples were positive for HGV/G/C including 15 HCV RNA (+) and 11 HCV RNA (-). Semen fractions: HGV RNA was detected in all patients after discontinuing Percoll Percoll. HGV RNA was detected in the seminal plasma of 6/24 (25%) tested, serum HGV RNA (+) individuals. Conclusions: Our results: 1) demonstrate that even in HCV-viremic subjects each semen fraction (SP, RC, Spz) results uninfected from HCV (confirming that sexual transmission of HCV is rare); 2) confirm the high prevalence of HCV/HGV coinfections in IVDU. 3) show that, HGV/G/C genotype can be detected in sperm raising the possibility of distinct modes of transmission from HCV.

90 Idiopathic Thrombocytopenic Purpura (ITP) and Viral Hepatitides: Is There Any Relationship?

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Purpose: Acute and/or chronic ITP has already been associated with various viral infections. This study was conducted to investigate the possible relationship(s) of viral hepatitides (A-E) with well defined ITP cases.

Methods: We determined the presence of various markers of infection from viral hepatitides (A-E) in serum samples from 23 patients (5 male and 18 female, range 17-74 years) with chronic ITP. Samples were collected before any treatment, transfusions of blood or blood products and platelet concentrate infusions. Patients with epidemiological, clinical or biochemical data suggestive for infections with viral hepatitides or with a past history of jaundice or first degree relative suffering from liver diseases were excluded from the study. 972 healthy volunteers (682 male and 303 female, range 20-80 years) were also studied. All subjects were investigated for the presence of anti-HAV, HBSAg, HBSAb, HBCAb, HBeAg, anti-HCV and anti-HV by commercial enzyme immunoassays. Results: None of the markers studied was statistically more frequent in patients compared to those of healthy controls (Table). Table. Frequency (%) of viral hepatitis markers in patients with ITP and in healthy controls.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-HAV IgG</td>
<td>65.2</td>
</tr>
<tr>
<td>anti-HAV IgM</td>
<td>0</td>
</tr>
<tr>
<td>HBSAg</td>
<td>0</td>
</tr>
<tr>
<td>HBSAb</td>
<td>0</td>
</tr>
<tr>
<td>HBCAb</td>
<td>30.4</td>
</tr>
<tr>
<td>Anti-HD IgG</td>
<td>0</td>
</tr>
<tr>
<td>Anti-HC</td>
<td>4.3</td>
</tr>
<tr>
<td>anti-HV IgG</td>
<td>1</td>
</tr>
</tbody>
</table>

*The only one patient reactive by third generation enzyme immunoassay was negative for Riba-III while HCV-RNA was not detected. **Both two patients reactive by enzyme immunoassay were negative by the inhouse immunoassay. NS = not significant by X² or Fisher's exact test.

Conclusions: Our findings suggest that at least in our region other immunopathogenetic mechanisms may be responsible for the development of ITP. Particularly for HCV, this study can neither confirm the findings observed by others nor indicate a trend for pathogenic link between HCV and ITP.

91 Infections from Viral Hepatitides and HTLV-II in Open-Heart Surgery in North Western Greece. A Preliminary Study

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Purpose: The aim of the present preliminary study was an attempt to determine the prevalence of various markers of viral hepatitides (B-E) and HTLV-II in patients (pts) from northwestern Greece who underwent an open-heart surgery. Methods: We investigated 104 pts (90 having coronary artery bypass grafting and 14 having replacement of mitral valve (prosthetic valve) and 113 healthy controls matched for age and sex. Forty six pts had been operated on before 1991, among them 83 pts had the open-heart surgery done in Greece and the remaining 21 pts abroad. The pts had at least a 6 month period from the time of the operation to the entrance the study. The serological determinations of the various viral markers were done using commercially available enzyme immunoassays as well as confirmatory immunoassay assays (for HCV and HEV). Results: We found that none of the pts was positive for the HBSAg or the HTLV-II antibodies. By contrast, the markers of a previous infection by HBSAg were found more frequent in pts (HBSAb: 46.1%, HBSAb: 37.3%, HBSAb: 37.3%) than in healthy controls (30.9%, 24.8% and 7.90% respectively). These differences were statistically significant (p = 0.05, p < 0.10 and p < 0.001 respectively). The presence of the markers of previous HBB infection among pts was not associated with age, sex, place of the operation or the number of transfusions, but with a longer duration from the day of the open-heart surgery (HBSAb: 70.7 ± 65.2 in positive vs 49.6 ± 38.4 in negative (p < 0.05), HBSAb: 75.3 ± 68.7 in positive vs 49.9 ± 39.1 in negative (p < 0.10) and HBSAb: 70.9 ± 70.3 in positive vs 52.5 ± 38.8 in negative (p < 0.001)). Anti-HCV and anti-HV antibodies were detected in 1.92% and 3.94% of pts respectively. This finding is not statistically significant when compared to healthy controls (0% for both antibodies). Conclusions: We conclude that: (1) this group of pts does not appear to be a high risk group for HTLV-II infection as we have already reported it for other groups; (2) although none of the pts was positive for HBSAg, the presence of significant increased incidence of markers of previous HBIV infection compared to healthy could suggest this group as a high risk one for HBIV infection and a intensive vaccination schedule against HBIV before the operation seems rationalize the presence of anti-HBB antibodies among the pts needs further evaluation, since, the oral fucal route of transmission of HBB may not be the only one.
Anagographic, Anthropometric and Metabolic Predictors of Ultrasonographic ‘Bright’ Liver

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Background & Aim Ultrasound (US) finding of a ‘bright’ liver (BL) is accurately predictive of fatty liver allowing non-invasive surveys of this common condition. Our purpose was to ascertain factors independently associated with a BL US finding in our series. The hypothesis was that a cluster of features characterize the so-called ‘Bright Liver Syndrome’ (Lonardo A, Endoscopy 1995; 27 A61. Lonardo A, Am J Gastroenterol 1996; 90: 2072-2074). Series & Method 97 subjects underwent liver US scanning, anthropometric and laboratory evaluation. Criteria for exclusion were: pregnancy, thyroidal disease, exposure to hepatotoxic drugs or chemicals, familial heterozygous hyperbetalipoproteinemia (Lonardo A, Gas. Metab. In Press). Each patient was registered: age (ys); alcohol consumption (g/day); body mass index (BMI, in Kg/m²); serum levels of GOT and GPT (mg/mL), albumin (g/dL), total cholesterol (ch), HDL-ch, apoB, fasting glucose (all in mg/dL). Based on the liver-kidney contrast (Yajima Y. Tohoku J Exp Med 1983; 139; 43-50) cases were classified as controls (n = 50; 40 males) or BLs (n = 47; 35 males). Data were analyzed through SPSS package. Results Mean values were as follows (control vs. BL): age 65.6 ± 56.4; BMI 25.8 ± 29.2; alcohol 26.1 ± 35.6; glomerular filtration rate 61.9 ± 50.9; ch 195 ± 225; HDL-ch 39 ± 43.2; apoB 1.23 ± 1.44; tg 134.6 ± 178.1; albumin 3.5 ± 3.9. Univariate F-ratio analysis selected BMI (P < 0.0001); serum albumin (P < 0.0001), ch (P < 0.0001), (P < 0.0001), apoB (P < 0.0001) and (P < 0.0001) as significantly different in cases with BL vs. controls. Using BMI as a dependent variable, logistic regression analysis (LRA) carried out through backward stepwise approach disclosed BMI (P < 0.0027), age (P < 0.0080), apo B (P < 0.0359) and (P < 0.0622) to be independent predictors of BL. However, when forward stepwise approach was adopted, BMI (P < 0.0072) ch (P < 0.1433) outturns to be significant. LRA allowed correct classification of cases with 74.22% accuracy. Conclusions Obesity, younger age, hypercholesterolemia, hypertyglicemia and elevated serum apo B levels are independent predictors of BL in our series. These findings support the existence of the “Bright Liver Syndrome” and highlight its metabolic significance.

Ultrasonographic Score (US) of Chronic Hepatitis

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Cut-off value Sensitivity Specificity

Williams score ≥ 6 66.7% 99.8%

US ≤ 18 83.4% 100%

An ultrasonographic score (US) has been prospectively correlated to the histological activity index (HA) in 20 patients with chronic hepatitis (10 CH, 6 CH+C, 4 CH+D, 1 CH+B, 1 CH+C) alcohol-related liver disease. US was determined with the same equipment, using the Spearmann Rank correlation coefficient.

US ≥ 24 had a positive value with the histological activity index (HA) in all controls: 24.6% had a positive US ≥ 24 and a negative HA, 10.4% had a negative US ≥ 24 and a positive HA, 18.1% had a positive US ≥ 24 and a positive HA. The accuracy of the US in the diagnosis of cirrhosis was evaluated: the US proved to correlate with the histological activity of hepatic disease and allowed the identification of patients with cirrhosis chronic liver disease. The sensitivity of the US for grading and staging of diffuse hepatic diseases is evaluated.

Nitrous Oxide Anaesthesia for Intercostal Liver Biopsies: Results of a Prospective Randomized Trial

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Pre-mixed 50% nitrous oxide and oxygen mixture (Entonox®) anaesthesia has been previously shown to be effective in pediatrics for painful acts. It is widely used during labour, is safe and can easily be administered by the patient. Liver biopsy is essential for the diagnostic and severity assessment of most chronic liver diseases, however it may be painful to the patient. Sedation is not routinely given before biopsy because no protocol has proven to be effective so far.

Aims: to study prospectively the effects of Entonox® on pain relief and comfort in comparison with a placebo in patients undergoing intercostal liver biopsy using a randomized study design.

Methods: 48 consecutive patients admitted since March 1996 to our unit for intercostal liver biopsy (chronic hepatitis C: 26, alcoholic liver disease: 12; others: 10) were enrolled after local ethical committee approval and written informed consent. They were randomized to receive during biopsy either (i) a breathing mixture of even parts of oxygen and nitrous oxide (Entonox®) from a face mask with a demand valve (group E, n = 23), or (ii) a breathing oxygen placebo from the same valve setup (group P, n = 25). Liver biopsy was performed by 2 senior operators according to the Menghini technique and the exclusion of the patient from analgesia with Xylocaine 1%. Pain after biopsy was assessed at the end of procedure (DO) and the next morning (D1) using visual analogues scales (VAS) scoring from 0 to 10. The following data were also assessed: comfort scored from 0 to 100 (VAS); anxiety scored from 0 to 100 (VAS); amount of antalgic drugs (paracetamol mg) required within the observation period (TA); comfort of biopsy; incidence and side effects observed.

Results: Pain scores at DO were 10.05 ± 2.66 and 29.72 ± 4.7 for groups E and P respectively (p < 0.002). Pain scores at D1 were 15.7 ± 4.6 and 35.4 ± 5.4 for groups E and P respectively (p < 0.01). Comfort scores were 84.4 ± 3.35 and 68.44 ± 5.28 for groups E and P respectively (p < 0.02). Anxiety scores were 31.3 ± 4.85 and 34.08 ± 6.27 for groups E and P respectively (p = 0.7). 38 patients agreed for new biopsy with the same conditions. No serious adverse effect was observed. Amount of antalgic drugs required was not statistically different between the 2 groups. The average cost of Entonox® used for 4 or 5 biopsies was 10.65 Euros.

Conclusions: Administration of Entonox® during intercostal liver biopsies resulted in a significant decrease in pain at DO and D1 and in discomfort of patients without influence on anxiety. If such results are confirmed on a larger group of patients, Entonox® could increase the acceptability of liver biopsy in the follow up of patients with chronic liver diseases.

Serum Erythropoietin Level in Patients with Chronic Normochromic Anaemia in Hepatic Fascioliasis

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In Egypt, human fascioliasis is an increasing health problem especially in the Nile Delta. This study was conducted on 35 anaemic* patients with fascioliasis, 20 anaemic patients with schistosomiasis (parasitic control group) and 10 persons (healthy control group).

Serum erythropoietin “EPO” and ferritin were measured using a sandwich ELISA technique, asto other ferrokinetic parameters were done. In patients with fascioliasis, EPO level (19.0 ± 13.3 mL/m²) was significantly higher than those of healthy control (7.23 ± 1.6 mL/m²) and of schistosomiasis (12.3 ± 2.85 mL/m²) groups (P < 0.001).

 Serum ferritin was significantly lower in fascioliasis group (384.8 ± 51.1 ng/mL) than in schistosomiasis (174.7 ± 83.4 ng/mL) and in healthy control (117.5 ± 29.5 ng/mL) groups (P < 0.001).

In a ferrokinetic studies showed that serum iron, intramural transferrin saturation, low iron total binding capacity, insipide of adequate iron stores shown by high serum ferritin, these findings together with normal reticuloctic count were similar to those found in the anaemia of chronic disorders.

The presence of anaemia inspire of high EPO level and the study iron stores may be explained by unresponsiveness of bone marrow to high EPO level which may be due to blocking the action of EOP by some cytokines as interleukin -1, tumour necrosis factor, and gamma interferon which were proved to be abundant in hepatic fascioliasis, it may be due to inhibition of iron mobilization by large amount of proline which is usually present in hepatic fascioliasis.

After treatment, cases of hepatic fascioliasis showed a good improvement of all parameters which were more or less comparable to those of healthy control.

* Normocytic Normochromic anaemia.

Cirrhosis and Cryoglobulinemia: Relation with Severity and Evolution of Liver Disease

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Recent studies have reported a relationship between presence of mixed cryoglobulinemia (CG) and cirrhosis in patients with alcoholic or viral liver disease. The aim of this study was to assess the prevalence and the prognostic significance of CG.

Patients and methods: 77 cirrhotic patients (53 men, 24 women) were enrolled in this study. All the patients were tested for anti-HCV antibodies by a third generation ELISA test, for HCV-RNA by PCR, and for HBS Antigen. Cryoglobulinemias were characterized by their immunological and immunofluorescent findings. The study of cirrhosis were done in 31 patients), HCV (30 patients) and miscellaneous
(16) In addition, 101 patients with chronic hepatitis caused by HCV but without cirrhosis were included. Results: CG was found in 56% of our patients with cirrhosis (58% in alcoholic cirrhosis, 76%), whereas the CG level was higher in HCV patients. There was no significant difference in age between patients with CG and without CG (56 ± 9 vs. 61 ± 7 yr. in alcoholics, 59 ± 12 vs. 53 ± 14 yr. in HCV-infected patients). There was no difference in prevalence of CG according to the Child’s score. Among the patients without cirrhosis, CG was found in 54.4%. There was no difference in age between patients with and without CG (42 ± 14 yr. vs. 41 ± 15 y).

Conclusion: These results confirm the high prevalence of CG in liver disease, and the role of HCV infection. The fact that CG is unbound with the Child’s score nor with early occurrence of cirrhosis suggests that it is an epiphenomenon without consequence on the evolution of the liver disease.

97 Child-Pugh-MEGX Score in Assessment of Prognosis in Cirrhotic Patients

R. Testa, S. Caglieris, E. Giannini, L. Arzani, S. Alvarez, G.L. Guido, A. Remagnino, G. Celle, D. Rasso 1, P.B. Lantieri 1, R. Pellicci 1, L. Mondello 2, G. Dardano 3, U. Valente 2. Gastroenterology Unit, DIMM, Italy. 2 Institute of Medical Statistics, Italy. 3 Transplant Center, University of Genoa, Italy

Prognostic evaluation of cirrhotic patients is a basic step for liver transplantation. The Child-Pugh’s score is the usual tool and monotherapy glycylide (MEGX, lidocaine metabolite) formation was proposed as an additional test. Aim of this study was to verify the prognostic usefulness of the combined score Child-Pugh-MEGX in cirrhotic patients. One hundred forty-three patients (104 males, 39 females, mean age 49 ± 9, consecutively admitted to our Units for hepatic functional evaluation, were studied. According to Child-Pugh’s score 32% patients were class A, 79% class B and 32 class C. Serum MEGX (ng/m) at 30 min after i.v. lidocaine (1 mg/Kg) was measured by TDX fluorescent polarization immunomassay. MEGX 30 min were scored as follows: score 1 to 30 ng/ml; 2 to 30 ± 10 and 3 to 10. The modified Child-Pugh-MEGX score was calculated using MEGX-score to Child-Pugh original score. In the follow-up period, ranging from 6 to 60 months, 20 patients died and thirty were transplanted. Kaplan-Meier survival curves were calculated for patients: 1) with Child-Pugh scores ≤ 9 and for those with scores > 9 (overall survival was 63% versus 65%, p = 0.0001); 2) with MEGX levels > 10 ng/ml and for those with levels < 10 (94% versus 70%, p = 0.001), and 3) for patients with Child-Pugh-MEGX scores ≤ 12 and for those with scores > 12 (92% versus 56% survivors, p = 0.0001). These whole survival rates reflect those at 12 months. Excluding Child-Pugh’s class A patients, Cox’s test significance p was 0.00008 for Child-Pugh score, p = 0.00049 for MEGX level and p = 0.00001 for Child-Pugh-MEGX score. These results suggest that MEGX test can improve prognostic evaluation only in Child-Pugh’s class B and C patients.

98 The Changes of the Lipid Profile in Hepatitis Patients


The liver has an important role in the metabolism of cholesterol, cholesterol esters, phospholipid and triglyceride. The transportation of cholesterol, triglycerid and phospholipid are carried by apolipoproteins. The lipid profile changes in acute and chronic liver disease. Activity of the plasma lecinin cholesterol acil transferase, esterified by the liver and esterified and free cholesterol decreases. So HDL and VLDL decrease; LDL and triglyceride increase. Some reports declared that annexin-V and apoprotein-H facilitate the bonding of protein-S of HbA to herapot. We studied lipid fractions in hepatitis-C, chronic active hepatitis (CAH), chronic persistent hepatitis (CPH).

<table>
<thead>
<tr>
<th>Cont (mg/dl)</th>
<th>HBV (n=30)</th>
<th>HCV (n=14)</th>
<th>CPH (n=13)</th>
<th>CAH (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholest.</td>
<td>208.11±31</td>
<td>190.16±54</td>
<td>203±53.7</td>
<td>192.8±39</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>150.52±84</td>
<td>155.83±93</td>
<td>163.46±93</td>
<td>186.1±50.4</td>
</tr>
<tr>
<td>HDL</td>
<td>37.5±6.6</td>
<td>40.56±11.1</td>
<td>49.4±5.2</td>
<td>38.6±4.9</td>
</tr>
<tr>
<td>LDL</td>
<td>138.92±27.4</td>
<td>119.33±58.6</td>
<td>122.6±54.6</td>
<td>133.3±39.3</td>
</tr>
<tr>
<td>VLDL</td>
<td>38.4±17.4</td>
<td>31.26±15</td>
<td>32.5±17</td>
<td>25.2±15.3</td>
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</table>

We found low cholesterol values in subjects, specially in CAH patients. It was nearly the same for triglyceride. HDL-cholesterol was the highest in active HCV infectious patients. LDL-cholesterol was lowest in CAH group. When we compared the CAH with CPH cholesterol, it was obtained that triglycerid and cholesterol values were lower at CAH but it was not statistically significant (p > 0.01). HDL lower at CPH group (p < 0.05). Cholesterol and triglycerid were lower HbV infection group (p > 0.05). LDL was higher at HCV group and LDL was lower at HbV group (p > 0.05). As a conclusion, We obtained high lipid levels at all of the subjects according to the other group. We explain these changes not only by some metabolic changes at the level of hepatoid (disorders of the Lechitin metabolism, decreasing LCAT enzyme activity) and disorders at the level of membrane receptors and changes at the metabolism of the hepatic synthesis. So we need more studies for explaining that pathogenic event.

101 The Assessment of Arylhydroxyegenic Side-Effect of Interferon-α 2A by Heart Rate Variability Tests in Chronic Active Hepatitis

A. Kadayifci, K. Aytemir, M. Arslan, S. Aksoyek, M.C. Savas, B. Sivri, B. Kayhan. Hacettepe University Medical School, Ankara, Turkey

Cardiac arrhythmia and sudden death have been reported recently in various clinical trials of interferon. In this study, the cardiac arylhydroxyegenic side-effects of recombinant interferon-α 2A (rIFN-α2A) were investigated prospectively with heart rate variability (HRV) tests, in a group of patients with chronic active hepatitis (CAH). Sixteen patients with CAH type B and 16 patients with CAH type C and one patients with CAH type D were included in the study and 4.5 MIU, 3 MIU and 9 MIU of rIFN-α2A were administered thrice weekly to these patients, respectively. The HRV analysis of all patients were
made with a standard record of 7 minutes at the beginning of the study and at the first and sixth month of interferon therapy and also 6 months later ceasing the therapy. Two-time domain variables were calculated as being the mean RR intervals (MRR) and the mean of squared differences between successive intervals (MSD). In addition, power of the low frequency peak (P1) and power of the high frequency peak (P2) were calculated for spectral analysis as the determinants of frequency-domain analyses. The mean values of data obtained in patients are shown in Table 1.

Table 1. The mean values of data obtained from HRV analysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MRR (ms)</th>
<th>MSD (ms)</th>
<th>P1 (m²Hz)</th>
<th>P2 (m²Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before therapy</td>
<td>71.2 ± 5.5</td>
<td>31.8 ± 3.7</td>
<td>962 ± 490</td>
<td>201 ± 180</td>
</tr>
<tr>
<td>First month</td>
<td>72.4 ± 4.8</td>
<td>30.2 ± 2.4</td>
<td>959 ± 515</td>
<td>196 ± 167</td>
</tr>
<tr>
<td>End of therapy</td>
<td>70.7 ± 5.2</td>
<td>31.2 ± 2.7</td>
<td>966 ± 390</td>
<td>188 ± 281</td>
</tr>
<tr>
<td>After 6 months</td>
<td>70.1 ± 4.7</td>
<td>32.3 ± 3.9</td>
<td>921 ± 339</td>
<td>187 ± 156</td>
</tr>
</tbody>
</table>

No significant difference was observed in all parameters of HRV analysis before, during and after the rIFN-α2a therapy.

Conclusion: These results indicate that rIFN-α2a alone does not produce an arrhythmogenic potential. Atrial and ventricular arrhythmia and cardiac sudden death attributed to interferon in previous reports are questionable in CAH patients.

102 Nocturnal Protein Turnover and Liver Glycogen Stores in Patients with Cirrhosis

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In patients with cirrhosis of the liver nocturnal administration of glucose has a sparing effect on the nocturnal protein breakdown. Suggesting an early onset of gluco-neogenesis because of glycogen depletion. To further study this phenomenon the nocturnal protein turnover using 15N-glucose as a label was studied simultaneously with measurement of the depletion of liver glycogen. For the latter we used a new developed technique of labelling the liver glycogen pool with naturally 13C-enriched dietary carbohydrate. Oxidation of the labelled glycogen was measured indirectly by 13C2O2-enrichment in breath. Concurrently insulin, glucagon and the glucose response to glucagon i.v. were studied in 12 cirrhotics (P) and 12 healthy volunteers (V).

Though bodyweight did not differ, the body cell mass (BCM) was 25.4 kg (SD,94) in P versus 33.0 kg (SD,70) in V, p < 0.01. The nocturnal protein breakdown was 0.78 g/kg h BCM9 hr (SD,38) in P vs 0.46 g/kg h BCM9 hr (SD,36) in V, p < 0.002. The protein balance did not differ between P and V. After 14 hours fasting the contribution of liver glycogen to 13C2O2 production was 44% of initial value in P vs 73% in V, p < 0.005. The molar insulin-glucagon ratio was 4.48 (SD,28) in P vs 3.21 (SD,22,4) in V, p < 0.005. The blood glucose rose 0.74 mmol/l (SD,38) in P vs 2.11 in V, p = 0.0001 in response to 1 mg glucagon i.v. after 15 hours of fasting.

In patients with cirrhosis the liver glycogen pool is smaller and depleted earlier. The degree of depletion correlates with the molar insulin-glucagon ratio, while in cirrhosis this ratio is significantly lower compared with healthy volunteers. The nocturnal protein breakdown is increased. No correlation between the two phenomena could be demonstrated.

103 Randomized Trial Comparing Intravenous Ceftriaxone with Oral Cefixime for Treatment of Spontaneous Bacterial Peritonitis (SBP) in Cirrhotic Patients: Pilot Study

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Background/Aims: SBP is a common and potentially fatal complication of cirrhosis. The use of antibiotics oral will contribute greatly to advance in the treatment of SBP. The aim of this study was to assess the efficacy of cephalosporin orally (cefixime) versus cephalosporin intravenous (ceftriaxone) for the treatment of non-severe SBP in patients with cirrhosis and ascites.

Methodology: A randomized trial comparing the molar ceftriaxone:cefixime ratio, while in cirrhosis this ratio is significantly lower compared with healthy volunteers. The nocturnal protein breakdown is increased. No correlation between the two phenomena could be demonstrated.

104 Type of Estrogen Receptors (ERs) Determines Response to Antiestrogen Therapy

E. Vilia, A. Dugani, L. Camellini, E. Fantoni, P. Buttafoco, A. Grotto, I. Ferretti, F. Manenti. Gastroenterology, University of Modena, Italy

Tamoxifen (TAM) for inoperable HCC has given contradictory results, possibly explained by the presence in many HCCs of variant ERs (vER) modified in the hormone binding domain [1]. These cases might respond to megestrol (MEG), which blocks estrogen action at post-receptor level. We therefore planned a pilot study in which TAM or MEG were used depending on ER transcript type. Methods: PNA, extracted from liver tumor of 7 pts with unresectable, multicellular HCC, was reverse transcribed and amplified by RT-PCR with primers localized in exons 4 and 6. Tumor growth was evaluated by magnetic resonance (MR) at baseline and after 3 months without therapy. Pts with vER were then started on TAM 80 mg/day while pts with vER were assigned to MEG 160 mg/day. MR was repeated after 9 months of therapy. Success was defined as a growth during treatment not exceeding that in the 3 months without therapy. Successful tumor growth was higher in vER tumors. After 1 year, 4/4 TAM-treated and 2/3 MEG-treated pts showed inhibition of growth. Incremental volume fraction by MR in the 2 periods of observation is indicated in the table:

\[ V_{TAM} - V_{BASELINE} \]

\[ V_{MEG} - V_{BASELINE} \]

Conclusions: 1. growth of tumors characterized by vERs is extremely aggressive; 2. the choice of antiestrogen in relation with type of ERs determined an overall high response rate, significantly improving the known response rate to tamoxifen.

[Supported by grant 60%]


105 Impaired Hepatocellular Integrity in Pre-eclampsia as Assessed by Measurement of Plasma Glutathione S-Transferase Alpha-1 (GSTA1)

T. Mulder, 1 M. Knapen, 2 R. Penders, 2 E. Steegers, 2 W. Peters 1.

1 Department of Gastroenterology, University Hospital Nijmegen, The Netherlands; 2 Departments of Obstetrics and Gynecology, University Hospital Nijmegen, The Netherlands

Pre-eclampsia is a complication of pregnancy characterized by elevated blood pressure and proteinuria. The term HELLP syndrome describes a group of pre-eclamptic women with Haemolysis, Elevated Liver enzymes, and Low Platelets. Deterioration of hepatic function is a crucial determinant as whether to terminate pregnancy. Glutathione S-transferase Alpha-1 (GSTA1) constitutes as much as 1% of the cytoxic protein in the liver, has a uniform hepatic distribution, and a plasma half-life of less than 2 hours. Seventy-five women during uncomplicated pregnancy, 26 women with pregnancy induced hypertension, 40 women with pre-eclampsia and 20 women with the HELLP syndrome were included in the study. Plasma GSTA1-1 levels were measured by ELISA.

Of the women with non-complicated pregnancy five subjects (7%) had elevated GSTA1-1 levels and two (3%) had elevated alaminotransferase (ALT) levels. Both ALT and GSTA1-1 were elevated in four (15%) patients with pregnancy induced hypertension. Fifteen patients with pre-eclampsia (75%) had elevated GSTA1-1 levels whereas 10 (50%) had elevated ALT activities. In the eight patients where both parameters were elevated, the median rise in plasma GSTA1-1 was more pronounced (5.7 times upper normal reference limit; UNRL) than the median rise in ALT (2.7 times UNRL). All patients with the HELLP syndrome had elevated ALT and GSTA1-1 levels. However, the median rise in plasma GSTA1-1 (3.4 times UNRL) was significantly (P < 0.01) higher than the median rise in ALT (8.3 times UNRL).

Conclusions: Plasma GSTA1-1 levels may provide an early and sensitive indicator of hepatocellular damage during pre-eclampsia and the HELLP syndrome.

106 Is Carbohydrate-Deficient Transferrin (CDT) a Valid Marker of Harmful Alcohol Intake among Surgical Patients?

H. Tannesen, M. Carstensen, P. Maia. Department of Surgery, Copenhagen County Hospital in Herlev, University of Copenhagen, DK-2730, Denmark

Alcohol abusers are at special risk at surgery. Daily consumption of at least 60 g (35 beverages/week) is related to 3–4 fold increased development of complication after colorectal resection. (Lancet 1992; 340: 334–337)

Purpose: Examine the validity of CDT as a trap for harmful alcohol intake in surgical patients previous to operations.

Methods: Consecutive adult patients admitted to the department of surgery were included after baseline measurements of CDT and 94% of patients treated with ceftriaxone were cured of their infections (p = 0.52). Side-effects could not be attributed to the drugs.

Conclusions: Our results suggest that ceftriaxone is an efficacious, safe and cost-effective measure to treat non-severe SBP in cirrhosis.

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Cyclosporine causes high LDL cholesterol levels to occur, but has not yet been clarified.

Methods: As the main part of LDL is catabolized in the liver, by its hepatocytes, an in vitro system using cultured hepatocytes was used. HepG2 cells, a hepatoma cell line, which is highly differentiated and with functional LDL receptors, was studied.

Conclusion: The results showed that Cyclosporine reduced cellular LDL uptake and degradation by mainly inhibiting the LDL receptor mediated pathway. HMG-CoA reductase inhibitors, which upregulate LDL receptor activity, reversed the Cyclosporine caused downregulation of LDL receptor activity.

Conclusion: Cyclosporine causes hypercholesterolemia in transplanted patients by inhibiting LDL receptor activity. This effect can be reversed by HMG-CoA reductase inhibitors. This argues for treating patients with Cyclosporine caused hypercholesterolemia with HMG-CoA reductase inhibitors.

107 Tissue Inhibitor of Metalloproteinases-1 in Liver in Patients with Chronic Liver Disease

Yoshikazu Murawaki 1, Yuto Ika 1, Yusuke Idohe 1, Yukiko Kitamura 1, Hironaka Kawatsuki 1, 2

Tissue inhibitor of metalloproteinases (TIMP)-1 is an important regulator of matrix metalloproteinase activity. To clarify its changes in diseased liver, we measured TIMP-1 concentrations in liver tissue samples from patients with chronic liver disease using an enzyme immunnoassay after the extraction with 2 M guanidine.

When the results were compared, the liver TIMP-1 level increased 2.2-fold in CAH 2A, 2.9-fold in CAH 2B and 4.1-fold in LC, but not in CPH. Liver TIMP-1 levels were closely correlated with the histological degree of perportal necrosis, of portal inflammation and of liver cell damage.

When we compared the level of TIMP-1 in diseased liver tissue extracts to liver TIMP-1 in normal liver, TIMP-1 was mainly found in hepatocytes, and the intensity was stronger in CAH and LC than in CPH.

Conclusion: TIMP-1 concentration increased with the progress of liver disease, where the degradation of extracellular matrix proteins would be decreased, resulting in the development of liver fibrosis.

108 Expression of a Non-mdr2-Coded Phosphatidycholine Membrane Transport Protein from Rat, Mouse and Human Liver in Xenopus Laevis Oocytes

L. Comaczchia, A. Locéhi, J. Mössner, F. Berr, Dept. of Medicine II, University of Leipzig, Germany

Phosphatidycholines (PC) are secreted into the bile via hepatocyte canalicular membrane transport protein(s). Evidence for ATP-dependent mdr2-encoded PC transport was recently demonstrated by expression of specific liver mRNA species/fractions. Aim: To test in Xenopus laevis oocytes, whether the functional expression of mdr2 is involved in the PC transport system and functional expression of liver mRNA. Transport was assayed using a water-soluble, radiolabeled PE homolog, 1.4-C-dibutyroyl-PC (diC4PC). Results: Functional expression of rat or mouse mdr2 cDNA in Xenopus laevis oocytes did not result in detectable uptake of diC4PC in presence or absence of ATP. By contrast, expression of rat, mouse and human liver total mRNA resulted in saturable, carrier-mediated and ATP-independent uptake of diC4PC with an apparent Km of 9.6 mM, 7.7 mM and 11.6 mM, respectively. Antisense inhibition of mdr2 expression increased diC4PC uptake by total liver mRNA from rat and by 45%–50%. In addition, a clear difference in mRNA size was shown between MDR2 and the diC4PC carrier, after size fractionation of rat liver mRNA. Conclusion: The data prove the existence of a specific mRNA for a non-mdr2-coded cell membrane carrier in mouse, rat and human liver which exhibits similar transport affinity for diC4PC as the PC carrier previously characterized (J Biol Chem 268: 3976; 1993) in rat liver canalicular membranes.

109 Cyclosporine and Hypercholesterolemia. Cyclosporine Down-Regulates Low Density Lipoprotein Receptors in Cultured Liver Cells

C-H. Flöten, O. Al Rayyes, A. Wallmark. Department of Internal Medicine and the Wallenberg Laboratory, Malmö University Hospital, Malmö, Sweden

Purpose: Cyclosporine is today one of the most widely used immunosuppressive drugs and is used in most transplantations to prevent organ rejection. Patients, who are transplanted, and use this drug are, however, prone to be afflicted by cardiovascular disease, due to the development of athrombogenicity caused by a decrease of low density lipoprotein (LDL) cholesterol levels. The mechanism whereby Cyclosporine causes high LDL cholesterol levels to occur, has not yet been clarified.

Methods: As the main part of LDL is catabolized in the liver, by its hepatocytes, an in vitro system using cultured hepatocytes was used. HepG2 cells, a hepatoma cell line, which is highly differentiated and with functional LDL receptors, was studied.

Conclusion: The results showed that Cyclosporine reduced cellular LDL uptake and degradation by mainly inhibiting the LDL receptor mediated pathway. HMG-CoA reductase inhibitors, which upregulate LDL receptor activity, reversed the Cyclosporine caused downregulation of LDL receptor activity.

Conclusion: Cyclosporine causes hypercholesterolemia in transplanted patients by inhibiting LDL receptor activity. This effect can be reversed by HMG-CoA reductase inhibitors. This argues for treating patients with Cyclosporine caused hypercholesterolemia with HMG-CoA reductase inhibitors.

110 Effects of Oltipraz, α-Tocopherol, β-Carotene and Phenethylisothiocyanate on Glutathione S-Transferases of the Rat Digestive Tract

E.M.M. Van Lieshout, W.H.M. Peters, J.B.M.J. Jansen. Dept. of Gastroenterology, St. Radboud University Hospital, Nijmegen, the Netherlands

Many studies have linked consumption of naturally occurring anticarcinogens, present in vegetables and fruits, to the prevention of gastrointestinal tumours. The mechanism is unclear, GSTs are a family of isozymes, divided into classes Alpha, Mu, Pi and Theta, which serve a major role in the detoxification of many substances, including carcinogens. In order to understand better their anticarcinogenic potential, four anticarcinogenic agents were tested, namely, oltipraz, α-tocopherol, β-carotene and phenethylisothiocyanate [PEITC], incorporated in the diet at 0.03, 0.02, 0.02, and 0.045% w/w, respectively, were studied with respect to their effects on oesophageal, gastric, colonic, and hepatic GST activity and GST isoenzyme levels.

In Wistar rats were fed normally or supplemented with these agents. Organs were removed and cytosolic fractions were prepared. Herein, GST activity towards 1-chloro-2, 4-dinitrobenzene was measured and GST isozymes were quantified using an immunoblotting assay. A high negative predictive value was used to assess statistical significance of differences. GST activity was significantly increased in oesophagus and colon by PEITC and in liver by oltipraz.

Oltipraz, α-tocopherol, and PEITC induced hepatic GST Alpha. GST Mu was increased by β-carotene and PEITC in stomach and liver, by oltipraz in liver, and by α-tocopherol in stomach. PEITC induced colonic GST Pi. In conclusion, oltipraz, PEITC, and to a lesser extent α-tocopherol and β-carotene, may exert their chemoprotective effects in liver, colon and oesophagus by enhancing GSTs, resulting in a more efficient detoxification of carcinogens.

111 Prospective Evaluation of Circulating Hepatocytes by Alpha-Fetoprotein mRNA in Humans during Liver Surgery

A. Lemogne 1, D. Azoulay 2, T. Le Bricon 1, M. Salucci 1, P. Pham 1, H. Bismuth 2, B. Deburel 1, 1 Biochemistry; 2 Hepato-Biliary and Liver Transplantation Center, Paul Broussais Hospital, Villejuif, France

“Tissue-specific” mRNAs have been used for the detection of circulating micrometastatic tumor cell populations. In human liver, expression of alpha-fetoprotein (AFP) mRNA, a prospective study in a random group of 64 consecutive patients (pTs) undergoing hepatic resection for various tumors (HCC: n = 20, secondary metastatic liver: n = 27 and non tumor etiologies: n = 17). AFP mRNA was evaluated in peripheral blood by reverse transcription-polymerase chain reaction (RT-PCR) before surgery and at 2 intraoperative time intervals during surgery. Prior to hepatectomy, AFP mRNA was detected in the blood of 17% of pts which included 5 out of the 20 pts with HCC. Intraoperatively, 53% of the pts (6/20 operated for HCC) had a positive result (null hypothesis for a non-transmortal etiologies) were found to be AFP mRNA positive, regardless of the disease, type of surgery or clinical parameters surrounding their disease. Thirty two pts (50% of which were positive intraoperatively) were examined 6 months after surgery and all but one were negative for AFP mRNA. None of the pts treated for HCC in this series had recurrence 6 months after surgery (2 died of liver failure). Although liver surgery seems associated with a transient release of AFP mRNA positive cells in the circulation, this gene transcript is not a specific marker of circulating micrometastases from HCC. It would be impractical to attempt clinical application of this marker until a more accurate test is devised which can link specific gene markers to circulating tumor foci.

112 Cholestatic Liver Injury Induces a Rapid Increase in Proliferation and Expression of β PDGF Receptor In Hepatic Stellate Cells

O. Goria, M. Maratrat 1, C. Rey, F. Ballet, R. Poupon, C. Housset. Unité d’Hépatologie et INSERM U 402, Hôpital Saint-Antoine, Paris; 1 Rhône-Poulenc Rorer, Aortville

The molecular mechanisms of hepatic stellate cells (HSC) activation have been well described in vitro but have not been fully elucidated in vivo, particularly
in cholestatic liver injury. Increased proliferation is one of the major aspects of HSC activation. PDGF has been identified as the most potent mitogen for HSC in vitro. However, the mitogenic effect of PDGF in vitro, requires up-regulation of β PDGF receptor in these cells. The aim of our study was to determine the kinetics of β PDGF receptor expression and that of β PDGF receptor expression in HSC, following cholestatic liver injury.

Materials and methods: Cholestatic liver injury was induced in male Sprague-Dawley rats by double ligation with sectioning of the common bile duct (BDL). Sham-operated rats served as controls. Liver injury induced by biliary obstruction was assessed by liver tissue histology and by the measurement of serum bile acids (Enzabile®) and of bilirubinemia. HSC proliferation was assessed by immunoperoxidase incorporation of bromodeoxyuridine (BrdU), which had administered intraperitoneally (50 mg/kg) one hour before cell isolation. HSC were isolated by in situ liver enzymatic dissociation and cell separation on density gradient, 1, 2, 3 and 7 days after bile duct ligation, respectively. Freshly isolated HSC were subsequently analyzed by flow cytometry, in order to quantify incorporated BrdU. In separate experiments, HSC plasma membrane expression of PDGF receptors was also analyzed by flow cytometry using an anti-PDGFR (R) β polyclonal antibody.

Results: Cell purity, as determined by fluorescence of retinoid-containing vacuoles under UV excitation, was > 95%. Biochemical determinations, semi-quantitative analysis of ductular reaction, and incorporation of BrdU in HSC are shown in the following table (M ± SEM, n = 3).

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<tr>
<th>D1</th>
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In addition, HSC proliferation was associated with a more than ten-fold increase in β PDGF receptor density on the plasma membrane.

113 Expression of Gap Junction Protein Connexin 32 in Experimental Rat Liver Cirrhosis

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Gap-junction-protein levels of connexin 32 (Cx 32) were reported to be decreased in neoplastic foci induced by hepatocarcinomas in rat livers. However, there was no report concerning the expression of Cx 32 was decreased in experimental liver cirrhosis. We examined immunohistochemically the expression of Cx 32 in cirrhotic rat livers induced by diethylnitrosamine (DEN).

Methods: Male Wistar rats were treated with DEN for 6 weeks. After sacrifice, the livers were resected, and immediately frozen by liquid nitrogen for immunohistochemistry and H&E stain. The immunohistochemical detection of Cx 32 was performed using an avidin-biotin complex peroxidase technique with a mouse monoclonal anti-Cx 32 antibody. The number of Cx 32 positive spots/100 μm² was counted at random in 15 photographic fields (magnification x1000).

Results: Macular Cx 32 spots were observed at intercellular borders of hepatocytes. The number of plaques in control and cirrhotic livers was 133 ± 30.7 spots and 79.4 ± 25.8 spots/100 μm², respectively. The number of spots in the cirrhotic livers was significantly decreased compared with that in the control livers (p < 0.05).

Conclusion: The expression of connexin 32 is decreased in cirrhotic rat livers treated with DEN when compared with that in normal livers.

114 Trimethylamine-N-Oxide (TMAO) – Important in the Pathogenesis of Hepatic Encephalopathy after TIPS?

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Background: About 20–30% patients become encephalopathic following TIPS. TMAO is a nutrition-related, diet related, produced by gut flora, crosses the blood brain barrier, is present in the portal circulation, is metabolised by the liver and inhibits Ca²⁺ ATPase. This study was designed to test the hypothesis that TMAO or other biogenic amines may contribute to the development of encephalopathy.

Methods: Plasma was collected prior to TIPS and at 3 months when the patients attended for routine follow up and stored at −70°C. Two patients were clinically encephalopathic prior to TIPS. In vivo ¹H magnetic resonance spectroscopy was performed on the plasma using a spin echo sequence on an 11.7 Tesla magnet (JEOL). Spectra were analysed on a Sun-Sparc computer. Amino acids were measured in the plasma using HPLC.

Results: The severity of encephalopathy worsened in the 2 patients who were encephalopathic before TIPS and 2 other patients had encephalopathic changes. The changes in TMAO correlated significantly with the changes in the portal pressure gradient (r = 0.58, p < 0.02). TMAO levels in controls and patients before and after TIPS is illustrated in Fig 1. No significant change was noted in capillary ammonia or amino acids.

TMAO levels in controls and patients

Conclusions: TMAO may be important in the development of encephalopathy and deserves further investigation.

115 Collagen Type IV and Laminin in Alcoholic and Nonalcoholic Liver Diseases. Relationship with Portal Hypertension

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Collagen type IV and laminin are markers of basement membrane formation and sinusoid capillarization. In this study we investigated the localization and expression of collagen type IV and laminin in alcoholic and nonalcoholic liver diseases. The expression of collagen type IV and laminin was studied by immunocytochemistry on liver biopsy specimens from 30 patients with different stage of alcoholic liver disease (15-fatty liver, 5-alcoholic hepatitis, 10-intracylinder cirrhosis), 20 nonalcoholic liver diseases (10 chronic active hepatitis, 5-viral liver cirrhosis, 12-PBC) and 3 controls. All patients were clinically follow-up for a period over 3 years about severity of liver damage and appearance of portal hypertension. The expression of collagen type IV and laminin was various intensity around blood vessels and bile ducts in the portal tracts, sinusoidal walls, areas with cell infiltration and liver cell necrosis, fibrotic septa and periportal and perisinusoidal spaces in all patients. The expression of collagen type IV and laminin in periportal areas in alcoholic cases was more intensive than other cases. In alcoholic patients there was also relationship between the intensity of staining reactions in periportal spaces and appearance of portal hypertension (r = 0.890, p < 0.001) for the follow up period and the verify of liver damage (x = 56.14, p = 0.001) for conclusion liver fibrosis and capillarization play a central role in liver function impairment and portal hypertension in chronic liver diseases, especially in alcoholics.

116 Longterm Outcome and Predictive Factors of Efficiency, Encephalopathy (EH) and Obstructions after TIPS Placement for Refractory Ascites (RA)

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The aim of this study was to assess the TIPS efficiency and to evaluate prognostic factors for EH, obstruction, efficiency and mortality after TIPS for RA. Methods: Between 04/92 and 11/95, 33 patients with RA followed 18 mo. (2–36 mo) underwent TIPS placement. A favorable response to TIPS was defined as an improvement of RA, with or without diuretics or occasionally paracentesis (-1 by 3 mo). 28 parameters of the pre-TIPS screening were examined as potential prognostic factors. Results: 12 mo after TIPS placement, RA was resolved or improved in 73% patients. Cumulative rates of obstruction were respectively 19, 50, 66 and 66% at 1, 2, 12 and 30 mo. After reinterventions TIPS free of obstruction were respectively 70, 80, 86 and 80%. 6 parameters were predictive of EH: pre-TIPS EH history (p = 0.05), hypoaalbuminemia (p = 0.04), hemoglobinemia (p = 0.0003), Child score (p = 0.03), Prothrombine Time (p = 0.002) and proacrolein (p = 0.003). 3 parameters were predictive for thrombosis: recent operators (p = 0.04), hypoaerialbinemia (p = 0.01), proacrolein (p = 0.004). For TIPS free of obstruction, only 2 parameters were predictive of efficiency: anticoagulant therapy (p = 0.04) and Child score (p = 0.006). None parameters were predictive for stenosis. 5 were predictive of mortality before 15 mo: Child score (p = 0.0004), hypoaerialbinemia (p = 0.003), proacrolein (p = 0.004), proacrolein (p = 0.02) and blood alcalin phosphatases (p = 0.02). One-year survival was 70%. After TIPS Child score improved 7.85 ± 1.73 vs 9.27 ± 0.88 before TIPS (p = 0.0001). Conclusion: During long term
follow-up, TIPS might be a treatment of RA and complications might decrease under the following conditions: 1) only Child B must be treated, 2) early reinversion in case of obstruction, 3) lactulose and antibiotics treatments should be prescribed before TIPS and anticoagulant therapy after.

117 Utility of Thoracentesis in Cirrhotic Patients


About 10% of cirrhotic patients with ascites have an associated pleural effusion, but the utility and safety of thoracentesis is not well established. A prospective study to evaluate complications of thoracentesis in cirrhotic patients with pleural effusion. Material and methods: From October 1994 to December 1995 a diagnostic thoracentesis (DT) was performed on all cirrhotic patients with pleural effusion (<200 ml) or when spontaneous bacterial peritonitis was suspected. A therapeutic thoracentesis (TT) was performed in patients with dyspnea secondary to the effusion. To detect complications, a chest radiograph and exhaustive follow up were done after the procedure. Results: 106 thoracenteses have been performed in 33 cirrhotic patients, 77 DT and 29 TT. Of the 77 DT, no pleural fluid was obtained in 4 (5.2%), pleural fluid analysis was compatible with hydrothorax in 75 (74%) and pleural fluid analysis provided a definitive diagnosis in 16 (20.8%); tuberculosis in 2 cases, malignancy in 2 and spontaneous bacterial peritonitis by 12. In 79.3% of the 29 TT pleural fluid could be evacuated (mean volume 1900 ± 685 ml). Major complications were pneumothorax in 6 (6.6%), 4 of them required chest tube insertion, and uncomplicated wall haemostasis in 4. Pneumothorax appeared in 2 (2%) of the 77 DT and in 4 (13.8%) of the 29 TT (p < 0.05, Fisher exact test).

Conclusions: 1. – In 20% of the thoracentesis performed in cirrhotic patients, pleural fluid analysis provides a definitive diagnosis other than hepatic hydrothorax. Thoracentesis in cirrhotic patients is a useful and safe procedure. 2. – Therapeutic thoracentesis is an effective procedure having greater morbidity than diagnostic one.

118 Helicobacter pylori Infection and the Risk of Peptic Ulcer among Cirrhotic Patients

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Helicobacter pylori and peptic ulcer are known to be associated and there is a high prevalence of peptic ulcer in cirrhotic patients. However, H. pylori is a risk factor for peptic ulcer in cirrhosis remain controversial. The aim of the present study was to determine whether there is a significant correlation between Helicobacter pylori infection and peptic ulcer in liver cirrhosis. In a prospective study, 49 cirrhotic patients were endoscoped regardless of symptoms. Another group of 75 controls without liver disease were also examined routinely. Both groups of patients were subdivided into ulcer and non-ulcer patients. The presence of H. pylori was assessed by culture, histologic findings, and rapid urease test of antral biopsy specimens. The prevalence of H. pylori in cirrhosis was significantly lower than in control group (49% vs. 68%, p < 0.05). The presence of H. pylori was more frequent in ulcer than in non-ulcer patients in controls (91.3% vs. 57.6%, p < 0.005), whereas this difference was not significant in cirrhosis (56.7% vs. 36.8%, p > 0.05). As for peptic ulcer between two groups, H. pylori was identified in 56.7% of cirrhotic patients compared with 91.9% of controls (p < 0.01). The positive rate of H. pylori in cirrhosis group is clearly related to the presence of serum hepatitis B surface antigen. There is no strong evidence to substantiate an etiologic role of H. pylori in the development of peptic ulcer among cirrhotic patients. H. pylori may frequently infect hepatitis B virus-related cirrhosis.

119 The Effect of Cefazidime on the Prevention of Peritonitis after Recurrent Ascites Paracentesis

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A common complication of recurrent paracentesis of ascitcs in cirrhics is the appearance of bacterial peritonitis.

Aim of the study: was to evaluate the efficacy of Cefazidime (Solveitan) on prevention of bacterial peritonitis due to recurrent ascites paracentesis in cirrhotic patients with tense ascites.

Materials and Methods: This study included 26 patients with non complicated cirrhosis and tense ascites. During their hospitalization (1-2 months) all the patients got recurrent paracentesis every once or twice weekly. After each procedure 15/26 patients (Group A) were received Cefazidime (Solveitan) 1 gr IV while the rest of them (Group B) received nothing. Clinical and laboratory investigation was done every day.

Results: Only one patient (6%) from group A appeared clinical symptoms of bacterial peritonitis (fever, local pain and increased blood and ascitic fluid white cell count). Patients of group B got typical clinical picture of bacterial peritonitis with positive ascitic fluid cultures in 2 of them.

Conclusions: Cefazidime (Solveitan), a third generation cephalosporin, is quite effective on the prevention of bacterial peritonitis due to recurrent evacuated paracentesis in cirrhitics with tense ascites.

120 Pattern of Dapsone Induced Liver Injury and Outcome

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Objective: Paucibacillary Hansen’s disease (PBD) is emerging as a major public health problem in developing countries. We studied the pattern of liver injury and their outcome in paucibacillary Hansen’s disease administered Dapsone.

Setting: Tertiary referral centre.

Participants: 382 cases of PBD administered Dapsone (100 mg), Ciofazime (50 mg) daily and Rifampicin (600 mg) monthly.

Study variables: Onset of jaundice, its temporal relationship with drug therapy, alcoholism, biochemical parameters (S. bilirubin, transaminases, alkaline phosphatase and S. albumin), tests for haemolysis, HBsAg and haematology.

Results: Of 362 subjects (190 males and 172 females) 15 developed jaundice (M:F = 2:1). Jaundice occurred 3 to 6 weeks after drug therapy; fever and erythematous rash with exfoliation 100%; body pain and malaise 50%; altered behaviour 20%; congestion of eyes 20%. Clinical signs were tender liver (100%), asterixis (14%), leg edema (14%) and lymphadenopathy (20%). Five males were alcoholics. Serum bilirubin and transaminases were elevated in all those with jaundice and HBsAg was negative. One patient progressed to hepatocellular failure and expired. None had evidence of haemolysis.

Conclusion: Multidrug therapy for PHD resulted in hepatocellular injury in 4.1% and has significant morbidity and mortality.

121 Helicobacter Pylori: Is it a Risk Factor for Hepatic Encephalopathy in Cirrhotic Patients?

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Objective: To determine whether Helicobacter pylori (hp) infection is a risk factor for hepatic encephalopathy in patients (pts) with liver cirrhosis. Methods: 108 cirrhotic pts undergoing upper GI endoscopy for detection of oesophageal varices were included in this study: 34 pts Child-Pugh grade A, 60 pts grade B and 14 pts grade C. The aetiology of liver cirrhosis was either posthepatic or mixed, Biliary fibrosis and postnecrotic cirrhosis. Diagnosis of Hp infection was done by histopathology using antral and fundal biopsies, Hp fast test and serologically by estimating Hp IgG antibody titers by ELISA (more than 20 u/ml). Estimation of serum NH4 using an enzymatic assay for all pts was done and the results were compared with that of 24 normal subjects as a control group.

Results: Serum NH4 is significantly higher in cirrhosis than in normal controls (p < 0.001) 86 cirrhotic pts with Hp +ve were similar 22 Hp –ve with regard to age, sex, aetiology of cirrhosis and Child score. Hp +ve had significantly high NH4 in comparison with Hp –ve pts (p < 0.01). Also significant high NH4 in pts grade C compared with grade A (p < 0.01) and grade B (p < 0.01).

Detection of Hp IgG antibodies by ELISA is a sensitive test as it was positive (more > 20 u/ml) in all pts with positive Hp fast test and positive histopathology for Hp. There was a significant positive correlation between serum NH4 levels and Hp IgG antibody titers in cirrhotic pts (r = 0.9, P < 0.001). Serum Hp IgG antibody levels can be considered as a risk factor for hepatic encephalopathy in cirrhotic patients and may warrant eradication.

122 The Reduction of Cerulean Blood Flow in Cirrhotic Patients Precedes the Appearance of Overt Encephalopathy

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Several studies have shown cerebral morphologic abnormalities by CT and MNR and reduction of cerebral blood flow by single photon emission tomography (SPECT) in cirrhotic patients. However, it is unknown if these abnormalities precede or accompany the appearance of overt encephalopathy. Aim of the study: to analyze whether alterations in cerebral blood flow evaluated by SPECT in cirrhotic patients is associated with or precedes the onset of hepatic encephalopathy. Patients and Methods: SPECT was performed in 20 cirrhotic male patients (50–70 years), without history of alcoholic abuse, diabetes, cardiovascualr and neuropsychiatric diseases. The presence of encephalopathy was assessed by clinical examination, EEG, ammonia blood levels, event related potentials (ERPs) and psychological tests. Latent encephalopathy was defined as absence of clinical signs, in the presence of normal EEG, normal blood ammonia levels, abnormal ERPs and psychological tests. Results: 620 patients showed clinically assessed encephalopathy and reduction of cerebral blood flow by SPECT; 1420 showed neither signs nor symptoms of encephalopathy but 714 showed ERPs, psychological tests and SPECT abnormalities. Two of these seven patients showed a specific SPECT alterations while five showed the same alterations of overt encephalopathy patients. Conclusion: Our data suggest that SPECT might be useful to detect subclinical encephalopathy in cirrhotic patients.
123  Effect of Somatostatin on Renal Functions in Cirrhotic Patients with Ascites

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Purpose: Somatostatin therapy is known to be effective in controlling bleeding oesophageal varices. Effects of somatostatin in renal functions in patients (pts) with cirrhosis is controversial. Purpose of the study is to evaluate the effect of somatostatin on renal functions in cirrhotic pts with ascites.

Methods: Twenty cirrhotic pts with ascites were studied. None of the pts had bleeding oesophageal varices and received no diuretic therapy at least 1 week before the study. During the control period Dextrose 5% solution (2 ml/min) for 2 hr was infused. 24 hr after control period somatostatin infusion (250 µg/hr in Dextrose 5%) was given for 2 hr. Urine was collected during the infusion and 2 hr postinfusion period in both control and somatostatin experiment. Basal and postinfusion blood samples were collected and mean arterial blood pressure (MABP) was monitored.

Results: The MABP changes in urine volume (V), urinary osmolality (Uosm), creatinine clearance (Ccr), free water clearance (Cw), urinary Na (Ua), fractional Na excretion (FENa) under somatostatin infusion were given in below table.

<table>
<thead>
<tr>
<th>V (m/min)</th>
<th>Uosm (mOs/mg)</th>
<th>Ccr (m/min)</th>
<th>Cw (m/min)</th>
<th>Ua (µM/m)</th>
<th>FENa (X10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control P</td>
<td>0.7 ± 0.6</td>
<td>406 ± 27</td>
<td>92 ± 14</td>
<td>100 ± 18</td>
<td>12.3 ± 3</td>
</tr>
<tr>
<td>Test P</td>
<td>1.2 ± 0.1</td>
<td>318 ± 29</td>
<td>153 ± 18</td>
<td>126 ± 18</td>
<td>21.6 ± 6</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: non-significant

Conclusion: Somatostatin therapy has no deteriorating effect on renal functions in cirrhotic patients with ascites. It increases creatinine clearance and urine volume and may be beneficial in this group of patients.

124  Relationship Between KLA Genotype and Phenotypic Expression in Irish Families with Genetic Haemochromatosis

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Introduction: The association between genetic Haemochromatosis (GH) and HLA locus has allowed early identification of affected siblings. The proportion of subjects vulnerable to iron overload that are homozygous (HH) or heterozygous (Hh) is unclear. Studies correlating clinical features with HLA type in families from Ireland – a putative source of this Celtic trait have not been described.

Methods: This study correlated clinical, biochemical and pathologic features of GH with HLA typing (lymphoproliferative assay) in 67 first degree relatives of 12 probands.

Results: Initial analyses identified 12 HH, 40 Hh and 15 nn (normal) individuals. Eleven of 40 individuals initially thought to be Hh had stainable iron on liver biopsy confirming GH and therefore HH status. Further HLA analysis revealed 7 homozygous x heterozygous matings and identification of all disease haplotypes in each pedigree allowed final classification of 30 HH, 25 Hh and 12 nn individuals.

Conclusions: (1) Initial HLA haplotype mismatching classified 18 GH homozygotes맞춤형으로 대조한 후 7 homozygous x heterozygous matings (7 in 12 families). (2) Correlation of HLA haplotype with hepatic saturation and biopsy findings identifies homozygotes and HH x Hn matings. (3) High frequency of HH x Hn matings in this Irish population supports a Celtic origin of the GH gene.

125  Activation of Human Neutrophil Phospholipase D (PLD) is Impaired in Alcoholic Liver Diseases (ALD)


It is well known that alcoholics have higher morbidity and mortality in bacterial infections. Defect in the superoxide anion production of polymorphonuclear granulocytes (PMN) has been suggested as pathogenic mechanism. PLD contributes to the production of phospholipidic acid (PA) that plays a second messenger role in the activation of NF-kappaB (nuclear factor kappa B). The PA can induce the p38 mitogen-activated protein kinase (MAPK) pathway.

The activity of PLD was assessed by measuring the phosphatidylcholine phospholipidase activity. The enzyme activity was assayed in patients with ALD and compared with healthy controls. The results showed that the activity of PLD was significantly reduced in patients with ALD as compared to healthy controls.

126  Cytokin (IL-1 beta, IL-2, IL-4, IL-6) Concentration in Patients with Chronic Hepatitis B during the Treatment with Interferon-Alpha

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In the present study we investigated the effect of interferon-alpha (INF-α) treatment on the concentration of IL-1 beta, IL-2, IL-4 and IL-6 in the serum of patients with chronic hepatitis. Serum levels of IL-1 beta, IL-2, IL-4 and IL-6 were measured by a specific immunoassay (ELISA) method in 46 patients (24 males and 22 females). The patients were treated with INF-α for 6 months. Statistical analysis was performed with use of the paired samples test. Serum levels of IL-1 beta, IL-2, IL-4 and IL-6 concentrations did not show significant changes in examined children. In adults an increase of the IL-6 level was observed (p < 0.02).

The assessment of the IL-6 concentration in serum in patients with chronic hepatitis who received INF-α may have some importance mainly in the adults.

127  Interest of a Higher Dose of Interferon Alpha in Non Responder Patients with Chronic Hepatitis C: A Prospective Randomized Study


The aim of our study was to evaluate the interest of a higher dose of interferon (IFN) in non-responder patients to a first treatment by INF 3 MIU TW 6 months.

Patients and Methods: A prospective randomized and biometric study concerned 23 patients (17 males, 6 females) with histologically proved chronic hepatitis C. Mean age was 38.7 ± 9.1 years. Non-response was defined by ALT ≥ 2 ULN during first treatment duration. Patients were randomized in two groups: group 1 (n = 14): INF α2b 10 MIU TW 2 months then 6 MIU TW 4 months; group 2 (n = 9): INF α2b 6 MIU TW 6 months. The 2 groups were similar for age, sex, mode of contamination, duration of disease, genotype, quantitative viremia (MONITOR), Knodell score (METAIR) and Metavir index (MAM), and viremia (AMPLICOR, MONITOR) were evaluated for each patient at M3, M12, M1, M12 D1, M12 D3 and Knodell score and Metavir index at M12 D1.

In the second group, patients were treated at M3, M12, D1, D3 and Knodell score (9.2 ± 2.5 vs 5.4 ± 3) (p < 0.05) and Metavir index was observed in group 2, particularly for activity index (1.8 ± 0.7 vs 0.9 ± 0.7) (p < 0.05). On the other hand, HCV RNA negativity was statistically more frequent at M7 D1 in non 1b (82%) than in 1b genotype patients (14%) (p < 0.01). The Knodell score (8.7 ± 6.4 vs 6.3 ± 5) and the Metavir index was significantly decreased (1.7 ± 1.8 vs 1.1 ± 0.8) in non 1b genotype patients.

In conclusion, our results suggest that it is possible to obtain an immediate biochemical and virological response in non-responder patients to first treatment by using higher dose of IFN, particularly in non 1 b genotype patients. Indeed, a significant histological improvement is observed in non 1b genotype patients.

Further studies should compare this schedule with a longer duration to IFN-Ribavirine association in this group of patients.

128  Influence of Flumazenil on Hepatic Coma: Clinical and Neurophysiological Study

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Purpose: It has been shown that flumazenil, a benzodiazepine receptor antagonist, has beneficial effect on cerebral function in patients with hepatic coma caused by liver cirrhosis. The aim of this study was to examine the influence of flumazenil on clinical and neurophysiological changes in 10 patients with hepatic coma as a consequence of underlying alcoholic liver cirrhosis.

Methods: Clinical and neurophysiological (electroencephalogram-EEG) and visual evoked responses ("flash" stimulation) parameters were analyzed after administration of flumazenil.

*IMPL*: 1.81 ± 0.27; 1.21 ± 0.13; 1.29 ± 0.11; 1.01 ± 0.22
*PMA*: 1.09 ± 0.16; 0.96 ± 0.17; 0.96 ± 0.13; 1.28 ± 0.22

*ALD vs Controls p < 0.03 Kruskal-Wallis analysis of variance

*Conclusions: IFM-PL induced activation of PLD is impaired in ALD whereas PLD activity in response to PMA is normal. This defect may be of significance for the enhanced susceptibility to infectious agents in ALD.*
Results. The initial dose of flumazenil was 0.2 mg, while the full dose varied from 0.2 mg in 24 patients to 0.3 mg in 17 patients. Positive effect of flumazenil was expressed by patients awakening and manifested in the first 6 hours by motory excitability, aggres-
siveness and mental confusion, followed by drowsiness, but without pyramid
deficit. After flumazenil administration, acceleration of average electrical ac-
tivity from 2.4-4.6 cycles per second was noted on EEG, and shortening of the lat-
ency period (P-100) on visual evoked responses from 69 to 61 msec (aver-
age values). In summary, flumazenil administration showed positive effect on awakening, EEG and visual evoked responses in 810 patients (80%). Survival rates were found to be 6 months and 40% (4/10 patients) at the end of one year.

Conclusion. Our results suggested that endogenous bezodiazepines have
significant role in pathogenesis of hepatic coma. The administration of flumaze-
nil should be recommended in patients with hepatic coma.

129 Epidemiology of Hepatocellular Carcinoma in the Department of Calvados (France). Risk Factors and Prognostic Factors in a Non-Selected Population

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The aim of this study was to determine the epidemiological characteristics of hepatocellular carcinoma (HCC), and to study risk factors and prognostic factors in a non-selected population.

We describe 186 patients between 1984 and 1990. Every case of HCC was registered by the registry of digestive tumors of Calvados. Standardized incidence rates were calculated for male and female. Prognostic factors were determined with the Cox’s multivariate method.

Results: 213 HCC have been registered. Diagnosis of HCC was based on: histology (50p.100), imaging + AFP ≥ 250 ng/ml (34p.100), imaging alone (16p.100). Standardized incidence rates were 7.5/100,000 in men and 0.2/100,000 in women. Sex-ratio was 37.1. Mean age was 66.5 ± 12.2 years. HCC was uncommon before age of 50 (30p.100). HbsAg was present in 10/119 cases (8p.100), anti-HCV antibodies (EIA 1) were present in 6/22 cases (27p.100). Presence or absence of an underlying liver disease was evaluated in 191 cases: normal liver (histologically proven) in 10p.100, cirrhosis in 86p.100, noncirrhotic liver disease (histologically proven) in 4p.100. The cause of cirrhosis was known in 150 cases: alcoholic 73p.100, cryptogenic 9p.100, viral 7p.100, alcoholic + viral 5p.100, hemochromatosis 5p.100. Sex-ratio was 2.9-4.6 for patients with liver cirrhosis, 1.1 for patients without.

Conclusion: In a french non-selected population, at least 10p.100 of HCC occurs on normal liver. Occurrence of HCC seems to be linked to cirrhosis, male sex and age ≥ 50, which could constitute main selection criterias for HCC screening. Unknown cirrhosis is associated with a better prognostic, suggesting the interest of early cirrhosis diagnosis.

130 HB X RNA and Antigen Detection and Sequence Analysis of the X Gene in the Tumorous and Non Tumorous Tissue of HBsAG Negative Patients with HCC

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Deleted HBV genomes persist in hepatocellular carcinomas (HCC) developed in HBsAg negative patients and the X region is often selectively transcribed [1]. In order to understand the role of HBV in these tumors we have: 1) looked for the X protein in the tumorous (T) and non tumorous tissues (NT) and 2) analysed by sequencing the X and the preC regions in HBsAg negative patients with HCC.

Methods: HBV-DNA PCR with 8 sets of primers covering the HBV genome; HBV-RNA analysis with primers on the S, X and C gene; X antigen detection by immunohistochemistry; sequence analysis of the X and, as a “control”, preC/region by a combination of direct and after cloning sequencing. The structure and RNA expression of the HBV genome were analysed in the T and NT of 9 HBsAg negative patients. HBV-DNA PCR revealed frequent genomic deletions. 8 and C RNAs were never expressed in the T and NT. Conversely, X specific transcripts were found in the T and NT tissues of 7/9 patients. Seven patients were tested for the HbsAg in the T and NT. 5 scored positive and 2 negative, the results being in agreement with RNA results. In 2 of these patients the sequence analysis of the X region showed a high T/TN tRNA nucleotide divergence rate in preC/sequences (0.28% and 0.34%).

Conclusions: Our study demonstrates the expression of both the X RNA and protein in the T and NT liver tissues of HBsAg negative patients and identifies mutations which might modify the X function. These results are consistent with a role for the X protein in the HBV related liver oncogenesis in HBsAg negative patients.

131 Mass Screening for Detecting Hepatocellular Carcinoma

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A total of 278,448 inhabitants of Yamanashi prefecture, Japan, were screened for hepatocellular carcinoma (HCC) by using ultrasonography (US) from April 1986 through March 1994. Among 80 HCC patients detected by US, 25 patients were screened annually (group A), the other 55 patients were found at the first screening or at screening two or more years interval (group B).

The detection rate of HCC was calculated as 28.7 per 100,000. The male/female ratio was 5.7:1. All patients were asymptomatic at the time of detection. In respects to important risk factors for HCC: 11 patients were positive for HBsAg from 79 examined, and 40 patients were positive for anti-HCV from 53 examined. Five patients were negative for both HBsAg and anti-HCV, but 79% of them were male.

As compared with group A, group B had a tendency to detect in more smaller size and at more earlier stage. Small HCC (≤ 2 cm) in group A and B were 9 patients (36%), 15 patients (33%) each. Solitary HCC in group A and B were 19 patients (76%), 32 patients (58%) each. For the survival rate of HCC, group A (1/35 year: 92/74%/31 %) was significantly higher than group B (73%/35%/0%).

In conclusion, repeated mass survey by US within one year interval was effective for detecting the HCC at early stage and for expecting good prognosis.

132 Albumin mRNA in Peripheral Blood a Poor Prognostic Marker for Recurrence of Hepatocellular Carcinoma after Orthotopic Liver Transplantation


Survival after orthotopic liver transplantation for hepatocellular carcinoma is limited by a high rate of tumor recurrence. A PCR-assay based on the detection of albumin mRNA expression in peripheral blood for detection of hepatocyte micrometastasis of hepatocellular carcinoma has been described, which may help to select candidates for orthotopic liver transplantation.

The prognostic value of a highly sensitive nested RT-PCR assay was evaluated in comparison to the TNM-classification of the UICC in a population of liver transplant patients.

Six patients with hepatocellular carcinoma and 11 of 59 patients with diseases of the liver were positive on albumin RT-PCR, making this assay a good indicator of ongoing liver damage without absolute specificity for hepatocellular carcinoma. Twelve patients with hepatoma were followed after liver transplantation and 7 of those patients had a tumor recurrence during 12 months. Six of these patients with recurrence had UICC stage IV tumors preoperatively, while only one of them was positive on albumin RT-PCR before transplantation. Only one patient with a stage I to III tumor had a recurrence within 12 months.

Albumin mRNA RT-PCR seems to be an unreliable marker for assessing hematogenous spread of hepatocellular carcinoma before orthotopic liver transplantation. UICC stage IV A tumors was a much better marker of tumor recurrence compared to albumin mRNA RT-PCR. The practical value of albumin mRNA RT-PCR for patients undergoing liver transplantation seems to be very limited.

133 Diagnostic Value of Color Doppler Ultrasoundography in Hepatocellular Carcinoma

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Hepatocellular carcinoma accounts for 80 to 90 percent of liver carcinomas. There is a wide variation in the incidence of hepatocellular carcinoma in different parts of the world, and a numbers of etiologic factors may be important.

Aim: A differential diagnosis of liver tumors was attempted on the basis of the pattern of blood within and around tumors on color Doppler flow images.

Methods: The study comprised 47 patients with liver masses: 22 patients with hepatocellular carcinoma, 15 had hemangiomas, 4 had metastatic liver cancer, 3 had liver abscesses, 3 had liver cysts. A separate group of color flow and Doppler flow velocity were established and compared according to the Tanaka classification.

Result: Color Doppler flow imaging was observed in masses of all 22 patients with hepatocellular carcinoma, of 3 patients with liver metastasis, of 3 patients with hemangiomas. Doppler signals were not observed in 1 patient with liver metastasis, in 3 patients with liver abscesses, in 3 patients with liver cysts. The mean of maximum blood flow velocity in hepatocellular carcinoma, 49.3 cm/s in metastatic liver cancers, 10.3 cm/s in hemangiomas. A basket pattern (a fine blood-flow network surrounding the tumor nodule) was observed in 77% (17/22) of hepatocellular carcinomas. An image of vessels within the tumor (blood flow that runs into and branches
within the tumor) was observed in 5 (23%) of the 22 hepatocellular carcinomas. These two findings were observed only in hepatocellular carcinomas. In three of 15 hemicirrhosis, a spindleshaped pattern (color-stained dots or patches in the central region of the tumor) was seen.

Conclusion: According to the results, hepatocellular carcinomas have some characteristic appearances on Doppler flow images. Therefore, color Doppler ultrasound can aid in the diagnosis of liver mass lesions.

134 Cholangiocellular Carcinoma: Pathological, Ultrasonographic and Angioechographic Correlation

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Correlation were sought among histopathologic, ultrasonographic (US) and angioechographic findings in 27 resected cholangiocellular carcinomas (CCC) cases. According to macroscopic appearance CCC was classified into three types: nodular, peruductal and intraductal. This classification was accepted because clinicopathologic features, as well as prognosis, differed among tumor types. A tumor mass was clearly visible in nodular type tumors. 17 were large tumors and 3 were small less than 3 cm. The large tumor were ill-defined (70%), showed hypoechoic rim (85%), had an echo pattern which tended to be more echogenic and were associated with bile duct dilatation (59%). Vascular structures within the tumor were of importance as US characteristics of CCC: Portal tract passing through the tumor (23%), disappearing portal tract (29%) and the "vesSEL like structures" sign (30%). Tumor mass could not be identified or was poorly visualised in periductal and intraductal type CCC. A disappearing portal tract sign was noted in four while bile duct dilatation was seen in all five periductal type cases. The two cases had intraductal type CCC. Tumor mass obliterating the bile duct was seen in one case.

In addition angioechography was performed in 9 of these CCC cases. After CO2 gas injection three angiographic patterns were discernible, peripheral enhancement in three cases, whole tumor enhancement in four cases and partial tumor enhancement in two cases. There was a tendency for the angiographic pattern to change from whole to peripheral enhancement as the tumor increased in size. The comparisons contribute to understand the significance of the different US and angiographie appearances of CCC.

135 Cytohistological Study of Localisation of Hepatitis B Surface Antigen in Hepatocellular Carcinoma Using Oฆริง Staining

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Objective: To test the hypothesis that oฆริง staining of cytologic specimen from hepatocellular carcinoma (HCC) can detect hepatitis B surface antigen (HBsAg) and to determine the observer variability and agreement.

Methods: 20 cases of suspected HCC were evaluated clinically and cytohistological examination of fine needle aspiration cytology specimen for malignancy by papanicolaou and oฆริง staining for HBsAg was carried. Modification of oฆริง stain was done to suit cytology. Using the oฆริง stain, HCC was diagnosed. HBsAg was detected in serum by ELISA. Observer variability and agreement were assessed on oฆริง positivity by two independent cytopathologists. Data were analysed using Kappa statistics for observer agreement.

Results: Of 20 suspected HCC, 13 had definite HCC. 8 HCC had HBsAg positivity and 8 had oฆริง positivity as judged by observer 1 and 7 by observer 2. A Kappa value of 0.837 was statistically significant.

Conclusion: A high degree of observer agreement between cytopathologists in the interpretation of oฆริง positivity was noted and there was excellent correlation with HBsAg status. This technique is cheap, safe and quick and need further evaluation as a test for laboratory diagnosis of HBV related HCC.

136 HCC Prevalence in Liver Cirrhosis: A Prospective Study on 121 Cases

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Prevalence of Hepatocellular carcinoma (HCC) in patients suffering from cirrhosis is high and its prognosis generally poor. The early detection of HCC could improve treatment chances and survival. We monitored 121 consecutive patients (73 men, 48 females ages, average age 58, ranging from 20-73) with semiannual controls of alfabetoprotein and ultrasonography. Cirrhosis was viral in 48 patients, ethanol related in 63 and due to other causes in 10. At the moment of enrollment 64 of the patients were classified in class A of Child, 39 in B and 18 in C. The average length of our observation was 48 months (range 11-102). There were 20 HCC diagnosed, 15 of them male. In relation to aetiology, 12 HCC developed in patients with viral cirrhosis (25%) and 8 in alcoholic disease (16.8%). In 11 HCC underlying cirrhosis was classified in Child grade A, in 8 B, and in one case in C. In 10 cases tumor size was smaller than 5 cm in diameter. The levels of Alfaetoprotein were high in 12 patients with HCC (range 7 to 149.7 µg/L). In 7 with cirrhosis, Cumulative incidence of HCC became greater with the length of follow up: 2.5% at the first year, 22.4 at the sixth, with annual incidence of about 4%.

In conclusion our results suggest that the semiannual screening of cirhotic patients permits the diagnosis of HCC in its early stages in 50% of the cases. Alphaetoprotein measurement alone is an unreliable index of HCC development for its low sensibility. In patients with Child grade C cirrhosis background liver disease itself determine the prognosis, so that screening for HCC development seems to be unnecessary.

137 Serum Carbohydrate-Deficient Transferrin in Patients with Nonalcoholic Liver Disease and with Hepatocellular Carcinoma

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Serum carbohydrate-deficient transferrin (CDT) test is a reliable and specific marker for detecting alcohol consumption. However, recent studies have shown false-positive of CDT test results in nonalcoholic liver disease. We examined the clinical significance of serum CDT in nonalcoholic liver disease and especially in hepatocellular carcinoma (HCC), using alcoholic liver disease as a positive control.

The subjects included 23 teetotalers, 56 patients with alcoholic liver disease (39 liver fibrosis and 17 LC), 64 patients with chronic viral liver disease (24 CPH, 33 CAH, 27 LC) and 67 patients with HCC. Serum CDT was measured with an % CDTRIA kit (Axis Biochemicals AS, Oslo, Norway) and expressed as percentages of the total transferrin (% CDT).

The % CDT was 1.2 ± 0.8% in the teetotalers. The mean serum % CDT value was increased 1.5-fold in patients with alcoholic liver disease and 3.6-fold in alcoholic LC compared with the teetotalers. The % CDT values in viral chronic hepatitis were similar to those in teetotalers, and were increased 2.0-fold in nonalcoholic LC. False-positive results were found in 10 (37%) of the 27 patients with nonalcoholic LC, but not in viral chronic hepatitis. The mean serum % CDT value was increased 2.5-fold in HCC, and false-positive results were found in 31 (46%) of the 67 patients. CDT was also recognized by isoelectric focusing followed by immunodetection of carbohydrate-deficient serum from patients with HCC. The % CDT value in HCC was related to the size of tumor and the grade of histological differentiation.

These results suggest that the ability of serum CDT measurement to detect chronic alcoholism may be reduced in patients with nonalcoholic liver cirrhosis and with hepatocellular carcinoma.

138 Precancerous Potentiality of Macrogenervative Nodules in Liver Cirrhosis

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Background: A close follow-up with ultrasonographic (US) examination and alpha-fetoprotein (AFP) measurement is the current policy for early diagnosis of hepatocellular carcinoma (HCC) in patients with cirrhosis. Large hepatic nodules, histologically defined as macrogenervative nodules (MRNs), are sometimes picked up by US. The significance of these lesions, whether they are hepatic neoplasms or simple reactive changes, is still debated. In the present study the pre-malignant potential of these nodules is evaluated, by following them up with regularly scheduled US examinations and US-guided fine needle biopsy (FNB).

Methods: 14 focal lesions (with an average size of 24 mm in diameter) in 11 cirrhotic patients (10 males and 2 females, age 52–75 years), with histological diagnosis of MRNs, made by US-guided fine needle biopsy, were followed from 1989 to 1996. Mean follow-up was 41 months (range 12 to 77). During the observation period a regular US and chemical evaluation at an interval of 4 months was carried out. When some changes occurred in nodules size, US pattern or AFP levels, a rebiopsy was performed. US and clinical features, including Child’s classification, alcohol intake, AFP and HBV/HCV positivity were investigated (chi-square test was used for statistical analysis of data).

Results: 12 out of 14 nodules (78.5%) increased in size during the follow-up period, and became histologically malignant (HCC) after 28.7 ± 10 months (range 7 to 54). There was no statistical correlation between neoplastic transformation and US pattern or clinical data at the time of entry into the study.

Conclusions: The pre-malignant nature of MRNs in cirrhotic liver is very likely and the treatment of these lesions should be encouraged.

139 Retroviral Thymidine Kinase Gene Transfer and Ganciclovir Therapy (GCV) Generates CD8+ Tumor-Mediated Immunity and Bystander Effect Against Non Transduced Liver Tumors Cells in Rat


Significant regression of liver metastases in rats was observed after a silent retroviral transfer of thymidine kinase gene from Herpes Simplex Type 1 (HSV1-TK), followed by GCV. However, few tumor cells were effectively transduced. This could be explained by bystander effect (intercellular crossing of toxic form of GCV) and/or anti-tumour immunity. The aim of this work was to study these two mechanisms in rat liver metastases and hepatoma.
Material and Methods: (a) bystander effect was studied in 16 rats after intraarterial injection of colon cancer cells mixed with variable rates of transduced TK+ (0, 25%, 50% and 100%) TK+ and TK− cells. After 5 days, rats were treated with GCV for 5 days. Rats were sacrificed at Day 12; (b) anti-tumour immunity was studied in 12 rats after intraarterial injection in 2 different-latitude-related tumor cell clones in each rat, left side treated with TK− cells and right lobe TK+ cells. At Day 14, 6 rats were treated with GCV for 5 days. The 6 others rats received no treatment. Two weeks later, all the rats were sacrificed.

Results: (a) significant tumour regression was obtained from tumors with 25% TK+ cells: mean tumor volumes were: 30 ± 16 mm³ (0% TK+), 15 ± 10 (25% TK+); p < 0.04 vs 4 ± (50% TK+) and 0.2 ± 0.2 (100% TK+); (b) in control rats with double liver tumors, mean tumor volume for TK− was 4895 ± 4300 and TK− − 6494 ± 1300 mm³ (N.S.). After GCV mean, tumor mean of TK− was 11 ± 13 (p < 0.006 vs control TK+) and TK− − 30 ± 41 mm³ (p < 0.004 vs control TK−).

Conclusions: (a) bystander effect was responsible of a significant anti-tumour effect even if only 25% of tumor cells were effectively transduced; (b) the unexpected significant regression of TK− liver tumors suggested a possible role of host immunity.

This study demonstrated that 2 different mechanisms could amplify the therapeutic effect observed after HSV1-TK retroviral gene transfer and GCV: the bystander effect and the generation of anti-tumour immunity against TK− cells after destruction of TK+ cells.

140 Assay of Trace Elements and Superoxide Dismutase Activity on Biopsic Specimens from Chronic and Neoplastic Liver Diseases
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Oxygen radicals play an important role in multistep carcinogenesis and antitumoral substances can inhibit the carcinogenesis. Hepatocellular carcinoma (HCC) is a neoplasia mostly arising on cirrhotic liver. The aim of our study was to evaluate on liver specimens obtained by echo-guide percutaneous biopsy from patients with neoplastic and/or chronic liver diseases: A) activity of the two types of antioxidant enzyme Superoxide Dismutase (Mn-SOD and CuZn-SOD); B) the level of some elements (Mn, Cu, Zn, Fe) involved in free radicals metabolism.

We evaluated the SOD activity on specimens from 8 normal livers, 17 chronic hepatitis (CH), 11 cirrhosis, 4 dysplastic nodules (DN) and 11 HCC; the element levels on specimens from 4 normal livers, 14 CH, 16 cirrhosis, 1 DN and 4 HCC.

In present study a reduction of SOD activity has been found progressing from chronic liver diseases to HCC; this reduction could be involved in the pathogenesis of liver dysfuction by the natural history of HCC. In our study the level of Mn, Cu and Zn (cofactors of the two types of SOD) aren't lower in liver diseases, thus they aren't involved in down regulation of SOD. On the contrary, the iron, element involved in free radicals production, is higher in all liver diseases.

141 Hepatocellular Carcinoma (HCC) in HVCL-Related Cirrhosis Treated and Not Treated with Interferon

Hepatocellular carcinoma (HCC) is a complication of long-standing cirrhosis, especially with viral etiology. In an Italian population the HCC incidence/100 patients was 1.0/10,000 in CH and 1.0/100,000 in CHHVCL-related cirrhosis is 3.6/2 (J. Gastroenterol. 1994; 26: 164). Recent reports suggested that IFN treatment can reduce the risk of HCC development in HVCL-related cirrhosis (Lancet, 1995; 346; 1051; J Hepatol, 1996; 24: 141).

Since we wanted to verify whether in our population IFN treatment can reduce the incidence of HCC in Child A cirrhotic patients, we studied two group of patients, prospectively followed up, and treated or not with IFN.

Methods: IFN treated group: 44 patients (26 M, 12 F, mean age 56 Range 29–84) enrolled in Alpha-INF trials between 1991–1994 and followed-up for at least 12 months (mean 37.6 Range 12–56) after the end of full treatment.

Control group: 157 non treated patients (81 M, 76 F, mean age 54.2 Range 30–72) enrolled in a prospective follow-up study between 1981–1994. All patients had well compensated cirrhosis (Child A), biopsy proven at the enrolment, and no statistically significant differences in demography were detectable. In the treated group 8 pts (18.2%) were responders, 27 (61.3%) non-responders and 9 (20.5%) responses were received for TK+ cells and right lobe TK+ cells. At Day 14, 6 rats were treated with GCV for 5 days. The 6 others rats received no treatment. Two weeks later, all the rats were sacrificed.

The results were statistically analysed by the Mantel Haenszel life table analysis.

Results: 5 (11.4%) patients in the treated group developed HCC. The respective incidences in responders, non responders and relapers were 2/5 (40%), 3/5 (60%) and 3/9 (33.3%). In non treated patients 32/157 (20%) developed HCC. After adjustment for the follow-up period, the Mantel Haenszel life table analysis showed that the probability of remaining HCC free was not statistically different between the two groups (p = 0.30).

In Conclusion our results, based on a prospective follow-up study of cirrhotics, treated or not with IFN, failed to confirm the above evidence that IFN treatment reduce the HCC incidence in well compensated cirrhosis.

142 Trial of a New Therapy Combining DDS with Hyperthermia for Liver Cancer
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We have been developing a new form of therapy to achieve more effective local control of liver cancer.

First, we designed a new anticancer drug delivery system (DDS) targeting liver cancer. Then, we developed a drug complex bound to hydroxyapatite (HAP) including doxorubicin hydrochloride (DOX) and buthionine sulfoximine (BSO). This drug complex was named the HAP system. DOX is an anthracycline anticancer drug, and BSO is a selective inhibitor of GSH (intracellular glutathione; γ-glutamyl-cysteinyl-glycine) biosynthesis. GSH is a scavenger of free radicals in tumor cells. We found in an in vitro experiment that DOX and BSO were eluted from the HAP system over the first 3 hrs of incubation, and that from 4 hrs onwards the remaining DOX was released continuously, showing a slow-release property. This property is likely to be favorable because it allows continuous attack on tumor cell DNA after the initial depletion of GSH by BSO.

Second, we performed an in vivo experiment using sarcoma 180 tumors transplanted into the thigh of the right hind leg of mice. After measuring the tumor volume every day we evaluated the inhibitory effect of the HAP system on tumor growth. The inhibitory effect of the system was remarkable, the tumor volume ratio (DOX-HAP complex-treated group/DOX & BSO-HAP complex-treated group) on the 31st day being 1.93.

We then examined experimentally the inhibitory effect on tumor growth of the HAP system + hyperthermia (42°C for 40 min) on the 7th day after HAP system transplantation. On the 29th day after the transplantation of this system, the tumor volume in the DOX & BSO-HAP group was 6.36 ± 10 mm³ whereas that of the DOX & HAP + hyperthermia group was 2.23 ± 10 mm³. The latter group showed significant suppression of tumor volume to 64%, as compared with the former group (t-test, < 0.05). Therefore, it was assumed that the HAP system + hyperthermia treatment was more effective for cancer therapy than the other treatments.

We also have been studying the inhibitory effect on tumor growth of the HAP system + hyperthermia using VX2-transformed livers (hepatic tumor) in Japanese white rabbits, and obtained results similar to those for sarcoma 180.

143 Arterial Infusion Chemotherapy Using in vitro Chemosensitivity Test for Unresectable Hepatocellular Carcinoma (HCC)
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We studied the clinical usefulness of hepatic arterial infusion chemotherapy based on a chemosensitivity test.

Twenty eight patients with unresectable HCCs were treated by hepatic arterial infusion chemotherapy using a percutaneously implanted reservoir. Before chemoinfusion, we selected an anticancer drug by in vitro chemosensitivity test using a biopsy specimen, in which karyologic changes of cancer cells were observed microscopically and an inhibition of cell growth.

Forty four patients were treated by chemosensitivity-positive drugs (group A). The remaining 34 patients (group B), failing to have chemosensitivity-positive drugs, were treated by randomly selected 1 of 4 drugs (piranubicin, epirubicin, carboplatin, mitoxantrone). The general response rate by WHO criteria was 46% in group A and 12% in group B. The 1-year and 2-year survival rates of group A were 69% and 42%, while, those of group B were 58% and 0%, respectively.

The arterial infusion chemotherapy based on the chemosensitivity test improved therapeutic results in the patients with unresectable HCCs.
High Preoperative Serum Alanine Transaminase Level Increases the Risk of Liver Rejection for Hepatocellular Carcinoma (HCC) in Children: A Chirurgical Patients

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Liver resection in patients with liver cirrhosis (even in the absence of overt liver insufficiency) is associated with a greater risk than in patients without underlying liver disease. Because the incidence of HCV-cirrhosis related HCC is expected to increase rapidly in the near future we have assessed, by multivariate analysis, parameters associated with in-hospital mortality and morbidity in a consecutive series (1984–1994) of 108 Childs’ grade A cirrhotic patients undergoing liver resection of an HCC (1 or less liver segment, 2 segments or 3 or more segments in 42, 43 and 43 patients respectively). Parameters entered for analysis included age, aetiology of cirrhosis, preoperative serum bilirubin, AST, ALT, GGT, albumin, creatinine levels as well as prothrombin time, presence or absence of pathological features of superimposed active hepatitis, ascites, in-hospital death and duration of vascular clamping and amount of intraoperative blood loss. Overall incidence of in-hospital death and major postoperative complications were 8.3% and 48.1% respectively. By univariate analysis, preoperative serum ALT levels (p = 0.001) and intraoperative transfusions (p = 0.01) were the only parameters significantly associated with in-hospital death. However, only serum ALT concentrations was an independent risk factor. In-hospital mortality in patients whose preoperative serum ALT was below 2 N (n = 77), comprised between 2 and 4 N (n = 23) and greater than 4 N (n = 7) was respectively 4, 13% and 37.5%. Increased ALT levels (> 2 N) was also associated with an increased incidence of postoperative ascites (56 vs. 32%, p = 0.01), kidney failure (16 vs. 0%, p = 0.0003) and UGI bleeding (41 vs. 0%, p = 0.02).

Conclusion: Preoperative serum ALT level is an independent and reliable predictor of in-hospital mortality and morbidity following liver resection in Child A cirrhotic patients. Our results suggest that cirrhotic patients with ALT > 2 N should undergo only a limited resection. If a larger resection is necessary other therapeutic option should be considered.

Low Mortality after Liver Resection with Ultrasound Dissector in Cirrhotic and Non-Cirrhotic Patients

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Aim of this study is to evaluate the postoperative (30 days) morbidity and mortality in a consecutive series of 144 liver resections carried out with the ultrasonic dissector (CUSA). From 1987 to 1996 144 patients (M/F: 90/54, mean age 64 years) underwent liver resection for hepatocarcinoma (n = 51), liver metastases (n = 62), bile duct carcinoma (n = 5), hemangiomas (n = 11), and other benign tumors (n = 15). Forty-six patients were cirrhotic and 98 patients had a normal residual liver. Hepatic resections included: 43 major resections (left, right or extended right heptectomy), 21 segmentectomies and 84 atypical or subsegmental resections. Surgical technique included the use of CUSA, which allowed the meticulous identification and ligation of major as well as minor hepatic vessels and ducts crossing the resection plane, thus preventing local surgical complications.

The morbidity and mortality observed are detailed in the table:

<table>
<thead>
<tr>
<th>Complications</th>
<th>All patients (n = 144)</th>
<th>Cirrhotic (n = 46)</th>
<th>Non cirrhotic (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver failure</td>
<td>6 (2%)</td>
<td>2 (5*)</td>
<td>4 (1*)</td>
</tr>
<tr>
<td>Liver bleeding</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td>2 (1*)</td>
<td>2 (1*)</td>
<td>0</td>
</tr>
<tr>
<td>Biliary leakage</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Septic shock</td>
<td>2 (1*)</td>
<td>1 (1*)</td>
<td>1</td>
</tr>
<tr>
<td>Hb, hnc, renal failure, MOF</td>
<td>11 (8*)</td>
<td>5 (1*)</td>
<td>6 (2*)</td>
</tr>
<tr>
<td>Total complications</td>
<td>24</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Total pts with complications</td>
<td>16 (11%)</td>
<td>7 (15%)</td>
<td>9 (9%)</td>
</tr>
<tr>
<td>Mortality</td>
<td>7 (5%)</td>
<td>3 (7%)</td>
<td>4 (4%)</td>
</tr>
</tbody>
</table>

*total outcome

In conclusion, our results indicate that liver resection can be done in cirrhotic and non-cirrhotic patients with low morbidity (11%) and low mortality (5%), by using the ultrasonic dissector, which allows a better hemostasis and control of bile leakage of the liver transaction surface, as compared to the finger dissection technique.

Quantification of Intra-Hepatic HCV-RNA According to Histology and to Genotypes after Liver Transplantation

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A relation between genotypes 1b and severity of recurrent liver disease due to HCV has been described after liver transplantation. However, the mechanism of such severity remains unknown.

The aim of this study was to analyse in liver transplanted patients remaining serum HCV RNA positive, the relations between the level of intrahepatic replication of HCV, the severity of liver disease and the genotypes.

Samples and methods: 98 post-transplant biopsies from 33 liver transplanted patients (21 were genotype 1b) were available. Intrahepatic HCV RNA was quantitated by competitive method using serial dilutions of HCV cDNA. For all biopsies, a similar competitive method was used for the quantification of 28S ribosomal RNA to normalize the quantification of HCV between the biopsies.

Results: Levels of intra-hepatic replication was higher in case of lobular hepatitis than in case of chronic active hepatitis, rejection, cholestasis and subnormal histology (10 competitor Unit (CU) vs 2; 1.8 and 2.2 p = 0.01). Low level of replication decreased with time after LT and was not related to genotype 1b.

Conclusions: Our results show that intra-hepatic replication of HCV 1) exist in liver graft with normal histology; 2) was only increased in case of acute lobular hepatitis; 3) decreased with time after transplant. 4) was not related to genotype 1b. These findings suggest a direct cytotoxic effect of HCV at the time of acute hepatitis and that pathogenicity of HCV type 1b is an intrinsic property not related to an increased level of replication.

Value of Transjugular Intrahepatic Portosystemic Shunt in Cirrhotic Patients Awaiting Liver Transplantation

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From November 1991 to January 1995, 34 transjugular intrahepatic portosystemic shunt (TIPS) were attempted in 34 cirrhotic patients (mean 48.4 ± 2.4; 22-66) candidates for liver transplantation (LT). Patients were classified Child class A in 5 cases, B in 11 cases, C in 18 cases. Indication for TIPS was schlerotherapy failure in 23 cases and intractable ascites in 11 cases. Two patients were excluded because of technical failures which were treated by OLT in one case and open calibrated porta-caval shunt in one case. The follow-up with LT as end point was 1 to 34 months (7.6 ± 1.6 M). Results: Early thrombosis (< 3 months) occurred in 8 cases: 6 were desobstructed via the internal jugular vein and 2 were desobstructed surgically together with calibrated porta-caval shunt. Late thrombosis occurred in 1 case with portal vein thrombosis and was treated by mesenterico-caval shunt followed by LT 6 months later.

Recurrence of hemorrhage occurred in 222 patients who underwent TIPS for schlerotherapy failure (one rupture of varices, one duodenal ulcer). Ascites disappeared in 7/10 patients who underwent TIPS for intractable ascites and was controlled together with diuretics in 2 patients. Ascites remained unchanged in 1 patient.

21 patients were transplanted following TIPS with a mean delay 6.4 ± 1.6 (range: 1–26 months). During the same period, 7 patients with cirrhosis and surgical open porta-caval shunt were transplanted. Comparison of patients with TIPS to patients with surgical open shunt showed a shorter duration of operation for patients with TIPS (332 ± 351 vs 467 ± 480 min, P = 0.05), less blood transfusion (3.5 ± 2.1 vs 7.3 ± 2.6 L, P < 0.05). Graft and patient survival among the two groups were similar. We conclude that TIPS controls the complications of portal hypertension in patients awaiting transplantation. TIPS diminishes blood requirement during liver transplantation procedure.

Prospective Evaluation of Serum GSTζ in Liver Transplantation

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The cytosolic enzyme, Glutathion S transferase ζ (GSTζ) has been proposed as a marker of acute graft rejection following liver transplantation (LT). GSTζ may offer significant clinical advantages over conventional liver function tests (LFT; wide hepatic distribution and shorter in vivo plasma half life). The value of this test over LFT remained to be assessed and was the impetus for the present study. A prospective daily evaluation of plasmatic GSTζ using a new enzyme immunometric assay (Hepkit, Biotrin, Ireland) was performed in 45 recipients (men: 46%, 46 ± 13 y), in the first 20 days following LT. The results were compared to conventional LFT and significant clinical events. Twenty patients experienced biopsy proven acute graft rejection episodes (only 15 were treated); 15/20 had an increased GSTζ vs 11/20 for transaminases. The sensitivity of GSTζ assay for the occurrence of treated acute graft rejection was 100% and the specificity 73% vs 73% and 88% for ALT, respectively. The positive predictive value was 75% and the negative one was 100%. GSTζ increased earlier than the conventional LFT before acute graft rejection and decreased also more rapidly with steroid treatment was efficient. GSTζ remained significantly elevated in case of steroid-resistant graft rejection. In the first 3 days of the post operative period, GSTζ was significantly correlated with the prothrombin time at D3 and DS (p < 0.007) and with the occurrence of acute graft rejection. The slower was the decrease, the higher was the occurrence of acute rejection. According to the early increase before acute graft rejection and the rapid decrease following efficient treatment of graft rejection, GSTζ assay seems to be a useful marker of acute graft rejection to use in combination with conventional LFT. Moreover the initial GSTζ release following LT was correlated with early graft function.
Liver Transplantation for Hepatocellular Carcinoma on Cirrhosis: Prognostic Impact of an Adapted Patient Selection

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Hepatocellular carcinoma (HCC) is an established but still debated indication of liver transplantation (LT). The high risk of recurrence and five-year survival rates significantly lower (0–50%) in different series than those of benign diseases have questioned the place of LT for HCC in the current period of organ shortage. We report in this study the consequences of a new selection of patients adapted from prognostic indicators established in the first phase of a same series. From November 1985 to December 1991, 109 patients with cirrhosis were transplanted for HCC. Of these 109 patients, only 95 patients with HCC diagnosed before LT were included in the study. The presence of extrahepatic deposits on pretransplant staging or pre-operative exploration was a contraindication to LT in 12 patients (11% of the first period of our series (November 85–December 91)), the selection criteria only included the absence of any extrahepatic tumour (60 patients). After assessment of prognostic factors in this first period (mainly tumour size > 30 mm, number of nodules > 3 and presence of a portal thrombosis), we proceeded to a more restrictive selection of those patients at very high risk of recurrence. Results in terms of patient selection and 3-year survival were as follows:

<table>
<thead>
<tr>
<th>1st period (85–91)</th>
<th>2nd period (92–94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 60</td>
<td>n = 35</td>
</tr>
<tr>
<td>No nodules &gt; 3</td>
<td>24 (40%)</td>
</tr>
<tr>
<td>Size &gt; 30 mm</td>
<td>29 (48%)</td>
</tr>
<tr>
<td>Portal thrombosis</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>No and Size &lt; 30 mm &lt; 3 nod</td>
<td>21 (35%)</td>
</tr>
<tr>
<td>&gt; 30 mm &gt; 3 nod</td>
<td>14 (23%)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>3 yr-Surv.</td>
<td>55% – 45%</td>
</tr>
</tbody>
</table>

Conclusion: An adapted selection of patients with HCC for LT allows a significant decrease of the recurrence rate and a trend to improved survival. This warrants the indication of LT for HCC even in the current period of organ shortage.

Impact of Cytomegalovirus (CMV) on Morbidity and Mortality after Liver Transplantation (LT)

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CMV is the predominant cause of viral infection after LT. The aim of this study was to analyse the impact of the CMV on the results of LT. From January 1987 to December 1992, 789 LT were performed in 688 patients. 76 CMV seronegative recipients (R-) received liver from CMV seronegative donors with CMV free blood transfusions (D-): group I: 34 M, 42 F; mean age 44.3 ± 16 years) and the 612 other patients were either seropositive (R+) or acquired the virus from the graft or from transfusions (D+): group II: 265 M, 247 F; mean age 46.3 ± 13 years). LT was performed in urgent for fulminant liver failure in 18.4% of the R-D- group and in 19.2% of the group D. ABO incompatible graft were used in 5.2% and 4.6% of group I and group II respectively. Among group II patients, 37.5% developed a CMV infection, 21% a CMV disease and two died of CMV disease. None of the R-D- patients developed a CMV infection. There was no statistical difference in the cumulative actuarial incidence of acute rejection between group I (36%) and group II (35%). The cumulative actuarial incidence of chronic rejection was comparable in the two groups (7%). There was a statistical difference in actuarial patient survival rate (group I: 81% vs group II: 70% at 5 years, p < 0.01). No statistical difference was seen in actuarial graft survival rate (group I: 79% vs group II: 64%).

In conclusion: Despite improvements in early diagnosis, prevention and treatment of CMV disease, morbidity is lower and survival better in R-D- patients than in R-D+ patients. Despite scarcity of organ donors, it is of the utmost importance to match CMV seronegative recipients with CMV seronegative donors.

RNA-HCV Higher Levels in Orthotopic Liver Transplantation Treated with Steroid because of Acute Rejection

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Aim: To study the influence of steroid treatment on hepatitis C virus (HCV) RNA titres in orthotopic liver transplantation patients (OLT) because HCV chronic hepatitis.

Materials and Methods: 26 OLT patients with HCV infection were studied. All the patients: a) HCV-RNA positive by PCR before OLT and in the 5-day determination after OLT; b) At least 3 months post-OLT survival; c) No receiving antiviral or autoimmune treatment after OLT. All the patients treated with immunosuppressive therapy (cyclosporin, azathioprine, steroids). Rejection episodes were treated with methylprednisolone pulses (1 grid). OKT3 was used in those who no response was observed. In the 6–7 months follow-up steroid doses were tapered. At 12th month all patients received 4–8 mg of steroid treatment and at 15th month has been suppressed in all patients. Acute rejection episode was observed in 14 patients. OKT3 was administrated to one patient. In 16 patients serum samples had been stored on 5th day post-OLT, 9 of them before the acute rejection episode. Another serum sample of those patients were stored between 15–30 days post-OLT. In all patients serum samples were stored 2 months after-OLT, in 23 at 6 months after-OLT, and in 8 patients after steroid therapy suppression. RNA-HCV titre was assessed by amplification. Mean age: 51 ± 10 (16 mates). Results: No differences were observed in relation to age, sex, mean ciclesporin levels, time of azathioprine treatment and infections in patients who developed not an acute rejection episode. RNA-HCV levels at 5 days post-OLT were 912 ± 1220 x 106 Eq/ml. Patients treated with 3 pulses of steroids had higher titres of RNA-HCV at second samples (4153 ± 7692 x 106 Eq/ml) than at 5 day (697 ± 393 x 106 p < 0.05). Titrates at 2 months (8435 ± 10866 ± 106 Eq/ml) and 6 months (8348 ± 8217 ± 107) were higher than titres at 5 day post-OLT (p < 0.05) and higher than RNA-HCV levels in patients without acute rejection episodes. After steroid withdrawal RNA-HCV titre fallen to 1296 ± 1029 x 106; no statistically difference was observed.

Conclusions: OLT-HCV positive patients treated with steroid pulses present higher levels of HCV viremia at 2 and 6 months after-OLT than immediately post-OLT levels and patients no treated with steroid pulses. Steroid suppression was followed by a decrease HCV viremia, although no difference was observed with 6 months values.

Transjugular Intrahepatic Portosystemic Shunt (TIPS) Is of Benefit to Orthotopic Liver Transplantation (OLT)

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Although patients waiting for a liver transplantation are considered as the better indication for TIPS, interest of this procedure has not been clearly demonstrated and results show some discrepancies.

Aim: to determine the benefit of TIPS in OLT, we have compared in a retrospective case-control study, patients who, during the same period, underwent OLT after TIPS (T+) and control OLT patients without TIPS (T–). Method: 15 Patients and 15 controls were matched for age (± 5 years), gender, Child-Hugh stage and cirrhosis etiology.

Results: No difference between the two groups were observed in Apache Scores, previous ascites, previous encapsulopathy episodes and previous abdominal surgery; delay between registration on the waiting list and OLT was identical in the 2 groups. Differences were significant concerning the presence of peritoneal adherences (0 (T+) vs 5 (T–) ; p < 0.02) and portal thrombosis (0 (T+) vs 1 (T–) ; p < 0.02). A trend toward a decrease in procedural time (5.6 h. ± 1.2 (T+) vs 7.2 h. ± 3.3 (T–); ns) and a decrease of amounts of transfusion (5.3 blood unit ± 3.5 (T+) vs 10 (T–); ns) was noticed. There was a significant decrease in the intensive care unit stay: (3.9 d. ± 1.2 (T+) vs 6.5 d. ± 4.5 (T–) ; p < 0.05).

Conclusions: Because of the reduction of portal hypertension, TIPS allowed to simplify the OLT procedure and to reduce the length of post OLT intensive care unit stay. A prospective randomized study is necessary to confirm these data for patients waiting for OLT with and without complications.

Bile Duct Reconstruction affects the Results of Orthotopic Liver Transplantation

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Aim of the study was to assess the effect of bile duct reconstruction (split technique according to Zimmermann) on the postoperative course, the liver function parameters and on the histomorphological changes in the liver parenchyma after orthotrophic liver transplantation (ORT) in three groups, each consisting of 10 syngenic rats, were compared: I: sham operation, II. bile duct reconstruction, III. ORLT. After the operation the clinical course and the serological parameters were monitored. 4 weeks postoperatively the histomorphological changes according to a semiquantitative score (scale: 0–103 points) and the proliferation rate (using the proliferation marker BrdU) were evaluated.

Survival rate was 100% in I and II, 90% in III; early postoperative elevation of transaminases was normalized after 4 weeks in all groups. Morphological changes as bile duct alterations, Kupffer cell proliferation, increase in fibrous tissue and hepatocellular necrosis and regeneration were minimal in I (2.5 points), accentuated in II (36.5 points) and prominent in III (45 points). Hepatic proliferation was low in sham operated animals, significantly increased after bile duct reconstruction, and again doubled after ORLT.

The lasting effect of bile duct reconstruction on the morphological results necessitates the inclusion of a mere bile duct reconstruction as a control group in ORLT-experiments.
154 HCV Recurrence after Liver Transplantation

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Recurrence of HCV infection is extremely frequent after liver transplantation (OLT) for HCV-induced cirrhosis. The incidence and severity of graft disease occurring after HCV infection recurrence is still controversial. Aim of our study was to evaluate the features of HCV recurrence after OLT. From November 1990 until November 1995, 41 patients (33%) were transplanted for HCV-related cirrhosis out of a total of 123 patients transplanted at our institution. Patients with previous therapeutic period were excluded from analysis. A total of 35 patients were included in the study: 28 males and 7 females, with a mean age of 49 years (range 25–61 yrs); 17 pts (49%) had HCV alone, 7 (20%) concomitant alcohol abuse, 5 (14%) HCC, 4 (11%) coexistent HBV infection, 2 (5.5%) diabetes, and 1 PSC. Mean age at OLT for patients with HCV alone was 52.5 ± 6.8 years, compared with 42.6 ± 12.6 years in patients with multiple etiology (p < 0.02). All patients were anti-HCV(+) prior to OLT, whether 29 (83%) were HCV-RNA by PCR(+). A total of 28 recipients (96%) remained HCV-RNA by PCR positive during the post-transplant follow-up. None of the 6 anti-HCV(+)HCV-RNA(−) recipients became HCV-RNA(+) after OLT.

155 Predictors of Survival in Acute Hepatic Failure

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Objective: To determine the prognostic factors and survival in hyperacut, acute and subacute liver failure.

Setting: Tertiary referral centre.

Study Design: Prospective prognostic and survival.

Participants: 60 subjects with hepatic failure (8 hyperacute, 29 acute & 23 subacute) selected as per Roger Williams criteria, 1993.

Study Variables: 32 clinical and laboratory variables which included liver span, IVC, splenic, collateral, laboratory and haematological parameters.

Outcome variables: Prognostic factors and survival or death in the hepatic failure subgroups.

Data Analysis: Descriptive, independent 't' test, proportions by chi square, survival rate by Kaplan Meier (KM) product limit method and univariate & multivariate analysis using Cox Proportional Hazard Model.

Results: 17 subjects expired (hyperacute 2, acute 6, subacute 9). Age, onset of hepatic failure, IVC diameter, platelet, transaminases and plasma levels of CP and di did not differ between survivors and non-survivors. Serum bilirubin (mg/dl) was 20.96 ± 7.43 among survivors & 24.95 ± 5.576 in non survivors (P = 0.048). KM survival probability for hyperacute, acute and subacute were 0.79 (95% CI 0.51–0.93), 0.70 (95% CI 0.59–0.90) and 0.61 (95% CI 0.38–0.77). Hazard ratio was 3.77 for those with encephalopathy grade 3 & 4 and prothrombin time > 25 sec (P = 0.001).

Conclusion: Acute and subacute hepatic failure have poor prognosis and grade of encephalopathy with abnormal prothrombin time and low platelet count are poor prognostic factors.

156 Cholestatic Liver Disease in Proteoprophia: Treatment with Cholic Acids and Liver Transplantation

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In erythropoietic protoporphyria with ferrochromea deficiency an unpredictable hepatobiliary course of the metabolic disease develop in a quarter of patients: orthotopic liver transplantation (OLT). The incidence of liver transplantation (OLT) for chronic hepatic failure (cholestasis) was estimated in patients with protoporphyria (PP) and in patients with cholestatic liver disease. Patients with protoporphyria (PP) were identified in a series of 265 patients (89% male; age range, 1–89 years) with protoporphyria (PP) and cholestasis. The cholestasis was defined as a transaminase level > 2 times normal and a total bilirubin level > 1.5 mg/dl. Patients were treated with UDCA (10 mg/kg) for 1–19 months. Porphyria were analyzed by HPTLC and HPLC.

Results: A decrease was observed concerning 1) hyperbilirubinemia (200–400 µmol/l) of 65% up to normal and 3) coproporphyrinemia of 70% (p < 0.001). In spite of improved bilary PP elimination a clinical remission occurred only in one case; 5 patients were liver-transplanted after UDCA therapy; the other died of liver failure. All patients were treated with UDCA after liver transplantation in two patients UDCA treatment was continued up to 5 years. Porphyrin parameters remain moderately elevated. The clinical status improved in 4 patients.

Conclusion: The association observed between metabolic and clinical course of protoporphyria and disease severity might lead to partial AA-PP accumulation in liver cells. A long-lasting remission of hepatobiliary involvement by UDCA therapy can be achieved only in the early phase of complicated protoporphyria. A pathologic coproporphyrinemia with isomer increase and red cell PP (> 20 µmol/l) are first states, with hepatic involvement in protoporphyria. This is the indication for starting UDCA treatment. The PP-reducing effect of UDCA may be due to mobilisation of accumulated PP crystals in liver cells and improved bilir. flow. However, the unchanged PP level in some patients after UDCA treatment might be secondary due to increased PP induced progressive toxic liver injury leading to reduced clearance of PP from the liver. In these patients with protoporphyria-associated irreversible cholestatic cirrhosis liver transplantation is the therapy of choice. (Supported by the Hans-Fischer-Gesellschaft, Munich)

157 Liver Transplantation (OLT) for Hepatocellular Carcinoma (HCC): Role of Selection and Chemotherapy

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The role of OLT for patients with HCC is still controversial because of high disease recurrence. The aim of our study was to evaluate if careful pre-O LT selection and multidimensional chemotherapim could improve the results after OLT. Twelve out 108 patients transplanted at our unit were affected by HCC. Our policy is to only transplant the patients in which a diagnosis of HCC is obtained while on the waiting list. In 5 out of 12 the diagnosis of HCC was incidental. One case, a 24 year old patient, with a 15 un irreparable HCC in HBV-related chronic hepatitis, was an exception to the rule. Pathologic TNM staging was T3 in 9 patients, T3 in 2 (both incidental HCC) and T4 in the last. Seven patients received pre-OLT lidoiplod-mediated arterial chemotherapy or chemomobilization with adriamycin plus at least three courses of systemic chemotherapy (5-fluouracil, folinic acid, carboplatin). After OLT the patients received systemic chemotherapy weekly for a total of 24 cycles. The same treatment was administered in 3 patients with incidental HCC. Two patients with incidental HCC refused chemotherapy. In one patient, a severe HCV-related hepatitis contraindicated the chemotherapeutic regimen. Therefore, a total of 9 patients underwent post-OLT chemotherapy. Mean follow up is presently 24 months (range 4–48 months). One patient died for HBV-related cirrhosis after 44 months after OLT, free of HCC recurrence. Overall and disease-free survivals are 100% and 92% at 36 months, respectively. The only recurrence was observed 3 months after OLT in one incidental HCC which did not receive adjuvant chemotherapy. Conclusion: 1) patients with small HCC are good candidates for OLT if proper selection is adopted; 2) once a patient with HCC is selected for OLT, or once HCC is an incidental finding at OLT, also multimodal chemotherapy can improve the results in overall and disease-free survival.

158 Liver Transplantation in Patients with Cirrhosis and Hepatocellular Carcinomas


It has been recently shown that liver transplantation (LT) is an effective treat-ment for small unresectable hepatocellular carcinoma (HCC) in patients with cirrhosis (NEJM 1999; 334: 693–9). In this study we report our experience on LT for HCC. Between January 1987 and December 1995, 244 LT were undertaken for patients among which 152 had cirrhosis (except for secondary biliary cirrhosis). Among these 152 patients, 48 (31.6%) had an HCC. Retrospectively, the indication for LT was HCC (group A, n = 34), HCC (group B, n = 13), and cirrhosis (group C, n = 14). In group C, HCC was incidentally discovered by pathological examination in resected livers not originally thought to contain tumors. As of May 1996, 29 patients (60.4%), with a mean age of 54.9 ± 1.8 years (range, 28 to 68 years) were alive without apparent recurrence, with an overall survival of 33 ± 3.9 months (range, 6 to 83). 14 patients (29.2%), with a mean age of 57.4 ± 1.4 years had died after LT complications without apparent tumoral recurrence within 1.6 ± 0.5 months (range, 0 to 2 months) (effect of stage 7), whereas 11 patients (22.4%) (range, 62.2 ± 1.7 years, range, 56 to 66) had died of tumoral recurrence within 16.5 ± 4.2 months. Group C had the highest survival rate (79%) compared to groups A and B (52% and 62%, respectively). Patients alive from Group C had tumors, at the time of LT, whose diameter never exceeded 1.7 cm. From 9 patients, of the three groups, with tumors > 5 cm, 6 died and 3 of them of tumoral recurrence. All the patients who died of tumoral recurrence belonged to group B while 4 of the 11 patients with tumors ≤ 5 cm died, of whom there were tumoral nodes in the hilum (1/5), or adherence of the tumor to the diaphragm (1/5) (lung metastasis after LT). Of seven patients, out of 11, who
had more than 3 tumor masses < 5 cm, except for 1 tumor, are alive. Hepatic resection (three cases) nor chemoembolization (12 cases) before LT seem to have an impact on survival, since all patients undergoing hepatic resection and 8 out 12 patients undergoing chemoembolization were alive. Among the 104 patients transplanted for cirrhosis without cancer, 76 were alive (73%) and 28 (27%) died. These patients had a mean of 50.8 ± 1.1 (range, 27 to 69) and 51.4 ± 3.3 (range 40 to 70) years, respectively, at the time of LT and had a survival time of 50.2 ± 3.2 months (range 7 to 102) and 11 ± 3.6 months (range 0 to 86).

In conclusion, it seems reasonable to propose LT to cirrhotic patients with a single or multiple HCC < 5 cm without loco-regional spread. However, in this study, patients with cirrhosis and HCC presented a high rate of mortality, possibly linked to the age of patients at the time of LT.

159 Diagnostic Accuracy of Sonography (US), Computed Tomography (CT) and Angiography in the Diagnosis of Hepatocellular Carcinoma (HCC): Comparison of Histopathological Findings in 189 Transplanted Patients

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Purpose: To assess the diagnostic accuracy of US, CT, and angiography in the diagnosis of HCC based on histopathological examination of explanted livers.

Materials and Methods: The presence or absence of HCC based on US, CT, and angiography performed in 192 patients before liver transplantation was retrospectively reviewed. The radiological findings were compared to the histopathological studies.

Results: Histopathological examination detected 81 nodules of HCC in 56/192 patients, whose diameter ranged from 0.7 to 9.0 cm (mean 2.65 cm). US, performed in 186/192 patients, diagnosed 59/79 nodules with a sensitivity of 82.1% (46/56 true positive patients), specificity of 90.9% and diagnostic accuracy of 88.3%. In 152/192 patients, CT detected 35/54 nodules (65/40 true positive patients; sensitivity 72.5%), with a specificity of 95.5% and diagnostic accuracy of 89.5%. The sensitivity of integrated diagnostic imaging studies was 89.6% with a specificity of 84.3% and diagnostic accuracy of 86%.

Conclusion: Our study, which supports the findings reported in the literature on explanted livers, underlines the low sensitivity of CT in detecting HCC. The integration of US, CT, and angiography, indispensable in the presurgical assessment when newer imaging methods (helical CT, angiography-MR) are not available, is more sensitive for the diagnosis and staging of HCC but has a slightly reduced specificity and diagnostic accuracy.

160 Radiological Treatment of Hepatocellular Carcinoma (HCC): Review of Results in 58 Transplanted Patients

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Purpose: To evaluate the results of radiological treatment performed in patients with HCC before liver transplantation.

Methods: Fifty-eight transplanted patients with a total of 81 nodules of HCC, whose diameter ranged from 0.7 to 9.0 cm (mean 2.6 cm) were studied. 43 lesions in 34 patients were previously treated with transcatheter arterial chemoembolization (TACE) (24), percutaneous ethanol injection (PEI) (10), and combined TACE + PEI (8). On histopathological examination of the explanted livers, tumor necrosis was judged to be complete, partial, or absent. The percentage of recurrence after transplantation was also calculated.

Results: After TACE, necrosis was complete in 6/24 lesions (25%), partial in 6/24 lesions (25%) and absent in 12/24 (50%). After PEI, necrosis was complete in 8/10 (80%) and partial in 2/10 (20%). After combined therapy, complete necrosis was obtained in 9/10 (90%). 434 patients (11.8%) had complications (2 vascular anastomosis (2 parenchymal)) without significant differences in transplant. 3/4 (11.8%) treated patients had post-transplantation recurrence; these patients previously underwent only TACE and explanted livers showed no tumor necrosis.

Conclusion: TACE alone is ineffective in treating HCC. The high efficacy of combined radiological therapy and the low incidence of recurrence in treated patients suggests using this approach even before liver transplantation.

173 Ultrastructural Characteristic of Papilla Vateri’s Carcinoma

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The microscopic picture of carcinoma of papilla Vateri makes its endoscopic diagnostics comparatively easy. However, sometimes even ERCP and histological examinations fail to diagnose papillary carcinoma of chronic otitis is too hampers.

Aim of this investigation was to define more accurately the ultrastructural characteristics of carcinoma through subcellular organelles features and hence the possibilities for electronmicroscopic examination in the differential tumor diagnostics.

Material and methods. Tumor material was received by clip biopsy during endoscopic duodenopapillotomy and ERCP of 14 patients, with following electronmicroscopic exploration.

175 Analysis of p53 Abnormalities in a Series of 34 Tumors of the Ampullary of Vater

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Background and Aims: As described in colon, epithelial tumors of the ampulla of Vater (AV) develop through a filiation from adenoma to adenocarcinoma with increasing grade of dysplasia. The diagnosis between adenocarcinoma and epithelial dysplasia of the AV may be difficult on endoscopic biopsies. p53 protein immunostaining has been proposed as a new marker of malignancy. Because controversies exist on the relationship between p53 protein immunostaining and the stage of resected ampullary tumors, we have investigated the presence of p53 abnormalities in AV.

Methods: We studied 29 invasive adenocarcinomas and 5 adenomas with foc of mild (n = 3) or severe (n = 2) dysplasia of the AV. Immunohistochemistry was performed on formalin fixed tissue with DO7 antibody. In 19 cases, DNA was extracted from frozen specimens or paraffin sections and analysed for mutations of the p53 gene (exon 5–8) by PCR/denaturant gradient gel electrophoresis (DGGE) and sequencing technique.

Results: One of the 5 adenomas (20%) and 16 of the 29 adenocarcinomas (55%) were positive on immunohistochemistry for p53. This positivity was present through all stages of ampullary adenocarcinomas. No significant difference in p53 protein expression was observed between adenomas and adenocarcinomas. A mutation of the p53 gene was detected on DGGE and confirmed by sequencing in cases with a good correlation with immunohistochemistry in all 19 cases but 2.

Conclusion: p53 gene mutation appears as a common event in ampullary tumors. It can be detected in most cases by immunohistochemistry. However our results suggest that its detection does not help to appreciate the stage of the tumors.

178 Surgical Treatment of Klatkin's Tumor: Our Experience

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Between January 1991 and February 1996 we operated ten patients with primary cancer originating in the hepatic duct confluence (Klatkin tumor). We got six males and four females. Average age was 59.6 years. The tumors were Type I in five, Type II in one, and Type III in two patients. Three of these patients, respectively. Three tumors were unresectable: two Type III and one Type IIIb. In these cases transmural stents have been placed. Stent in right, left and the both ("Y" prosthesis) hepatic ducts were placed in each case. In this group the average postoperative survival was 11 months. At three patients with the tumor Type I and II, tumor excisions in hepatic parenchyma and reconstructions with hepatico-jejuno-anastomosis Roux-en-Y were done. In the last four patients with the tumor Type III, liver resections were done. Right hepatectomy and left hepatectomies were done in three and one case, respectively. From this group of patients, one patient had liver cirrhosis and died twenty days after operation, because of liver insufficiency. Other patients did not have complications in the postoperative course. In this group, one patient died 2.5 years after the operation, and other patients are still alive. The longest postoperative survival has a patient who 3.5 years after operation has no signs of the tumor recide. Other patients are alive and without any recidive disease among 0.5 and 2 years. Our experience supports an aggressive surgical approach in patients with Klatkin tumor, and enables longer survival rate, than when only transmural stent is placed.

179 Hepatic Intraarterial Chemotherapy in the Treatment of Pig Experimental Biliary Cancer: A Comparative Study

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Human cholangiocarcinoma (CHC) is one of the most dismal biliary tree cancers to cure. In Central and Eastern Europe, resectability of CHC does not exceed 6%. In general, if unresectable, the median survival is less than 1 year. Aims: This study was performed to assess the feasibility of hepatic intraarterial (HIA) chemotherapy by FUdR to experimental pig CHC in comparison with those by FUdR conjugate (FUDR-Cathepsin B-like).
Severe Colonic Ischemia in Chronic Hemodialysis Patients: CT Scan Allows an Early Diagnosis

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1. Department of Surgery, Hôpital Laennec, Paris; 2. Department of Radiology, Hôpital Laennec, Paris

Colonic ischemia may be related to chronic hemodialysis, causing a high mortality and morbidity. This ischemia is distinctive due to its non occlusive character and to the frequency of right colon involvement. Clinical diagnosis may be difficult since patients symptoms are usually non specific. We report retrospectively the value of CT scan for the diagnosis of severe colonic ischemia in hemodialysis patients.

Patients and methods: Seven severe colonic ischemia was suspected in four chronic hemodialysis patients on clinical findings (mean age 59 years); symptoms were abdominal pain of variable intensity starting just after an hemorrhoidal session. CT scan with water soluble enema was performed within 12 hours after the onset of the symptoms.

Results: In all cases, CT scan showed a ring thickening of the bowel wall located in the right colon (mean 17.5 mm; 10-20), measuring 55 mm in length (30-90). In 3 cases a parietal pneumatosis was observed. These features were exactly correlated with operative and histological findings. Parietal pneumatosis was predictive of colonic gangrene.

Conclusion: CT scan allowed an early diagnosis of severe colonic ischemia in chronic hemodialysis patients. Parietal pneumatosis is the main finding reflecting colonic necrosis and indicates immediate surgery.

185 The Ischemic Bowel and Myoelectric Activity an Experimental and Clinical Studies


Evaluation of the intestinal electrical control activity (ECA) has been proposed as a tool for determining of the bowel viability. However its diagnostic value has not been definitively established. The purpose of the present investigations was to evaluate intestinal myoelectric activity in the normal and ischemic bowel in experimental animals and humans. Experiments were performed on 15 cats anesthetized with pentobarbital. After laparotomy total intestinal blood flow (BF) was measured in superior mesenteric artery (SMA) with ultrasonic flow probe and microcirculatory laser Doppler flowmetry (LD). Myoelectric activity was recorded simultaneously with BF and LD during three monopolar silver electrodes implanted on the serosa surface of the jejunum and Dynograph Recorder R-611. Bowel ischemia was induced by perfusion of SMA with saline or by ischemia induced by SMA occlusion. In experimental studies ECA was analysed in 10 patients undergoing abdominal surgery due to bowel ischemia, using EMG technique. In patients comparison was made between ECA in ischemic and normal bowel. In experimental conditions induced reduction in amplitude and frequency of ECA by 50%, whereas in patients about 60% reduction in amplitude and frequency of ECA was observed. Most sensitive parameter appears to be phase lag of ECA which even in small degree of ischemia changes to the bowel was doubled. Most dramatic changes with reduction of amplitude and frequency were observed during simultaneous SMA and SMV occlusion. Our results indicate that amplitude, frequency and the coupling of ECA are sensitive myoelectric parameters which could be used in clinical assessment of bowel viability.

186 Nitrate Reducing Bacteria in the Excluded Colon: A New Clue to Diversion Colitis

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Background: Argues: have accumulated favouring a pathogenic role of nitric oxide (NO) in intestinal inflammation. NO is metabolized in the colon in nitrate and nitrite and may influence the colonic flora. There is no data about nitrate-reducing bacteria in the colon of patients with chronic inflammation. In this work, we evaluated the nitrate-reducing flora in patients with diversion colitis which is a model of inflammation with no confirmed nitrate and nitrite-reducing bacteria. Patients and methods: Thirty patients (17 M, 13 F, mean age 45 yrs) having an excluded colon for various reasons (inflammatory bowel disease, n = 15; colon cancer, n = 5; diverticulitis and abscess; microscopical, n = 3) were studied. Presence of diversion colitis was assessed using endoscopic and histologic criteria. Fecal material was collected by rectal swabs. Bacteriological analysis was performed in anaerobic conditions. Results: were compared to those of 30 healthy controls (11 M, 19 F, mean age 28 yrs). Results: The percentage of nitrate-reducers among the total count of subculturable bacteria was 46 ± 41% (mean ± SD) in patients with diversion colitis as compared to 19 ± 24% in healthy controls (p < 0.05). In patients with diversion colitis, 75/254 (29.5%) different isolated bacterial strains were nitrate-reducers as compared to 61/254 (21%) (p < 0.05) in controls. Among the 75 nitrate-reducing strains isolated from patients with diversion colitis, 55 (73%) were aerobes as Pseudomonas, Proteus, Providencia and Morganella. In healthy controls, nitrate-reducing anaerobes were nearly as frequent as aerobes. Conclusion: The bacterial flora of patients with diversion colitis is characterized by a quantitative and qualitative enrichment in nitrate-reducing bacteria. NO synthase might produce a bacterial substrate increasing the growth of bacteria with a high pathogenic potential creating conditions for chronic inflammation in patients with an excluded colon.

187 Sodium Cromoglycate in the Treatment of Eosinophilic Colitis


Eosinophilic colitis is (EC) an uncommon inflammatory process marked by colonic eosinophilic infiltration. Despite of the discrepancy in the treatment, Sodium cromoglycate (SCG) seems to be effective. We report 3 cases of
eosinophilic colitis with good response to SCG treatment. Patient 1: A 30 year old woman, with recurrent abdominal pain, bloody diarrhoea, tenesmus, and weight loss for 3 years treated with Salazopyrine (SPZ) because of the diagnosis of Ulcerative Colitis. Eosinophilia was observed. By rectosigmoidoscopy, colitis with hyperemia, edema and friability was observed. Colonic biopsy showed severe colitis, remarkable infiltration of lamina propria with the presence of a regular crypt structure with depletion of goblet cells. The disease was limited only to the colon. The prick test was normal. After the diagnosis EC which developed during the SZP therapy, she was treated with SCG 300 mg twice daily. After 2 weeks of therapy, her symptoms and endoscopy findings improved. Patient 2: K C 33 year old woman with recurrent abdominal pain, diarrhoea, artralgia and weight loss for 14 months. By her admittance only IgE and IgG were elevated. The colonoscopy showed edema, hyperemia, friability and ulcers in the entire colon including terminal ileum. Crohn disease with a possible sclerodermatisation of the bowel showed chronic colitis with significant infiltration of the mucosa with eosinophils, intraepithelial eosinophils and regular crypt structure. Like patient 1 the colitis was limited to the colon. By the time the diagnosis was established, the patient had been treated with SCG 400 mg twice daily. The biopsy result was the same as the one of patient 1. Like the previous patients the disease was limited only to the colon and the patient responded well to SCG 300 mg daily after 2 weeks. The results provided evidence of SCG efficacy in the treatment of EC, suggests its application as a first choice of drug.

188 Treatment of Radiation Proctitis with Sucralfate Suspension Enema

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We conducted a pilot study to evaluate the utility of sucralate suspension enema for the treatment of radiation proctitis.

Subjects and method: Seven cases with radiation proctitis after irradiation for prostate cancer were included. Per administration of 10% suspension of sucralfate was performed twice a day for at least 3 months. Effectiveness of treatment was determined endoscopically.

Results: Treatment with sucralfate enema for three months, improve-ment of endoscopic finding was observed in six (85.7%) out of seven cases. In five cases, further treatment was continued for six months and more improvement of the mucosa was observed in four cases. No adverse effects were encountered.

Conclusion: Sucralfate suspension enema was useful for the treatment of radiation proctitis.

190 Intestinal Neurofibromas with von Recklinghausen's Disease: Report of a New Case

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Multiple neurofibromas (MN), which may occur either on familial basis with autosomal dominant inheritance or sporadically, were first described by von Recklinghausen (vR) in 1882. MN is a mesodermal and ectodermal dysplasia with a broad spectrum of clinical and radiological findings. The gastrointestinal tract, and in particular the small intestine, is involved in 10%–20% of the including cholelithiasis improved after 2 weeks therapy with SCG 400 mg twice daily. Patient 3: N B 39 year old woman with bloody diarrhoea, nausea for 2 months. She had allergy to various food, animal products and pollen. The prick test was positive. Eosinophilia was determined. We observed colitis with erosions at rectosigmoidoscopy. The biopsy result was the same as the one of patient 1. Like the previous patients the disease was limited only to the colon and the patient responded well to SCG 300 mg daily after 2 weeks. The results provided evidence of SCG efficacy in the treatment of EC, suggests its application as a first choice of drug.

191 Dermatitis Herpetiformis Duhring in Long-Term Clinical Follow-Up

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Dermatitis herpetiformis Duhring (DHD) is nowadays considered one of the variants of gluten enteropathy. The prevalence of the disease has been increasing considerably, affecting even younger persons and if its gastrointestinal symptoms remain hidden it may be source of serious complications. Group of 92 patients (52 men, 40 women) has been followed up for a long time, at the average follow-up time 7.4 years (2–24 yr). Even if 83% of patients have never had any digestive troubles and physical examinations have been normal in almost all of them, 16% had anaemia, 52% light signs of malabsorption and in 73% of them various degrees of jejunal mucosa damage were observed at the time of first diagnosis. In addition, 62% of patients had repeated biopsy (n = 44) the intestinal as well as skin changes have improved in 94% of persons. Malignant tumors occurred in 4 patients (2% death). Evaluation of the changes in jejunal mucosa is impossible by current laboratory findings or functional tests, but only using enteroscopy. In patients with proved changes permanent gluten-free diet is necessary, with a view to the usual absence of difficulties, however, the motivation to observing it is markedly lower than in the coeliacs. The course of DHD, complications and the risk of malignancy are the same as in coeliac disease and prove the necessity of steady follow-up by the gastroenterologist.

192 Collagenous Colitis Versus Inflammation of Collagenous Type as Nosologic Entity Versus Reaction Form

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In years 1993–1996 we examined 1340 patients with colonicoscopy. Among them we found 96 cases with mostly mild mucosal hyperemia in macroscopic picture and with broad subepithelial collagenous band in histology, mainly in the sigmoid, in 6 cases in other part of colon. All of them presented only with moderate symptoms not with watery diarrhoea which is characteristic of collagenous colitis. 22 had abdominal pain, 16: diarrhoea, 6; diarrhoea/constipation, 3: constipation, 10: meteorism, 2: without any abdominal complaint. In history 13 had chronic gastritis, 2 ulcerative colitis, 1 m. Crohn, 2 ascitic colitis. 3 had simultaneously colon erosions, 2 ulcers, 6 diverticulosis, 4 lactose intolerance, 2 statorrhoea, in 2 patients the collagen layer appeared in the subepithelial part of a polyp. 14 were treated with Salazopyrin. We had opportunity to follow up 10 cases, in all the collagen disappeared within 6–12 months. In 4 cases we observed a transitional phase with fragmented collagen layer.

Conclusion: on the basis of the data of the literature and our findings, we raise a hypothesis: it must be distinguished between

1. a collagenous colitis as a pathologic and clinical entity and
2. a collagenous inflammation reaction type which is manifested in certain person’s colon to various stimuli (per analagism: lupus erythematoses vs. lupoid reaction).

193 Malabsorption of Lactose (25 G), Fructose (25 G) and Sorbitol (5 G) In Patients with Irritable Bowel Syndrome (IBS): Effect of Ethanol On Saliva

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The aim of this study was to determine whether the prevalence of fructose (F) and sorbitol (S) malabsorption was dependent on ethnic origin as is the case for lactose (L). The effect of sex and age were also analysed. Methods: 520 ambulatory patients with IBS all underwent H2 breath testing after challenges of L, F and S. Using criteria of > 20 ppm H2 rise for L and > 10 ppm for F and S, 56.3%, 57.2% and 57.5% were malabsorbers of L, F and S resp. Results: Tests for equality of prevalence for L malabsorption across 6 ethnic groups was significant at p < 0.005 with Northern Europeans (n = 58) and French Canadians (n = 92) clustered as one group with a prevalence < 35% compared to another group consisting of Arabs (n = 35), Greeks (n = 60), Italians (n = 53) and Jews (n = 167) with an average prevalence exceeding 60%. Equivalency testing among F and S malabsorbers was not significant. Prevalence of malabsorption for all ethnic groups ranged between 41.5–55.4% and 47.2–63.0% for F and S resp. L malabsorption was greater among males: 63.0% male vs 52.8% female < 0.029. Females predominated among F and S malabsorbers; 55.8% and 60.8% female vs 45.7% and 50.0% males resp. < 0.033. The effect of age could be analyzed among Jewish patients (n = 167). 78.6% were negative. There were no differences in the ratios of L, F and S in the group aged 25–54 years. A progressive decline in L malabsorption was noted between 25–55 followed by a significant rise in later years. For F and S, prevalence progressively fell to 51.9% and 48.2% resp by 75–84 years. Conclusions: In contrast to L, ethnic origin does not influence F and S malabsorption. The trend of L malabsorption of L was more common among males, the reverse with F and S. While L malabsorption increased after 55 years, that for F and S fell progressively in the ethnic group analyzed.
Quality of Life (QOL) of Irritable Bowel Syndrome: Patients in the Community

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Although irritable bowel syndrome (IBS) is common, little is known about the quality of life (QOL) in IBS patients in the community. 181 patients, aged 19–74 yrs, with a diagnosis of IBS were randomly selected from lists of 3 general practices in Lothian. Patients were contacted by letter or telephone and invited to complete a postal questionnaire. Part 1 was the Mayo Bowel Questionnaire and part 2 was the Medical Outcome Survey SF36. A single reminder was sent to non-respondents. Responses were coded for analysis and results compared to known values for the general population, taken from a sample of 6212 residents of Lothian and a sample group of patients with angina pectoris.

110 questionnaires were returned: a response rate of 61% of whom 53% were female. Symptoms fulfilling the Rome criteria for IBS were reported by 79 patients. These patients were classified by the severity, Group 1 with mild or moderate pain and symptoms (39 patients (23 F 16 M) mean age 41 yrs) and Group 2 with severe pain and symptoms (40 patients (30 F 10 M) mean age 43 yrs). The remaining 31 patients (10 F 21 M), mean age 49 yrs, no longer had active IBS and appeared to be largely symptom free. MOS questionnaires showed the symptom free IBS patients had a QOL similar to that of the general population. MOS questionnaires showed that the mild or moderate group, QOL was minimally affected whereas in the severe group, QOL was markedly affected.

The severity of abdominal pain in patients with IBS living in the community is significantly associated with lower scores in all domains of the MOS. Even relatively well IBS patients have impaired QOL. Patients with severe abdominal pain have a reduction in QOL comparable to that observed in patients with angina pectoris.

Is There a Gender Difference in the Natural History of Irritable Bowel Syndrome (IBS)?

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IBS is a common functional gastrointestinal disorder in both primary and secondary care, with a reported prevalence of 14–20%, incidence of 9% and a predominance of 2:1. Little is known about the gender difference in relation to the progression and severity of the condition in the community.

181 patients, aged 19–74 yrs, with a diagnosis of IBS in the last five years were randomly selected from the lists of 3 general practices in the Edinburgh area. Patients were contacted by telephone or letter and invited to complete the Mayo Bowel Questionnaire. A single reminder was sent to patients not responding. Questionnaires were coded for analysis.

Of the 181 questionnaires sent out, 110 were returned (a response of 61%) of whom 53% were female and 47% male. Symptoms fulfilling the Rome criteria for IBS were reported by 79 patients (72% of responders, 33 F 26 M). The remaining 31 patients (mean age 49 yrs) appeared to be substantially symptom free. The mild or moderate group 10 were female, 21 male. The 79 females with active IBS were classified according to the severity of their condition. Group 1 with mild/mild/moderate pain and symptoms comprised 39 patients (23 F 16 M) mean age 41 yrs: Group 2 with severe pain and symptoms comprised 40 patients (30 F 10 M), mean age 43 yrs. There was a statistically significant association between female gender and symptom severity: $x^2 = 13.3, P < 0.01$.

These data show that among patients in the community diagnosed as IBS, symptom resolution is more common in men than in women. To our knowledge, this gender difference in the natural history of the disorder has not previously been reported and emphasizes the importance of community-based population studies.

Colonization with Extra-Colonic Symptoms of 1032 Patients with Irritable Bowel Syndrome

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Aim of this survey was to describe demographic and clinical features of patients complaining of irritable bowel syndrome (IBS) and consulting a non-hospital gastrointestinal department.

Materials and methods. Patients included had abdominal pain related to defecation associated with altered bowel habit and/or bloating. From September 1994 to February 1995, 271 French gastroenterologists filled-in 1032 questionnaires during a consultation.

Results. Average age of patients was 49 ± 16 years, with a high percentage of women (sex ratio: 2:3). Average duration of IBS was 11 ± 11 years; for only 1% of patients, symptoms appeared less than one year before the study, but for 40% of patients, symptoms were present since more than 5 years. At the time of the study, 96% of patients had abdominal pain and 93% a bloating, both symptoms for which intensity was mostly scored 3 to 5 on a 6 point-scale, and 64% of patients had constipation. The frequency of onset of symptoms was at least once per week for 81% of patients; for 30% of them, symptoms occurred daily. Most of patients (93%) had at least one other digestive symptom such as excess of gas, borborygms, flatulence, erosion or nausea, 65% of patients complained of one or several non-specific symptoms such as fatigue, insomnia or headache. 67% of patients described symptoms reflecting psychological disorders, mostly anxiety and depression. Number of consultations for IBS during the last 12 months was 3 ± 3, but 19% of patients had more than 5 consultations. 7% of patients were out of work and 9% were hospitalized during the last year because of IBS. Rest and holidays alleviated symptoms for half of patients; stressful events, food, job and family worsened symptoms of a majority of patients. 83% of patients went under at least one diagnostic procedure, a colono-scopic most frequently. 90% of patients had already at least one digestive drug prescription and 34% a sedative or anti-depressant drug.

Conclusion. IBS, expresses by many and frequent symptoms which occur during many years. IBS is associated with anxiety and depressive symptoms. Thus IBS is surely impaired to quality of life.

This research was funded by Laboratoires Vedin (UCB-Pharma).

Descriptive Study of Quality of Life in 1032 Patients with Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS), through its frequent, intense and chronic symptoms, affects the daily life of patients. This is not explored correctly by current clinical criteria such as abdominal pain. The aim of this survey was to evaluate health related quality of life in patients with IBS consulting a non-hospital gastroenterologist.

Methods. Patients had to present abdominal pain related to defecation and altered bowel habit and/or bloating. Self-questionnaire was filled-in in waiting-room. It consisted in 34 items reflecting different domains. Answer for each item was a 4-point Likert scale. This 30% of them, symptoms occurred specifically for this study, and it has not been validated for evaluative purpose in therapeutic trials.

Results. Among 1032 patients included by 271 gastroenterologists, 1021 (99%) completed the questionnaire. 68% of patients are afraid of not knowing when the next bout of IBS will arise. 69 to 85% patients declare having difficulties in the different aspects of their daily life (daily activities, job, social events, family, affections) because of IBS. There is a significant correlation ($p < 0.001$) between the severity of pain and bloating (but not for bowel habit) and the impact on the daily life, diet and health perception. 57% of patients consider their health to be good and 51% to be identical to other people. 81% of them are unsatisfied, anxious or afraid by their health. 66% of patients think that their health did worsen during the last years and 21% that it will worsen during the next years. They are only 23% to be convinced that IBS will improve in the next years. 83% of patients declare having sleeping disorders because of their health, 55% feel fatigue and 7% declare to be exhausted. 86% of patients judge their mind as medium to very bad and 65% of them take hypnotic or anxiolytic drugs. 75% of patients follow a diet, which is judged cumbersome or unbearable by 65% of them and not efficient by 49%.

Conclusion. Quality of life is daily altered in patients with IBS, in terms of discomfort, poor health perception, unsatisfaction and fear. For an optimal therapeutic management of patients with IBS, quality of life should be evaluated as well as symptoms.

This research was funded by Laboratoires Vedin (UCB-Pharma).

Health Status Questionnaire (SF-36) in Dyspepsia and Irritable Bowel Syndrome

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In a validation study of a specific quality of life questionnaire in functional digestive disorders (FDD) (results presented elsewhere), patients from France, Great Britain and Germany were asked also to fill-in the general health status questionnaires SF-36. Materials and methods. From June to November 1995, 187 practitioners recruited 401 patients: 197 with dyspepsia, 210 with irritable bowel syndrome (IBS). Patients were asked to fill-in alone the questionnaire SF-36. SF-36 has been given also to 97 control patients: among them, 12% had hypertension, 19% arthritis, 12% rheumatism, 9% insomnia. The SF-36 questionnaire consists of 36 items. The score contains 8 physical problems (RP), body pain (BP), general perception of health (GH),
vitality (VT), social functioning (SF), emotional problems (RE), mental health (MH). Period recall is the last 4 weeks. For each domain, items answers are summed to form a scale from 0 (best health) to 100 (best). Results. The acceptability of SF-36 is good, a missing data being found in 11.5% of questionnaires (IBS and French patients are less compliant). Median scores of each domain are significantly different between control group and patients IBS, and symptomatic and IBD. Conclusion. Quality of life is impaired in patients with FODD, in comparison to a control group of patients having other chronic diseases. SF-36 is a useful general tool to compare health status between patients with different clinical conditions.

This research was funded by Laboratoires Jouvenal.

199 European Psychometric Validation of a Specific Quality of Life Questionnaire in Functional Digestive Disorders O. Chassany, P. Marquis, B. Fragaie, B. Scherrer, C. Sapea, B. Geneve
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Through several studies, we developed a French specific quality of life questionnaire in functional digestive disorders, translated in English and German, consisting in a self-administered questionnaire of 68 items with answers on a five-point Likert scale, reflecting 12 domains. Aim of this study was to assess the final psychometric properties (acceptability, validity, reliability) of the questionnaire.

Materials and methods. From June to November 1995, 187 general practitioners and specialists recruited 401 patients in France, Great Britain and Germany. (193) Dyspeptic (D), 210 with irritable bowel syndrome (IBS). Patients were asked to fill-in all the specific questionnaire and the general health survey questionnaire SF-36.

Results. Average age of patients was 50 ± 15 years, with 52% (D) and 70% (IBS). 60% (D) were women compared with 46% (IBS) of patients present since more than 1 year. Disease was severe as 53% (D) and 46% (IBS) of patients complained of more than 10 symptoms. Handicap scored by doctor was at moderate to huge for 59% (D) and 69% (IBS) of patients. Acceptability of our questionnaire is good and similar to the SF-36 as 1 missing data occurring in 10.9% of questionnaires. Construct validity was assessed by a factor analysis. It resulted through several multivariate scaling analysis in a reduction of the questionnaire to 43 items in 8 domains having a good convergent and divergent validity. Reliability is shown by the overall a Cronbach coefficient at 0.94. Clinical validity is good as “food”, “discomfort” and “stress” domains discriminate dyspepsia from IBS patients. IBS patients and women report a lesser quality of life. There is a correlation between quality of life score and the length of the disease. Finally, correlation with SF-36 is high especially with the “daily activities” and “copying” domains of the specific questionnaire.

Conclusion. The good psychometric properties of this specific quality of life questionnaire in functional digestive disorders, translated in French, English and German, allow to use it in comparative therapeutic trials.

This research was funded by Laboratoires Jouvenal.

200 Kruis Scoring System and Manning's Criteria in Diagnosis of Irritable Bowel Syndrome: Is It Better to Use Combined? Ü.B. Doğan, S. Unal, Gaz University, Faculty of Medicine, Department of Gastroenterology, Ankara, Turkey
Irritable bowel syndrome (IBS) is characterized by abdominal pain and alteration of bowel habits. Manning et al. have reported that certain symptoms distinguish the irritable bowel syndrome (IBS) from other functional gastrointestinal disorders (FGID); these were partially relieved by defecation, looser or more frequent stools at the onset of pain, abdominal distention, mucus, and a feeling of incomplete evacuation. Another simple scoring system for discriminating IBS from FGID that incorporates historical data, physical examination findings, and basic investigations was first devised by Kruis et al. In differential diagnosis of IBS from FGID, to evaluate the reliability of Manning's criteria and Kruis scoring system when used apart or combined; we studied 347 outpatients who completed a bowel disease questionnaire. Manning's criteria objectively measured Manning's criteria and scoring system of Kruis. The group included 165 patients with IBS and 182 patients with OGD. The Manning's criteria discriminated IBS from OGD with a sensitivity of 90% and a specificity of 87% if three or more items were regarded as positive. Also the Kruis scoring system discriminated IBS from OGD with a sensitivity of 81% and a specificity of 91%. When used together, these systems discriminated IBS from OGD with a sensitivity of 80% and a specificity of 97%. Manning's criteria and Kruis scoring system had a strong correlation when compared in IBS, but not in OGD.

201 Impaired Quality of Life in Irritable Bowel Syndrome as Compared to Inflammatory Bowel Disease M. O'Sullivan, 1 M. Mamhud, E. Lovett, C.A. O'Morain
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Introduction Chronic bowel diseases such as IBS and IBD can have a profound effect on patients' Quality of Life (QoL). Poor QoL may be more pronounced in IBS than IBD. Disease and non-disease related factors can impact significantly on QoL. The conventional methods for assessing QoL were compared in IBD patients using validated interview based and self administered questionnaires as follows: QoL, using the Schedule for the Evaluation of Individual Quality of Life (SEIQoL); Symptom scores (Tally’s modified); Psychological – Hospital anxiety and depression scale (HAD); Disease Knowledge and support (visual analogue). Results 110 patients, IBS (n = 40), Ulcerative Colitis (UC) (n = 40), Crohn’s Disease (CD) (n = 30) were studied. QoL (SEIQoL) was significantly (P < 0.01) poorer in IBD 51.56 ± 27.0 (UC 67.03 ± 24.7). Anxiety, depression and somatic symptom scores were significantly (P < 0.01) greater in IBS. Disease knowledge and support were significantly (P < 0.01) poorer in the IBD group. Preliminary data suggests impaired IBS QoL is not directly associated with any symptom severity. Conclusion QoL is significantly impaired in IBS as compared to IBD and does not appear to be directly associated with symptom severity alone. Other aspects such as psychological non-colonic symptoms and poor disease knowledge and support are likely to be important.

202 Symptoms and Colonic Transit Time in the Irritable Bowel Syndrome Treated with Psyllium and Cisapride or Placebo R. Meier, Ch. Beglinger, R. Brignoli, L. Lariboisiere, V. Lyon; Lariboisiere, Paris; L. Lariboisiere, Paris; W. Lariboisiere, Paris; Lariboisiere, Paris; Lariboisiere, Geneva; Lariboisiere, Geneva
From june to August, 1996, 101 pts. were randomized to either receive cisapride (CIS) (n = 51) or placebo (P) (n = 50). The primary end point was not reached, however lower abdominal pain significantly improved more in the CIS treated group (P < 0.05).

Delta CTQ and daily scores of Target Symptoms (Mean ± 2.5 D.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Cisapride</th>
<th>Intergroup</th>
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<tbody>
<tr>
<td>Abdominal pain</td>
<td>0.85 ± 0.43</td>
<td>0.57 ± 0.32</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Flatulence/meteorism</td>
<td>0.77 ± 0.37</td>
<td>0.76 ± 0.52</td>
<td>n.s.</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.81 ± 0.48</td>
<td>0.74 ± 0.50</td>
<td>n.s.</td>
</tr>
<tr>
<td>Urgo to defecate</td>
<td>0.34 ± 0.42</td>
<td>0.36 ± 0.41</td>
<td>n.s.</td>
</tr>
<tr>
<td>Incompl evacuation</td>
<td>0.32 ± 0.32</td>
<td>0.41 ± 0.58</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Conclusions: The adjuvance of CIS to Psyllium translated in a significantly larger pain relief. This effect can not be explained by an effect on colonic motility.

203 Experimental Gut Pain in Man A.M. Drewes, L. Arendt-Nielsen, J.B. Hansen, H.B. Krarup, U. Tage-Jensen. Department of Medical Gastroenterology, Aalborg Hospital, DK-9000, Aalborg, Denmark; Laboratory for Experimental Pain Research, Aalborg University, DK-9000, Aalborg, Denmark
Visceral pain is a substantial clinical problem. Experimental pain stimuli may elucidate physiological aspects of the pain, but only few human models are available. In the current study the findings using electrical stimuli of the gut mucosa were evaluated in patients with ileocolic stenotomy. Nine patients participated. Four had an ileostomy and five had sigmadostomy. In all subjects the stoma was normally functioning. A flexible catheter containing six stimulation electrodes separated by 4 mm was introduced into the stoma. The gut mucosa was stimulated, single, five repeated and continuous electrical stimuli. The sensation threshold, pain detection threshold (PDT) and pain tolerance threshold (PDT) was determined. Also the location and size of the referred pain area was characterized. Finally, brain potentials to single stimuli were measured. PDT and PTT to single stimuli were difficult to determine whereas these thresholds were easily found when repeated stimuli were used. The pain thresholds to single stimuli were as high as thresholds to repeated stimuli indicating the importance of central temporal summation in visceral pain. During continuous stimulation the pain intensity as well as the referred pain area gradually increased. Also the amplitude of the brain potentials increased for increasing pain intensity.
In conclusion, the model demonstrated the importance of repetitive stimuli for eliciting visceral pain. The brain potentials may be useful in the study of basic pain physiology. Visceral stimuli seem adequate to evoke referred pain with profiles similar to those found in patients with different gastrointestinal diseases.

204 Irritable Bowel Syndrome (IBS) Observatory: Clinical and Therapeutic Aspects in 1571 Patients Treated by Town GPs

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Purpose: the prevalence of irritable bowel syndrome (IBS) among the general public is high. Similarly, it presents in a wide range of ways and is treated by various means. The objective of the present work was to describe the characteristics, prevalence, and symptoms of patients consulting a GP for symptoms suggesting IBS, and to measure developments in the medium term.

Methods: a representative sample of GPs from the whole of France was randomly selected, and their patients consulting for IBS symptoms were included. The clinical and therapeutic data were collected at this consultation and a second time three months later. Prescription of treatment or further examinations was left to the GPs discretion, and recorded.

Results: Between February 1995 and January 1996, 463 GPs recruited 1571 patients aged 15 to 88 (mean ± SD: 53 ± 14), with women predominating (64%). 49% of the patients were in employment. 36% had had their appendix removed, and 12% their gall bladder. The IBS had existed for a mean of 8.9 years, chronically in 40% of cases and intermittently in 54%. During the initial consultation, 94% of patients reported pain, for 30.2% on a daily basis. Stool frequency was > 3 per day in 10% of patients, and < 1 per three days in 10.6%. Stool consistency was considered normal in 18%, soft or liquid in 42%, and hard in 41%. 30% of patients alternated between diarrhoea and constipation, and 86% exhibited meteorism.

At three months, the pain had disappeared in 13% of patients, and occurred on a daily basis in only 8.6%. The intensity of pain had significantly decreased. Stool frequency and stool consistency were generally lower, and normal in 30.6% and 3.4% of patients. Stool consistency was considered normal in 48% soft or liquid in 46%, and hard in 4.12% of patients alternated between diarrhoea and constipation, and 52.9% exhibited meteorism.

During the three months preceding the study, 93% of patients were already on a treatment and 62% on a diet for their IBS. At the end of the first consultation, 95% of the patients were prescribed a medical treatment and 84% a diet. The mean number of medicines prescribed was 2.36 before the consultation and 2.16 after; the reduction largely concerned prescriptions involving four or more medicines. The most commonly prescribed therapies were intestinal dressings (diocsmectite), antispasmodics of the muscolotropic (mebeverine) and non-anticholinergic (phloglucotin) types, and modifiers of digestive moticity (trimetopine).

Conclusion: the IBS observatory enabled a profile of patients consulting for IBS out-patient treatment to be established. Symptoms at three months were significantly improved. The therapy given, the patterns of disease development or even the conditions of observation could be responsible for this improvement.

205 Economic Aspects of Irritable Bowel Syndrome in 1571 Patients Treated by Town GPs

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Purpose: irritable bowel syndrome (IBS) is a common, benign pathology. The economic impact of its treatment is not exactly known. The objective of the present work was to assess consultation for treatment of patients presenting with IBS under usual non-hospital practice.

Methods: a representative sample of GPs from the whole of France was randomly selected, and their patients consulting for IBS symptoms were included. The entire medical consumption over the three months preceding the consultation was recorded as was any prescription of examinations or treatment during this and/or another consultation three months later.

Results: 1571 patients were recruited over 463 GPs. During the three months preceding the consultation, 1364 patients stated having consulted a GP and 335 a gastro-enterologist for IBS at least once out of respective totals of 3348 and 403 consultations (mean of 2.1 and 0.36 per patient per three months). 49 patients were hospitalised, four of which twice and one three times (mean stay in hospital 3.1 days). 94 patients were on sick leave at least once over the same period for a mean of 8.7 days. 534 patients stated having had at least one extra examination. The examinations included 336 coloscopies, 90 abdominal echographies, 46 barium enemmas, 6 rectoscopies, 5 abdominal scanners, 38 coprocultures and 6 thyroid checks. 1461 patients were on medical treatment (including less than 4 medicines in 8% and 7% were on a diet.

At the end of the first consultation, a medical treatment was prescribed to 1449 patients, with less than 4 medicines in 92.3% of cases, and combined with a diet in 1229 patients. Further examinations were prescribed for 93 patients. During the three months of the study, 36 patients saw a gastro-enterologist for a total of 2130 and 126 consultations. 18 patients were hospitalised for a mean of 3.3 days, and 39 were put on sick leave for 7.2 days.

Conclusion: IBS treatment may be improved through an analysis of its various components, taking their corresponding cost: efficiency ratios into account.

206 Analysis of the Effect of Irritable Bowel Syndrome (IBS) on the Well-Being of 1571 Patients Treated by Town GPs

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Purpose: irritable bowel syndrome (IBS) is one of the most common pathologically conditioned conditions encountered in day-to-day practice. The effect of colopathy on patients’ everyday life has rarely been examined. The objective of the present work was to estimate the physical well-being of patients consulting a GP for symptoms suggesting IBS and to measure its progress three months later.

Methods: a representative sample of GPs from the whole of France was randomly selected, and their patients consulting for IBS were included. A questionnaire asking for answers formulated on a four-point scale (enormously, fairly, a little, not at all) and analogue visual scales (AVSs) were filled in by the doctor and patient during the first consultation and at a second one three months later. Prescription of treatment or further examinations was left to the GPs discretion, and recorded.

Results: Between February 1995 and January 1996, 463 GPs recruited 1571 patients aged 15 to 88 (mean ± SD: 53 ± 14), with women predominating (64%). The IBS was chronic in 40% of cases and intermittent in 54%, and caused discomfort in various aspects of the patients’ lives; according to the aspect, the IBS bothered the patients “enormously” in 7.4 to 19.5% of cases. It bothered 69.3% of patients “fairly” or “enormously” for social life, 63.1% for leisure, 62.6% for family life, 57.4% for domestic life, 49.3% for work, and 30.2% for sex. During the initial consultation, stress was accused of triggering abdominal pain by 80.5% of patients and transit disorders by 69.3%. At the end of the study, no matter what the aspect, less than 2% of patients were “enormously” bothered by IBS and over 72% a little or not at all.

Assessment of discomfort by AVS demonstrated that the results changed on average between the two consultations from 4.7 to 2.6 for pain, 5.1 for swelling, 3.3 to 1.7 for diarrhoea, 3.2 to 2 for constipation, and from 5.2 to 3 for an AVS assessing overall discomfort. Age, sex and patterns of disease development were not significantly associated with differences in disease stage at three months.

Conclusion: measuring the effect of IBS by AVS and discreet-answer questions were in agreement. IBS deteriorated various aspects of physical well-being in day-to-life and, to improve care, must be taken into consideration. The improvements observed during the study could be due to the treatments given, but also to the patterns of disease development or even the conditions of observation.

207 Enterocyte Covering Agent Versus Intestinal Motility Inhibitor in the Irritable Bowel Syndrome

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Purpose: Irritable bowel syndrome (IBS) is a rather frequent disease in adults, with a complex pathogenic mechanism. Our aim was to assess whether enterocytes are more involved than smooth muscle cells in IBS with accelerated bowel transit.

Methods: Our study was a prospective monocentric study. We had 2 groups of participants: group D had 50 patients with IBS who were treated with loperamide, 9 g/day. Group L had 32 patients and was treated with loperamide 4 mg/day. Both groups were treated for 30 days and had a definite IBS diagnosis based on clinical data and laboratory exclusion of organic, infective or systemic diseases who could mimic IBS. All the 62 patients were evaluated at the beginning, and at days 10, 20, 30. Statistic analysis evaluate the median, maximal, and minimal values for quantitative variables, and Wilcoxon's test for qualitative variables.

Results: The number of stools had reduced from 3.4 ± 1.0 to 1.0 ± 0.7 in the D group compared with a reduction from 3.6 ± 1.0 to 1.8 ± 0.3 in the L group. The frequency of abdominal pain was reduced from 6.5 ± 2.4 to 1.6 ± 2.1 and from 5.8 ± 1.8 to 2.9 ± 2.2 respectively. Flatulence was absent in 16.7% more treatment and 63.3% after 30 days in group D compared with 21.9% and still 37.5% in group L. Global efficacy at 10 days was 46.7% very good and 26.7% good in the D group compared with 31.25% and 17.85% in group L.

Conclusion: Global efficacy at 30 days was 96.7% with diosmeetie and 62.5% with loperamide which may mean that usually in IBS smooth muscle hypermotility may be in some conditions more dependent upon the enterocyte and intraluminal status than upon the nervous system.

220 Comparison of Pantoprazole and Omeprazole in Acute Reflux Esophagitis

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Pantoprazole, a new proton pump inhibitor, was compared with omeprazole in the treatment of reflux esophagitis in a large multicenter trial.
Adverse events (AEs) were comparable in both groups with respect to type (most common: diarrhea, headache), frequency (pantoprazole: 16 patients, omeprazole: 19 with possible or definite relation to medication) and intensity. No clinically relevant changes in laboratory parameters including TSH, AST, ALT, and blood count were observed on either treatment.

Conclusion: Pantoprazole and omeprazole are similarly effective and safe in the treatment of acute reflux esophagitis.

221 A Double-Blind, Randomized, Placebo-Controlled Study of Cisapride in Pediatric Gastroesophageal Reflux

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Gastroesophageal reflux is a common condition affecting infants and may lead to serious complications such as aspiration, pneumatic esophagus, stricture and failure to thrive. The present study was designed to assess the efficacy and safety of oral Cisapride suspension in the treatment of pediatric gastroesophageal reflux disease. A randomized, prospective, double-blind, placebo-controlled clinical trial was conducted at three study sites. After a one-week run-in period for evaluable infants (aged 6 weeks to 2 years) were randomized to receive a 6 week double-blind treatment with Cisapride (0.2 mg/kg q6h) or a placebo suspension. Efficacy was assessed with 24 hour esophageal pH monitoring, esophageal manometry, esophageal biopsy before and after the treatment period. A diary was kept by parents of regurgitation frequency and severity, and global evaluation by parents and physician were performed at every visit. Safety was assessed by means of adverse event monitoring and standard laboratory measurements. Compared with placebo, Cisapride significantly (p < 0.05) reduced the mean duration of upright and supine reflux episodes by the end of the trial. Compared to baseline, Cisapride significantly reduced the duration of the longest reflux episode, and placebo increased the number of reflux episodes > 5 minutes. Cisapride was not significantly different from placebo for the following measurements: % total time pH < 4, number of reflux episodes, lower esophageal sphincter pressure, swallowing pressure, regurgitation frequency or global evaluation scores. The incidence of adverse events was comparable between groups. No serious adverse events were reported during the study. Cisapride is a safe, well-tolerated prokinetic agent which improves some parameters of 24 h pH measurements in children with GERD under the age of 2 years. It may be effective in reducing secondary complications of GER in pediatric patients.

222 Reflux Esophagitis: A Complication of Helicobacter Pylori (HP) Eradication Therapy?

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Recently some authors reported in a preliminary study an high incidence of reflux esophagitis patients in after HP eradication therapy. Aim of our study was to evaluate this phenomenon in our endoscopic population.

Methods: 276 patients on the antiulcer therapy using HP eradication were treated with different therapeutic regimens (various antimicrobial agents: Omeprazole or Ranitidine and various antibiotics: Claritromycin (C) + Tinidazole, C + Metronidazole (M), Amoxycillin (A) + Bismuth, A + M. The patients were investigated endoscopically and clinically at 1-6 months after therapy and when dyspeptic symptoms occurred. HP status was assessed by urease test and histology in antrum and body of stomach and in third inferior of esophagus as well.

Results: 16 patients (61.2%) were eradicated at 6 months after therapy: 24 of them developed an endoscopically proven reflux esophagitis which was mild (grade I) in all patients.

Conclusion: Our study confirmed the evidence of reflux esophagitis developing in patients treated for HP infection. Such evidence can be explained by different theories: altered eating and drinking habits that can reduce lower esophageal sphincter pressure or the interruption of a chronic therapy with antisecretory drugs for peptic disease. Any evidence of HP has been found in esophagus as in stomach as well. We believe that this phenomenon isn’t casual finding in patients without previous evidence of reflux esophagitis and that further studies are needed to clarify this phenomenon.

223 Treatment of Candida Esophagitis in the Acquired Immunodeficiency Syndrome: Evaluation of Long-Term Therapeutic Efficacy of Fluconazole and Itraconazole

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Introduction. Little information is actually available regarding the long-term response of Candida esophagitis to antifungal therapy. Aim of the study has been to assess the long-term therapeutic efficacy of fluconazole and itraconazole in the treatment of Candida esophagitis in a selected population of HIV-positive patients.

Methods. The study has considered 2213 HIV-positive patients at first episode of esophageal candidiasis diagnosed by endoscopy. 1105 received fluconazole (100 mg b.i.d., 1106 received itraconazole (100 mg b.i.d.) for 2 week. The patients who presented, after 2 weeks of treatment a partial endoscopic response (Kodi’s grade I), even if asymptomatic, continued the pharmacological treatment up to week 5. Then 5 week they underwent endoscopic examination and only the patients with a complete cure were considered for long-term follow-up. The other patients were withdrawn from the study. Beginning from week 5, clinical examination was performed every week up to month 3, every two week up to month 6 and then every month up to month 12. Endoscopic examination was performed also at relapses of esophageal symptoms were observed during the follow-up period, in order to assess if Candida infection was responsible or not for symptomatic disease.

Results. At week 2, endoscopic cure occurred in 81.2% of patients treated with itraconazole and in 65.6% of patients treated with itraconazole (relative risk: 1.22; 95% C.I.: 1.08–1.35; p < 0.001). Clinical cure was observed in 61.5% of patients treated with fluconazole and in 75.2% of patients treated with itraconazole (relative risk: 1.08; 95% C.I.: 0.95–1.18; p < 0.001). A total of 2158 patients were blindly and endoscopically evaluable at week 5 and were considered for long-term follow-up. At the end of follow-up, endoscopic and clinical cure were observed in 96.2% of patients treated with fluconazole and in 96% of patients treated with itraconazole (relative risk: 1.00; 95% C.I.: 0.87–1.08; p = 0.816). By intention-to-treat analysis endoscopic and clinical cure were observed in 93.6% of patients treated with fluconazole and in 93.3% of patients treated with itraconazole (relative risk: 1.00; 95% C.I.: 0.87–1.08; p = 0.857). Symptomatic relapses of endoscopically-demonstrated esophageal candidiasis were observed in 6% of fluconazole-treated patients and in 8.5% of itraconazole-treated patients, and were evaluated by a clinical cure at week 5 (relative risk: 0.92; 95% C.I.: 0.79–0.99; p = 0.650).

Conclusions. Both fluconazole and itraconazole are provided with a good long-term therapeutic efficacy in the treatment of Candida esophagitis in HIV-infected patients. Fluconazole group has a higher rate of endoscopic and clinical cure than itraconazole in short-term treatment.

227 TP53 Gene and MTS1 Gene Alterations in Oesophageal Squamous Carcinomas

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The TP53 gene is the most commonly mutated gene in human cancers. Another tumor suppressor gene called MTS1 encoding for p16 protein is frequently mutated in melanoma and other tumors. Recent data have reported homozygous and heterozygous deletions involving 9p21. The aims of this study were: 1) to establish the prevalence of TP53 gene mutations, 2) to analyze the rate of deletions of 9p21 and 3) to detect MTS1 mutations in a large series of oesophageal squamous cell carcinomas (SCC).

Material and Methods: One-hundred tumors were studied. TP53 mutations were identified using a GC clamp Denaturing Gradient Gel Electrophoresis (DGGE) and DNA sequencing. Deletion mapping of 9p21 was performed using microsatellites. MTS1 mutations were identified using DGGE and DNA sequencing.

Results: TP53 gene mutations were detected in 78 patients (78%). The mutations identified were transitions (48.5%), transversions (51.5%) and frameshift mutations (16.7%). Five patients had a germline mutation of the TP53 gene. Loss of heterozygosity of 9p21 was investigated in 82 samples. Allelic loss involving at least one of the 2 microsatellites was detected in 11 of 75 informative cases (14.7%). We found 76 deletions of exon 2; 3 consisted in deletions of 5, 10, and 33 base pairs and an identical missense mutation (A1407T) was detected in the 3 other cases. Two patients had a genomic point mutation identical to that found in somatic DNA, and a loss of the other allele in the tumour.
Conclusion: this study shows that TP53 gene alterations occur frequently in oesophageal SCC. On the other hand, somatic mutations of MTS1 gene are rare during oesophageal tumorgenesis.

228 Sensory Nervous Fibers and CGBR in Gastric Adaptation to Stress
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Gastric mucosa is capable to adapt to ulcogenic action of stress but the mechanism of this phenomenon is unclear. Stimulation of capsaicin-sensitive sensory nerves and calcitonin gene-related peptide (CGRP) exhibit protective effects against mucosal damage in various models of gastric lesions, and for this reason, we investigated the influence of sensory nerves and sensory neuropeptide such as CGBR on gastric adaptation to stress. Acute gastric lesions were produced with water immersion and restraint stress (WIRS) in rats. WRS was applied either once or repeatedly every other day for up to 8 days in animals with intact or capsaicin deactivated sensory nerves. It was found that WRS applied once produced multiple gastric erosions (19.0 ± 0.9 per rat) accompanied by an increase in basal gastric acid secretion (n = 20%), a decrease in gastric blood flow (determined by laser Doppler technique) and a 50% and a reduction of DNA synthesis by 57% as compared to intact rats without WRS. Repeated WRS resulted in a significant reduction of number of gastric lesions. This adaptation to stress ulcerogenesis was accompanied by a gradual decrease in gastric acid secretion, an increase in gastric blood flow and a return of mucosal DNA synthesis to the control value. Capsaicin-induced deactivation of sensory nerves eliminated adaptive adaptation to WRS as manifested by a failure to decline of gastric lesions after repeated WRS and sustained decrease in GSF. Pretreatment with CGBR in capsaicin denervated rats prevented the formation of acute gastric mucosal lesions by WRS exposure to WRS in CGBR-pretreated rats resulted in a decrease of gastric lesions number to the value similar to that observed after gastric adaptation to stress but repeated WRS insults did not result in any additional significant reduction of gastric lesions. We conclude that the stomach is able to adapt to repeated stress insults due to enhancement of GSF and mucosal cell proliferation, and this adaptation depends upon the activity of sensory nerves and probably the release of CGRP.

229 Clinical Guidelines for Dyspepsia in Primary Care
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Background Clinical guidelines have been suggested as a means of improving the quality and uniformity of patient care, though are easier to produce than to implement successfully. We have developed a local consensus-based management guidelines for dyspepsia in co-operation with local general practitioners (GPs), and report a prospective audit of their implementation in a primary care setting.

Methods A heterogeneous group of 9 GPs from 3 practices were recruited to plot the guidelines. consecutive consultations for dyspepsia were recorded on a dyspepsia management record (DMR), kept in each patient's casenotes. The DMR was designed to summarise clinical information, facilitate guideline compliance and enable audit.

Results 217 DMR's were completed, relating to 396 consultations for dyspepsia. Prescribing patterns (%) and guideline compliance are summarised for selected subgroups (274 consultations):

Sub-group N N Nil Ant Mot H2R PPI HP E %
New patient
29
27 7 48 38 (24) 3 (0) 0 73
Past dyspepsia, "empirical"
47
47 30 4 36 (21) 0 0 20
Past reflux, "empirical"
37
37 46 0 19 32 0 100
Non-ulcer dyspepsia
42
42 5 14 43 29 (24) 5 (5) 71
Reflux (ve investigations)
32
32 16 0 36 44 0 100
Reflux oesophagitis
37
37 0 5 22 (3) 68 5 92
Duodenal ulcer disease
50
4 2 62 (36) 8 (4) 24 60

Conclusions Although dyspepsia guidelines were well received by GPs, with overall compliance relatively good, some therapeutic agents were still used "inappropriately", and H₂-pump eradication therapy was under-prescribed in DU disease. Even when guidelines are introduced under "ideal" conditions, with ongoing audit, prescribing practice may prove difficult to modify. Continuing medical education, targeted at specific aspects of disease management, is vital, particularly if guidelines are to be viable on a larger scale.

230 Ultrastructural Evidence of H. Pylori Penetration into the Gastric Cells
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H. Pylori is considered as exerting its damaging effects to the gastric mucosa by adhering to the gastric mucosa cells without invading them. Aim of the study was to examine infrastructurally the sequence of H. Pylori interaction with the gastric mucosa. Twenty-eight patients with active chronic gastritis and peptic ulcer disease were diagnosed to have been infected with H. pylori. by 13C urea breath test, histology, culture and rapid urease test (CLO-test). Biopsy specimens from these patients were randomly selected and were fixed in 2.5% glutaraldehyde and sodium cacodylate and then post-fixed in osmium tetroxide. After dehydration and gold coating, samples were examined under a Scanning Electron Microscope (SEM). A second set of biopies were embedded in epoxy resin and cut to ultra thin sections which were stained with uranyl acetate and lead citrate for enhancing electron scattering for Transmission Electron Microscope (TEM) analysis. Both SEM and TEM showed that, initially, bacteria adhere to the glycoloyl layer of microvilli, destroying them. Then the bacteria attach directly via flagella to the gastric cell surface, where the phospholipase A₂ activity of H. pylori degrades the external phospholipid membrane of gastric cells especially around the areas of tight junctions. Subsequently, the bacteria penetrated into the cytoplasmic region of the gastric cells where bacteria were observed in small cytoplasmic vacuoles leading to cellular disintegration. Some bacteria were still adhered to cytoplasmic parts and granules which were floating free in the gastric milieu. Furthermore, generation of complement products and cytokines released after urease induced the chemotaxis of polymorphonuclear leukocytes which migrated through the epithelium into the gastric lumen, phagocytosing H. pylori and inducing inflammation to the gastric tissue. H. pylori strains having the GspA gene detected by Western Blotting showed greater polymorphonuclear leukocytes chemotaxis and hence induced more severe inflammation. The above observations reveal the ultrastructural mechanism under which H. pylori causes gastric and peptic ulcer disease by actually invading the gastric cells.

232 Serorepology of H. Pylori Infection and Helicobacter A. in Turin. Evidence Against a Common Mode of Transmission

Background: Recent studies showed that the age-specific prevalence of H. pylori infection in a parallelis Hepatitis A (HAV) suggest a common mode of transmission. Aim: To investigate risk factors for H. pylori and HAV and the possibility that the two infections could be associated. Methods: Between January and September 1995, a random sample of 705 resident subjects who attended the outpatient medical center of the rural county of Cirò (11,000 inhabitants, Southern Italy) for blood testing were recruited. All subjects completed a questionnaire for general demographic details, height, weight, current and childhood socio-economic circumstances, history of cardiovascular diseases, diabetes, dyspepsia, smoking and alcohol. Serum sample was drawn from each subject and stored at −20°C. Blood pressure was measured. All sera were assayed for H. pylori specific IgG by an in-house ELISA using a crude H. pylori sonicate as antigen (sensitivity and specificity 97% and 91%). In 466 subjects, antibodies to HAV were detected by means of a commercial ELISA kit (Behring, USA). In subsets of subjects, serum fasting cholesterol, triglycerides and glucose were also measured. Data were analysed by multiple logistic regression analysis, Spearman’s r statistic and expressed also as odds ratio (OR) and 95% confidence intervals (CI). Results: Of the 705 (273 M, 480 F; age range 1–87, median 50) subjects, 446 (63%) were positive for H. pylori. Among the 466 (163 M, 303 F; age range 1–87, median 49) subjects screened for HAV, 76 were positive only for HAV (OR = 0.07 (87%)) for HAV. Cross-tabulation of this data showed that 275 (59%) were positive and 43 (9%) negative for both H. pylori and HAV, 16 (3%) were positive only for H. pylori and 132 (28%) were positive only for HAV (OR = 5.8, CI: 3–10). There was an age-specific increase in the prevalence of the two infections and a fair correlation (r = 0.287) whereas the association was more weak (r = 0.21) or not significant (r = -0.064) when assessed in the first two decades. In multivariate analysis, current and childhood socio-economic features were differently associated with H. pylori dynamics (OR = 1.6, CI: 1.1–2.3, occupation (OR = 0.7, CI: 0.6–0.9) and HAV [n of siblings (OR = 1.3, CI: 1.05–1.7), refrigerator (OR = 5.6, CI: 1.3–24)]. Conclusion: The correlation between H. pylori and HAV reflects the age-specific high seroprevalence of both infections more than a true association. The fecal-oral spread of H. pylori is unlikely.

233 Regression of Lymphoid Follicles in Gastric Mucosa of Helicobacter Pylori Infected Patients 12 Months after Eradication
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Introduction. Only few studies, on small samples, demonstrated that eradication of Helicobacter pylori (Hp) infection causes a decrease of number of lymphoid follicles (LF) and a regression of low grade B cell gastric MALToma.
234 Helicobacter Pylori Infection and Antral Intestinal Metaplasia: One Year Follow-Up after Eradication

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Purpose of the study: To evaluate Helicobacter pylori (HP) significance in development of intestinal metaplasia (IM) and how HP eradication modifies IM evolution.

Methods: In 99 consecutive IM histological diagnosis in specimens obtained during upper endoscopic investigation we performed four biopsies, two from angular mucosa and two from fundic mucosa and detected HP presence histologically by Giemsa stain. All patients were endoscopically re-investigated one year later. The group of patients presenting IM and HP positivity underwent eradication therapy immediately after diagnosis. Statistical analysis was performed by chi-square method.

Results: 82 (82.6%) out of 99 patients had HP positive and 17 (17.2%) were HP negative. IM and HP positive 11 patients underwent eradication by triple therapy (omeprazole 20 mg daily for one month, amoxicillin 1 g twice a day for one week, clarithromycin 250 mg twice a day for one week). 69 patients out of 82 resulted eradicated by immunological method two months after therapy. All patients with HP positive and IM positive dropped out of the study. 14 (87.5%) out of 16 patients (87.5%) HP positive and HP negative at time 0 showed IM in histological specimen and one of them was HP positive to Giemsa stain. At the same time only 56 (64.5%) patients out of 80 treated demonstrated intestinal metaplasia on histological specimen; 40 patients out of 53 were HP negative and 13 resulted HP positive. Among 29 patients IM positive at time 0 and IM negative after four months later 27 were IM positive and HP negative and 2 were IM positive and HP positive. Comparing the IM and HP positive patients before and after eradication, data analysis by chi-square method showed statistical significance (P < 0.005).

Conclusions: 82.8% patients affected from IM is HP positive. HP eradication allows regression of histological intestinal metaplasia in 30% cases one year after eradication. According to Correa’s gastric carcinogenesis model, eradication HP positive patients with proven IM could be suggested.

235 13C-Urea Breath Test – A Reliable Diagnostic Technique for Assessment of Eradication


Introduction: The sensitivity of any diagnostic test for H. pylori is most rigorously test-posted treatment. Here post-treatment results are presented from 7 multinational studies conducted to standards of Good Clinical and Laboratory Practice.

Methods: 1029 patients, who had active DU and a positive CLOtest® pre-treatment, were evaluated post-treatment in a total of 1815 visits. H. pylori was assessed by 13C-Urea Breath Test (UBT) and at least one other test, [CLOtest, histology negative, culture (C), before and at 3, 6, or 12 months post-treatment, dependent on study. UBT, Hx and Cx were processed by central laboratories. Antral and corpus biopsies were taken. The cut-off of a cut-off of ≥ 5 compared to ≥ 3.5 excess 13CO2 per mil for the UBT is examined. H. pylori status was assigned from the combined results of at least two tests. Single positive tests or otherwise anomalous results were reviewed using data from previous and subsequent visits.

Results: see table.

Conclusion: The UBT is a convenient, non-invasive test which yields similar sensitivity for assessment of eradication as histology or CLOtest alone (using multiple biopsies). A cut-off of 3.5 excess 13CO2 per mil is recommended for increased sensitivity but the test is robust even at 5.0 excess 13CO2 per mil.

236 Cost-Effectiveness of Clarithromycin Plus Omeprazole Compared to Traditional Therapies for Treatment of H. pylori Associated Duodenal Ulcers

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Introduction: The NIH Consensus Development Conference recommended a comprehensive economic analysis of the impact of treating or not treating H. pylori (HP) associated ulcers. Patients were enrolled in a multicenter (n = 132), controlled clinical trial to determine cost savings of eradicate HP with clarithromycin plus omeprazole (C + O) versus conventional anti-ulcer therapy (omeprazole (O) or ranitidine (R) alone). Methods: Adult patients with HP and active duodenal ulcer were randomized to double-blind treated (Rx): 1) C 500 mg tid + O 40 mg qd for 14 days followed by 20 mg qd for 42 days; 2) C 20 mg qd for 28 days; or 3) R 150 mg bid for 28 days. Visit were performed at pre-Rx (EGD + biopsies post-Rx (eradication). After the third protocol directed visit, investigators followed patients for one year by monthly telephone calls to assess ulcer symptoms and collect economic data. Additional management was to be "standard of care" for the investigational site. Results: Of the 151 patients enrolled, 75 patients were eligible (confirmed ulcer and HP infection) for economic analysis. The demographics of the three groups were similar. Analysis of health resource utilization is given in the table below:

<table>
<thead>
<tr>
<th>Resource Utilization (by protocol)</th>
<th>C + O</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGDs</td>
<td>31</td>
<td>76</td>
</tr>
<tr>
<td>Ulcer-related clinic visits</td>
<td>84</td>
<td>136</td>
</tr>
<tr>
<td>Ulcer days lost from work</td>
<td>116</td>
<td>122</td>
</tr>
<tr>
<td>All hospitalizations</td>
<td>26</td>
<td>47</td>
</tr>
<tr>
<td>All ulcer hospitalizations</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>All hospital days</td>
<td>158</td>
<td>318</td>
</tr>
<tr>
<td>Overall ulcer hospital days</td>
<td>24</td>
<td>37</td>
</tr>
</tbody>
</table>

Conclusion: Our is the first prospective study to show that using antibiotics to eradicate H. pylori in patients with duodenal ulcer results in decreased utilization of health care resources, overall and ulcer related, when compared to conventional anti-ulcer therapy with omeprazole or ranitidine.

237 A Management Plan for Upper Gastrointestinal (GI) Disease in Primary Care

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The International Gastro Primary Care Group (IGPCG) was formed in May 1994 and includes primary care physicians from seven European countries, the USA and Australia. Based on their collective clinical and research experience, they devised a practical guide for the management of upper gastrointestinal (GI) disease.

In the absence of alarm symptoms or use of Non Steroidal Anti-inflammatory Drugs (NSAIDs) the IGPCG management plan allocates patients based on predominant symptoms into three subcategories: motility disorder likely (flatulence, bloating, anorexia, constipation, nausea), ulcer disease likely (localised epigastric pain), gastro-oesophageal reflux disease (GORD) likely (heartburn, regurgitation).

For each sub category particular management is recommended. The motility group is treated with a prokinetic. In the ulcer group the Helicobacter pylori (Hp) status is checked: Patients positive for Hp are referred for endoscopy and receive eradication treatment in an ulcer is confirmed. For GORD patients a step-up approach is recommended, starting with a prokinetic and H2 antagonists. Treatment failure receive proton pump inhibitors (PPIs) or combination therapy are referred or are untreated.

A pilot project in the form of a survey was set up to test the practical application of the IGPGC upper GI management plan in primary care. The age range was 20 to over 80 [mean 45 years] and the male/female ratio was close to 50:50. Of the 58 patients included, 25 (44%) were allocated to the motility group, 22 (40%) to the ulcer group and 12 (21%) to the GORD group. Only three patients (5%) could not be placed into a specific group. Overall a satisfactory response was obtained in 75 patients (12/56 (84%) in the motility group, 9/22 (41%) in the ulcer group and in 5/12 (42%) in the GORD group).

The SPCC protocol postulates the use of predominant symptom in primary care management. This is contrary to symptom clustering which has been shown to be impractical in the management of upper GI disease. However, prospective validation of this plan is required to evaluate its cost-effectiveness.
Has Spiral Computed Tomography Improved the Staging of Patients with Gastric Carcinoma?


Much controversy exists as to the value of computed tomography (CT) in the pre-operative staging of gastric cancer, due to its limited ability to correctly identify lymph node (LN) metastases, adjacent organ invasion and peritoneal metastases. The third generation of spiral CT scanners has a number of potential advantages including: minimal respiratory misregistration, image reconstruction smaller than scan collimation and optimization of intravenous contrast enhancement.

The aim of this study was to assess the sensitivity and specificity of spiral CT, compared with both a formal operative staging and the final pathological (TNM '87) staging.

105 consecutive patients who underwent both spiral CT and operative assessment were reviewed, median age 71 years (range 33-91 years). A single radiologist reviewed all scans which were assessed for LN metastases, adjacent organ invasion and hepatic and peritoneal disease. A similar assessment was made at the time of surgery. Both were then compared with the final histological staging.

Results:

<table>
<thead>
<tr>
<th>Spiral CT staging</th>
<th>Operative staging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>N1 nodes</td>
<td>24%</td>
</tr>
<tr>
<td>N2 nodes</td>
<td>43%</td>
</tr>
<tr>
<td>Mesocolon</td>
<td>76%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>50%</td>
</tr>
<tr>
<td>Hepato</td>
<td>57%</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>70%</td>
</tr>
</tbody>
</table>

There has been some improvement in spiral CT ability to detect both adjacent organ invasion (mesocolon) and peritoneal disease. Moderate sensitivity with high specificity in detecting spread means that a positive spiral CT result can be relied upon. Spiral CT has the potential to identify those patients who would be suitable for neo-adjuvant chemotherapy before surgery.

Incidence of Inflammatory Bowel Disease Across Europe: Is There a Difference between North and South?

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Background: It has been suggested that the incidence of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is higher in the North than in the South of Europe. The aim of this European Collaborative study was to investigate the hypothesis of the North-South gradient.

Methods: Over 2 years (1 Oct. 1991 to 30 Sept. 1993) all new patients with IBD were identified in 20 European centres according to a standard protocol for case ascertainment and definition.

Findings: 2201 patients aged 15 years or more were identified, of whom 1392 (63%) were women. UC and CD (including peripheries) accounted for 70% as CD and 116% as indeterminate. The overall incidence per 100,000 at ages 15-64 years (standardised for age and sex) of UC was 10.4 (95% confidence interval): 7.6-13.1) and of CD was 5.6 (95% CI: 2.8-8.3). Rates of UC in northern countries were higher than those in the South (ratio rate [RR] = 1.4, 95% CI: 1.2-1.5) and for CD they were 80% higher (RR = 1.8, 95% CI: 1.5-2.1). For UC, the highest incidence was in Iceland (24.5) and for CD, in Maastricht (The Netherlands) (9.2) and Amiens (NW France) (4.7). The lowest incidence of UC was in Almada (S. Portugal) (1.6) and of CD in Lozarnia (NW Greece) (0.9). An unexpected finding was that UC in the incidence in women but not men declined with age. The higher incidence rates in northern centres was not explained by differences in tobacco consumption or education.

Conclusions: The magnitude of the North-South difference for both conditions was less than expected which may reflect recent increases in the incidence of IBD in southern Europe. Nevertheless there are substantial differences in incidence of UC and CD across Europe which are not readily explained by differences in case ascertainment.

Inhibition of Cytokine Formation by the Novel Thiol Modulating Agent OR-1394

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Elevated levels of proinflammatory cytokines, (IL-1, IL-8 and TNF-α), have been associated with the pathogenesis of inflammatory bowel disease (IBD). The key regulatory proteins in the signalling cascade leading to the induction of cytokine genes with UC (short sensitive, OR-1394 (4-methyl-sulfonylphenyl)-methylene)-2,4-pentanenedione) is a novel thiol modulating agent, which forms reversible adducts with thiol groups. The aim of this study was to evaluate the effect of OR-1394 on the formation of the major proinflammatory cytokines. We also tested the efficacy of OR-1394 in experimental colitis induced by a hapten, TNBS.

Methods: Isolated human monocytes were used for the in vitro cytokine studies. The monocytes were stimulated with lipopolysaccharide (50 ng/ml) and incubated with different concentrations of OR-1394. IL-1, TNF-α and IL-8 were measured from the incubation medium by specific ELISAs. Collits was induced in mice and rats by a single intracolic administration (i. col.) of 5 and 15 mg of TNBS, respectively. In rats, 3-30 mg/kg of OR-1394 was administered i. p. 24 h before and in mice 30 mg/kg was given i. col. 24 h and 1 h before TNBS. The rats were killed 96 h and the mice 4, 8, 16, 24, 48 or 72 h after the induction of colitis. Colonic inflammation was assessed by macroscopic and histological scorings and by measurement of tissue myeloperoxidase (MPO) activity. In mice, IL-1, IL-6 and TNF-α were measured in mucosal homogenates of the colon.

Results: OR-1394 inhibited the formation of IL-1, TNF-α and IL-8 in human monocytes the IC50 values being below 10 μM. In rats, OR-1394 protected against TNBS-induced injuries and decreased the MPO activity significantly and dose-dependently, maximally by more than 70%. In mice, the colonic injuries were visible already 4 h after TNBS, but they were most severe at 48 h. IL-1, TNF-α and IL-8 levels, however, increased several fold reaching the maximum at 16 h. OR-1394 inhibited the formation of these cytokines and protected against the lesions and inflammation.

Conclusion: OR-1394 was shown to effectively protect against hapten-induced colonic injuries and inflammation. The marked suppression of the key inflammatory mediators IL-1, IL-8 and TNF-α suggests the anti-inflammatory activity of OR-1394 to be cytokine mediated. The locally acting OR-1394 offers a new alternative for the treatment of IBD.

Interleukin-8 in Inflammatory Bowel Disease

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Background and purpose: Increasing evidence points to various pathogenic roles for cytokines in inflammatory bowel disease (IBD). Interleukin-8 (IL-8) is a major cytokine for recruitment and activation of neutrophils. Neutrophils play a central role in the active stages of IBD. This study aimed to characterize secretion of IL-8 by mucosa in vitro and to visualize its distribution and its positive cell types in the affected intestine of patients with IBD.

Materials and methods: Biopsies or resected segment were obtained from 12 patients with active Crohn's disease (CD) and 18 with active ulcerative colitis (UC). In 11 patients, macroscopically normal portion was obtained from large bowel resected because of colorectal carcinoma. IL-8 content of organ culture supernatants was determined by enzyme linked immunosorbent assay, and IL-8 gene expression was analyzed by in situ hybridization with IL-8 DNA probes.

Results: The secretion of IL-8 (ng/ml) in 24 hours from inflamed mucosa of patients with CD (median 135.4, range 52.8-352.4) or UC (median 179.5, range 74.7-385.5) was significantly higher than that from normal mucosa (median 51.9, range 35.3-107.5; p = 0.0006 vs. CD, and 0.0001 vs. UC), and correlated with the number of neutrophils infiltrating in the affected intestinal mucosa of IBD (r = 0.760). In situ hybridization with IL-8 DNA probes revealed strong signals in the involved mucosa. The number of cells expressing IL-8 mRNA correlated with histology. The grade of disease activity. Most of IL-8 mRNA producing cells were focally distributed in erosive or ulcerative intestine of patients with CD, whereas they were diffusely distributed over the entire inflamed mucosa of UC. The expression of IL-8 was mainly expressed by macrophages, neutrophils, and epithelial cells in the involved intestine of IBD.

Conclusion: The results of this study suggest that IL-8 play an important role in the pathogenesis of IBD. The distinction of IL-8 gene in CD and UC may indicate that there exists a difference of inflammatory responses between these two forms of IBD.

Intestinal Cytotoxic CD4+ Th1-Like Cells in Crohn's Disease

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Cytotoxic function of isolated lamina propria T lymphocytes (LP-CTL) in inflammatory conditions is not well established. Our previous results show that LP-CTL, mediated by TCR-CD3, is significantly increased in Crohn's disease (CD). Aim of our study is to evaluate which kind TH subset is involved in inflammatory conditions. We functionally characterized the toxic cytokine and cytokine profile (IFN-γ, IL4 and IL5) of CD4+ enriched or CD4- depleted LPL, from terminal ileum of CD pts (n = 10) and controls (n = 10). LPL are isolated using DTT-EDTA-Collagenase digestion followed by discontinuous Percoll density gradients and CD4+ cells were purified by Dynal beads. The cytotoxic activity of freshly isolated LPL is assessed against the NK-resistant B7 P815 cell line; anti-CD3 (5 mcg/ml, TR66), anti-CD2 (T163) and PHA-P (1 mcg/ml) were added to control and CD4- depleted cultures for 6 h at 37°C. The results are shown in the figure.

The present data unexpectedly indicate that CD4+ have cytotoxic activity and TH1 profile (γ-IFN concentration 8 ng/ml). The CD4+ function is significantly altered from the tissues of all CD pts by TCR-CD3 engagement. Furthermore showing that both CD4+ and CD4- mediated lysis are independent from the pathogenesis of all CD pts by TCR-CD3 engagement.
costimulatory signal. Therefore, the present findings could be of interest because they point out the relevance of activated CD4+ subset with an inflammatory function in the pathogenesis of CD.

244 High Validity of Transabdominal Bowel Sonography for Detection of Complications in Crohn’s Disease

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Aim: Transmural bowel inflammation with alteration of the echocarotidge of the bowel wall and luminal narrowing in Crohn’s disease (CD) can be located and visualized by transabdominal bowel sonography (TABS). The significance of these findings has been demonstrated in previous studies regarding the diagnosis of CD (Eur J Gastroent Hepatol 1992; 4: 173–182).

Since TABS is a noninvasive, radiation-free method that is well tolerated by the patients, repeated investigations can be easily performed during the follow-up. This study was initiated to investigate the validity of TABS in detection of disease specific complications in CD. Methods: Between 1994 and 1996 repeated TABS were performed by two investigators (GC, MG) using 3.5 and 7.5 MHz transducers (Ultramark 9, ATL Inc.) in the follow-up of 213 CD patients. In this study all patients who underwent bowel resection were evaluated (n = 25). The presence and location of intraabdominal fistulas, abscesses or bowel obstruction was assessed by TABS and compared with results obtained by surgery and histopathology. Results: Per-intestinal hypoechoic lesions were considered to be fistulas and with a diameter > 2 cm to be abscesses. Bowel obstruction was defined by luminal narrowing and evidence of prestenotic dilatation. TABS turned out to have a high validity in detection of such complications: In 14/17 patients, fistulation was correctly detected by TABS (sensitivity 82%). 7/8 patients were identified to have no fistulas (specificity 88%). Intraabdominal abscesses were detected in 6/6 patients (sensitivity 100%) and excluded in 17/19 patients (90% specificity).

Intestinal obstruction was detected in 19/19 and excluded in 6/6 patients (sensitivity and specificity 100%). Conclusion: TABS is a valid method to detect specific intraabdominal complications in patients with CD. We therefore recommend TABS for monitoring patients with CD.

245 Maintenance Therapy May Be Discontinued in Ulcerative Colitis Patients in Remission for Over 2 Years with Saliycates

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Aims To compare the efficacy of a delayed-release 5-ASA (ASACOL) against placebo in patients with ulcerative colitis (UC) in remission and to verify if duration of disease remission affects the relapse rate. Patients and methods 112 patients (66 M, mean age 35), with intermittent chronic UC in clinical and endoscopic remission with salicylates for at least one year were included in a double-blind, double-dummy, randomized, with 5-ASA (1.2 g/day) versus placebo (PI), for a follow-up period of one year. Assuming that a minor duration of remission may be associated to higher relapsing risk, the patients were stratified according to the length of their disease remission, in groups (A) (5-ASA 29, PI 35, in remission from 12 to 24 months) and (B) (5-ASA 28, PI 23, in remission over 2 years, median 4 years). Clinical, endoscopic and histologic findings were assessed every 6 months. “End point” of the study was considered the finding of clinical and endoscopic relapse. A Kaplan-Meier life table analysis was used to calculate the relapse rate. Cox model was used to identify predictive factors of relapse.

Results Fifty-four patients were treated with 5-ASA and 58 with PI. The relapse rate was similar in both groups after 6 months (5-ASA 8/64 (15%), PI 14/58 (24%), p = 0.15, IC95 ± 0.03), while a statistically significant difference was found after 12 months of therapy (5-ASA 11/54 (20%), PI 23/58 (40%), p = 0.016 IC95-0.35–0.02). 5-ASA was significantly more effective than PI in preventing relapse at 12 months in group A (5-ASA 6/26 (23%), PI 17/35 (49%), p = 0.037, IC95 –0.48–0.02). In contrast, no statistically significant difference was observed between the two treatments, either at 6 months (5-ASA 2/28 (11%), PI 5/23 (22%), p = 0.24, IC95 0.31 ± 0.09) or 12 months (5/28 (18%), PI 6/23 (26%), p = 0.37, IC95 0.31 ± 0.14) of follow up, in group B. Patients in group B are older and have had the disease longer than those in group A. The probability of relapse was independently affected by the 5-ASA treatment and remission duration of disease. In contrast, the relapse rate was not affected by age, sex, age at onset of symptoms, duration of disease, extent of disease, familial aggregation.

Conclusions This study shows that 5-ASA prophylaxis is really necessary for preventing UC relapses in patients in remission for less than 2 years, and may be discontinued in those with a remission duration longer than 2 years.

246 Efficacy of 5-ASA Suppositories (Pentasa®) 1 g Three Times a Week to Prevent Relapse of Ulcerative Proctitis. A Double Blind, Randomised, Placebo Controlled, Multicentre Trial

P. Marteau, J. Crand, M. Foucault, J.C. Rembou, Gastroenterology Unit, St-Laurence Hospital, Paris; Ferrin GA, Gentilly, France

The efficacy of daily administration of oral or rectal formulations of 5-aminosalicylic acid (5-ASA) in the prevention of cryptogenic proctitis. Subjects and methods: 95 patients (44 M, 51 F, mean age 41 y) with cryptogenic proctitis were randomised immediately after remission (clinical remission + endoscopy score 0 or 1) to receive for 1 year or till relapse 3 suppositories per week of either 1 g 5-ASA or PI. Follow-up was performed at 1, 3, 6, 9 and 12 months or in case of relapse. The major end point was finding of relapse. Data were analysed in intention to using ANOVA, Chi2 tests and Kaplan-Meier life table analysis. In case of relapse, the patients received blindly 1 sup/tilt remission. Results: The comparable demographic and proctitis severity variables existed between the 2 groups. The figure depicts survival curves for time to relapse (log rank: p = 0.06). A significant reduction of the recurrence risk was observed for the following time intervals: 0–20 0 (19% relapse in the 5-ASA group vs 38%, p = 0.035), 0–180 d (29% vs 54%, p = 0.017), 0–270 d (38% vs 62%, p=0.031). The risk of recurrence was not influenced by the endoscopy score at entry. Treatment of relapse was significantly better in the group treated with pentasa® 1 g: 61% of the pts had benefit vs. 8% with PI (p = 0.001).

Adverse events were reported in 12% and 10% of the 5-ASA and placebo group respectively. Conclusion: 5-ASA suppositories 1 g 3 times per week are effective for preventing relapses of cryptogenic proctitis, and well tolerated. Pentasa® 1 g/d is effective in the majority of subjects who relapse with the 3 week schedule.

247 Gut Permeability Test in Subjects with and without Exercise-Induced Gastrointestinal Symptoms

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Introduction: Up to 30% of endurance athletes suffer from gastrointestinal symptoms during physical exercise. Splanchnic blood flow drastically decreases during exercise, and may lead to gut hypoxia or ischemia. The aim of this study was to investigate whether the permeability of the gut changes as a result of strenuous exercise.

Methods: After an overnight fast, 5 well-trained subjects with exercise-induced intestinal symptoms, and 5 well-trained controls ingested a test solution on 3 different occasions; at rest, during a 90 min. running period on a treadmill at 70% of their previously determined Vmax, and 24 h post-exercise, respectively. The test solution consisted of 10 gram lactulose (L) and 1 gram mannose (R) in 65 ml water. Before the solution was ingested, the subjects emptied their bladder. Urine was collected for 5 h, and during this period the subjects were not allowed to eat or drink. The L and R excretion was determined by a validated, sensitive, newly developed fluorescent detection HPLC system. Glucose excretion was determined as well. Data are presented as mean ± SEM. Statistical analysis was performed by using a two-way ANOVA (time and group).

Results: During exercise, both the L and the R recoveries were increased, compared to pre-and post-exercise: (L: pre = 0.15 ± 0.05%, exc. = 0.21 ± 0.04% and post = 0.14 ± 0.04%, R: pre = 8.9 ± 1.2%, exc. = 12.2 ± 1.0%, post = 8.4 ± 1.0%). R recovery shows a significant difference (p = 0.033).
between the three periods. Comparing these periods, no significant difference was found in the L/R ratio. The increase of the R recovery was higher (p = 0.04) in subjects with exercise-induced symptoms. This increase was related to urinary glucose excretion (p = 0.008).

Conclusion: Alterations in gut permeability seem not to occur during strenuous exercise on a treadmill in our experiment. Rise in diarrhoea recovery coincided with an increased glucose excretion. Whether this observation is related to fluid balance differences in these subjects remains to be established.

Supported by grants of Sandoz Nutrition and the Dutch Olympic Committee

248 The Influence of Small Bowel Bacterial Overgrowth in Patients after Total Gastroctomy


Aims: To analyse B-PGIL (PGL 143 cases, PIL 45 cases) with the purpose of: 1) differentiate behaviours between themselves and between different tumour phenotypes, 2) histopathological reclassification after therapy, 3) staging (Ann Arbor/Musthoff), 4) conventional statistical analysis (X2, log-rank test, etc.).

Methods: 1) Records review; 2) histopathological reclassification after therapy; 3) staging (Ann Arbor/Musthoff); 4) conventional statistical analysis (X2, log-rank test, etc.).

Results: The significantly more common features in LG PIL (84 cases) were male sex, infiltrative endoscopic/pathologic aspect and localised stages (IE-IIE1) and in HG PIL (51 cases) they were weight loss, vegetative endoscopic/pathologic aspect, serosa invasion and an advanced stage (IIIE-IIIV). The following were favourable prognostic factors: LG, character, localised stages, normal LDH, complete surgical resection (cSR) and achieving a complete remission (CR). The significantly more common features in LG PIL (20 cases) were a long pre-diagnostic history, diarrhoea/steatorrhoea, finger clubbing, malabsorption, hypoprothrominaemia/edema, multisegment involvement and a nodular radiologic pattern and in HG PIL (25 cases) they were a palpable mass, surgical emergencies, ileo-coecal involvement and a high cell rate. Favourable risk factors were: female sex, normal LDH, uncinert tumour, lowPC/positive cell rate, use of polychemotherapy (+/- cSR) and achieving a CR.

Conclusions: B-PGL and B-PIL show different clinicopathological features between themselves and when compared with their nodal counterparts. The different behaviour of the LG and HG seems to translate unequal biological entities, nonetheless united by the mixed histology forms (LG/HG) that are more proximal to HG in PGL and to LG in PIL. Finally, in the group with PIL a small patient subgroup was discovered suffering a form of lymphoma which resembled the so-called "immunopro liferative small bowel disease".

250 Bone Mineral Density in Adult Coeliac Disease

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The association between overt coeliac disease (CD) and clinical bone disease has long been recognized. However, evidence is mounting that bone demineralization may occur even in relatively young patients with latent gluten sensitivity. We examined bone mineral density (BMD) and biochemical markers of bone metabolism in a group of otherwise healthy, treated, adult CD patients, and compared these results with those obtained in untreated CD patients. Subjects and methods: 36 CD patients (31 F, 5 M) diagnosed in adult life and already established on a gluten free diet participated in the study. The average age of the men was 28.2 yr (range 23-65 yr) and that of the women 36.8 yr (range 20-69 yr). BMD of total skeleton was measured by dual energy x-ray absorptiometry (DEXA), and serum and urinary parameters of mineral metabolism (serum and urinary calcium and phosphorus, serum and urinary 1,25-D3, alkaline phosphatase, PTH, osteocalcin, and urinary deoxypyridinoline) were determined by standard methods. BMD was expressed both in terms of absolute values (g/cm2) and as the Z-score, calculated from age- and sex-matched control subjects. Results: 20% of 36 (36/5% 36) asymptomatic untreated adult patients in the CD group showed BMD values of BMD, defined as a Z score < -1 SD; of note, severe osteopenia defined as BMD > 2 SD below mean normal values for sex and age, was found in 4 young patients (3 F, 1 M) aged 20-26 yr. Overall, reduced BMD was found in 29 out of 36 (80.5%) newly diagnosed/untreated patients; however, while 23 out of 25 (92%) symptomatic patients presented osteopenia, reduced BMD was found only in 6 of 11 (55%) symptomatic patients. No difference in BMD was found between treated and asymptomatic untreated CD patients. Serum and urinary markers of bone metabolism did not show conclusive abnormalities. Conclusions: Our findings provides evidence that osteopenia is common in adult CD, even in treated asymptomatic patients, and emphasize the importance of early diagnosis and treatment.

251 Evaluation of the Effects of 2 'Spasmolytic' Agents on the Motility of the Jejunum in a Prolonged Ambulant Small Bowel Manometry (PSBM)


Small bowel motility can be evaluated by PSBM during waking and sleeping, during fasting and after food, at rest and under mental or physical stress, and in response to medication; its precision is increased by computer analysis. In the past, PSBM has shown that oral medications do not always have the effects predicted from in vitro studies or "bolus" i.v. administration. We have previously shown that the "prokinetic" cisapride does not alter the amplitude and incidence of contractions, and the main action of trimetidine, a gut-selective enkephalinergic agonist, is the preservation of normal motility during exposure to stress.

We used PSBM to study the effect of 2 'spasmolytic' medications – alverine citrate 60 mg and phloroglucinol 80 mg – on small bowel motility in healthy volunteers. PSBM was carried out twice for 24 hours in 6 subjects, once with twice daily dosing with one drug, and again with twice daily dosing with the other. After intubation on Day 1 of the study, recordings were started. Subjects were freely ambulant and went home after an evening meal. On Day 2 they were exposed to 2-2.5 hours of mental stressors separated by a 1-2 hour rest period before exubuation. Efficacy of stressors was monitored by serial measurements of heart rate and blood pressure. After computer analysis, motility variables were compared for the 2 drugs against established control values.

Stress significantly increased heart rate (p < 0.001). Neither agent had any effects on the incidence, or amplitude of stress induced contractions. No effects of the drugs were seen on any aspect of motor activity were detected. Alverine blocked the effect of stress on migrating motor complexes (MMC) (p < 0.05) and on blood pressure (p < 0.001); it also blocked (p = 0.02) the normal diurnal lengthening of the MMC cycle. These findings are consistent with an effect of either agent on gut smooth muscle; the effects of alverine are better explained by blockade of the central modulation of gut motility during CNS arousal in waking and under stress.

252 Discrimination of Irritable Bowel Syndrome by a Hybrid of Linear and Nonlinear Analysis of 24 H Jejunal Motility

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Background: Conventional analysis of ambulatory long-term manometry of the small intestine has revealed an abnormal result in only 43% of patients with the irritable bowel syndrome (Gastroenterology 1995; 108: 8605), mainly during phase 2. The aim was to use methods from non-linear dynamics to discriminate motility in IBS from healthy subjects.

Methods: Ambulatory 24 h jejunal motility was obtained with digital data logging and catheter-mounted pressure transducers and standard transcutaneous intake in 30 diarrhea-predominant IBS patients and 30 controls. The variability and dynamics of the amplitudes of successive phasic contractions during phase 2 was described as a symbolic dynamical system, which means that the dynamics of a system is represented by a sequence of symbols. The sequence was quantified with the entropy, a specific complexity measure,
253 Diagnosing Whipple’s Disease in Feces
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Whipple’s disease is caused by the bacteria Tropheryma whippelii that still cannot be cultured. The diagnosis is based on the examination of a double biopsy. Part of the bacterial 16S RNA gene sequence is known. We investigated the possibility to diagnose Whipple’s disease non-invasively by analysing bacterial DNA isolated from feces.

DNA was extracted from stool of an untreated patient with Whipple’s disease and from 18 healthy control subjects. Since the known sequence of T. whippelii shows a high homology to other bacteria, we increased both sensitivity and specificity of the subsequent PCR by using three nested primers: 5’-AGA CAT AGC CCC CCC GA (position 956 of the 16SV1S sequence in the EMBL GenBank), PS: 5’-ATT GCG TCC ACC TTG CGA (position 1214), PS: 5’-CGG CAA CGG GCG CAC CCC CC (1046), PS: 5’-AGC CGT GAA GCC CAA GAC CG (1163), PS: 5’-CGG CTT GTG TTG CGG ACA GCG (1065), PS: 5’-CGC ACC AAG GAA GGG GGA (1153). The annealing temperatures were 55°C (PS2-PS3), 52°C (P5-P6) and 58°C (P6-P7).

After the third PCR, the product was analysed by gel electrophoresis. A single strong band was visible using DNA extracted from the patient’s stool and in 10/18 samples from control subjects. Sequencing the PCR product of the patient resulted in the known T. whippelii sequence. In control samples superposing sequences were seen representing DNA from various bacteria.

This result shows that Whipple’s disease can be diagnosed by detection of DNA of T. whippelii in the stool. As long as only part of a highly conserved gene of this bacteria is known, the PCR product has to be sequenced to confirm the diagnosis. However, as soon as more specific sequences of the bacteria are known, the diagnosis can be based on PCR with DNA from stool and subsequent specific tests such as restriction analysis or dot blot tests to exclude amplification of DNA of other bacteria.

254 Multicenter Double Blinded Randomized Placebo Controlled Study to Assess the Effect of Sucralfate in Intermittent Stool Eosinophilia Secondary to Pelvic Irradiation
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1. Purpose of the study: To demonstrate the efficacy of Sucralfate in the prevention of the acute enteric toxicity induced by pelvic irradiation with linear accelerator in patients with primary diagnosis of gynecological (cervix and endometrium), prostate or urinary bladder neoplasia, compared with placebo.

2. Methods: 120 patients (without metastasised neoplasia) between 18 and 80 years old, with a Karnofsky index > 80% and usual defecation range (3-10 defecations/week) undergoing whole pelvic irradiation, were included. The whole duration of the study was 7 weeks, following weekly controls. The first week all the patients received placebo. In the second week the patients were randomized into two groups: Sucralfate (61 patients, 2 g tablet p.o. before meals) and placebo (59 patients). The pelvic radiotherapy started in the beginning of the third week after the patient inclusion and lasted until the end of the study. All patients received a total dose of 50 Gy divided in 5 days/week with the "box technique". The principal variable was percentage of diarrhoeal stools per week. The statistical analysis of the clinical records was carried out by means of a two way analysis of variance with a repeated measurements design over the groups Sucralfate and placebo.

3. Results: Intention to treat analysis of the main variable showed a statistical significance in favour of Sucralfate vs placebo (p = 0.03) concerning the evolution of this study variable from the baseline (first week) to the finalization of the radiotherapy treatment (fourth week). Per protocol analysis also showed a statistical significance in favor of Sucralfate vs placebo group (p < 0.03) in this study variable.

4. Conclusion: Sucralfate is effective in the prevention of acute enteric toxicity induced by pelvic irradiation with linear accelerator compared to placebo.

255 Microsatellite Instability in Sporadic Colorectal Carcinomas

Background: Microsatellite instability (MIN) has been observed in the large majority of tumors from patients with Hereditary Non Polyposis Colorectal Cancer (HNPCC) as well as in 10 to 15% of sporadic colorectal cancer (CRC). Aims: To evaluate the prevalence of MIN tumors in a series of sporadic CRC as well as to analyse their clinical and pathologic characteristics. Patients and Methods: Sixty three patients with sporadic CRC were included in the present study. Mean age was 64 (36-83) years, 36 were male and 27 were female. MIN was detected by evaluating the length of CA repeats sequences at 12 loci: D1S216, D2S19, D3S1201, D5S404, D5S620, D17S783, D21S268. DNA was amplified in a radioactive PCR, the products were run in a 6% polyacrylamide denaturing gel and autoradiography was performed. MIN was defined as the presence of an extra band in one or in the two alleles in DNA from tumor as compared to DNA from normal colonic mucosa. Results: MIN in one or more cases was found in 15 (24%) patients, while 7 (11%) patients displayed MIN in at least 2 markers. We found that among patients with MIN+ tumors, familial history was more prevalent (33% vs 11%) although not significant. MIN+ and these tumors were more frequently located on the right side of the colon (P = 0.01). From a pathological point of view, MIN+ tumors were more frequently mucinous (29% vs 17%) and a higher percentage of them presented at an earlier stage (TNM staging stage I: 57% vs 30%). When considering solely patients with right sided neoplasms, the differences were even more striking. Patients with MIN+ tumors were younger (59 ± 14.4 vs 70 ± 8.5); familial history was found in 50% of the cases as compared to 0% in patients with MIN- tumors. Also, right sided MIN+ tumors relapsed in 50% cases but no relapses were detected in those MIN-.

Conclusions: MIN seems to identify a subset of patients in whom familial history is more prevalent, tumors are more frequently located on the right colon and despite a more aggressive morphology, they seem to have a better prognosis. Mutation analysis of the mismatch repair genes are needed to elucidate whether these patients are part of the HNPCC syndrome.

259 Non Specific Anal and Complex Fistulae: Results of MRI in the Diagnosis and Follow-Up
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Purpose: to assess the interest of MRI in the surgical management of preterminal anal fistulae in ano and in the follow-up of the cases treated by long term seton drainage with or two three stage fistulotomy.

Method: (1) 24 patients, crohn disease excluded, with recurrent high trans-sphincteric or supra-sphincteric fistulae (n = 20) and/or important anal or rectal stenosis (n = 11) and/or active suppuration (n = 5) were prospectively assessed with MRI before treatment [pre-op MRI]. Surgery consisted in excision (n = 24) eventfully completed by fistulotomy and long term seton drainage (n = 14).

(2) In the later cases a second MRI [post-op MRI] was systematically performed in order to assess the result of the drainage (n = 14).

(3) Long term (> 6 months) overall results were obtained in 20 patients (18 month mean follow-up).

Results: (1) [pre-op MRI]: the systematic comparison with surgical findings showed an overall accuracy of 84% for the visualization of internal opening, primary and secondary tracts, and 100% for the detection of horse-shoe fistula tract (77%).

(2) post-op MRI: in 5 cases, MRI modified the planned treatment indicating the necessity of a prolonged seton drainage or a new surgical excision by demonstrating the evidence of persistent infection.

(3) Long term clinical follow-up showed no evidence of recurrence for 19/20 patients (95%).

Conclusion: MRI accurately demonstrates the extension of high complex fistulae in ano and is helpful to assess the healing in cases treated with seton drainage and two three stage fistulotomy. Therefore it appears to be useful in the conservative surgical management of fistula in ano.

260 Immunohistochemical Study About the Neuronal Intestinal Dysplasia Type B in Whole Mounts of the Human Colon
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Neuronal intestinal dysplasia (NID) is well known, but its diagnosis is a topic of debate. The histopathologic diagnosis of NID is based on traditional enzyme-histochemical methods such as the acetylcholinesterase and lactate-dehydrogenase reaction on native sections. In this study, we have investigated the enteric nervous system in whole mounts of resected intestinal segments affected by NID of the plexus submucosus (type B). The plexuses of the tunica mucosa and tunica submucosa were visualized by immunohistochemical methods using a polyclonal antibody to protein gene product 9.5 (PGP 9.5). PGP 9.5 is a novel general cytoplastic marker specific for the
neurovascular. The morphology of the plexuses is revealed in full, making possible changes easily discernible. Known pathological changes of the NID can be identified and judged more precisely with this method. Numerous enlarged nerve trunks run within the tunica submucosa and tunica mucosa.

In the present study is shown that PGP 9.5 immunostaining overcomes many problems observed with other neuronal and glial markers. It has been possible to demonstrate the histopathological features of NID with PGP 9.5 in whom many patients with at least one ulcer of index ≥ 2, D14 being the main endpoint. The biological safety was assessed at each session.

Results: The two groups were comparable for age, sex, Child's score, % of active bleeding at inclusion and total dose of injected sclerotherapists at each visit. 39 patients (in gp 1 and 20 in gp 2) were withdrawn before D70 mainly due to complications of the underlying disease. % of patients with at least one ulcer of index 2 on D14 were 31.7% (13/41) in gp 1 and 23.1% (9/39) in gp 2 (p = 0.38). At D7 this criteria was: gp 1 = 1% (0/45) vs gp 2 = 14.6% (6/41); p = 0.006. At D28, D49 and D70 no significant difference was found between groups 12 and 14 severe adverse events were declared in groups 1 and 2 respectively. One of them was related to treatment in each group (2 cases of ulcer bleeding). For each liver and kidney function test, % of patients with a clinically relevant change was similar in the two groups. 0/3 (gp 1) and 3/30 (gp 2) patients had a gasrin level ≥ 2 N at the end of the treatment.

Conclusion: Anti-secretory treatment is not likely to prevent or accelerate healing of O.V.S.-induced ulcers after the 2nd sclerosis session. In cirrhotics, clinical and biological safety of Lansoprazole was excellent.

### 264 Percutaneous Microwave Coagulation Therapy for Liver Cancer

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Aim: Percutaneous ethanol injection therapy (PEIT) is widely performed as a percutaneous local treatment for liver cancer. It has been reported by many that the effects of PEIT were satisfactory. However, PEIT is occasionally ineffective for intracapular or extracapular invasion of cancer cells. In some cases, injected ethanol flows into the vessels around the tumor, instead of causing tumor necrosis. There is therefore a need for a more effective treatment to destroying liver cancer.

We designed aultrasonically guided percutaneous microwave coagulation therapy (PMCT) as a new method of percutaneous local treatment for liver cancer. In this presentation, we introduce the technique of PMCT and report the effect of PMCT comparing with that of PEIT.

**Subjects and Method:** Percutaneous local treatments were performed for the 94 patients having a single liver cancer (tumor size ≤ 3 cm) between Ja. 1990 and Ap. 1996. There were 46 patients treated by PMCT alone (Group M) and 48 who were treated by PEIT alone (Group E).

The microwave electrode (2.0 mm in thickness, 25 cm in length) was inserted through a guide needle (13G) to be placed in the tumor area which was then imaged by ultrasound. PEIT was performed conventionally with a 21G fine needle. The therapeutic results of these two groups were evaluated on the basis of survival rate, disease free survival rate, pattern of recurrences, and kind of re-treatment for recurrent cases.

**Results:** For Group M and E, the 5 yr-survival rates (4 yr-disease free survival rate) were 65% (39%) and 30% (17%), respectively. The recurrent rate of Group M and Group E at treated subsegment area within one year after treatment was 4% (2 cases) and 13% (6 cases), respectively. In recurrent cases of Group M and E, TAE was performed for 3 cases and for 8 cases, respectively.

### 265 Long Term Ganciclovir Therapy for Hepatitis B Virus Infection after Liver Transplantation

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HBV infection of liver graft is characterized by a severe outcome leading to graft rejection and is associated with high level of HBV replication. It has been suggested that GANICLOVIR (Roche-Syntex, USA) is active against HBV. We have studied the efficacy of long term GANCICLOVIR therapy for HBV infection on liver graft. 17 patients (pts): 12 with HBV reinfection and 5 with de novo HBV infection were studied. HBV DNA was positive in all pts, HBeAg in 8. Pts received IV GANCICLOVIR 10 mg/kg/day for 14 days, 5 mg/kg/day for 30 days or 1 mg/kg/day for 3 to 5 times a week after a month (0, 3, 5, 7, 9, 11, 13). HBeAg and HBV DNA (Digeno Hybrid Capture System; Murex, France), were tested for every month. At time of onset of treatment, mean HBV DNA titer was 756 pg/ml (12-2000), liver graft histology showed no specific changes (n = 1), acute hepatitis (n = 5), chronic active hepatitis (n = 8), cirrhosis (n = 3). GANCICLOVIR was well tolerated. During therapy, HBV DNA negativation (complete response (CRI)) was observed in 11 pts, decrease of more than 50% of HBV DNA values (partial response) in 3 and absence of response in 3. Among the 11 complete responders, 4 pts relapsed under (n = 1) or after (n = 1) therapy. This last pt presented a CR after a second course of GANICLOVIR. HBeAg clearance occurred in 3 pts. A dramatic clinical improvement was observed in 4 pts. Last histology showed cirrhosis in 6 pts, CAH in 3 and submassive hepatitis in 2 who were retransplanted. One pt died from variceal rupture. Among the 3 partial responders, none died or was retransplanted, last histology showed cirrhosis in 2 and CAH in 1. Among the 3 non responders, 1 pt suspected for HBV related graft failure and 2 developed chronic active hepatitis.

Conclusion: GANICLOVIR is effective to inhibit HBV replication after LT and is well tolerated. Despite HBV DNA negativation and clinical improvement, an histological deterioration may be observed.

### 266 Prevention of Hepatitis B Reinfection after Liver Transplantation by Post Transplant Long-term Administration of Ganciclovir IV and Anti-HBs Immunophrophylaxis in Patients at High Risk of Viral Recurrence

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The risk of HBV recurrence after liver transplantation (LT) is high in patients (pts) with HBV replication despite use of long-term anti-HBs immunophrophylaxis even associated with preLT Interferon (IFN). The aim of this study was, in HBV DNA positive pts, to assess the effect of a single preLT, post-LT long-term administration of anti-HBs immunoglobulins and GANICLOVIR (Roche-Syntex, USA) 9 pts (8 M, 1 F, mean age 45.8 yrs), Hbs Ag and HBV DNA positive were included. The initial diagnosis was: HBV recurrence on first graft (4), acquired HBV infection post-transplant (1), HBV cirrhosis (3), and subacute hepatitis HBV (1). Each patient received antiviral therapy prior to LT: ARA AMPS then GANCICLOVIR (4), GANCICLOVIR (2), IFN (2), APA AMP then IFN (1). LT recipients long-term anti-HBs immunophrophylaxis to achieve anti-HBs Ab titer above 500 mIU/ml were tested every week from day 2 to day 120. Graft histology was available in 7 pts at 1 year and was normal. Tissue antigen detection of HbsAg and HbsAb was negative. One pt died at 2 years of carcinoma unrelated to HBV infection. Conclusion: This open study demonstrates the efficacy of a combination of post-transplant long-term anti-HBs immunophrophylaxis and IV GANCICLOVIR in pts at high risk of HBV recurrence.

### 267 Hepatitis G Virus Infection in France: Preliminary Epidemiologic Data and Analysis of Viral tropism

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**Goal:** The two main goals of the present study are: 1) to evaluate the prevalence of the newly discovered hepatitis G virus (HBV G (GBV-C) in different groups of French patients 2) to analyze, in the patient group displaying the highest prevalence, the cellular tropism of GBV-C viruses.

**Methods:** Prevalence of infection was assessed by means of RT-PCR amplification of viral sequences from the NS3 region (nestest amplification) using degenerated primers (adapted from Simons et al., Nature Medicine

Conclusion: Compared with PEIT, PMCT is an effective liver cancer in producing local necrosis.
HVG (GB-C) and HCV Coinfections in French Intravenous Drug Users: Prevalence and Histological Impact

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We have analyzed 77 anti-HCV positive intravenous drug users (IVDU) to 1) define on a large series the actual prevalence of HGB-C (coinfected in this population and 2) evaluate the impact of these coinfections on liver biological tests and histology.

Methods and patients: 77 anti-HCV positive French IVDU (63 males and 14 females), aged 32 ± 5 years, including 24 anti-HIV positive. Serum HCV RNA was tested by PCR in 79/100 and HGV (GB-C) RNA was tested by PCR with NS5 (GB-C) and NS3 (HGV) sequences.

Results: HCV: RNA was detected in 67/77 and HCV typing showed genotypes 1a (22), 1b (9), 3a (16) and 4/15. HGV (GB-C): HGV (GB-C) RNA was detected in 16/77 (20.7%), 14 males and 2 females. For 14 of these 16 were infected by HGV. HGV (GB-C) RNA positive and negative individuals showed similar duration of drug abuse (11.5 and 10.2 years, respectively).

There was no significant differences between HGV (GB-C) RNA positive and negative patients for ALAT/AST and GGT levels nor histology Knodell scores (5.1 ± 3.2 vs. 5.2 ± 3.2) (NS), respectively. Finally HCV genotype distribution did not differ in the 2 groups.

Conclusions: Our study demonstrates a high prevalence of HCV/HGV (GB-C) coinfections in French IVDU, whether or not coinfected by HIV and 2) shows no evidence for worsening of liver lesions due to these coinfections.

Is Interferon Alpha Atherogenic?

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Interferon-α (IFN) is the treatment of choice for Chronic Hepatitis C (CHC). Recent report has suggested that serum triglycerides (TG), cholesterol (XOL), cardiac risk indexes (XOL-LDLXOHL), Apo A1/Apo A1) increased during IFN therapy (Malaguarnera M, Hepatology 1995, 38/5). This study was performed to define the effect of IFN on serum TG, XOL, XOL-HDL, XOL-LDL, Apo A1 and Apo B in adults with CHC. Methods: 33 patients were evaluated (mean age 48 ± 28 years; 46% were male). All had (+) anti-HCV by third generation antibody assay and RIBA-4, (+) serologies for all other causes of chronic hepatitis, and liver histology compatible with CHC. All patients were treated with 3-6 MU of IFN TIW for six months. The following parameters were determined and monitored using routine laboratory tests before and during treatment (3 to 6 months): Serum XOL, TG, XOL-HDL, XOL-LDL was determined by Friedwald's formula: Apo A1 and Apo B using the nephelometric method. The Apo B/Apo A1, XOL-LDL/XOL-HDL ratios were considered. Statistical analysis was used conducting Student's test for paired data. Results: Mean serum apo A1 and HDL decreased from respectively 1.30 ± 0.20 g/l ± 0.18 g/l to 1.26 ± 0.30 mmol/l to 1.02 ± 0.52 mmol/l (p < 0.001). No statistical difference was found for serum apo B, XOL, XOL-LDL. Mean serum TG increased from 1.11 ± 0.50 to 1.93 ± 2.8 mmol/l (p = 0.019). A (p < 0.001) and B/Apo A1 and XOL-LDL of XOL-LDL was analyzed for the presence of cardiac risk index increased respectively from 0.71 ± 0.26 to 0.85 ± 0.24 (p = 0.005) and from 2.63 ± 2.1 to 3.1 ± 1.1 (p = 0.005). Conclusion: 1) Serum TG increase and Apo A1 decrease during IFN therapy, the mechanism of which is unknown. 2) Interferon-α therapy should be explored. A) an increase of cardiac risk index by reduction of lipoprotein-lipase (LPL) activity. B) an increase of TG hepatic synthesis by IFN and/or C) a decrease of Apo A1 hepatic synthesis 4) In the future patients with basal increase of cardiac risk index must be considered carefully regarding IFN therapy.
resulting in the formation of fibrosis and tissue repair. EGFR was shown to promote cellular growth and the regeneration of pancreatic tissue and the influence of TGF-f1 and EGF on the course of acute pancreatitis has not been studied. In the present study we investigated the changes TGF-f1 and EGFR expression as well DNA synthesis, pancreatic blood flow (PBF), protein content and plasma amylase concentration in the course of human-induced acute pancreatitis. Methods: Wistar rats weighing 200-250 g were infused with supramaximal dose of caerulein (10 mg/kg/h s.c.) for 5 h to induce pancreatitis. Rats infused with saline served as a control. Animals were killed at 1, 2, 3, 4 and 5 h after the start of infusion. The PBF was measured using laser Doppler flowmeter and blood was collected to determine serum amylase concentration. The pancreatic tissue was removed and biopsy samples were taken for measurement of the protein content, DNA synthesis (by incorporation of H thymidine) and TGF-f1 mRNA expression. TGF-f1 and EGFR mRNA was studied by reverse-transcriptase polymerase chain reaction (RT-PCR) and assessed semiquantitatively as undetectable (−), minimally expressed (+) or strongly expressed (++). Results: Caerulein infused at 1, 2, 3, 4 and 5 h caused a time-dependent decrease in DNA synthesis as compared to vehicle-controls, by 11%, 15%, 45%, 49%, and 54%, respectively. This was accompanied by gradual decrease of PBF by 29%, 36%, 43%, 50%, and by 54%, respectively, and a significant increase in pancreatic weight reaching after 3-5 h 157%, 163% and 173% of control value, respectively. The protein content and plasma amylase concentration showed progressive increase with the peak achieved after 5 h of caerulein infusion. Histological analysis of the tissue, strong cell vacuolization and prominent leukocyte infiltration starting after 3 h of caerulein infusion. Following the development of pancreatitis, TGF-f1 mRNA was strongly expressed at each time interval beginning from the 1 h after the start of caerulein infusion. By contrast, EGF mRNA was first detected 5 h after induction of pancreatitis. Conclusions: 1) During the development of pancreatitis is observed an inhibition of pancreatic tissue growth and PBF accompanied by enhanced expression of TGF-f1. 2) The expression of EGF at the end of the pancreatitis development may indicate the initiation of pancreatic repair. 3) TGF-f1 seems to lead to sequential induction of EGF that stimulates the regeneration of injured pancreas.

278 The Role of TGF-f1 and IL-6 in Pancreas Regeneration

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Some reports suggest that low doses of cholecystokinin octapeptide (CCK-8) have inhibitory effects on the development of pancreatitis, TGF-f1 and EGFR mRNA expression was studied by reverse-transcriptase polymerase chain reaction (RT-PCR) and assessed semiquantitatively as undetectable (−), minimally expressed (+) or strongly expressed (++). Results: Caerulein infused at 1, 2, 3, 4 and 5 h caused a time-dependent decrease in DNA synthesis as compared to vehicle-controls, by 11%, 15%, 45%, 49%, and 54%, respectively. This was accompanied by gradual decrease of PBF by 29%, 36%, 43%, 50%, and by 54%, respectively, and a significant increase in pancreatic weight reaching after 3-5 h 157%, 163% and 173% of control value, respectively. The protein content and plasma amylase concentration showed progressive increase with the peak achieved after 5 h of caerulein infusion. Histological analysis of the tissue, strong cell vacuolization and prominent leukocyte infiltration starting after 3 h of caerulein infusion. Following the development of pancreatitis, TGF-f1 mRNA was strongly expressed at each time interval beginning from the 1 h after the start of caerulein infusion. By contrast, EGF mRNA was first detected 5 h after induction of pancreatitis. Conclusions: 1) During the development of pancreatitis is observed an inhibition of pancreatic tissue growth and PBF accompanied by enhanced expression of TGF-f1. 2) The expression of EGF at the end of the pancreatitis development may indicate the initiation of pancreatic repair. 3) TGF-f1 seems to lead to sequential induction of EGF that stimulates the regeneration of injured pancreas.

283 Overexpression of ICAM-1, VCAM-1 and ELAM-1 in Colorectal Carcinomas

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Purpose of the Study: The pathogenesis of colorectal cancer and the mechanisms which contribute to metastases are still poorly understood. Adhesion molecules are cell-surface-bound glycoproteins which are important in cell-to-cell attachment. Changes in the expression of adhesion molecules may increase the risk for local invasion and hematogenic metastases of colorectal cancer cells.

Patients and Methods: Cancerous tissue samples were obtained from 5 female and 19 male patients with a mean age of 67 years (range 46-84 years) undergoing colonic resection due to carcinoma of the colon or rectum. Tumors were classified according to the TNM-system (UICC): 5 stage I, 10 stage II, 1 stage III and 8 stage IV. Normal colonic tissues from the same patients served as controls. Tissues destined for RNA extraction were frozen in liquid nitrogen immediately upon surgical removal. In addition, freshly removed tissue samples were fixed in Bouin solution and paraffin embedded for histological analysis. Expression of ICAM-1, VCAM-1 and ELAM-1 was analyzed by Northern blot analysis using specific cRNA probes. In addition, immunohistochemical analysis using specific monoclonal antibodies was performed.

Results: By Northern blot analysis ICAM-1, VCAM-1 and ELAM-1 mRNA were increased in 16/24 (67%), 12/21 (57%) and 15/24 (63%) carcinomas, respectively in comparison with the normal tissue samples. Densitometric analysis of Northern blots revealed a 2.1-fold increase of ICAM-1 (p = 0.006), a 3.4-fold increase of VCAM-1 (p = 0.02) and a 2.2-fold increase of ELAM-1 (p < 0.002) in cancerous tissues compared to controls. Linear regression analysis showed co-expression between ICAM-1 and VCAM-1 (r = 0.8) and ICAM-1 and ELAM-1 (p > 0.05). These findings are consistent with previous reports of enhanced ICAM-1, VCAM-1 and ELAM-1 immunoreactivity in endothelial cells of cancer blood vessels. Furthermore, the intercellular matrix of cancer samples exhibited more intense ICAM-1-immunostaining than the stroma of controls.

Conclusion: Our findings suggest a role of adhesion molecules in tumor pathogenesis and disease progression. The overexpression of these factors might increase the ability of colonic cancer cells to attach in blood vessels and distant organs and thereby contribute to tumor invasion and metastasis.

284 Overexpression of Cyclin D1 is a Common, Important and Early Event in Gastrointestinal Tumorigenesis Process

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Cyclin D1 is a cell cycle regulator essential for G1 phase progression. Our laboratory has shown that: a. Cyclin D1 is amplified in about 30% of esophageal squamous cell carcinomas, b. A cyclin D1 antisense construct introduced into human esophageal and colon cancer cell lines reduced their tumorigenesis. To further determine the importance of Cyclin D1 in gastrointestinal tumor formation, we have extended our previous studies and examined the expression of cyclin D1 in expression was determined from nuclear immunoreactivity in 719 samples.
Organ | Normal | Inflammation | Adenoma | Carcinoma
--- | --- | --- | --- | ---
Esophagus | 0/94 | 0/39 | 31/99 (41) | 26/37 (67)
Stomach | 0/87 | 0/32 | 26/37 (67) | 16/33 (48)
Small bowel | 0/88 | 0/32 | 26/37 (67) | 16/33 (48)
Large bowel | 0/88 | 0/32 | 26/37 (67) | 16/33 (48)
Pancreas | 0/85 | 0/32 | 26/37 (67) | 16/33 (48)

Number of positive/total (%), *Barrett's esophagus, Both squamous and adenocarcinomas.

Cyclin D1 immunoreactivity was not seen in hyperplastic polyps nor in inflammatory tissues and did not correlate with the mitotic index; implying that cyclin D1 expression is not merely a marker of increased proliferation. Cytoplastic immunostaining was seen in about 25% of the tissues, sometimes without nuclear staining, possibly representing a novel role of cyclin D1. Increased expression was associated with advanced age, well differentiated tumors and smoking status. Cyclin D1 overexpression was found in 70% of the intestinal type of gastric cancers and only 8% of the diffuse type. It was also significantly higher in the left colon than the right colon (48% and 11%, respectively).

We conclude that increased nuclear expression of cyclin D1 occurs in many gastrointestinal tumors; as an early event during the multistage process of carcinogenesis. It was also seen in adenomatous polyps and Barrett's esophagus. Increased expression of cyclin D1 may perturb cell cycle control and thereby enhance tumor progression. These findings suggest that cyclin D1 may be a useful target in cancer therapy.

825 Increased Retinoblastoma Protein (pRb) Expression Occurs Throughout the Adenoma-Carcinoma Sequence in the Colon, and is Associated with, but does Not Parallel Increased Proliferation

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In colonic mucosa, as in other normal tissues, pRb expression is G0 and G1 is believed to mediate growth inhibition. In tumors, deregulation is typically associated with a decrease in pRb expression. In colorectal carcinoma (CRC) pRb expression is increased. We investigated the hypothesis that pre-invasive adenoma epithelium is associated with increased pRb expression, thus confirming increased pRb expression as a feature of the entire adenoma-carcinoma sequence in CRC.

To evaluate pRb expression in colorectal adenomas and correlate this with proliferation.

Normal colorectal mucosa, 11 hyperplastic polyps, 52 adenomas (31 low grade dysplasia (LGD), 21 high grade dysplasia (HGD), (30 tubular, 22 villous)) were immunostained using monoclonal antibodies to pRb and PCNA, by standard immunoperoxidase techniques.

Colorectal adenomas were confined to the proliferative compartments of colonic crypts (< 10% cells staining). In hyperplastic polyps 10/11 (91%) showed < 10% pRb expression in a pattern resembling that seen in normal crypts. 45/52 (87%) of adenomas showed increased pRb expression throughout the epithelium, (mean 45 ± 25, range 10-90%). 32-52 (62%) of adenomas showed increased PCNA expression throughout the epithelium, (mean 50 ± 22 range 10-90%). In adenomas, individual pRb expression did not correlate with increased PCNA expression and discordant percentage of expression was seen in 46/52 (88%) cases. Increased pRb expression does correlate with the grade of dysplasia, but does not correlate with growth pattern.

Increased pRb expression (1) occurs in the earliest stages and throughout the adenoma-carcinoma sequence in CRC from tubular adenomas to large villous adenomas; (2) is not a feature of hyperplastic polyps; (3) shows increased pRb expression in HGD compared with LGD; (4) is associated with, but does not parallel increased proliferation.

826 Deregulated Apoptosis Contributes to the Development of Human Colon Cancer

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It has been shown that cells of the luminal surface epithelium exhibit fragmentation of the nucleus, suggesting that programmed cell death, apoptosis, was involved in the superficial loss of intestinal cells. Normal tissue homeostasis requires the physiologic deletion of cells by activation of apoptosis. Inhibition of apoptosis by the deregulation of certain oncogenes results in clonal expansion. The search for potential endogenous regulators of apoptosis (e.g. Apo, p53, DCC, MCC, and RAS) has been frustrated by the traditional approach of attempting to inhibit apoptosis using pharmacological agents (e.g. thymidine kinase mutant adenovirus, Targeted Gene Therapy, TUNEL).

There are two main apoptosis pathways: the mitochondrial pathway and the death receptor pathway. The latter involves the activation of the death receptor protein, Fas, which triggers a cascade of events leading to the activation of caspases and the cleavage of DNA. The pro-apoptotic Bcl-2 family members, such as Bax and Bad, promote cell death by interacting with anti-apoptotic members, such as Bcl-2 and Bcl-xL, which can block the release of pro-apoptotic proteins from the mitochondria. The pro-apoptotic Bax protein is a substrate for caspase-8, a key enzyme in the death receptor pathway.

828 Effect of Octreotide Acetate and 5-Fluorouracil on the Human Rectal Neuroendocrine Carcinoma (Adenocarcinoid) Xenograft in Nude Mice


Colorectal neuroendocrine carcinoma (NEC) is an aggressive tumor with a high tendency to metastasize and its prognosis is poor. At the 4th UEGW (1995), we have reported the establishment of human rectal adenocarcinoma in nude mice xenograft. Here we examined the effect of a somatostatin analog, octreotide acetate (OA), and 5-fluorouracil (5-FU) on the growth of this tumor.

Materials and Method: Tumors of a 3 mm cube were implanted bilaterally into the flank of nude mice. Mice were randomly divided into 4 groups (n = 10 mice/group) and agents were intraperitoneally administered as 2 weeks for group 1: saline, group 2: OA (300 μg/kg), group 3: 5-FU (10 μg/kg), group 4: OA + 5-FU (OA 300 μg/kg + 5-FU 10 mg/kg). The bodyweight of mice and tumor size were measured weekly.

Results: 1) Both OA and 5-FU, administered as single agents, inhibited the tumor growth compared with control group, and 5-FU was more effective than OA; however, after stopped administration of these agents, tumors had grown as well as the control group. 2) OA and 5-FU treated in combination, most significantly inhibited tumor growth, and inhibition continued to the time of killing. 3) There were no difference in body weight of mice between the experimental groups and control group.

Conclusion: OA and 5-FU inhibited growth of NEC in nude mice. This tumor will be a useful experimental model to elucidate the biological behavior of human NEC and further study of various therapeutic agents on this tumor is important.

829 Importance of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) in Duodenal Secretion

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Surface epithelial bicarbonate is an important factor in preventing acid-peptic
Injury, as well as facilitating pancreatic enzyme function. HCO₃⁻ is likely transported with CI⁻ by CFTR. However, the role of CFTR in duodenal secretion has been ignored. Thus, our aims were to determine if CFTR regulates basal and acid-stimulated HCO₃⁻ transport, as well as CAMP- and Ca²⁺-mediated secretion. The cystic fibrosis (CF) murine model (cfr⁻/⁻munc) was used with genotyping confirmed by PCR. Normal littersmates (25–46 d, 17 ± 3 g) were compared to CF [CFTR (−/−)] mice (22–39 d, 13 ± 3 g). Anesthesia was induced and maintained with hypnomidazolam (i.p.); animals (37°C) were hydrated with saline. The proximal duodenum (~4–7 mm) was cannulated and perfused with 154 mM NaCl (0.17 mM/l min). Stimulation was accomplished with either intrasegmental perfusion of HCl (10 mM/l, 5 min), PGE₂ (10⁻⁶–10⁻⁴ M), forskolin (10⁻⁴–10⁻⁴ M), carbachol (10⁻⁶–10⁻³ M), or VIP (5 pmol/g, i.p.) to activate either CAMP- or Ca²⁺-stimulated secretion; N = 4 for each series. HCO₃⁻ secretion was measured by a validated micro-balloon titration method. Initial studies demonstrated that animals could be maintained under these conditions for 3 h (stable basal HCO₃⁻ secretion, respiration rate, plasma [HCO₃⁻]). Basal HCO₃⁻ secretion was diminished significantly (P < 0.001) in CFTR (−/−) mice. HCO₃⁻ secretion was significantly (P < 0.01) impaired in response to all secretagogues (Figs). Furthermore, normal littersmates demonstrated net fluid secretion during basol which increased in response to stimulation, whereas CFTR (−/−) expressed net absorption (P < 0.05 vs. normal) and was largely unresponsive to agonists. We conclude that CFTR plays a key role in regulating epithelial bicarbonate transport, as well as duodenal secretion, processes likely mediated by CAMP.

### 290 Pantoprazole Versus Famotidine in the Treatment of Acute Duodenal Ulcer Disease


**Objective:** Pantoprazole is a new, precise proton pump inhibitor. It was the aim of the present clinical study to compare the efficacy and tolerability of pantoprazole (40 mg) and famotidine (40 mg) in outpatients with acute duodenal ulcer under the conditions of routine gastroenterological practice.

**Methods and patients:** Open, 2:1 randomized, controlled, multicenter study. 456 Outpatients with acute and uncomplicated duodenal ulcer (diameter 3–20 mm) were recruited by 72 investigators to receive either pantoprazole (n = 307) 40 mg o.d. (morning) or famotidine (n = 149) 40 mg o.d. (evening) for 2 weeks, or 4 weeks if endoscopic healing was incomplete by 2 weeks. Demographic data and ulcer size were comparable in both treatment groups.

**Results:** Endoscopically proven healing rates (primary study end-point, intent-to-treat analysis [per protocol]):

<table>
<thead>
<tr>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoprazole</td>
<td>82% [97%]</td>
</tr>
<tr>
<td>Famotidine</td>
<td>69% [76%]</td>
</tr>
</tbody>
</table>

Among the patients with pain prior to treatment, complete freedom of pain after 2 weeks was achieved in 87% of the patients in the pantoprazole group and in 72% of the patients in the famotidine group (p = 0.01). Both drugs were well tolerated.

**Conclusions:** Pantoprazole (40 mg o.d.) was highly effective and well tolerated in the acute treatment of duodenal ulcer. Clinically relevant superiority of pantoprazole over famotidine was achieved with respect to healing and pain relief, while the tolerability of both drugs was comparable.

### 291 Comparison of Pantoprazole and Ranitidine in Acid-Related Diseases


**Objective:** It was the aim of this phase-IV clinical trial program to assess the efficacy and safety of pantoprazole in the acute treatment of acid-related diseases in outpatients under the conditions of routine gastroenterological practice.

**Methods:** 3 open, 2:1 randomized, controlled, multicenter trials (duodenal ulcer [DU]: 73 centers; gastric ulcer [GU]: 56 centers; reflux esophagitis [GERD] grade III according to Savary-Miller: 130 centers) were performed. Pantoprazole (P) 40 mg o.d. (morning) was compared to ranitidine (R) 300 mg o.d. (evening) for 4 weeks (DU: 2 weeks), or 8 weeks (DU: 4 weeks) if endoscopic healing was incomplete after 4 weeks (DU: 2 weeks).

**Patients:** In total 1473 patients with uncomplicated disease were enrolled (DU: n = 476; GU: n = 274; GERD: n = 723). 991 patients were treated with P and 482 received R. For each of the three clinical trials, baseline and demographic data were comparable for patient groups treated with P and R, respectively.

**Results:** Endoscopically proven healing rates (intention-to-treat analysis):

<table>
<thead>
<tr>
<th>Indicator</th>
<th>P vs. R at 4 weeks</th>
<th>P vs. R at 8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>DU</td>
<td>87% vs. 67%***</td>
<td>96% vs. 91% (p = 0.06)</td>
</tr>
<tr>
<td>GU</td>
<td>82% vs. 70%*</td>
<td>91% vs. 82%*</td>
</tr>
<tr>
<td>GERD</td>
<td>75% vs. 54%***</td>
<td>87% vs. 66%***</td>
</tr>
</tbody>
</table>

P provided better pain relief compared to R. Both treatments were very well tolerated, without any relevant differences in the frequency or severity of reported adverse events.

**Conclusion:** Pantoprazole (40 mg) was significantly more effective than ranitidine (300 mg) in terms of healing rates as well as pain relief in patients with an acid-related disease, confirming the results from previous phase-III trials. Both treatments were well tolerated.

### 292 Clinical Tolerability of Pantoprazole Compared to Ranitidine and Famotidine


**Objective:** The German pantoprazole phase-IV clinical trial program was designed to assess not only the efficacy but also the safety of pantoprazole in acute treatment of acid-related diseases in outpatients.

**Methods:** Six open, 2:1 randomized, controlled, multicenter trials (duodenal ulcer [DU]: 145 centers; gastric ulcer [GU]: 96 centers; reflux esophagitis [GERD] grade II and III: 259 centers) were performed comparing pantoprazole (P) 40 mg o.d. (morning) with ranitidine (R) 300 mg o.d. (evening) or famotidine (F) 40 mg o.d. (evening). Treatments were for 4 weeks (DU: 2 weeks), or 8 weeks (DU: 4 weeks) if endoscopic healing was incomplete after 4 weeks (DU: 2 weeks). Patients: In total 2842 patients were enrolled (DU: n = 932; GU: n = 453; GERD: n = 1467). 1915 patients received P, 482 received R and 445 received F.

**Results:** Most frequently reported adverse events (cut-off incidence: 0.4%, overall safety analysis of the six studies, intention-to-treat):

<table>
<thead>
<tr>
<th>Pantoprazole symptom</th>
<th>Ranitidine symptom</th>
<th>Famotidine symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>0.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Hyperlip./hyperchol.</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>GPT/GOT increase</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Conclusion:** Pantoprazole (40 mg), ranitidine (300 mg) and famotidine (40 mg) were found to be equally well tolerated in the collective of 2842 patients studied.

### 293 Pantoprazole in Long-Term Management of H₂-Blocker Refractory Acid-Peptic Disease

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**Aim:** The efficacy and tolerability of the H₂⁻K⁺-ATPase inhibitor pantoprazole (PAN) in H₂-blocker refractory acid-peptic disease was investigated.

**Methods:** Patients with acute reflux esophagitis or peptic ulcer refractory to extended high-dose H₂-blockers were treated with 40–120 mg PAN daily for 4–12 weeks, depending on healing, in an open-label trial. Healed patients were admitted to maintenance treatment for up to 5 years. Upper GI endoscopy was performed at admission, every 4 weeks during the healing phase, and every 6 months during long-term treatment. Intermediate results are presented.

**Results:** Healing of the acute lesions was achieved in 129/141 patients (91.5%) after 4 weeks and 140 (99.3%) after 12 weeks. In one patient with severe esophagitis, the lesion took more than 6 months to heal. By the time of this analysis, 115 patients were on maintenance treatment for at least one year, 89 for 2 years, 60 for 3 years, 27 for 4 years, and 9 for 5 years. Most patients were kept in remission with 40–80 mg PAN daily; 20 patients required higher doses up to 320 mg. Most frequent treatment-related adverse events were puritus and tiredness (in two patients each). Four patients with reflux disease and full-blown liver cirrhosis tolerated this treatment without any side-effects up to five years. Routine laboratory tests remained without significant changes throughout the entire period of treatment. Median serum gastrin levels were already elevated at baseline due to pretreatment with H₂-blockers (72 pg/ml, 68% range 41–191) and increased to 120.0 pg/ml (68–260) after one year of maintenance treatment without any further consistent increase thereafter. Median ECL cell density in the oxyntic mucosa increased slightly from 0.3% to 0.5% after 3 years.
Conclusions: The data demonstrate that pantoprazole is highly effective and safe in acute healing and long-term treatment of H₂-blocker refractory peptic ulcer and reflux esophagitis and also in patients with advanced liver disease.

Methods and patients: Between October 1994 and December 1995, 71,906 patients (female 38.3%, male 61.7%) were enrolled in this PMS program (DU 40.9%, GU 22.2%, reflux esophagitis 28.7%, other 8.2%). The majority of patients presented with a first episode of their disease (56.5%). 88.9% of patients received pantoprazole in the recommended daily dose of 40 mg for a mean treatment period of 22.75 days (SD ± 14.08).

Adverse events n %

Diarhoea 136 0.19
Nausea 132 0.18
Headache 112 0.16
Dizziness 93 0.13
Gastrointestinal 65 0.09
Exanthema/Urticaria 52 0.07

295 Rabeprazole Effectively Inhibits 24 Hr H⁺ Activity and Nocturnal Acid Secretion in Healthy Subjects

H.G. Dannmann 1, F. Burkhardt 1, N.E. Bell 1, T. Bjäland 1 2. 1 Institute for Clinical Research, Hamburg, Germany; 2 Eklas Europe, London, UK

In humans rabeprazole has produced a dose-dependent, potent and long lasting inhibition of acid secretion. No significant incremental effect was noted with doses greater than 20 mg. Purpose: The purpose of this study was to determine the effect of an oral dose of rabeprazole 20 mg on 24 hr intragastric pH, 24 hr H⁺ activity and nocturnal acid secretion in healthy subjects following a 14 day dosing period. Methods: 12 young healthy male subjects (mean age 27.5 yrs) were investigated in this single centre, double-blind, randomised, 2-period crossover comparison of rabeprazole 20 mg and placebo given orally once in the morning for 14 days. On Days 1-8 intragastric pH was monitored and nocturnal acid secretion measured by continuous aspiration (00.00 to 06.00 hrs) and titration of gastric contents. The pH measurements were extended for a 72 hr period after the last dose of medication (Days 14, 15 and 16). pH measurements were collected using a Synectics Mk II system and a nasogastric tube with pH electrode.

Results: In comparison to placebo, rabeprazole 20 mg significantly inhibited 24 hr H⁺ activity (table) and nocturnal acid secretion (36.6 ± 6 vs ± 2 mmol/00.00-06.00 hrs).

24 hr H⁺ activity (AUg = 24 ± mmol/l/h, means ± SE)

<table>
<thead>
<tr>
<th>Day</th>
<th>Placebo</th>
<th>Rabeprazole</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>503 ± 71</td>
<td>72 ± 27</td>
<td>86</td>
</tr>
<tr>
<td>14</td>
<td>343 ± 102</td>
<td>44 ± 12</td>
<td>87</td>
</tr>
<tr>
<td>21</td>
<td>501 ± 121</td>
<td>126 ± 31</td>
<td>75</td>
</tr>
<tr>
<td>28</td>
<td>379 ± 74</td>
<td>146 ± 29</td>
<td>61</td>
</tr>
</tbody>
</table>

Conclusions: Rabeprazole 20 mg produced a significant and long lasting inhibition of acid secretion. Inhibition of up to 87% was observed. The half-life for recovery of acid secretion was longer than 72 hours. Based on published data rabeprazole 20 mg appears to be at least as potent and as long acting as omeprazole and lansoprazole.

296 Tolerability and Safety Profile of Pantoprazole Based on 71,906 Patients. Results of a German Post Marketing Surveillance Program

E.G. Hahn 1, H. Bossecker 1, H.G. Dannmann 1, M. Schlander 1, Erlangen, FRG; 2 Berlin, FRG; 3 Hamburg, FRG; 4 Konstanz, FRG

Purpose: Pantoprazole is a novel proton pump inhibitor (PPI) with precisely defined pharmacological properties, profound antisecretory capacity and high clinical efficacy in the treatment of acid related diseases. This PMS program was designed to determine the tolerability and safety profile of pantoprazole.

Methods and patients: Between October 1994 and December 1995, 71,906 patients (female 38.3%, male 61.7%) were enrolled in this PMS program (DU 40.9%, GU 22.2%, reflux esophagitis 28.7%, other 8.2%). The majority of patients presented with a first episode of their disease (56.5%). 88.9% of patients received pantoprazole in the recommended daily dose of 40 mg for a mean treatment period of 22.75 days (SD ± 14.08).

Conclusion: The pantoprazole PMS program proves the favourable tolerability and safety profile of this novel PPI.
between the three doses. Dosing with rabeprazole resulted in a significant dose-related increase of mean 24-hour plasma gastrin – the differences between the 10, 20, and 40 mg doses, and the 20 and 40 mg doses, being significant (p = 0.002 and 0.037, respectively). Conclusion: Rabeprazole is a potent gastric acid antisecretory drug: a single daily dose (10 mg, 20 mg or 40 mg) causes a significant decrease of 24-hour intragastric acidity with reciprocal rise of plasma gastrin concentration.

Purpose: PD-136,450 is a selective ligand for the CCK-B receptor in vitro with binding affinity for mouse cortex = 500-fold greater than for rat pancreatic CCK-A receptors. This study characterises gastric acid and pancreatic bicarbonate secretions together with anxiolytic activity of PD-136,450 in the rat. Methods: Gastric and pancreatic secretions were measured by titration at 10 min intervals. Basal acid output and secretion stimulated by s.c. injection of 32 µg/kg gastrin-17 or 10 mg/kg dimaprit were determined in anaesthetised rats or conscious animals fitted with indwelling fistulae. Pancreatic secretions were collected via a catheter inserted into the main duct of anaesthetised rats. Anxiolytic activity was assessed by a standard black and white two-compartment activity assay. Results: As anticipated, PD-136,450 inhibited gastrin-stimulated acid output in anaesthetised or conscious rats (IC50 of 1 mg/kg s.c.). Doses up to 10-fold higher had no effect on dimaprit-induced acid output (255 µmol/hr before and 243 µmol/hr after PD-136,450). Of note, PD-136,450 increased pancreatic secretion. At a dose of 4.5 mg/kg it showed similar efficacy to CCK-B with bicarbonate output rising from 36 to 207 µmol/hr 60 min after s.c. dosing and remaining elevated for > 3 hr. This action was inhibited by 75% after pretreatment with the CCK-B antagonist L364,718 (bicarbonate output 55 µmol/hr) but was not affected by the CCK-B antagonist L365.60 (output 211 µmol/hr). Time spent in the dark compartment by rats pretreated with 10 mg/kg PD-136,450 was reduced 36% compared with control (p < 0.01, n = 6). This response was similar to the effect of 5 mg/kg diazepam (41% inhibition). Latency for movement from the light to the dark compartments increased similarly with PD-136,450 and diazepam.

Conclusions: PD-136,450 is a selective inhibitor of gastrin-stimulated acid secretion in vivo. The drug also stimulates pancreatic bicarbonate secretion and displays anxiolytic activity comparable to diazepam. While PD-136,450 has weak antisecretory activity compared with H2 blockers or PPIs, it may have utility as an adjunct therapy in peptic ulcer disease by counteracting the actions of gastrin and increasing acid neutralization.

**301** Evaluation of the Antisecretory Activity of Pantoprazole in Duodenal Ulcer Patients by Continuous 24-hour pH Monitoring

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The extent and duration of acid suppression are considered of critical importance for healing of acid-related disorders and optimizing activity of anti-inflamatory drugs in H. pylori eradication. Pantoprazole (Pan) is a novel proton pump inhibitor that shows a unique selective binding to the H+/K+-ATPase of gastric parietal cells. Its main distinctive features are represented by minimized potential for interactions with human cytochrome P450 enzyme system, the stability and lack of activation in neutral environment and a predictable pharmacokinetic profile. The aim of our study was to evaluate the effects of Pan on 24-h intragastric acidity in duodenal ulcer (DU).

Pan 40 mg man was orally administered for 5–7 days to 20 patients with DU in clinical remission. 18 out of them were H. pylori positive.

Continuous 24-h intragastric pH-meter was performed in basal conditions and after treatment, according to well established procedures. Mean 24-h, nighttime and daytime pH values, and mean times above pH thresholds of 3.0, 4.0, and 5.0 have been evaluated as acidity indexes. Two-way ANOVA was used for statistical analysis.

A significant reduction of gastric acidity was induced by Pan compared to basal levels (p < 0.001), in terms of both mean pH in the different time intervals and times spent above several pH thresholds (see Table).

**302** Comparison of Intravenous Famotidine and Ranitidine in Suppression of Gastric Acid Secretion in Reflux Esophagitis and Duodenal Ulcer Bleeding

M. Tuner, A. Dobrucal, I. Yurdakul, C. Davutoglu, N. Bagatur, F. Harmisoglu, A. Cekil, E. Oktay, Gastroenterology Department of Cerrahiaga Medical Faculty of Istanbul University, Istanbul, Turkey

Purpose: We investigated the efficacy of H2-receptor blockers in raising gastric pH to above 4 in patients with reflux esophagitis and duodenal ulcer bleeding and we compared the efficacy of intravenous famotidine and ranitidine in this subject.

Methods: 44 patients with endoscopically proven reflux esophagitis (grade II, III, Savary-Miller classification) and duodenal ulcer bleeding. 44 patients (28 males, 16 female, 28–68 yrs) were assigned randomly to receive intravenous bolus doses of either famotidine 20 mg every 12 hours (n = 22) or ranitidine 50 mg every 8 hours (n = 22) for mean 5 days. Gastric juice was aspirated before the start of treatment (base-line) and six times during each 24 hour period; pH was measured by a pH meter.

Results: Measured base-line pH was not significantly different between the two groups. Famotidine raised gastric pH to higher level than did ranitidine, reaching statistical significance (p < 0.05) for 28 of 44 collection periods. In Conclusion; When given by intermittent intravenous bolus, famotidine 20 mg every 12 hours is more effective than ranitidine 50 mg every 8 hours in raising gastric pH to above 4 in reflux esophagitis and duodenal ulcer bleeding.

**303** Efficacy of Ebrotidine vs Ranitidine in the Treatment of Benign Gastric Ulcer

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Purpose. To assess the efficacy of Ebrotidine 400 mg compared with Ranitidine 300 mg nocte, in the treatment of benign gastric ulcer.

Methods. A randomized phase III parallel double-blind trial. 104 patients from 4 hospitals were enrolled in the study. 98 of them (94.23%) completed the treatment and were evaluated. Patients gathering the entry criteria were randomized to receive Ebrotidine or Ranitidine until the endoscopic cure of ulcer, or until a maximum of 12 weeks of treatment. Basal endoscopy was repeated after 6 and 12 weeks of treatment. The chi-square test was used to compare the number of patients with a complete remission of the ulcer, and the ANCOVA test was used to compare reduction in the size of the ulcer.

Results. Both groups were homogeneous and without differences for the basal parameters studied. In the intent to treat analysis Ebrotidine was effective in 88.2% of the patients and Ranitidine in 78.7% (N.S.). There were significant differences (p = 0.017) concerning the size of the ulcer at six weeks of treatment (95% CI, 0.5589 mm to 1.8724 mm for Ebrotidine and 1.6337 mm to 4.9621 mm for Ranitidine). Adverse events were absent in both groups of patients.

Conclusions. Ebrotidine appears to be significantly faster than Ranitidine in the healing of the benign gastric ulcer. Ebrotidine is a new drug with anti-secretory profile, anti-H2 and gastroprotective activity, thus conferring a significant advantage versus Ranitidine and possibly other types of antisecretors drugs.

**304** A Comparison of Intragastric Acidity Following Low Doses of Ranitidine and Cimetidine

M.R. Hamilton 1, J. Sercombe 1, R.E. Pounder 1, C.C.L. Snell 2, 1 Royal Free Hospital School of Medicine, London, NW3, UK; 2 Glaxo Wellcome Research & Development, Middlesex, UK

This randomised, 3-way, crossover study compared the effects of low doses of ranitidine and cimetidine on intragastric pH.

Methods: Thirty healthy subjects (18 male, 12 female) took part in the study. On three separate occasions single oral doses of placebo, ranitidine 75 mg (one Zantac 75® tablet) and cimetidine 200 mg (two Tagamet 100® tablets) were taken after lunch at 12:30 h. The pH of gastric aspirates was measured for 20 hours after the dose (Day: 12:30–22:30 h, night: 22:30–08:30 h). Subjects ate standard meals (lunch and supper) and snacks on each study day.

Results: The decrease in intragastric acidity, relative to placebo, was 58.7% after ranitidine and 35.4% after cimetidine during the day and 18.3% and 2.0%, respectively, during the night. The decrease in acidity after ranitidine was significantly greater than cimetidine during the day and night. Both study
drugs were well tolerated. Only one event (itchy rash) was considered related to study drug (cimetidine).

Conclusions: The increase of acidity after ranitidine was significantly greater in magnitude and of longer duration than that following cimetidine. A linchtime dose of ranitidine 75 mg caused a significant decrease of nocturnal acidity.

305 Trough Plasma Bismuth Concentrations during Long-Term Treatment with Ranitidine Bismuth Citrate
Ranitidine bismuth citrate (RBC, PYLORID®) is used for the healing of duodenal and gastric ulcers and, when co-prescribed with certain antibiotics, for the eradication of Helicobacter pylori (H. pylori). In acute studies, bismuth absorption was found to be of no clinical concern. In this 6 month study, trough plasma bismuth concentrations (i.e. approximately 12 hours after dosing) were monitored as a measure of chronic bismuth exposure.

Bismuth concentrations were determined in 190 symptomatic H. pylori-positive patients randomised to receive RBC 400 mg bd or comparator (ranitidine 150 mg bd), by inductively coupled plasma mass spectrometry.

Median, 95th percentile and maximum plasma bismuth concentrations (ng/mL) in the RBC group were:

- Week of dosing: 4 13 26
- No of patients: 91 80 77
- Bismuth concentration (ng/mL)
  - Median: 3.07 5.14 5.65
  - 95th percentile: 10.57 20.80 20.49
  - Maximum: 22.81 54.20 74.49

Median bismuth concentrations for patients receiving ranitidine were below quantification limit (< 0.2 ng/mL).

Long-term administration (6 months) of RBC 400 mg bd resulted in extremely low trough plasma bismuth concentrations.

306 Role of β-Adrenoceptors and Nitric Oxide in the Circulatory, Metabolic and Protective Effects of Epidermal Growth Factor (EGF) in the Stomach
EGF is considered to play an important role in the maintenance of gastrointestinal mucosal integrity. The evidence exists that pointed to the trophic and vascular effects of EGF in the mechanism of its gastroprotective activity. The aim of this study was to investigate the involvement of beta adrenergic receptors in the vascular and protective actions of EGF in the stomach. Two series of dogs and rats were performed. In anesthetized dogs with fundoplication preparation, total gastric blood flow (GBF) was determined ultrasonically and mucosal blood flow (MBF) by laser Doppler flowmetry. Gastric oxygen consumption (GVO2) and systemic arterial pressure (AP) were also determined. EGF administered i.a. at dose 2.0 mg/kg increased GBF, MVO2 by 58 ± 9, 123 ± 22% and 37 ± 8% respectively, not change AP. Pretreatment with propranolol (5 mg/kg i.v.) significantly reduced above and metabolic responses induced by EGF. In rats acute gastric lesions were induced by 100% ethanol. Mucosal blood flow (LDF) was measured by laser Doppler technique area which was also determined in mm. Ethanol induced mean lesion was 110 ± 12 mm and reduction in LDF by 75%. Pretreatment with EGF (100 mg/kg i.v.) decreased the lesion area by 86 ± 10% (p < 0.05) and increased LDF by 72 ± 8% (p > 0.05). Pretreatment of rats with propranolol (5 mg/kg i.p.) abolished the protective effect of EGF. Propranolol alone was without any effect on the ethanol induced gastric damage. These data provide evidence that EGF is a potent vasodilator of gastric circulation and modulator of gastric tissue oxygenation. This peptide possess also protective properties which, at least in part, may depend on its diatery activity. Both, vascular and protective effects of EGF appear to be mediated by beta adrenergic receptors.

307 Inhibition of the Gastric H,K ATPase and Acid Secretion by a New Anti-Ulcer Drug
W.A. Simon, R. Boer, C. von Bödingen, K. Munson 1, G. Sachs 1, Byk Gulden, Konstanz, Germany; 2 UCLA, Los Angeles, USA
Background: A new anti-ulcer drug, the imidazol 1,2x pyridine BY841, that inhibits gastric acid secretion, is in clinical trial for treatment of acid related diseases.

Aims: To correlate the binding of the drug with inhibition of the gastric H,K ATPase and to determine the relative efficacy of this inhibitor compared to a H,K ATPase antagonist.

Methods: Purified hog gastric vesicles were used to compare inhibition of the ATPase with binding of BY841 and determine K competition with BY841. Isolated rabbit gastric glands were used to compare the effects of ranitidine and BY841.

Results: The Ki for inhibition of the ATPase by BY841 was 6 nM and the 

308 Deposition of Bismuth from Different Compounds. An Advantage in Using Ranitidine Bismuth Citrate (RBC)?
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Purpose: To determine if treatment of rats with bismuth compounds can lead to their deposition in several organs after therapy, 30 days after stopping treatment and to see if this is affected by gastric pH.

Methods: 48 male wistar rats were gavaged twice daily, during 15 days, with placebo (PL, n = 14), bismuth subcitrate alone (B, n = 14)-13.7 mg/Kg/day, or in association with ranitidine (8.6 mg/Kg/day)-(BR, n = 8) and with RBC, (n = 12):22.8 mg/Kg/day. After 2 weeks 6 rats of PL, B and 6 of RBC groups were euthanized and samples of blood, liver, kidney, brain and lung were removed. After 30 days, remaining rats were killed and organs were collected. Intragastric pH was assessed at the 10th day with a glass electrode connected to a pH measuring device. Bismuth was assessed by Particle Induced X-ray Emission and concentrations are expressed in µg/g of dry weight.

Results: Intragastric pH were: PL:1.7 ± 0.2, B:2.3 ± 0.1, BR:3.6 ± 0.2, RBC:3.5 ± 0.1. In PL group all analysed samples showed bismuth levels below detection limit (2 µg/g). After 15 days bismuth concentration was:

- B: < 0.01 µg/g
- BR: 0.29 (100%)
- RBC: 0.29 (100%)
- * p < 0.001 (t-student). Blood values above 2 µg/g were found in 75% of B group, in 35.7% of BR group and in 83% of RBC group. After 30 days all rats had values below 2 µg/g. Conclusions: Treatment with bismuth can lead to its deposition in several organs and it is not influenced by gastric pH. Bismuth deposition was lower in brain and blood levels correlated poorly with organ deposition. One month after stopping therapy bismuth deposition was not detected and this is a clear advantage in using RBC as far as organ deposition is concerned.

309 Safety and Efficacy on Symptom Relief of Pantoprazole: Interim Analysis of a French Prospective Study
H. Licht, G. Giret-d’Orsay, R. Samoyeau on behalf of the Eupantol study group. Hôp. Delafontaine 93 St Denis-Lab. Byk France 77 Le Mée/Seine
Objective: Pantoprazole is a potent inhibitor of acid secretion which binds precisely to the key cysteines of the gastric H+/K+ ATPase. This trial was conducted to assess the safety and efficacy on symptoms of pantoprazole 40 mg/day given for the acute treatment of acid-related diseases.

Methods: Open, prospective, multicentric trial. The general practitioners (n = 900) selected the patients and the gastroenterologists (n = 300) included them after the endoscopic evidence of ulcer or erosions. Patients were treated for 1 or 2 months.

Results: In duodenal ulcer patients (n = 166), the most frequently reported symptoms at entrance were characteristic ulcer pain (70%) and nausea and vomitting (40%). 89% of patients with characteristic ulcer pain and 97% of patients with nausea and vomitting were free of symptoms at day 7. Smokers, age under 50 and male gender seem to be predictive factors for rapid symptom relief.

Gerd patients (n = 620) were classified as follows: stage I: 56%, stage II: 30%, stage III: 8%, stage IV: 6%. The most frequently reported symptoms at entrance were: heartburn (78%), acid regurgitations (73%), non characteristic epigastric pain (35%), dysphagia (23%). 87% of patients with characteristic Gerd symptoms were asymptomatic at day 7. The median time to be free of symptoms without intake of antacid was 4 days independently of the original stage. Non smoker status, age over 50, and the male gender seem to be predictive factors for a rapid pain relief.

Safety was analysed on 1020 patients. The adverse events possibly or certainly related to the test medication were: diarrhea (2.4%), headache (2.3%), abdominal pain (1.6%), dizziness, asthma. These rates correspond to those described with the other PPIs.

Conclusion: On a large population representative of acid-related disease patients, pantoprazole is effective on symptoms relief. The tolerability was excellent. These results have to be confirmed by the final analysis.
310 Influence of the H+, K+-ATPase inhibitor Pantoprazole on Blood Ethanol Levels in Healthy Humans

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Background: Alteration in gastric first pass metabolism of ethanol during administration of several H2 receptor antagonists has been previously reported. In the H+, K+-ATPase inhibitor omeprazole interplay interaction was not detected. Aim: To study the effect of the H+, K+-ATPase inhibitor pantoprazole on blood ethanol levels after taking a moderate dose of ethanol in healthy humans. Methods: 16 healthy volunteers (12 male, 4 female; mean age 27 years) received either 40 mg pantoprazole or placebo orally at 8.00 AM for 7 days in a double blind, randomized, cross over design, separated by a 14 day wash out. On day 7 a standardized breakfast was given at 8.00 AM, directly after administration of pantoprazole. At 10.00 AM, 200 ml of orange juice containing 5.0 g/kg body weight pure ethanol, were given within 5 min. Blood samples were taken at 10–30 min intervals for 4 h and then hourly until 8.00 PM. Ethanol concentrations were determined by a modified ADH enzymatic assay (ALC, Du Pont). For confirmatory analysis the area under the curve (AUC) of blood ethanol levels over 8 hours (10.00 AM–6.00 PM) was calculated. Lack of interaction was handled as an equivalence problem (Steinjäger et al., 1991). Results: The seven day administration of pantoprazole caused no significant change in peak ethanol concentration (Cmax, %) and in 8 h integrated (AUC) blood ethanol levels (% h) after ingestion of 0.5 mg/kg ethanol as compared to placebo. Independent from the sequence of treatment regimens, no side effects were reported.

Table: Peak ethanol concentration (Cmax, %) and in 8 h integrated (AUC) blood ethanol levels (% h) after ingestion of 0.6 mg/kg ethanol

<table>
<thead>
<tr>
<th>Pantoprazole</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (% h)</td>
<td>1.277 (0.855–1.485)</td>
</tr>
<tr>
<td>Cmax (%)</td>
<td>0.435 (0.339–0.558)</td>
</tr>
</tbody>
</table>

Values are geometric means and 68% ranges; the 90% confidence intervals of the pantoprazole/placebo ratio (AUC: 0.91–1.2; Cmax: 0.94–1.11) were within the equivalence range (0.8–1.25).

Conclusions: 1.) A therapeutic dose of pantoprazole does not alter the pharmacokinetics of orally administered ethanol. 2.) Sporadic intake of ethanol does not influence the safety and tolerability of pantoprazole when concomitantly taken.

311 Activity of γ-Glutamyl-Transferase (γ-GT) in Blood Serum and Mucosa in Helicobacter Pylori Infected Subjects

Grzyna Kuprińska, Dept. of Infectious Diseases and Gastroenterology MAA, Kloniowski I/5, 93-347 Lodz, Poland

γ-Glutamyl-transferase (γ-GT) is a heterogenic enzyme. In physiological environment hepatic fractions (iso-enzymes) make only about 40% of the whole activity of this enzyme and the remaining part comes from other organs. The increase of γ-GT is assumed to be a sensitive factor of tissue damage and neoplasia. The purpose of this study was estimation of γ-GT activity in serum and gastric mucosa in Hp-infected subjects. The studies were performed in 26 men, aged 42–66 years, whose histopathological diagnosis showed active, chronic gastritis. Hp infection was confirmed by microscopy and enzymatic examination (urease test). Other diseases particularly of liver and pancreas were excluded. The patients were not administered any drugs or alcohol. Before and six weeks after treatment (lamotidine + amoxycylcline + metronidazol) γ-GT was determined in serum according to kinetic method and in fundic and antral mucosa – by method of Cormay γ-GT – TRIS no cat. 1-033 acc. to F. Hoffmann-La Roche. The following results were obtained:

<table>
<thead>
<tr>
<th>γ-GT</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (µl/mg protein)</td>
<td>7.78 ± 7.3</td>
<td>61.3 ± 8.2</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fundic</td>
<td>14.3 ± 5.2</td>
<td>9.1 ± 5.4</td>
</tr>
<tr>
<td>antrum</td>
<td>16.7 ± 8.0</td>
<td>10.4 ± 5.9</td>
</tr>
</tbody>
</table>

It has been concluded that, Hp infection is the reason of the increase of γ-GT activity in gastric mucosa and serum. The role of Hp infection in gastritis pathogenesis also proves the necessity of antibacterial treatment.

312 Profferative Response to Helicobacter Pylori (Hp) CagA Protein in Cultured Blood Lymphocytes from Infected Subjects

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In organ specific infections the significance of local immune reactivity can be different from that of peripheral blood lymphocytes (PBL). The organ draining blood could represent an intermediate compartment between the mucosa associated and the systemic immunity.

Aim of our study was to investigate if the PBL were able to proliferate to Hp CagA protein and if this assay had any specificity in Hp infected patients. Moreover we compared the data with those obtained testing the lymphocytes isolated from gastric vein blood (GVBL). Patients supposed to undergo abdominal surgery (not for neoplastic diseases) underwent a thorough study for Hp infection. During the same operation the susceptibility of PBL and GVBL to P-19 was obtained by puncture of the gastric draining veins and the antecubital vein. Lymphocytes were purified and cultured in the presence of several mitogenic stimulus (anti CD3, anti EAGEN IgG) and the proliferative response was measured by means of transformed thymidine uptake. Hp CagA protein induced lymphocyte proliferative response in a high percentage of Hp infected subjects (71.5% GVBL and 57% PBL) while it did not in the Hp negative patients. Comparing the Hp positive subjects, the mean lymphocyte proliferative response to CagA was significantly higher in the GVBL (p < 0.05) while no difference was detected for the other mitogenic stimulus. Analysing the patients as a whole, GVBL showed a significantly higher response than P-19 PBL.

It is known that the Hp CagA protein can induce a humoral immune response. We have detected a different functional behaviour of GVBL compared to PBL. We have demonstrated that T lymphocytes from Hp infected subjects specifically proliferate when challenged with this antigen and that GVBL of such patients show a more frequent and higher response.

313 Detection of Cytotoxic Associated Gene a (CagA) as Serodiagnostic Marker in Diagnosis and Treatment of Duodenal Ulcer (DU) and Non-steroidal Anti-inflammatory Pseudopy (NSAP)

E. Karczewicz, A. Bobrynski, S. J. Kortunek, H. Kaendar, Immunologie, Univ Sch Med, Krakow, Poland; OrVax Inc, Cambridge, MA, USA

Purpose: It has been proposed that about 60% of Helicobacter pylori (Hp) isolates express CagA and that this protein induces serum IgG antibodies. Infection with Hp expressing CagA was suggested to increase the risk of DU but no comparative studies have been made regarding the expression of CagA in DU and NUD during anti-Hp therapy.

Methods: This study included 50 Hp-positive (by 14C-UBT, CLO, histology and culture of gastric biopsy) DU patients with active ulcers, 50 symptomatic NUD patients and 25 Hp-negative healthy control. Serum samples were obtained at day of initial endoscopy, 2 wk after triple therapy (omeprazole 20 mg bd, amoxicillin 750 mg bd and metronidazole 500 mg bd) and 4 wk after completion of this therapy. The presence of serum IgG antibodies to Hp was determined by ELISA using EAGEN HP IgG test. The patients to CagA sera were detected by ELISA using recombinant CagA (ORV220) as antigen.

Summary of results: All tested Hp-positive DU and NUD patients but none of healthy controls had positive serology for IgG. Serum IgG antibodies to CagA were positive in 80% of DU, in 40% of NUD and in none of healthy controls. After 2 and 6 wk of anti-Hp therapy, which succeeded in 95% eradication of Hp of both in DU and NUD and complete healing in DU, there was significantly gradual decrease in IgG and CagA titre both in DU and NUD, reaching ~80% of patients.

Conclusions: 1) Expression of Hp is strongly associated with Hp infection and is about twice higher in DU than in NUD patients suggesting that CagA expression increases the risk of DU and may serve as serodiagnostic marker to implement anti-Hp therapy. 2) Gradual decrease in serum IgG and CagA titre may be useful in documentation of the progress in DU healing.

314 Suppression by Sulglycotide of H. Pylori Protease Activity towards PDGF and TGβ3

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Sulglycotide, a chemically sulfated derivative of duodenal mucin, is a potent cytotoxic agent also recognized for its remarkable inhibitory activity towards Helicobacter pylori (Hp). Since, Hp is known to undermine the gastric mucosal integrity through a variety of enzymes capable of rapid destruction of gastric mucosal defense potential, in this study we assessed the effect of sulglycotide on the susceptibility of PDGF and TGβ3 to Hp protease. The experiments were carried out with Hp, strain ATCC 45304. The plates with grown colonies were washed with 0.9% NaCl, filtered (0.2 µm) to retain the bacteria, and the filtrate was used as an enzyme source. The incubation mixtures for Hp protease assays consisted of 15 labelled PDGF or TGβ3, enzyme protein (50–100 µg), sulglycotide (0–100 µg) and 0.22 µl phosphate buffer, pH 7.0. After 1 h incubation at 37°C, the incubates were chromatographed on an agarose-Gel-P-2 column and the introduced 125I labelled peptide fragments were measured in a gamma counter. The results of analysis of the eluted fragments revealed that Hp protease caused extensive degradation of growth factors. Under the assay conditions Hp protease evoked a 6.3% 32P 49% degradation of PDGF and a 62.3% degradation of TGβ3. The presence of sulglycotide to the reaction assay system caused a dose dependent inhibition in PDGF and TGβ3 proteolysis by Hp enzyme. The maximal inhibitory effect was obtained with sulglycotide at 100 µg/ml, at which dose an 84.4% decrease in PDGF and 88.3% decrease in TGβ3 degradation was achieved. These results demonstrate that sulglycotide by exerting a strong inhibitory activity on the Hp protease towards PDGF and TGβ3 is capable of promoting the proliferative action of these peptides in mucosal repair process.
Mucosal Levels of Prostaglandins E₂ (PGE₂) and F₂₀α (PGF₂₀α) in Helicobacter Pylori (HP) – Positive Chronic Gastritis

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The interaction between mucosal levels of PGE₂ and PGF₂₀α and histological features of CG HP-positive still remain to be elucidated.

Aim: to assess the relationship between the CG HP-positive activity and concentration of PGE₂, PGF₂₀α in the gastric mucosa.

Methods: 73 patients (pts) with CG HP-positive (22 men, 51 women age 43.7 years) were randomized into three groups. Group 1 (n=21) – pts with histological features of superficial gastritis and neutrophil polymorph infiltration (active metaplasia); Group 2 (n=33) and group 3 (n=19) – without polymorph infiltration (non-active gastritis), but with temperate and severe mucosal atrophy conformity. Group 4 – control (n=20) included healthy volunteers. Multiple gastric antral and corpus biopsies were performed in every subject. HP was sought by histology and biopsy urease test. PGE₂ and PGF₂₀α concentrations were investigated by RIA.

Our results are presented in the table:

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration PG (ng/ml)</th>
<th>Concentration PGF₂₀α (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E₂</td>
<td>corpus</td>
</tr>
<tr>
<td>1</td>
<td>1.54 ± 0.54*</td>
<td>1.88 ± 0.64*</td>
</tr>
<tr>
<td>2</td>
<td>1.64 ± 0.54*</td>
<td>1.81 ± 0.34*</td>
</tr>
<tr>
<td>3</td>
<td>1.02 ± 0.34*</td>
<td>1.1 ± 0.54*</td>
</tr>
<tr>
<td>4</td>
<td>6.02 ± 1.1*</td>
<td>6.37 ± 1.2*</td>
</tr>
</tbody>
</table>

*p < 0.05 with above mentioned conclusions

Discussion/Conclusion: The found disbalance between concentration of PGE₂ and PGF₂₀α may be very important in the pathogenesis of CG-associated with HP.

Emerging Patterns of Helicobacter Pylori (H. Pylori) Antimicrobial Susceptibility in Europe -

Q.N. Karim 1, R.P. Logan 2 for the GloxWellcome H. pylori Study Group. 1 St Mary’s Hospital, Paddington; 2 University Hospital, Nottingham

Introduction: H. pylori antimicrobial susceptibility is an important determinant of the efficacy of antimicrobial therapies [1]. The prevalence of antimicrobial resistance varies within Europe and is likely to increase given the diverse number of regimen and dosages currently used. This multicentre study assesses the prevalence of H. pylori antimicrobial resistance in the United Kingdom.

Methods: H. pylori was isolated from antral biopsies of patients undergoing routine endoscopy and cultured according to standard microbiological methods (blood/chocolate agar in a microaerophilic environment, incubated for up to 10 days). Antimicrobial resistance was determined using "E-tests" or disc tests (tindazole only) and breakpoints were taken from previous studies.

Results: H. pylori has been isolated from 32% (1222/3823) of biopsies and antimicrobial susceptibility determined in 90% (1077/1122) of isolates. The percentage of resistant to average resistances (ranges) for the most widely used antimicrobials are as shown in the table below:

<table>
<thead>
<tr>
<th>Metronidazole</th>
<th>Tindazole</th>
<th>Clarithromycin</th>
<th>Tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.6 (14.6-65)</td>
<td>28.2 (7.2-41)</td>
<td>4.8 (1.3-12.5)</td>
<td>2.7 (2-6.3)</td>
</tr>
</tbody>
</table>

The prevalence of resistance to metronidazole was greater in isolates from inner city centres (45.1%, n = 488) compared with rural centres (17.7%, n = 273). The same was shown with resistance to clarithromycin (4.8% vs. 1.7%).

Nineteen isolates showed resistance to both clarithromycin and metronidazole. Multiple resistance was therefore seen in 5.3% of isolates.

Discussion/Conclusion: The results demonstrate the wide variation of antimicrobial resistance to H. pylori. In the UK metronidazole resistant H. pylori is endemic. Multiple antimicrobial resistance seen in approximately 5% of H. pylori positive isolates, underlines the importance of establishing local patterns of antimicrobial resistance.


Nitric Oxide Synthase Activity in Gastric Mucosa: Influence of Helicobacter Pylori Colonization and NSAID Administration

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Purpose: Nitric oxide (NO) is related to several gastric processes including protective and aggregative effects. In this sense, NO synthetized by constitutive enzyme has protective effects such as an increase of mucus production and a major proliferative index of epithelial cells. These effects are mediated by cGMP. As opposed to this, NO released by inducible enzyme is related to appearance and maintenance of inflammatory processes. On the other hand, Helicobacter pylori (Hp) and NSAID administration have the ability to induce gastric damage by different mechanisms: Hp increases aggregative factors and NSAID decreases protective effects. In this sense, we have studied the influence of Hp colonization and NSAID administration on the NOS activity and the CGMP levels in gastric mucosa.

Methods: Determination of NOSI and NOSc activity by measuring 14C-Citrate formation (pmol of tissue/1 min) and cGMP levels (pmol of tissue/1) in gastric biopsies of 78 patients of Gastroenterological Service of Virgen Macarena Hospital, Seville. The sample study was conducted by 11 normal subjects, 7 patients with NSAID-induced gastric damage, 35 with gastritis Hp+, 6 patients with gastritis Hp−, and 19 patients Hp+ and with duodenal ulcer. Results: NOS activity, both constitutive and inducible are not significantly different when normal, and gastritis patients (Hp+ and Hp−) were contrasted (normal NOS: 17.2±4.24 and NOSI: 20.89±3.81; gastritis patients Hp+: NOS: 35.67±12.26 and NOSI: 31.24±6.27). Similarly there were no significant differences when cGMP gastric levels were compared (normal: 100.5±5.27 and cGMP Hp−: 76.59±21.85; and gastritis patients Hp+: 81.57±14.28). On the contrary, the NOS activity in patients with duodenal ulcers was significantly increased in comparison with normal and gastritis patients (NOSI: 14.53). The cGMP concentration is similar to normal. In patients with NSAID-induced gastric damage, the NOS activity is significantly lower than normal (NOS: 27.5±12.7). The same result was observed when cGMP was determined (22.08±5.24).

Conclusions: NO can be related to gastrectic ability of Hp through the NOSI activation and perpetuation of inflammatory processes. On the contrary, the NSAID-induced gastric injury can be related with a fall in NOS activity and decrease of cGMP levels in gastric mucosa. This last results is in concordance with others experimental results obtained in rats.

Effect of Helicobacter Pylori Infection on Gastric Endocrine Cell Behavior of Duodenal Ulcer Patients

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Aim. This study investigated the influence of H. pylori infection on gastrin-immunoreactive cell (G-cell) and histamine-immunoreactive cell numbers in stomachs of duodenal ulcer patients.

Method: Endoscopic biopsy specimens from antrum of 22 duodenal ulcer patients were fixed by Carnoy's fluid and immune-stained using antibodies of gastrin and H. pylori. Another specimens from gastrin bodies were fixed by 4% [1-ethyl-3 (3-dimethyl-aminopropyl)-carbodiimide] (EDCDI) and 4% paraformaldehyde (PFA), and they were immune-stained using antibodies of histamine.

The numbers of gastrin and histamine cell were counted. The degree of H. pylori infection was classified to three groups according to the ratio of the numbers of gastric pits where H. pylori exist in 50 pits at random.

Conclusions: The result suggests that H. pylori infections has a stimulatory effect on gastrin cell numbers, but the severe infection in antrum seemed to induce the damage and decrease of gastrin cells. Histamine cell numbers were also increased according to the degree of H. pylori infection. The increase of gastrin-immunostained cell in gastrin body could be caused not only the increase of enterochromaffine-like cells but also those of mucosal mast cell.

Induction of Gastric Epithelial Apoptosis by H. Pylori Lipopolysaccharide and Its Suppression by Sucralfate

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The preservation of gastric mucosal homeostasis is a highly complex biological process that involves the programmed cell death. Under normal physiological conditions the mucosal integrity is maintained by a dynamic equilibrium of cell loss, by apoptosis with that of cellular proliferation, while the enhanced cell apoptosis is a prominent feature in HP-associated gastritis. In this study we assessed the effect of HP lipopolysaccharide (LPS) on the induction of gastric epithelial cell apoptosis. The experiments were conducted with rat gastric mucosa in intragastric surface epithelial treatment with a dose of 50 µg HP LPS or LPS preincubated with 100 µg sucrafate. The animals were sacrificed 2 days following the treatment and their stomachs subjected to quantification of apoptotic epithelial cells. The cells undergoing apoptosis were identified using terminal deoxynucleoaid transferase-mediated dUTP-digoxigenin nick endlabeling assay. The slides were developed with diaminobenzene reagent and counterstained with methyl green. The sections were subjected to counting and the number of positive cells was expressed as the apoptotic index (AI %).

The results of microscopic assessment revealed only occasional presence of apoptotic cells in the surface epithelium from the control group (AI 2.6%), while in the LPS group a number of apoptotic cells were identified not only in the superficial epithelium but the deeper gastric glands. The presence of HP LPS with sucrafate prior to animal treatment led to a marked reduction in the epithelial cell apoptosis (AI 4.9%). The findings demonstrate that HP induces apoptosis through its LPS and that sucrafate is capable of suppressing this untoward effect of HP.
Corporal Lymphoid Folicles (LF) Do Not Discriminate Autoimmune or H. Pylori Related Chronic Atrophic Gastritis (CAG)

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CAG is a condition characterized by atrophy of oxyntic mucosa hypoachlorhydria and fasting hypergastrinemia. It has been considered an autoimmune condition, usually associated with latent or overt pernicious anemia (PA), but it has recently been observed that a small proportion of CAG patients are Hp infected. However, since the progression of corporal gastritis is accompanied by disappearance of H. pylori, the persistence of immunological memory, expressed by the presence of IgG to Hp, could indicate past exposure to the bacterium. Presence of LF in gastric biopsy specimens have been described as a constant feature of H. pylori-associated gastritis. Aim of this study was to investigate in a consecutive series of newly diagnosed CAG patients, the prevalence of present or past infection and the presence of LF as a histological marker of H. pylori infection. 104 consecutive hypergastrinemic CAG patients (84 F, 20 M aged 22–81) were divided in three groups as follows: Histo+ = histology, colt, IgG negative. Histo+ histology, colt, IgG positive; at least two of these tests positive. IgG+ = histology and colt negative, only IgG positive (± 40 Uf; Elisa, Biorad). Corporal atrophy was defined as focal or complete replacement of oxyntic glands by metaplastic pyloric or intestinal glands. LF were defined as intramucosal, basally located lymphoid aggregates with or without germinal centers.

<table>
<thead>
<tr>
<th>CAG groups</th>
<th>% Positive lymphoid follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum</td>
<td>Corpus</td>
</tr>
<tr>
<td>Histo– (n = 58; 55.8%)</td>
<td>16.7</td>
</tr>
<tr>
<td>Histo+ (n = 20; 19.4%)</td>
<td>50.0</td>
</tr>
<tr>
<td>IgG– (n = 21; 20.2%)</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Results: In a consecutive, newly diagnosed series of CAG patients, autoimmunity accounts for 55.8%, whereas active or past H. pylori infection for 44.2%. Corporal LF are widely present but do not discriminate the two different etiologic causes. In the antrum of Histo+ pts, LF are present in the 50%, being significantly higher than the other two groups (Fisher test p < 0.005). Conclusions: These data show that 44.2% of CAG pts have been infected H. pylori and that corporal LF are not exclusive markers for the presence of H. pylori infection.

Helicobacter Pylori Biotypes in Patients with Peptic Ulcers

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Objectives: We isolated Helicobacter pylori from the collected gastric mucosa in patients with peptic ulcers. The obtained strains were classified into biotypes according to the presence or absence of activity of some enzymes, and the differences of these biotypes among peptic ulcers was investigated.

Methods: The subjects were 164 patients with peptic ulcers, who had undergone endoscopic examination including H. pylori. They consisted of 82 with gastric ulcer, 61 with duodenal ulcer, 21 with gastric and duodenal ulcer. In upper gastrointestinal endoscopy, gastric mucosal samples were collected from the greater curvatures of the antrum and the gastric body incubated under microaerophilic conditions (5% O2, 10% CO2 and 85% N2) for 5–7 days. The produced colonies were allowed to grow in blood agar medium for 3–7 days. A bacterial solution was used for experiments using API ZYM Kit (Bio Meriux S.A., France) for determination of H. pylori biotype. H. pylori was classified into biotypes I, II and III according to Kung’s classification.

Results: Investigation of the clinical isolates from 164 patients with peptic ulcers showed that 154 (94%) were positive for incubation (with Squiraw medium). Biotypes I, II and III were revealed in 7, 48 and 46 cases, respectively. There were some patients who showed different biotypes in the antrum and the gastric body of the same patient. This type was designated as mixed type. The patients who showed such mixed types consisted of one with mixed type I + II, one with mixed type I + N.D. (non-differentiated), and 18 patients with mixed type II + III. As a result of assessment of biotypes according to each disease, the frequencies of biotypes II and III were high in each peptic ulcer. There was no significant difference between disease and biotype using the chi-square test of independence.

Conclusions: The frequencies of Kung’s types II and III were high on the evaluation of H. pylori biotypes in patients with peptic ulcers. There were some patients who showed different H. pylori biotypes in the stomach of the same patient. There were no significant relationships between various peptic ulcers and biotypes.

Enhanced Cellular Proliferation and PS5 Accumulation in Gastric Mucosa Chronically Infected with Helicobacter Pylori

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Aims: The purpose is to evaluate whether the increased risk of gastric carcinoma development due to Helicobacter pylori (H. pylori) infection might be linked with cellular proliferative activity and oncoprotein overexpression. Subjects: Forty-eight patient undergoing therapy for H. pylori positive gastroduodenal ulcers were separated into not eradicated (NE:23 cases) and eradicated (E:25 cases) group 6 months after the treatment.

Methods: Histological changes in gastric mucosa and antrum, assessed according to modified Sydney System, as well as epithelial cell kinetics (mitosis, Ki 67, PCNA), and expression of oncoproteins (p53, bcl-2) were examined before and at 3 months and 6 months after treatment for H. pylori. Results: Chronic persistent H. pylori infection was associated with increased inflammation and activity score, as well as elevated proliferation, as evidenced by the Ki677 and PCNA labeling indices and the mitotic index in NE group. Overexpression of p53 protein continued to be observed in the NE group after treatment but was significantly decreased in the E cases. Conclusions: Persistent H. pylori infection causes gastritis, with epithelial degeneration and regeneration that result in accentuation of epithelial cell proliferation and overexpression of p53 protein, this presumably heightening the risk of gastric carcinoma development.

Effect of Helicobacter Pylori (HP) Infection on Oxygen Metabolism in Gastric Mucosa

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In pathogenesis of gastrointestinal tract diseases more and more often the role of active forms of oxygen is taken into consideration especially as they cause the direct damage to mucosa. The overproduction of free oxygen radicals is stimulated by many pathologic factors which include also Hp infection. The aim of this study was the estimation of selected parameters of aerobic metabolism in subjects with Hp dependent gastritis before and after infection eradication. Investigations were carried out in 30 subjects, aged 38–73 years, with histopathological diagnosis of chronic gastritis. Hp infection was confirmed by urease test and histological examination. In gastric mucosa bioplate frozen to −70°C, there were determined: – malonic dialdehyde (MDA) – by Yagi method; – glutathione peroxidase (Gpx) – by Paglia and Valentine method; – superoxide dismutase (SOD) – by Minami and Joshicara method. The tests were performed before and six weeks after the successful anti-bacterial treatment (famotidine + amoxicline + metronidazol). The following results were obtained:

<table>
<thead>
<tr>
<th>Condition</th>
<th>MDA (nmol/mg protein)</th>
<th>Gpx (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>0.976 ± 0.239</td>
<td>0.0369 ± 0.0173</td>
<td>6.61 ± 2.11</td>
</tr>
<tr>
<td>After eradication</td>
<td>0.985 ± 0.250</td>
<td>0.0249 ± 0.024</td>
<td>4.29 ± 1.25</td>
</tr>
</tbody>
</table>

Conclusions: The decrease in SOD activity in gastric mucosa after Hp eradication indicates the bacterial origin of this enzyme.

Helicobacter Pylori (Hp), Gastric Mucous and Endocrine Cells in Duodenal Ulcer

E.V. Bespalova, Y.A. Gaidar, E.V. Stepanova, Ukrainian Scientific Research Institute of Gastroenterology, Dnepropetrovsk, Ukraine

The aim of the study was to investigate the interrelation between Hp and gastric mucus producing, gastrin- and somatostatin in producing cells in patients with duodenal ulcer (DU).

Material and Methods: 94 patients with DU were observed. Hp-infection was revealed in 86% cases. The estimate of gastric mucin production based on quantitative and qualitative analysis of intraluminal mucus in surface epithelium and mucus in gastric juice using biochemical, histochemical and morphometric methods. The condition of G- and D-cells was determined by immunomorphologic method and electron microscopy. The generally accepted methods of statistical analysis were used.

Results: It was determined, that persistence of Hp in patients with DU was accompanied by considerable decrease of intraluminal mucus quantity in surface epithelium (p = 0.01) with simultaneous increase of mucoprotein concentration in basal gastric juice (p < 0.03). That testified to the endocytosis and exocytosis exudation. These changes were increased in correlation with the increase of Hp-infection degree (r = 0.82). The considerable decrease of fecose and sialic acids testified to qualitative changes of mucus. The morphologic confirmation of direct contact of Hp with G-cells was received. The different degree of...
G-cells hyperplasia accompanied with D-cells hyperplasia was noted in 23% patients with DU associated with Hp.

Conclusions: Therapy was capable of modifying the action of Hp on protective mechanisms and neuroendocrine function of the stomach in DU patients.

1. Shimmizu, T. Ando, Y. Yamaguchi, M. Sakakibara, M. Shinoda, T. Konageya, K. Kyokan, M. Ohsuga, N. Kasuga, K. Kusugami. First Department of Internal Medicine, Nago University School of Medicine, Nago, Japan

**Purpose:** In Helicobacter pylori (H. pylori) infected gastric mucosa, there is an increase in the number of polymorphonuclear neutrophils (PMN) and mononuclear cells (MNC). H. pylori is known to stimulate the production of chemokines involved in recruitment of PMN and MNC. This study aimed to evaluate infiltrating cells and chemokine activity (IL-8 and GRO-a) in H. pylori infected gastric mucosa.

**Methods and Material:** Ten duodenal ulcer patients were studied before and after 1 week eradication therapy with omeprazole, metronidazole, and clarithromycin. H. pylori infection was confirmed by bacterial culture, histology, and urea breath test before the treatment. Three mucosal biopsies were taken from the antrum and body, respectively: one was for immunohistochemical staining with the antibody against H. pylori, myeloperoxidase (MPO), CD11b, CD68, IL-8, and GRO-a, one for HE staining to assess according to the pycnographic value and one for 24-hr culture.

**Results:** H. pylori eradication was achieved in all 10 cases (100%). Before eradication, there was a significant increase of mucosal PMN and MNC especially in the antrum. Most MPO-positive PMN were also positive for CD11b. After eradication, the number of MPO-positive PMN (antrum: 103 ± 25.2 ± 5.1 ± 2.0 mm²; body: 56.8 ± 21.3 ± 1.0 ± 1.0 mm²) and MNC was significantly decreased. The infiltrating IL-8 and GRO-a-positive cells were mostly CD68-positive macrophages and their number decreased after eradication to 5.9 ± 12.8 ± 14.7 ± 6.9 mm² (GRO-a: 43.4 ± 9.2 ± 10.6 ± 5.3 mm²) in the antrum. Before eradication, the gastric epithelial cells were also positive for IL-8 and GRO-a. In the organ culture stomach, IL-8 and GRO-a activity decreased significantly after eradication.

Conclusions: Chemokines may play an important role in the pathogenesis of H. pylori-infected gastric mucosa.

2. **Salivary and Gastric Epidermal Growth Factor (EGF) and Gastric Mucosal EGF Expression in Duodenal Ulcer (DU) Patients before and after Eradication of Helicobacter Pylori (Hp)**


EGF is released mainly by salivary glands and promotes gastric mucosal growth and repair, but the influence of Hp infection on EGF release and its mucosal expression have not been evaluated. In this study, basal and pentagastrin-induced salivary and gastric luminal EGF release (radioimmunoassay) as well as gastric mucosal expression of EGF (RT-PCR) have been examined. Hp-negative (by *C. caviae* breath test) controls and Hp positive active duodenal ulcer (DU) patients were tested before and after 4 weeks post 2 week triple therapy (amoxicillin 500 mg qd, metronidazole 500 mg bd and omeprazole 20 mg qd). There was no difference in basal salivary and gastric luminal EGF contents between healthy controls and DU patients. Infiltration of pentagastrin (2 mg/kg/h) raised by 3 folds salivary and gastric luminal concentrations and outputs of EGF both in control and DU subjects but following successful eradication of gastric Hp (confirmed by histology and culture of endoscopic biopsy samples) and complete healing of DU, there was 3-4 fold higher gastric EGF release in basal state and after pentagastrin than before the therapy. Salivary basal and pentagastrin-stimulated EGF was not significantly affected by Hp infection and was detected in gingival pouches in 18 out of 20 DU patients using Hp culture and PCR technique. Gastric mucosal EGF expression was negligible in healthy controls but was 2-4 times higher in DU patients and triple therapy had no influence on this enhanced expression. We conclude that (1) the stomach itself is capable to release large amounts of EGF that is augmented by pentagastrin, (2) gastric Hp eradication by triple therapy results in DU healing and further increase in gastric luminal EGF release and mucosal EGF expression but does not affect salivary EGF release possibly due to the failure to eradicate oral Hp.

**3. Is there a Correlation between the Mean Values of IgG and IgA Specific Antibodies Against H. Pylori and Severity of Chronic Active Gastritis?**

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**Aim:** To evaluate the correlation between specific IgG and IgA values to H. pylori according to the severity of chronic active gastritis.

3. **Innemohistochemische Study in Gastric Mucosa of Helicobacter Pylori-Positive Duodenal Ucer**

3. **Correlation between Helicobacter Pylori Gastric Infection and Plasma Levels of Fibrinogen, Plasminogen Activator Inhibitor (PAI) and Von Willebrand Factor (vWF) Antigen**

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There is evidence suggesting that patients at risk for coronary heart disease (CHD) are more likely to suffer from Helicobacter Pylori (Hp) gastric infection than controls. In addition, preliminary data suggest that patients with Hp infection may have high levels of plasma fibrinogen. Aim: to determine whether Hp infection is associated with increased plasma levels of fibrinogen and other risk factors for CHD such as PAI and vWF antigen. Methods: consecutive patients undergoing upper gastrointestinal endoscopy at our Institute were studied. Hp infection was diagnosed by the histological (modified Giemsa) and biochemical (CLO-test) methods. Concomitant inflammatory conditions were excluded by clinical examination and by measurement of ESR, WBC, PCR, alpha-1-acid-glycoprotein. Fibrinogen levels were measured by Claus’ technique, while vWF antigen and PAI were assayed by ELISA. Statistical analysis was carried out by means of Student’s t test. Results: 130 patients (66 HP positive and 64 HP negative) were enrolled. There was no difference between the two groups in sex, age, smoking history, hormonal therapy in females, arteri hypertensión, dyslipidemia, values of acute phase reactants, family history of CHD. Plasma fibrinogen levels (mg/dl) were 318 ± 76 in the HP+ group vs 291 ± 65 in the HP- group; plasma PAI levels (ng/ml) were 41.2 ± 29 in the HP+ and 35.8 ± 24 in the HP- patients; vWF antigen levels (U/dl) were 138 ± 53 in the HP+ group vs 114 ± 52 in the HP- group. Differences between the two groups were statistically significant for vWF plasma levels p = 0.01 and fibrinogen plasma levels p = 0.037. Conclusion: Plasma levels of vWF and fibrinogen were significantly higher in HP+ than in HP- patients, PAI levels were also increased in HP+ patients although the difference did not reach statistical significance. Further studies on larger patients populations are required to clarify whether HP+ patients are indeed at higher risk for CHD.

3. **Helicobacter Pylori Infection and Serum Pepsinogen I Concentration**

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**Purpose:** In order to clarify the relationship between H. pylori (Hp) infection and pepsinogen I (PGI), we have compared fasting serum PGI levels and after the eradication of Hp infection in patients with peptic ulcer. Methods: Serum PGI levels were measured by RIA in 511 Hp+ (+) and 225 Hp- (-) patients. 110 out of 511 Hp+ (+) patients were given TDB, metronidazole, ranitidine, and amoxicillin. 97 Hp+ (+) and 54 Hp- (-) patients were treated only with ranitidine and antidepressants. Results: Fasting serum PGI levels were significantly higher in infected patients 1243 ± 46.9 vs 77.9 ± 25.8 mg/ml, p < 0.001. Hp eradicated in all the patients who received 4-weeks 4-bacterial therapy and serum PGI were significantly decreased from 128.8 ± 43.0 to 82.4 ± 24.0 ng/ml (p < 0.001).
Pre- and post-eradication PGI levels of both Hp (+) patients, not receiving antibacterial therapy, (120.8 ± 40.9 vs 128.3 ± 40.4 ng/ml) and Hp (-) patients (75.1 ± 8.0 vs 77.3 ± 24.5 ng/ml) were not changed. Conclusion: We have confirmed that infection increased PGI secretion and eradication of Hp results in significant fall in PGI levels.

### 330 Serum Caga IgG Antibodies in Korean Patients with Helicobacter Pylori Infection and Their Relation to Gastric Pathology and Serum Pepsinogen

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**Purpose:** To examine serum IgG response to Caga protein of Helicobacter pylori (Hp) in Hp+ Korean patients with functional dyspepsia (FD) and peptic ulcer disease and evaluate its effect on gastric pathology and biochemical changes (changes of serum gastrin and pepsinogen (PG) concentrations).

**Methods:** Sera from 74 patients with FD (n = 50), gastric ulcer (GU, n = 12) and duodenal ulcer (DU, n = 12), all of whom were Hp+ by histology and rapid urease (CL0) test, were assayed by ELISA for Caga IgG antibodies using a recombinant fragment (50 kDa) of Caga as antigen. The cut-off level for seropositivity was determined as 2 standard deviations above the mean reactivity of Western blot negative sera. The degree of gastric inflammation was studied in patients with FD using gastric inflammatory scores (modified method of Marshall et al. and Rugger et al., total score 0-8). The serum levels of gastrin, PGI, PG II were also studied in patients with FD using radioimmunossay kits.

**Results:** Percentage Caga seropositivity in HP+ patients with DU, GU and FD was 92%, 83% and 77% respectively (p = 0.28). The magnitude of Caga IgG response was higher in patients with DU than those with GU and FD, but the difference was not statistically significant (mean optical density (OD) ratio: 0.41 ± 0.25 vs 0.26 ± 0.18 vs 0.28 ± 0.21, p = 0.12). The IgG titers (OD ratios) of Caga in patients with FD (n = 32) correlated well with the degree of gastric inflammation (r = 0.6558, p < 0.001). The mean inflammatory score was 5.67 ± 1.61 in Caga+ patients and 2.26 ± 0.19 in Caga− patients (p < 0.01). The IgG titers of Caga in patients with FD (n = 48) also correlated with serum PGI II level (r = 0.3133, p < 0.05) and PGI/III ratio (r = -0.4056, p < 0.05), but not with serum levels of gastrin or PGI I. Conclusion: Levels of Caga seropositivity in HP+ ulcer patients and HP+ DU patients did not differ in this Korean population and may not be useful to predict peptic ulceration. However IgG titer of Caga correlated well with gastric inflammation and serum PGI II level.

### 333 Decrease in Basal and Stimulated Gastrin and Pepsinogen Levels after Eradication of H. pylori: A One-Year Follow-Up Study

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**Purpose:** To determine the influence of H. pylori eradication on basal and stimulated gastrin (G) and pepsinogen I (PG) levels in duodenal ulcer patients, at an early stage and for a one-year follow-up period.

**Methods:** 31 patients (81% males, mean age 51 ± 12 years) with a duodenal ulcer and successful H. pylori eradication were studied. In all patients biopsy (H&E) and a CagA-urea breath test were performed both at diagnosis and 1 year after completing the therapy (triple therapy with bismuth, or co-treatment plus one or two antibiotics). Serum samples were obtained at diagnosis and at 1 month, 6 months and 1 year, to measure basal and stimulated G (10 and 20 min) and PG (30 and 60 min) levels after ingestion of beef and PG levels after eradication are summarized in the table.

<table>
<thead>
<tr>
<th>G 0 m</th>
<th>G 10 m</th>
<th>G 20 m</th>
<th>PG 0 m</th>
<th>PG 30 m</th>
<th>PG 60 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial 45 ± 12</td>
<td>94 ± 42</td>
<td>92 ± 42</td>
<td>101 ± 30</td>
<td>106 ± 33</td>
<td>116 ± 36</td>
</tr>
<tr>
<td>1 min 40 ± 10</td>
<td>62 ± 22</td>
<td>62 ± 21</td>
<td>83 ± 23</td>
<td>96 ± 27</td>
<td>95 ± 29</td>
</tr>
<tr>
<td>6 min 38 ± 10</td>
<td>60 ± 26</td>
<td>60 ± 24</td>
<td>75 ± 24</td>
<td>77 ± 25</td>
<td>86 ± 26</td>
</tr>
<tr>
<td>1 year 39 ± 8</td>
<td>66 ± 27</td>
<td>62 ± 22</td>
<td>74 ± 26</td>
<td>77 ± 27</td>
<td>87 ± 30</td>
</tr>
</tbody>
</table>

**Conclusions:** A significant histologic improvement both in the antrum and body (p < 0.001) was observed after finishing treatment. Decrease in G and PG levels after eradication are summarized in the table.

### 335 Gastric Metaplasia (GM) and Helicobacter Pylori (HP) Infection in Normal Men (N) and Patients with Duodenal-Ulcer Disease (DU)

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GM has been considered a necessary condition for the infection of duodenal by Helicobacter Pylori and the development of DU (Marshall 1988).

**Purpose of the study:** To assess the prevalence of GM (Toluidine, PAS+) on median bulb N or marginal ulcer DU biopsies (n = 3), Gastric acid secretion was collected by aspiration in basal state (1 hour) and after pentagastrin stimulation 6 pg/kg/m (1 hour): PAO (mmol/l) (stimetric method); Histological detection of bulb and antral mucosa (biopsies = 4 in all N and DU).

**Endoscopic findings:**

<table>
<thead>
<tr>
<th>N</th>
<th>DU</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>15/5</td>
<td>15/10</td>
</tr>
<tr>
<td>18</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**Results:** Percentage GM presence; --: absence, R: normal, AG: Acute gastritis. CG: chronic gastritis, p-value: p < 0.05.

**Conclusion:** This study confirmed the high incidence of GM in DU (GM+ = 75%) compared to N (GM+ = 5%) and its strong link with hyperacidity (82% of GM+ = PAO > 30 bulb HP+ (95% of GM+ = HP) and CG Antral mucosa (100% of MG+ = CG) in DU.

### 336 H. Pylori Colonization of Gastric Metaplasia in Proximal Duodenum (DMG) Is Not An Obligatory Condition for a Duodenal Ulcer (DU) Development and a Preferentially One

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According to the accepted cascade of pathophysiological events in DU disease, H. pylori colonizes DGM resulting in active duodenitis and ulceration. In fact DGM is a pre-existing obligatory condition for DU development independent of any associated risk factor (Gastroenterology 1996; 110: 4: 232), in contrast Hp is inconsistently found in DGM areas. To establish duodenal Hp prevalence and location in DGM areas, and determinants of duodenal Hp colonization, 55 active DU pts with Hp positive gastritis were evaluated prospectively.

**Methods:** Duodenal Hp was detected by histology and immunohistochemistry (IHC) (antibody-DAOK B0471) from multiple biopsies (4–8 quadrantic in 1st duodenum and 3 on 2nd margins). PAS (DGM prevalence and extent [as % of total epithelial surface measured in biopsies] and HPS (grading of gastritis in 6 Sydney system biopsies) and duodenitis) stains were performed. Results: Prevalence: Duodenal Hp was detected in 17/55 (30.9%): Hp was detected by histology and IHC in 13 cases and by IHC only in 4 cases. Location: In all pts with Hp detected in duodenum, Hp was located on DGM areas harboring the niches (100%) and concomitantly in DGM areas outside the ulcer in 23% only. In Hp+ve DGM areas, active duodenitis was present in 100% on ulcer margins and 50% in extra DGM areas. Determinants of the preferential duodenal Hp colonization: a) larger extent of Hp colonized DGM vs non colonized DGM: % median (range): 85 (70–100) vs 50 (10–100) respectively p = 0.03. b) significantly higher association with Hp gastritis in duodenal Hp+ve (12/17) vs duodenal Hp–ve (11/38) p = 0.03. c) significantly higher association with antral atrophic gastritis and intestinal metaplasia in duodenal Hp+ve (7/17) vs duodenal Hp–ve (5/38) p = 0.02. No other significant differences were significant concerning age, sex, familial history, smoking, number of relapses, location and type of ulcer or previous treatment between duodenal Hp+ve vs. Hp–ve group. Conclusion: In DU pts Hp colonization of DGM was found in only 31%, located in the most extended areas of DGM and preferentially in patients presenting with both Hp gastritis, antral atrophic gastritis and intestinal metaplasia.

### 338 Structure of Gastric Mucous Membrane When an Ulcer Disease is Present Associated with Helicobacter Pylori (HP)

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**The purpose:** study of the role HP colonization in progression of structural changes of gastric mucosa.

**Materials and methods:** There were 162 patients observed with Ulcer disease, including 94 with gastric Ulcers and 68 with duodenal ulcers. Endoscopy with biopsy and histological examination of gastric mucosal biopsies were performed as well as detection of HP. Cytogetic smears and the presence of HP in them were studied also.

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Results: Endoscopic gastritis was diagnosed in 87% of gastric Ulcers, and 95% of duodenal ulcer cases. In histological examination, it was found in 100% of the patients, surface gastritis in 13.6%, atrophic gastritis in 23.4%, atrophic gastritis with intestinal metaplasia in 38.8%, atrophic-hyperplastic in 6.2%, erosive gastritis in 8%, diffuse gastritis in 10.5%. The cytoplastic picture was characterized by marked cell changes: there were signs of inflammation and proliferation in 100%, dysplasia in 85%, dysphorie in 53%, intestinal metaplasia in 65% and infiltration by lymphocytes in 38.8% of all cases. In all investigated smears, neutrophil infiltration was revealed. Cell dysplasia was characterized by expression of proliferation, illegible borders, pyknotic cells, changes of a nucleo-cytoplasmatic ratio. Dysplasia of the first degree was revealed in 9%, second degree in 55.6%, and third degree in 20.4% of all cases. The results listed above testify the significant structural changes of gastric mucous in HP colonization. HP was detected by standard histologic method in gastric ulcers in 66%, and in duodenal ulcers at 87% of the time. In cytoplastic smears HP was detected in 67% of gastric Ulcers and in 89.8% of duodenal ulcers.

Conclusions: The degree of HP colonization correlates with structural changes of gastric mucous in relationship with the length of time needed for healing from ulcer damage. Structural changes and time for healing seem to be closely related.

339 Helicobacter Pylori (HP) and Free Oxygen Radicals
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Free radicals (FR) can easily intake and give out electrons from molecules around them due to the fact that they contain in the last orbit only one electron. These radicals come into the being either during normal cell functions which are toxic, or materials produced by defence cells. They cause the degeneration and the membrane loss of organs functions and that of our work was searching the relation between the action of HP on gastric mucosa inflammation caused by FR and to know the condition by patients given omeprazole (om.) or om. + amoxicillin (amox.) before or after treatment in the basis of FR.

The study has begun with 20 female, 27 male (totally 47) patients in our clinic with peptic complaints, who had taken no medicine for last month. (Age range 16-63, average 35.9 years). Endoscopic results have shown duodenal ulcers 10, antral gastritis 25, bulblits 7, bulblits + gastritis 5 cases. The patients were divided into two groups at random. Group A: (n: 16), HP (+), omep. Group IB: (n: 24), HP (+), om. + amox. Another group was build up of HP’-s; Group II: (n: 16), HP (-), omep. Omeprazole was given. 40 mg/day orally every morning by the patients. Amox. 1000 mg/day for 10 days orally. From each case 9 biopsies have been taken from antrum on days 0 and 30. HP searched by Clo test, FR were searched through chemiluminescence method. For H2 O2, OH’, hypochlorous and peroxynitrite “Luminall”, for O2* “Luminenn” are used. The results are given as in the table.

<table>
<thead>
<tr>
<th>Luminall (Cpm/mg-tissue)</th>
<th>Luminenn (Cpm/mg-tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0. Day</td>
<td>30. Day</td>
</tr>
<tr>
<td>0. Day</td>
<td>30. Day</td>
</tr>
<tr>
<td>Group IA</td>
<td>Group IB</td>
</tr>
<tr>
<td>2.25</td>
<td>0.60</td>
</tr>
<tr>
<td>1.57</td>
<td>0.30</td>
</tr>
<tr>
<td>2.40</td>
<td>0.82</td>
</tr>
<tr>
<td>1.61</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Conclusion: The FR level before the treatment in both HP (+) and HP (-) groups was very high. This indicates that HP is increasing the gastric mucosa inflammation through the FR. By single or combined treatment a considerable decrease of FR has been observed. (Those decreases are more obvious by the patients who take om. + amox. related to the patients who take only om.) So the treatment of peptic diseases causes to decrease of FR and consequently the decreasing of destructive action of HP.

340 Condition of Hormonal System and Mucosal Immune Response of Antral Section in Women of Different Age Categories with Helicobacter Pylori: – Associated Duodenal Ulcer

Aim: To investigate the relationship between the hormonal system and the local immune response of the gastric mucosa (GM) in women of the reproductive and postmenopausal age groups with duodenal ulcer (DU) associated with Helicobacter pylori (H.p.).

Methods: Basal Levels of pituitary (F SH, LH, STH, ACTH, TSH) and peripheral hormones (estradiol, progesterone, testosterone, cortisol, T3, T4, gastrin, C-peptide, insulin) in 98 women with DU in both phases of their menstrual cycle plus 20 control subjects and also in 76 elderly women as well as 16 comparable controls were assessed by RIA. In antral biopsies the number of IgA, IgG, IgM-producing cells and Tf-helper (Th-1), and T-suppressors (Th-2) by the indirect immunofluorescence method with the aid of mononal antibodies. H.p. was detected according to L. Watters et al.

Results: It has been found that DU distorts the feedback between the central and the peripheral parts of the endocrine system. The prevalence of confirmed H.p. was 98.2%, the number of IgG was 37.13 ± 4.44 ± 0.54 against 10.29 ± 0.14 in younger women (N 900 magnification). Elderly women manifested a statistically reliable decline in the number of IgA-producing cells (p < 0.01) and T-H. Their immunoregulatory index was reduced to 0.71 against 0.81 in younger women. Women of the reproductive age group had a high correlation between the amount of progesterone and IGM in the first phase of the menstrual cycle, such correlation disappeared in the second phase; in elderly women there was a positive correlation between IgG and STH and a negative correlation with the number of H.p. (R = 0.74, D = 0.55).

Conclusion: Depending upon their age and phase of menstrual cycle, women suffering from DU associated with H.p. display marked differences in the relationship between the hormonal system and the local immune response of GM.

341 Gastric Muscoco Lymphoid Hiperplasia in Helicobacter Pylori (HP) Infections
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A long time infection with HP can evolve in some people from diffuse lymphoid hiperplasia towards malignant lymphomas, although the lymphoid tissue is not extremely abundant in the gastric mucosa.

We studied 54 surgical removed stomachs, 48 from patients with complications: gastric ulcer and 8 from patients with endocrine aspect as lobulated, polyploid mass in the distal half of the stomach. We made a search from gastric mucous and stained them with Giemsa for HP; the removal pieces were embbeded in parapipn and stained with hematoxilin eoxine and PAS hematoxilin. We also applied immunohistochimical methods.

39 chronic gastritis associated with chronic gastritis and HP infections have been found. In 7 cases the gastric ulcer was accompanied by a diffuse mucosal and submucosal inflammatory hiperplasia with the presence of clearly reactive germinal centers throughout the lesions. In the gastric hiperplasia HP was present. One case was a marginal extra nodal lymphomas with small cleaved cells, accompanied with chronic diffuse gastritis with active foci and a high HP infection. We found also 2 large polymorphous B cell lymphomas and 4 cases were mixed diffuse form, small and large B cell linhoma. In the 2 cases of large cell lymphomas in the gastric mucous microplasma flora was polymorph and HP was absent. Interesting was the case of an unferilated gastric carcinoma accompanied with a high diffuse hiperplasia, HP being also present.

Our observation suggest that the HP infection could develop an important lymphoid hiperplasia, sometimes liable to turn into lymphoma. The establishement of policolial or monoclonal nature of the lymphoid hiperplasia by immunohistochemical methods is essential in finding out the reactive or neopla-

342 DNA Fingerprinting of Helicobacter Pylori Isolated from Patients with Peptic Ulcer

The gastric pathogen, Helicobacter pylori establishes long-term chronic infection that can lead to gastritis, peptic ulcer, and gastric cancer. Ulcerase might allow the survival of the bacteria in an acidic environment, a prerequisite for colonization. Helicobacter pylori is cytotoxic to cultured human gastric epithelial cells and this toxicity is due in part to ammonia produced by hydrolysis of urea. In a previous study by Foxall et al., they suggested that Hae III digest patterns of PCR-amplified UreaA and UreaB genes might serve as a sensitive epidemiological tool for the typing of clinical isolates of Helicobacter pylori. We obtained the PCR-amplified UreaA and UreaB genes from the 18 clinical isolates in Korea and compared the Hae III digest patterns. In methods, clinical isolates of Helicobacter pylori were obtained by endoscopic biopsy from 18 patients with gastric or duodenal ulcer. Biopsy tissues were inoculated onto blood agar plates containing 5% horse serum and skim milk's supplement, and the plate were cultured for 3 days at 37 °C under microaerobic conditions. Chromosomal DNA of Helicobacter pylori were extracted from harvested colonies and PCR amplification were performed to amplify the urease subunit gene (UreaA and UreaB) (Labigne et al., 1991). PCR products, digested with Hae III, were run on 1.5% agarose gels. In results, the 2.4 kb PCR products were amplified from all 18 Helicobacter pylori isolates. From the restriction enzyme digestion pattern of these PCR products, we could classify on the basis of RFPL patterns by Hind III restriction endonuclease digestion produced 11 distinct patterns on agarose gel, with five patterns occurring within two or three isolates. In conclusion, the urease genes of Helicobacter pylori had genetic heterogeneity, therefore it could be of considerable tool for epidemiological studies.
434 Low Gastric Glutathione and Glutathione S-Transferase Levels in Patients Infected with Helicobacter Pylori


Introduction: Infection with Helicobacter pylori (HP) is strongly associated with peptic ulcer disease. In addition, chronic infection with HP may increase the risk of gastric cancer. The mechanism of carcinogenesis, however, is not yet clarified.

Glutathione (GSH) and glutathione S-transferases (GSTs) represent an important detoxification system in epithelial cells of the gastrointestinal tract. Toxins or carcinogens are inactivated by GSH, catalyzed by GSTs. High levels of GSH and GST have been correlated with a low risk of developing gastrointestinal cancers.

Methods: GST and GSH levels were measured in biopsies taken from the gastric antrum of 1) patients with gastric complaints without HP infection (n = 57; age 43.1 ± 13.5 yrs.), 2) patients who became HP-negative after eradication of HP (n = 22; age 49.4 ± 2.3 yrs.) and 3) patients with proven HP infection (n = 24; age 49.5 ± 2.6 yrs.). Results are given as means ± SD and were tested for significant differences with the Wilcoxon rank sum test.

Results: GSH and GST levels in both groups of patients negative for HP (groups 1 and 2) did not differ and were 32.8 ± 10.8 vs. 27.2 ± 11.1 mmol/l protein, and 775 ± 291 vs. 860 ± 206 mmol/min. mg protein, respectively. GSH and GST values in the HP-positive patients (11.6 ± 11.8 mmol/mg protein and 591 ± 176 mmol/min. mg protein, respectively) were significantly lower as compared to values in both groups of HP-negative patients (all p-values < 0.01).

Conclusion: The antral mucosa of patients infected with HP contains significantly lower amounts of GSH and GST, which results in a decreased capacity to detoxify toxins and carcinogens. This finding may contribute to the increased risk of development of adenocarcinomas in these patients.

434 Short-Chain Fatty Acids Produced by Helicobacter Pylori

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Short-chain fatty acids (SCFA) are produced by various bacteria and principle fermentation products are different depending on individual groups of bacteria. There have been few studies which demonstrate what kinds of short-chain fatty acids to be produced by Helicobacter pylori (Hp). Therefore, we analysed the short-chain fatty acids produced by Hp.

Methods: The five strains of Hp which had been isolated from the mucosa of gastric ulcer patients were cultured in burrella broth supplemented with 10% horse serum under a microaerophilic atmosphere without antibiotics, at 37°C for 7 days. After incubation, the cells were removed from the cultured broth by centrifugation and filtration. The supernatant was analysed by HPLC.

Results:

<table>
<thead>
<tr>
<th>SCFA</th>
<th>Amount (mean ± SD, µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malic acid</td>
<td>0.458 ± 0.034</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>1.955 ± 0.407</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>4.97 ± 0.911</td>
</tr>
<tr>
<td>Formic acid</td>
<td>2.65 ± 0.747</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>17.4 ± 12.8</td>
</tr>
<tr>
<td>Levulinic acid</td>
<td>1.01 ± 0.065</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>12.9 ± 4.14</td>
</tr>
<tr>
<td>i-Butyric acid</td>
<td>6.52 ± 0.555</td>
</tr>
<tr>
<td>n-Butyric acid</td>
<td>3.27 ± 2.96</td>
</tr>
<tr>
<td>Bi-Valeric acid</td>
<td>3.04 ± 1.96</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50.5 ± 19.6</td>
</tr>
</tbody>
</table>

Citric and pyruvic acids were not detected.

Conclusion: Acetic acid and propionic acid were the principal SCFA produced by Hp.

435 Degradation of Growth Factors by Helicobacter Pylori: Effect of Sucraflate

B. L. Slomiany, J. Piotrowski, A. Slomiany. Res. Ctr., UMDNJ, Newark, NJ USA

Infection with Helicobacter pylori (HP) is now recognized as a major factor in the pathogenesis of gastric disease, and the bacterium is known to elaborate a number of enzymes capable of rapid compromise of gastric mucosal homeostasis and the repair mechanisms. Among the factors implicated in the control of gastric repair are bioactive peptides that exert their effects by activating specific cell surface receptors which often contain an intrinsic tyrosine kinase activity. The purpose of this study was to assess the susceptibility of EGF, bFGF TGFp and PDGF to degradation by HP protease. The effect of an anticytotoxic agent, sucraflate on this pathogenic activity of HP was also investigated. The experiments were carried out with HP protease obtained from the filtrates of saline washes of the bacterium cultures. The incubation assays for growth factors susceptibility to HP protease consisted of 125I-labeled EGF, bFGF, TGFp and PDGF, enzyme protein (50 µg), sucraflate (0-200 µg), and 0.22 ml of phosphate buffer, pH 7.0. The reaction mixtures were maintained at 37°C for 1 h, and then subjected to chromatography on Bio-Gel P-2 column and the produced 125I-labeled peptide fragments were quantitated by counting in a gamma counter. The results established that under the assay conditions HP contain only 5-7% degradation of EGF and bFGF. However, the HP protease evoked a 61.7% degradation of PDGF and a 62.3% degradation of TGFp.

Introduction of sucraflate to the assay system caused the inhibition in the extent of growth factors proteolysis by HP enzyme. This inhibitory effect of sucraflate was dose dependent and reached a maximum value at 200 µg/ml sucraflate, at which concentration a 79.7% decrease in PDGF and 82.7% decrease in TGFp degradation occurred. These results provide strong evidence for the effectiveness of sucraflate in the protection of gastric mucosal growth factor pool against degradation by HP.

436 Increasing Surface Hydrophobicity in Transformation to the Coccoid Form of Helicobacter Pylori. A Pathogenic Factor?

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It has been suggested that the coccoid form of H. pylori facilitate the survival of the microorganism in the stomach. This may contribute to the relapses of infection following eradication therapy. Our aim was to examine the contact angles during transformation of H. pylori to the coccoid form, to estimate potential changes in surface hydrophobicity which could affect bacterial attachment and the protection of the microorganism in an acidic environment.

Methods: H. pylori strains 88-23, AS and knock-out mutants lacking acid-soluble protein (AS vac A), the case-negative strain 4, and the mutant strain 69 A lacking flagellae were investigated. Aliquots were grown on agar from 2 to 15 days. Samples of viable bacteria were obtained at regular intervals, and evenly spread on glass covers. After 30 minutes of drying, a droplet of saline was applied and the contact angle was measured using a goniometer.

Results: In all examined strains the contact angle was significantly increased from day 2-3 and on, compared to control values (p < 0.001). Thus, the contact angle of the strain 88-23 was 50% higher after 14 days, the maximal increment for the urease negative strain 46.15% and for the flagellae-negative mutant 28.3%, respectively. Similarly, an increment in contact angle of 37% and 21.5% were observed in the AS and the AS vac A strains, respectively.

Conclusions: Development of coccoid forms of H. pylori is associated with increased surface hydrophobicity, which may facilitate the attachment of the microorganism to the gastric mucosa. Possibly, it may also act as a protective mechanism against hydrophobic agents such as gastric acid.

437 Gastric Production of Inflammatory Cytokines in Patients with Helicobacter Pylori Infection with and without Duodenal Ulcer

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An enhanced mucosal production of interleukins 8 (IL-8) and 6 (IL-6) has been observed in Helicobacter pylori (Hp) infected patients, while few data are available on interferon gamma (INF-γ) and INF-4. However, the correlation between cytokines levels and presence of duodenal ulcer (DU) is unknown.

Aim: to study gastric mucosal production of IL-8, IL-6, IL-4 and INF-γ in patients with Hp infection with DU or non ulcer dyspepsia (NUD).

Methods: We studied 13 patients with Hp+ DU, 8 with Hp+ NUD and 4 with Hp− DU. 10 DU patients underwent repeat endoscopy after treatment. Hp was assayed by rapid urease test and histology. IL-8, IL-6, IL-4 and INF-γ concentrations were measured in the homogenate supernatant of 2 antral biopsies by commercially available ELISA kits. Results: (see table)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Mean ± SD (pg/ml)</th>
<th>Hp+ DU</th>
<th>Hp+ NUD</th>
<th>Hp− DU</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>29.7 ± 39.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-6</td>
<td>0</td>
<td>1.4 ± 2.0</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>INF-γ</td>
<td>7.1 ± 3.1</td>
<td>0.8</td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Conclusions: Our data confirm a strong correlation between IL-8 production and Hp infection, regardless of the presence of DU. A weaker correlation exists between Hp positivity and INF-γ levels. IL-6, by contrast, appears to be produced only in patients with DU, either active or healed.
Antral G-Cell and D-Cell Numbers in Helicobacter pylori Infection

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Purpose: It has been recognized that H. pylori infection induced abnormal regulation of serum gastrin secretion. We examined whether there was a relationship between H. pylori infection and G- and D-cell numbers. Methods: The numbers of antral G- and D-cells and serum gastrin levels were compared between 37 peptic ulcer patients infected with H. pylori and 33 patients without infection. G- and D-cells in antral mucosa were examined immunohistochemically using antibodies specific for the gastrin and somatostatin. Results: While the number of G-cells per gastric gland was similar in infected and uninfected patients (7.1 ± 3.1 vs 7.3 ± 3.9), that of D-cells was significantly less in infected patients (1.3 ± 0.4 vs 2.5 ± 1.6, p < 0.001, G/D-cell ratio: 5.7 ± 2.7/1.0 vs 3.5 ± 19/1.0, p < 0.001). Serum gastrin level was also significantly higher in infected patients (80.3 ± 23.5 vs 47.6 ± 14.1 ng/ml, p < 0.001). Conclusion: There was an inverse relationship between the number of D-cells and the serum gastrin level. However, the degree of D-cell under-population was not sufficient to account for the depressed gastrin secretion in H. pylori infected patients. The lower number of D-cells in the antral mucosa can be the result of infection-induced inter- or intra-cellular adhesion.

Helicobacter Pylori (Hp) and Domestic Cats

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Handt et al isolated (Infection and Immunity 62: 2376-2374) Helicobacter pylori from domestic cats in 1994. Because the exact mode of transmission is not fully understood, it was suspected that patients with H. pylori infection may be living in close contact with cats in child- or adult-life. Hence, it is relevant to study whether the number of whatever endoscopic diagnosis was made. Two from antrum and corpus for histology and one for the CLO-test from antrum to determine if they were infected with Hp. These patients were asked if they had been in close contact with cats in child- and adult-life and whether they were born and lived in the Netherlands. The same questionnaire was handed out to patients who visited the department for colonoscopy. Excluded from this study were patients who were treated recently for eradication of Hp, had undergone gastric surgery, or were suffering from bleeding during examination or came for an acute endoscopy. We decided that a patient was Hp positive if both CLO-test and histology turned out to be positive. Likewise a patient was free of Hp, if both assays didn’t show Hp.

In a period of 4 months 185 patients were enrolled in this study. In 25 of the cases the CLO-test outcome was not equal to histology. From the remaining 160 patients 38 were Hp positive (23.8%) and 122 negative. From the 40 patients who never had cats all 18 (45%) were contaminated with Hp. The "ever" group is a combination of people who had cats all their lives 10.7% (3/28) or only in child- or adult-life resp. 28.1% (9/32) and 22.2% (6/27) were Hp positive. The overall Hp positive status of Dutch patients in our study turned out to be 4.3% (29/672). The patients who were Hp negative showed an equal behavior in owning cats as the gastritis group. With only 22.8% of our patients suffering from Hp, the incidence in our patient group is low compared to the 30-50% which is the average nation-wide. In our material we didn’t find over-presentation of Hp positive patients whom were in close contact compared to the "never" group. Although the group of people who were in close contact with cats all their lives is small with only 28 patients, this group shows a remarkable low percentage of 10.7% infected.

So in our patient group we couldn’t find support for our speculation that owning a cat leads to a higher rate of Hp.

Induction of Nitric Oxide (NO) Synthase in Helicobacter Pylori-Associated Gastritis in Duodenal Ulcer Patients


Purpose: Previous studies showed the presence of constitutive NO synthase (NOS) in gastric epithelial and endothelial cells and NO was found to mediate mild irritant-induced gastroprotection and mucosal hyperemia in rats but no study was undertaken to identify the role of inducible NOS (iNOS) in mucosal damage associated with Helicobacter pylori (Hp) infection in men. Methods: The immunohistochemistry, which selectively stains iNOS has been used to detect the iNOS in the antral mucosa obtained by endoscopic biopsy from Hp positive (14-C urea breath test, histology and culture) duodenal ulcer (DU) patients in Hp and the iNOS derived from the culture of antral mucosa. These bacteria were checked that they are members of the group of Gram-negative, spiral curved microaerophilic, oxidase-positive-rod. A sterile swab having all culture from previously sub-cultured bacteria was immersed in a microaerophic, oxidase-positive-rod. The density of bacteria suspended in saline was 10 colony forming units and 3 μl of this suspension was smeared on glass and air dried. The immunoreactivity of iNOS was examined using primary antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA) diluted at 1:50. The reaction was developed with APAAP Dako kit (Dako, Copenhagen) using fast red as chromogen. Summary of results: The iNOS immunoreactivity was found in the antral mucosa of 5 biopsies obtained during endoscopy from 50 active DU patients who were Hp-positive (with 14-C urea breath test, CLO-test and culture). The iNOS were detected in histiocytic cells and in mucosal microvesel cells. The epithelial cells in the pits and glands were negative. The isolated bacteria from these DU patients were strongly stained by the antibody used in all tested samples. Conclusions: Hp is capable of expressing iNOS and NO produced in excess in gastric mucosa by this enzyme may contribute to the pathogenesis of Hp-associated gastritis

Inhibition of Gastric Mucosal Somatostatin Receptor by H. Pylori Lipopolysaccharide: Effect of Ebrotidine

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Among the consequences of Helicobacter pylori (HP) infection, the loss of inhibition of gastrin release and subsequent hypergastrinemia. This apparent pathological effect of HP has been linked to the impairment in feedback inhibition by somatostatin. Recently, we provided evidence that HP through its cell wall lipopolysaccharide inhibits the binding of somatostatin to its mucosal cell membrane receptor (Biochem. Mol. Biol. Int. 1995; 36; 491). The purpose of this study was to assess whether an antiinflammatory agent, ebrotidine, is capable of countering this untoward effect of HP. The study was conducted with rat gastric mucosa. A rat model of gastritis was produced with the use of various gastric epithelial cell membranes by affinity chromatography on a column consisting of covalently coupled D-Trp@5-GRIF-14 to Affi-Gel 10. The receptor protein displayed on SDS-PAGE a band of 61 kDa and showed specific affinity towards 125I labeled somatostatin. The binding of somatostatin to the isolated somatostatin receptor was inhibited by HP lipopolysaccharide and reached a maximum of 94.1% inhibition at 50 μg/ml. Preincubation of HP lipopolysaccharide with ebrotidine caused a dose-dependent reversal of HP inhibitory effect, and at the optimal concentration of 20 μg/ml ebrotidine produced an 84% restoration in somatostatin-mucosal receptor binding. The interference by HP lipopolysaccharide with the receptor binding site for somatostatin could account for the observed deficiency of negative feedback from D-cells to G-cells with HP infection. Further, our results demonstrate that ebrotidine possesses the ability to counteract HP interference with somatostatin acid secretion regulatory effects. Hence, ebrotidine offers a new potential choice in ulcer therapy.
No significant differences were observed comparing H. pylori prevalence in the control group with other groups. Conclusions: 1) The overall H. pylori prevalence was in agreement with that observed in other western countries, and H. pylori infection was not a higher prevalence of H. pylori infection than the control group. 3) Performing gastrointestinal endoscopies and the gastroenterology specialty itself are not associated with a higher risk for developing H. pylori infection in Spain.

354 Does Helicobacter Pylori Infection Have a Role in Primary Sjögren’s Syndrome?  
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To investigate a possible relationship between primary Sjögren’s syndrome (pSS), an autoimmune disease of unknown etiology, and Helicobacter pylori (Hp) infection 14 patients with pSS (13 women and 1 man, mean age: 48 ± 13.32) were studied. The diagnosis of pSS was based on San Diego criteria. Hp infection was assessed by histology, urease test and culture of gastric biopsies and detection of IgG antibodies for Hp. All cases were treated with omeprazole 20 mg b.i.d. for 1 month and amoxicillin 1 g b.i.d. and tinidazole 500 mg b.i.d. between 30-60 minutes beforeHp testing. Clinical and endoscopic examinations were repeated one month after completion of the therapy. Hp eradication was defined by negativity of all these tests, except anti-Hp IgG.

Active Hp infection was diagnosed in 11 (78.4%) cases. In 2 (14.3%) patients anti-Hp IgG was present alone. Hp eradication was achieved in all cases. After Hp eradication xerostomia disappeared in 8 (57.1%). But, xerostomia was not improved in any of Hp(-) cases, given the same therapy. Mean serum IgG level decreased from 2036.5 ± 725.9 mg/dl to 1817.9 ± 577.6 (p = 0.06) and Chisholm score, showing the degree of mononuclear cell infiltration on lip mucosal biopsy, from 3.45 ± 0.2 to 2.7 ± 0.3 (p = 0.1). Shrimler’s test improved from 6.0 ± 5.4 mm to 13.9 ± 9.4 (p = 0.0006). In Hp(-) –3 cases, given the same therapy, mean IgG level changed from 2984 ± 1084.2 mg/dl to 2028.3 ± 896.7 and Chisholm score from 3.0 ± 1.7 to 2.3 ± 1.5. Shrimler’s test also increased from 11.3 ± 8.1 mm to 14.0 ± 7.9 (Because of small number of patients statistical evaluation could not be performed). Conclusions: 1) The prevalence of Hp is high in patients with pSS. 2. Hp eradication results in a significant improvement in the signs and symptoms of pSS. 3. It seems that Hp infection may have a role in the pathogenesis of pSS. 4. Probable beneficial effect of the therapy should be tried in more Hp(-) patients.

355 Different Topical Duodenal and Gastric Mucosal Protection of Interleukin-1 beta, Interleukin-6, Interleukin-8, Tumor Necrosis Factor Alpha and Interleukin-2 Soluble Receptor in Helicobacter Pylori Positive and Negative Patients. A Pilot Study  
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Purpose of the study was to evaluate topical differences of mucosal cytokine production in Helicobacter pylori (HP) positive and negative patients.

Methods. Seventeen patients [6 men, 11 women, aged 21–74] entered the study. Five biopsy specimens for in vitro culture were taken from each patient during routine gastrointestinal endoscopy, if HP was postulated. Aim: To determine the prevalence of H. pylori infection among a wide group of health care workers compared with a control group and its possible relationship with inherent endoscopy risks.

Materials and Methods: A group of 224 medical workers (48 females; mean age: 41 ± 8 years; range: 25–70 years) and a control group of 84 persons (36 females; mean age: 36.3 ± 10.4 years; range: 19–62 years) -not working in health area- were studied. Subjects with history of previous peptic ulcer or digestive disease were excluded. All health care workers were asked for their relationship with gastrointestinal and endoscopy activities. In all subjects H. pylori status was assessed by the 13C-urea breath test following the European Standard Protocol. A positive result was defined as an excess of 14CO2 excretion > 5%.

Results: The overall (medical and non health care workers) H. pylori prevalence was 50%.

There was a correlation between antil-IL-1β and IL-8 (p < 0.0001), IL-1β and TNF-α (p < 0.0001), IL-6 and IL-8 (p = 0.0078). There was a correlation between fundal IL-6 and IL-8 (p = 0.0009). Duodenal sIL-2R production was significantly higher than antil one (p = 0.0038).

Conclusions. A great deviation of values (both personal and topical difference) suggests the significance of several factors (including Hp) influencing the consequence of the local inflammatory reaction.

356 DNA Typing of HLA Class II Genes in Japanese Patients with Helicobacter Pylori Infection  
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Purpose: The aim of this study is to investigate the HLA-linked genetic predisposing factors in H. pylori infection.

Subjects and Methods: The study group consisted of 58 Japanese patients with H. pylori positive gastric ulcer and 44 Japanese patients with H. pylori positive duodenal ulcer and 44 Japanese patients with H. pylori positive gastric. Control subjects were selected without H. pylori infection. The biopsy specimens taken from the antrum and the body were used for the bacterial culture. We compared HLA class II genes between control and patients groups. HLA class II genes was analyzed by the PCR-SSOP typing of each class.

Results: 1) The allele frequencies of DRB1*1502, DQAI*0102 and DBPI* 0901 were significantly higher in H. pylori positive gastric ulcer patients than in controls, whereas those of DRB1*1501, DQAI*0102 and DBPBI*0601 were significantly lower in H. pylori positive gastric ulcer patients than in controls. The same results were observed between H. pylori positive gastritis patients and controls, except for DPAI*0102. 2) The allele frequencies of DRB1*0405 were significantly higher in H. pylori positive duodenal ulcer and H. pylori positive gastric patients. DRB1*0401 were significantly lower in H. pylori positive duodenal ulcer patients than in controls. 3) There were no significant differences in the allele frequencies between H. pylori positive gastric ulcer patients and H. pylori positive gastritis patients. 4) The allele frequency of DRB1*0901 was significantly lower in H. pylori positive duodenal ulcer patients than in H. pylori positive gastritis patients.

Conclusions: These observations suggest that H. pylori infection is associated with HLA class II genes and the HLA class II genes are involved in the pathogenesis of peptic ulcer and gastritis via different mechanisms.
Cytokine Gene Expression on Helicobacter Pylori Infection in Gastric Epithelial Cells

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Infection of Helicobacter pylori (H. pylori) activates infectious response on gastric epithelial mucosa by monocytes and neutrophils. This cellular response probably represents a primary immune defense mechanism against a microbial pathogen. H. pylori produces various factors which will attract or activate neutrophils. Infection with H. pylori also results in increased gastric mucosal production of cytokines, interleukin-6 and -8, which is a potent activator and chemoattractant for neutrophils. As gastric epithelial cells express interleukin-6 and -8, they may have an important role in regulating primary host defense mechanisms and be functionally involved in the neutrophil response to H. pylori infection. In this study, we compared IL-6 and -8 gene expression by reverse transcription-PCR of human gastric epithelial cells, Kato-III, to H. pylori infection with special reference to neutrophil modification. Human IL-6 and -8 specific mRNA was detected when Kato-III cells were incubated with H. pylori. H. pylori did not express IL-6; IL-8 mRNA, whereas Kato-III cells and neutrophils showed expression of IL-6 and -8 mRNAs. IL-6 mRNA expression by Kato-III cells was further enhanced when they were co-incubated with both H. pylori and neutrophils compared to cells with neutrophils only. We conclude that human gastric epithelial cells, Kato-III expresses IL-6 and -8 mRNA which can be further enhanced by neutrophils. These results indicate that neutrophil may upregulate IL-8 mRNA expression in H. pylori-infected gastric epithelial cells.

Familial Occurrence of Helicobacter Pylori Infections: Contribution to the Studies of Treatment Failures
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In children with persistent complaints associated with the alimentary tract it is necessary to look for Helicobacter pylori (Hp) infection, also in their family environment. The study was carried out in a group of 50 children with suspected inflammation of the upper alimentary tract mucosa. We used two methods: endoscopic examination of upper portion of the alimentary tract; 2/Histopathological evaluation of mucous membrane of the stomach and duodenum (according to the Sydney Classification); 3/Quantitative determination of Hp antibodies performed by Enzyme Immunoassay tests (Boehringer) in all the children and members of the families of patients with Hp infection confirmed by means of histopathological or immunological techniques.

In the investigated group it was demonstrated that among 50 children with suspected inflammation of the upper alimentary tract mucosa on the basis of endoscopy suggesting Hp etiology the coincidence was confirmed histopathologically in 44 children. In 26/50 children there was a positive result demonstrating the anti Hp antibodies (range 24-240); simultaneous presence of serological and histopathological markers of Hp infection were observed in 22 out of 50 patients. In the investigated group infection markers were present in 34 family environments, including 16 cases in which anti-Hp antibodies were detected in more than one member of the family, more frequently in parents than in brothers or sisters.).

The above observations may indicate horizontal transmission in the family environment and make a contribution to the studies of the causes of failures of the currently recommended alternatives of therapy in the infected patients.

Seroprevalence of Helicobacter Pylori in Instituted Intellectually Disabled and Employees
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Instituted intellectually disabled an acquisition rate of Helicobacter pylori infection (Hpi) of 60%–75% is indicated (Berkowitz, 1987/Lambert 1995), while in the normal Dutch population a prevalence rate of 5–50% is found. Therefore we analysed the seroprevalence of Hpi in 2 institutes with 1987 inhabitants and 1404 employees. Randomly, in 338 intellectually disabled Hpi was assessed by retrospective analysis of sera with an Ela-Ig-g test (Orion), and after voluntary venapuncture in 254 employees. A level of > 300 IU was defined as evidence of Hpi. Subjects with Hpi were defined as patients and compared with the total Dutch population evaluated by Loffeld (thesis 1988).

In 280 (82.8%, mean age 51 yrs) intellectually disabled Hpi was found, compared with 51.4% in the total normal intellectual Dutch population (mean age: 55 yrs; p < 0.0001). Riskfactors in intellectually disabled for developing Hpi were: male gender; the duration of institutionalisation, an IQ < 50, rumination, and a history of upper abdominal symptoms. 75 (29.5%, mean age: 29.5 yrs) employees showed Hpi, compared with 25% (mean age: 30.5 yrs) in the total Dutch population (ns). Employees with intensive physical contact with the intellectually disabled, with a long duration of employment, and with upper abdominal symptoms had Hpi more frequently.

In conclusion: instituted intellectually disabled have a higher frequency of Hpi, especially if they are male, with a long stay in an institute, with an IQ < 50, with rumination or with a history of upper abdominal symptoms. Employees with intensive physical contact with intellectually disabled, and after longer duration of employment are at higher risk for Hpi.
363 Role of Helicobacter Pylori in the Pathogenesis of Gastroduodenal Lesions in Patients with Cirrhosis: A Prospective Evaluation

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Although gastroduodenal ulcerated mucosal lesions in patients with cirrhosis are common, their pathogenesis remains unclear. The aim of this prospective study was to determine the pathogenic factors associated with gastroduodenal mucosal lesions in patients with cirrhosis and especially to assess the role of Helicobacter pylori (Hp).

Patients with histologically proven cirrhosis and not recently treated by antibiotics, antisecretory or anti-inflammatory drugs, were enrolled and referred for upper gastrointestinal endoscopy. Upper digestive tract bleeding within the last week was an exclusion criteria. Age, gender, smoking habit, recent alcohol intake, etiology of cirrhosis, Child-Pugh grade were recorded and basal gastrinemia was determined. Esophageal varices were graded from 0 to 3. Severely of hypertensive gastropathy in the body and the antrum was graded from 0 to 3 for erythema, edema and snake-skin mosaic pattern (maximum score: 18). Helicobacter pylori status was determined from rapid urease test and histology on biopsy samples or 13C urea breath test when biopsies were impossible.

Sixty four patients were included. There were 52 males and 12 females, mean age ± SD: 55 ± 11 years. Cirrhosis was alcoholic in 47, grade A, B and C in the Child-Pugh classification in 19, 21 and 24 respectively. Thirty five (55%) were positive. One or several mucosal lesions were present in 24 patients (37%): gastric ulcer in 8, duodenal ulcer in 7, gastric erosions in 9, duodenal erosions in 4. Univariate analysis showed that mucosal lesions were not significantly related with age, gender, smoking habit, etiology of cirrhosis, Child-Pugh grade, esophageal varices grade, Hp positivity (12/24 vs 23/40, p = 0.74), and basal gastrinemia. Univariate and multivariate analysis showed that they were significantly related to a recent alcohol ingestion (62.5% vs 27.5%, p < 0.006) and to a high hypertensive gastropathy score (10.9 ± 4.4 vs 7.4 ± 5.3, p = 0.02).

Conclusion: Ulcerated lesions in cirrhosis are unrelated to Hp despite high prevalence of infection. They are significantly and independently related to recent ingestion of alcohol and to hypertensive gastropathy severity.

364 Variation in Immunoblot Patterns According to Geographical Origin of Patients Infected by Helicobacter Pylori (Hp)

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Hp infection is generally acquired early in childhood. The antigenic pattern of Hp may be related to the geographical area of origin. The aim of this study was to compare, by using Hp serological test by Western Blot (Helicob Blot 2.0 from Genelabs), the three different antibodies present in patients according to the area of birth and childhood.

Hp infection was found in 136 patients by mean of positive culture, histology or 13C urea breath test and positive Hp Elisa serological test. Country of birth (and rural/urban area at birth and childhood) were recorded. Countries were distinguished as France and Southern country (Southern Europe and Africa). By using Western Blot serology, the presence of antibodies against different molecular weight antigens (19.5, 26.5, 30 or 35 Kd) and against VacA (89 Kd) and CagA (116 Kd) was compared in the different groups.

Fifty-eight percent of patients were born or spent their childhood in France and 30% in rural area. Antibodies against antigens of 19.5, 26.5, 30, 35, 89 Kd were found in 46, 88, 61, 63, 45 and 67% respectively. Antigens of 26.5, 35 Kd were significantly more frequent in patient born in France (53% vs 25%; P < 0.03). Antibodies against antigens of 19.5 and 89 (VacA) were more frequent in patients from urban area (51% vs 28%, P < 0.02 and 49% vs 32%, P < 0.05 respectively). Cag-A was found similarly whatever the patient origin (66% in France vs 61%; 67% in Urban area vs 68%).

Western Blot patterns suggest that the antibodies directed against antigens of 26.5, 35 Kd are more frequent in South of Europe and in Africa than in France. By contrast Vac A seems more frequent in France and in urban areas. The results evoke that differences in Hp strain are associated with geographical origin of patients.

365 Serological Assessment H. Pylori Infection in Children and Adults with Chronic Gastritis and Gastroduodenitis

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Despite of available commercial serologic tests for Hp pylori (Hp). the diagnostic value of this method is still controversial.

The aim of the study was to assess the serologic exponents of Hp in children and adults. 27 and 23 dyspeptic children and adults with chronic gastritis or gastroduodenitis (diagnosed endoscopically and pathomorphologically according to Sydney System) with Hp infection (confirmed by culture test, histology, and microbiologic methods) and 14 adult volunteers as well as 13 children excluded of Hp were examined by serologic methods. The sera were tested for the presence of lgG, IgM and lgA specific antibodies to the surface acid-glycine extract (GE) obtained from cells of the reference Hp strain (CUG1 17874) and cagA antigen of Hp.

In 70% of children and in all adults with Hp. but only in 9% of uninfected controls lgG, IgM and lgA antibodies were found. In contrast antigE were detected only in 30% infected children and in 55% adults with Hp. The highest frequency of IgM antiGE was found in infected adults (70%), but antiCagA IgM only in 25% of individuals of this group were seen. IgG antibodies were detected only in 2% of children, while in adults they were present in 52% with Hp. and in 30% of the volunteers. Also titres of these antibodies were much higher in adults.

The presence of IgG antibodies (in high titres) against the surface extract antigens of Hp. in both infected groups irrespective of age was seen. Lower frequency of the antibodies to cagA suggests the possibility of cross reactions with other bacterial antigens and rather excludes monitoring Hp. infection by only serologic tests alone. Serologic test IgA of Hp. has no much usefulness in children.

366 An Audit of the Management of Helicobacter Pylori Infection in a District General Hospital

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Carefully controlled clinical trials indicate Helicobacter pylori (HP) eradication rates of 80–90% but it is important to know whether there rates are translated to clinical practice on an intention to treat basis.

Aims of Audit: 1. Is Hp eradication being used appropriately? 2. What method is used for Hp diagnosis pre-treatment. 3. Which treatment regimes are being used and evaluate their efficacy. 4. To check the timing of follow up breath tests.

Method: Information was collected prospectively over a 14 month period. Subjects who attended for a post eradication 13C UBT to confirm the success of eradication were included. The original diagnosis, the initial method used to confirm Hp infection, the Hp eradication regime used and the timing to the follow up breath test after completing eradication therapy were noted.

Results: There were 92 post-eradication breath tests in the study period in 85 patients. The overall eradication rate was 85%. The initial diagnosis was gastric or duodenal ulcer in 91% of cases but eradication was also offered to subjects with NUD (5%), eosinophilis (1%) and gastritis (1%). The CLO test was used most frequently to make the diagnosis of Hp infection pre-treatment. Five eradication regimes were used in the period. The best results were obtained with omeprazole (O) 20 mg bd, Clarithromycin (C) 250 mg bd and metronidazole 400 mg bd with an eradication rate of 96% (n = 25). Dual therapy with O 20 mg bd and C 250 mg tds was second at 93% (n = 27) and traditional triple therapy regimes gave a rate of 85% (n = 25). 96% of post-eradication breath tests were done more than 4 weeks after completing treatment.

Conclusion: In the DGH setting on the basis of intention to treat with no exclusion criteria, eradication rates were comparable to those of randomised controlled trials. Most patients received Hp eradication for PUD but 7% were treated inappropriately. The best results were achieved with low dose OCM (cost 29.88 £). The CLO test was used most frequently to make the initial diagnosis and the timing of a follow-up breath test was appropriate in 96% of cases.

367 Seroconversion of Helicobacter pylori: A Five Years Follow-Up In Asymptomatic Donors Living in a Western Country

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Purpose: Infection rate for Helicobacter pylori (H pylori) in Western countries is reported to be very low: 0.5–2/y year. We aimed to assess the infection rate, as determined by seroconversion from H pylori IgG seronegative to H pylori IgG seropositive, in asymptomatic blood donors resident in an urban area in the North of Italy over a follow-up-period of 5 years (1990–95).

Methods: From a blood donors population screened for H pylori in 1990–91 (N = 1010; M/F: 556/454, age: range 18–65, mean years) a total of 588 (58%) volunteers were invited for a randomised controlled eradication assessment in 1995–96. Specific anti-H pylori antibody were evaluated in duplicate by an “in house” ELISA assay validated in endoscoped patients (sensitivity and specificity of 94%). For each participant a repeat ELISA on the original serum sample (stored at −20 °C) was also carried out (i.e. for follow-up assessment).

Results: Until now 324 donors have been re-evaluated (M/F: 191/133, age: range 23–65 years). At the second measurement of original serum a total of 19/324 (6%) were found to be seropositive, and confirmed seropositive at follow-up. The remaining 305/324 (94%) were confirmed as seronegative. A