Detection of Helicobacter pylori Infection in Patients on Proton Pump Inhibitors

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The xanthine–guanine phosphoribosyltransferase (XGPRTase) is a bacillary bacterium that converts into coccoid form under various stress conditions. Coccoid forms are eliminated in feces, and can be present in drinking water. So they may transit in gut of an individual that already carries another strain. The significance of coccoid forms remains unclear: they are viable, nonculturable, and their infectivity is still under debate. Their DNA is a potential source of exogenous DNA for an implanted strain.

The aim of this work was to test the possibility of DNA transfer from nonculturabale coccoid forms and to characterize this transfer.

We compared the frequency of clarithromycin resistance transfer in vitro natural transformation between a resistant donor strain under spiral or coccoid form and a susceptible receiver strain. We enumerated culturable and nonculturable bacteria using a quantitative real-time polymerase chain reaction (qPCR) method calibrated with a traditional viable count method. We studied by electron microscopy the morphological aspects of the transfer.

Our results show that the transfer of clarithromycin resistance is possible from nonculturable-resistant coccoid forms to culturable bacillary susceptible strain. The fragmented DNA of coccoid form still transfers even after several weeks of culture and the transfer rate is the same whatever the age and the form of the bacteria. TEM data suggest that the transfer requires a contact between the coccoid donor cell and the bacillary receiver. Nonculturabale coccoid forms represent therefore a source of DNA for acquisition of antibiotic resistance and genetic diversity of H. pylori.

Abstract no.: 01.01
DNA Transfer from Coccoid to Bacillary Forms of Helicobacter pylori

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Helicobacter pylori is a bacillary bacterium that converts into coccoid form under various stress conditions. Coccoid forms are eliminated in feces, and can be present in drinking water. So they may transit in gut of an individual that already carries another strain. The significance of coccoid forms remains unclear: they are viable, nonculturable, and their infectivity is still under debate. Their DNA is a potential source of exogenous DNA for an implanted strain.

The aim of this work was to test the possibility of DNA transfer from nonculturable coccoid forms and to characterize this transfer.

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Abstract no.: 01.02
The Potential of Helicobacter pylori XGPRTase as a Therapeutic Target: An In Silico Investigation

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The xanthine–guanine phosphoribosyltransferase (XGPRTase) is an enzyme of purine nucleotide salvage synthesis. The glutamic-pyruvic transaminase (gpt) gene of Helicobacter pylori has been annotated as encoding an XGPRTase and proposed as essential for survival of the bacterium in vitro. The aims of this work were to establish the role of gpt in the bacterium, and to assess the potential of the H. pylori XGPRTase as a therapeutic target by investigating bioinformatically the effects of known inhibitors of purine PRTases.

XGPRTase activity was measured in the cytosolic fraction of five H. pylori strains by 31P-NMR spectroscopy, and also in recombinant XGPRTase produced by cell-free expression. Bioinformatics was employed to analyze the phylogeny of XGPRTase, to compare it to equivalent prokaryotic and eukaryotic enzymes and to build a structural model of the enzyme using threading techniques. The model served to identify key residues of the enzyme involved in catalysis. The interactions of purine phosphoribosyltransferases with inhibitors were used to assess their potential against the H. pylori XGPRTase.

It was demonstrated that the gpt gene of H. pylori encodes a functional XGPRTase enzyme. Analyses of the XGPRTase sequence revealed that the enzyme is significantly divergent from equivalent mammalian enzymes. A XGPRTase model revealed that while parts of the enzyme were similar to those observed of other PRTase enzymes, there were significant differences between H. pylori XGPRTase and purine salvage PRTases. Understanding of these differences will be an important contribution to the development of therapeutic agents against H. pylori XGPRTase.

Abstract no.: 01.03
Disulfide Reductases of Campylobacterales: Are They Involved in Drug Resistance and Response to Environmental Stresses?

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Disulfide reductases of pathogenic bacteria are involved in the resistance to drugs and the elimination of compounds toxic to the pathogen, functions that are crucial to the success of infections. CXXC- or CXXC-derived motifs characterize disulfide reductases and are involved in the catalysis of redox reactions.

Genome searches of CXXC- and CXXC-derived motifs were performed to identify putative disulfide reductases in the epsilon-proteobacteria Campylobacterales Campylobacter jejuni, Helicobacter pylori, and Wolinella succinogenes. Genes encoding thioredoxin, ferredoxin, and methionine sulfoxide reduction were identified in the genomes of the three species, with the exception of genes encoding methionine sulfoxide reduction in W. succinogenes. Phylogenetic trees of the three reductases suggested common clades for these enzymes. For instance, peptide methionine sulfoxide reductase A and ferredoxin oxidoreductase subunit A were significantly closer to one another than to their respective B proteins. In addition, the thioredoxin reductases of Campylobacterales were more closely related to those of Firmicutes than to the corresponding proteins of other proteobacteria.

The enzyme activities of the three Campylobacterales species were measured employing proton nuclear magnetic resonance spectroscopy and spectrophotometry. This was the first time that several of these activities were measured in C. jejuni and W. succinogenes.

These investigations demonstrated that the disulfide reductases of Campylobacterales have characteristic phylogenetic features and enzyme activities. Features that support a special role for these reductases in the physiology of these bacteria, and their response to environmental stresses, such as resistance to drugs and detoxication of xenobiotics.
Non-Animal-Derived Medium for Transport and Growth of Helicobacter pylori

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Introduction. The fastidious nature of Helicobacter pylori burdens the simple exchange of H. pylori isolates between laboratories. In addition, all media that are commonly used for the shipment of live H. pylori strains contain animal-derived materials, and as this is linked with the risk of spreading infectious diseases (like bovine spongiform encephalopathy), their use may be subject to regulations preventing the spread of such diseases.

Aim. The formulation of a transport and growth medium for H. pylori that does not contain animal-derived components associated with the transfer of infectious agents.

Methods. Non-animal-derived medium (NADM) was based on Columbia medium. Beef extract and peptic digest of animal tissue were replaced by soja pepton, serum components were replaced by β-cyclodextrin, and pancreatic digest of casein was replaced by acid-hydrolyzed casein.

Results. 1. Growth: NADM-supported growth of 4/4 reference strains and 16/16 clinical isolates to similar optical densities as obtained with bruccela media; 2. transport: NADM with 0.5% agar supported transport and storage of strains as 4/4 tested reference strains and 11/11 tested H. pylori strains were successfully cultured from vials stored for 3 days at room temperature.

Conclusion. NADM can be used both as transport and as growth medium for H. pylori. The formulation of NADM may allow future certification allowing international transport of H. pylori and other bacterial pathogens.

Ciprofloxacin–Bismuth Complexation: A Novel Approach for Helicobacter Therapy

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Helicobacter pylori is a curved, spiral Gram-negative motile organism. It infects the gastric antrum and causes gastritis. H. pylori infection results in an acute, then chronic inflammation of the gastric mucosa. The inflammation regresses following antimicrobial treatment.

Bismuth Compounds for Eradication of Helicobacter pylori. Helicobacter pylori is highly susceptible to bismuth, a heavy metal with antimicrobial activity linked to its effect on bacterial iron uptake. Despite these findings, bismuth monotherapy often fails to completely eradicate these bacteria. A number of studies have linked the antimicrobial activities of many heavy metals, including bismuth, against Helicobacter.

Fluoroquinolones possess a broad spectrum of activity. They show activity against a wide variety of aerobic Gram-negative and Gram-positive bacteria. The mechanism of their action involves inhibition of bacterial DNA gyrase, which is essential for DNA replication, and it has been proposed that metal complex intermediates are involved in this process.

Experimental Work. Present work involved the synthesis of an organometallic complex of ciprofloxacin with bismuth. The previously mentioned complex was purified and characterized by various spectral techniques like UV, IR, NMR, DSC, AAS.

Preliminary antimicrobial evaluation confirmed the activity of the synthesized complex against various Gram-negative and Gram-positive organisms.

In vitro anti-H. pylori studies were performed and the MIC values for the complex was determined. The complex was found to be active against H. pylori with a MIC value of less than 0.25 µg/l. Also, the activity was compared against the standard drugs (ciprofloxacin alone and also with the bismuth salt alone).
**Abstract no.: 01.07**

The Lectin Cascade and Killing of *Helicobacter pylori*

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**Introduction.** *Helicobacter pylori* survives in the stomach despite a good systemic response but also manages to circumvent the innate immune defenses by coating itself with host CD59, which is anticomplementary. The role of manno-binding lectin (MBL) in killing *H. pylori* has not been investigated. Thus, we have studied the effect of serum components including MBL on *H. pylori* survival using an in vitro system.

**Material and Methods.** Anti-*H. pylori* antibody positive and negative normal serum, serum heated at 50 °C or 56 °C for 30 minutes, C2-deficient serum and three sera deficient in C1q were tested against six clinical isolates of *H. pylori* and NCTC strain 11637. A suspension of *H. pylori* (1.0 × 10^8 final concentration) was mixed with sera or serum supplemented with 100 microg/ml purified MBL and samples taken every 15 minutes for 1 hour and dilutions plated on Colombia blood agar with 5% horse blood and incubated for 4 days at 37 °C in CO₂.

**Results.** Five percent antibody-positive and -negative sera killed *H. pylori* within 15 minutes. Sera heated at 50 °C or 56 °C and sera deficient in C2 or MBL did not kill *H. pylori*. MBL alone had no activity on the viability of *H. pylori*, C2-deficient sera with added MBL did not kill *H. pylori*. The three MBL-deficient sera with added MBL did kill *H. pylori* to the same level as the control sera.

**Conclusion.** *H. pylori* activates the lectin cascade resulting in killing.

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**Abstract no.: 01.08**

Sodium Deoxycholic Acid and Native Pig Bile Tolerance of *Helicobacter pylori* and Enterohepatic *Helicobacter* Species

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*Helicobacter pylori* and enterohepatic *Helicobacter* species (EHS) have been associated with hepatic diseases in man. Previous reports demonstrated antibacterial activity of bile acids against *H. pylori*. *H. pylori* as well as EHS tolerance to native bile has not been reported.

We determined survival and growth kinetics of *H. pylori* (CCUG 17874), *H. pullorum* (CCUG 33838), and *H. bilis* (CCUG 38995) using brucella blood agar (BA), brucella broth (BB), and a defined (F12) liquid medium with or without supplementation with various concentrations of sodium deoxycholic acid (Sdoc) and native pig bile (sterile filtered pool from healthy pigs).

The *Helicobacter* strains were cultured on BA, harvested, suspended in PBS (OD₅₄₀ 1.0), and inoculated onto BA supplemented with pig bile or Sdoc. Aliquots of exponential phase F12- or BB-cultures were supplemented with pig bile or Sdoc and CFU determined at different time-points. *H. pylori* grew on BA and in BB with up to 15% of native bile or 0.05% of Sdoc. Supplements prolonged the lag-phase at higher concentrations. Bacteria grown in F12 were more sensitive to both bile and Sdoc; growth was possible in 0.5% bile and survival (24 hours) in 3% bile. *H. pullorum* was extremely tolerant to bile and Sdoc and grew in pure bile and in BB or BA containing 1.25% Sdoc. Cultivation in F12 decreased the tolerance to Sdoc (2.25%), but not to native bile. Growth of *H. bilis* in BB was not inhibited by supplementation with 60% of native bile. *H. pylori* and EHS survive and replicate in natural bile.

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**Abstract no.: 01.09**

Inhibition of *Helicobacter pylori* by Probiotic and Dairy *Lactobacillus Strains*

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In recent decades, much attention has been paid on health-promoting probiotic properties of lactobacilli. Our aim was to investigate the growth inhibitory activity of seven probiotic and three dairy *Lactobacillus* strains against *Helicobacter pylori*.

Lactobacilli were grown overnight in MRS broth in anaerobic atmosphere. The bacteria were collected and resuspended in fresh MRS broth to 10^9 cfu/ml. *H. pylori* ATCC 43504 grown on brucella–horse blood agar was collected in PBS and 10^9 cfu was plated on the brucella–horse blood plates. Three filter paper discs (diameter 0.5 cm) were set on the plate and 25 µl of MRS broth, 1/100 diluted *Lactobacillus* suspension or undiluted *Lactobacillus* suspension was added on discs. After 3 days cultivation in a microaerophilic atmosphere, the diameters of the inhibition zones were measured.

Of the 10 tested lactobacilli, five (2 dairy and 3 probiotic) inhibited the growth of *H. pylori*. The diameters of the inhibition zones varied between 0.8 and 1.7 cm. Two probiotic strains caused a very narrow (< 1 mm) inhibition zone around the filter paper. The rest of the tested lactobacilli (one dairy and two probiotic) did not have any effect on *H. pylori* growth.

In conclusion, there were both probiotic and dairy strains that inhibited the *H. pylori* growth. The inhibitory activity was strain-specific – strains of same species behaved differently. Half of the tested strains showed potential in *H. pylori* growth inhibition in these preliminary in vitro experiments and are therefore interesting for further characterization.

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**Abstract no.: 01.10**

HYPERCHEM RELEASE 6.0, A Novel Approach for the Design of *Helicobacter pylori* Inhibitors

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The explosive development of computer technology and of methodology to calculate molecular properties have increasingly made it possible to use computer techniques to aid the drug development process with the aim of making it more rational.

**Objectives.** To explain how the software tools (HYPERCHEM release 6.0) help in designing some novel organometallic compounds, which were found to be specifically active against
Helicobacter pylori, a prevalent human pathogen responsible for more than 90% of the cases for ulcer.

Experimental Computation and Model Build. Quantitative structure–activity relationship (QSAR) properties like atomic partial charges, Vander Waals, solvent accessible surface area, Vander Waals's surface bounded molecular volume, mass, log p, molar refractivity, and polarizability were computed for the previously mentioned compounds using HyperChem release 6.0. Atom-wise contribution for the QSAR properties was carried out to find the role of each atom for the change in physicochemical parameters. Model build studies were performed to find the most stable confirmation of the synthesized compounds. Bond-length comparison was performed as a basis for drug receptor studies in details.

Also antimicrobial and anti-Helicobacter (in vitro) studies were performed.

Conclusion and Achievements. These calculations suggest that hydration energy in the range of 22.0 to 28.0 kcal/mol, log p, 1.004–2.07, refractivity, 86–214 Å^2, polarizability, 46–89 Å^3 are the minimum requirements for exhibiting anti- H. pylori activity. Model build studies suggest that the compounds have achieved energy minima and the most stable confirmation. Thus, HyperChem release 6.0 has been found to play a major role in the development of some new generation anti-ulcer agents.

Abstract no.: 01.11
Effect of Probiotic Lactic Acid Bacteria Isolated from Foods Against Helicobacter pylori

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Helicobacter pylori is an etiological agent of gastritis and peptic ulcer disease. Lactobacilli bacteria present in foods have been reported to exhibit antimicrobial activities against human pathogens. Even the oral administration of lactobacilli as supplements has been successfully used for pathogen eradication.

Objective. To evaluate the in vitro antimicrobial effects of lactobacilli isolated from commercial fermented products on H. pylori strains.

Methods. Eighteen commercial food samples, four probiotic lyophilised concentrates, and seven milk samples were analyzed after enrichment in MRS broth. Isolates were identified by CH50 API system. Two reference and two H. pylori clinical strains were used for inhibition assays. Inhibitory activity was assayed with a well test by adding 100 µl of the lactobacilli culture in each well, and by directly using agar discs with lactobacilli on a solid H. pylori culture. Antibiotic resistance was measured by E-test system. Urease activity was detected with a urease media containing phenol red and measured by spectrophotometric analysis at 550 nm. Detected H. pylori NCTC11637 was inhibited by all the lactobacilli tested. The different isolates belonging to L. casei, L. paracasei, L. plantarum, L. acidophilus, L. johnsonii, L. rhamnosus, and L. pentosus species presented antimicrobial activity against all H. pylori strains. Probiotic products containing species different from the previously mentioned were not active against H. pylori. Urease activity was affected by lactobacilli presence. The inhibitory activity was present always when lactobacilli cells were confronted to H. pylori cells, whereas supernatants without lactobacilli cells did not inhibit H. pylori growth.

Abstract no.: 01.12
Comparison of DNA Fingerprints of Single-Colony Isolates of Helicobacter pylori from Corpus and Antrum by RAPD–PCR

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Background. Chronic infection of Helicobacter pylori leads to gastric ulcer and cancer. Bacterial genetic profile is one important factor that determines the consequences of the infection. Genetic fingerprinting of H. pylori isolates indicates remarkable heterogeneity within H. pylori populations worldwide. In this study, genetic fingerprints of H. pylori isolates cultured from corpus and antrum of two cancer patients were compared.

Methods. Gastric biopsies from corpus and antrum of two patients with adenocarcinoma were cultured on brucella blood agar and incubated microaerobically at 37 °C. Twelve single colonies from corpus and antrum of each patient were subcultured for DNA extraction. Random amplified polymorphic DNA–polymerase chain reaction (RAPD–PCR) was optimized using designed primers. The generated DNA fingerprints were analyzed on agarose gel.

Results. DNA fingerprints of 12 colonies from corpus and antrum of each patient showed identical band profiles with 100% homology. Although isolates from two patients exhibited totally different genetic fingerprinting.

Discussion. The results of this study suggest that cancer patients under study were infected with a genetically predominant strain throughout the stomach. It appears that young individuals are commonly colonized with multiple strains of H. pylori, whereas adults become predominantly infected with one strain. These changes might convert a chronic benign to a malignant and detrimental malady.

Abstract no.: 01.13
Two-Phase Medium for Long-Term Storage of Helicobacter pylori Cultures

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The purpose of the work: To elaborate an effective and accessible medium permitting sustained vitality of the Helicobacter pylori cultures in laboratory conditions for long periods.

Medium consisting of semifluid and dense phases was elaborated. A semifluid phase is prepared of brain–heart broth (“HiMedia”, India) with addition of hemin, vitamin K, and agar (0.75 g/l). The dense phase represents egg emulsion coagulated at 85 °C. In each test tube with a dense phase we pour out 2 ml of a semifluid phase. If it is necessary, for addition of selective properties to medium we add amphotericin B, vancomycin, and nalidixic acid in the generally accepted concentrations. Then in test tubes with medium, we inoculate the H. pylori cultures or gastric juice or gastric biopsies. After incubation within 3–4 days at 37 °C, we study the grown microorganisms by microscopy of gram preparation, doing urease and other tests and if it is necessary reinoculate them on other mediums.
The *H. pylori* cultures in a given medium remain viable without reinoculation for not less than 20 days, and with weekly reinoculations – up to 2 years and more with maintenance of basic biological properties.

Thus, elaborated medium allows storing *H. pylori* cultures in laboratory conditions for a long time, at their regular reinoculations from one test tube to another. The combination of dense and semifluid mediums permits to grow microorganisms without the use of anerostats, mineral oil, and shakers, etc., that essentially reduce complexity and financial costs at work with *H. pylori* cultures.

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**Abstract no.: 01.14**

**Probiotics Against Helicobacter pylori. Is There Any Effect?**

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**Objective.** To determine the effect of six probiotics against 16 *Helicobacter pylori* clinical strains.

**Methods.** A prospective study was made from January 2004 to January 2005. *H. pylori* strains were isolated from 16 gastric biopsies. They were plated on blood and pylori agar and incubated under microaerobic atmosphere at 37 °C for 3–5 days. Identification was made by Gram stain and by the presence of oxidase, catalase, and urease. Probiotics were obtained from five commercial products and one blood culture and were identified by Gram stain and Api (BioMérieux). We also tested them for susceptibility to vancomycin by disc diffusion. We obtained three *Lactobacillus* spp., vancomycin-resistant, one *Lactobacillus reuteri* vancomycin-resistant, one *Lactobacillus acidophilus* vancomycin-susceptible, and one *Lactococcus lactis lactis* vancomycin-susceptible. To determine the effect of these probiotics we made the “striation” method. On a blood agar plate we made a striation with the maximum colonies of *H. pylori* that was crossed by another one of the probiotic after a 0.5 MacFarland suspension. Plates were incubated at 37 °C for 3–5 days under microaerobic conditions.

**Results.** Three *H. pylori* strains were inhibited by three probiotics. Two were *Lactobacillus* spp. and one *Lactococcus lactis lactis* and they had an inhibitory effect. After incubating the plates we could see no growth of the *H. pylori* but it was clear for the probiotics.

**Conclusions.** We found an inhibitory effect of three probiotics on three clinical *H. pylori* strains. This effect needs confirmation by other techniques, but keeps the possibility to an adjuvant treatment against *H. pylori*.

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**Abstract no.: 01.15**

**The Growth Inhibitory Effects of Cranberry Extract on Helicobacter pylori – In Vitro Analyses**

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**Background and Aim.** Cranberry is a fruit that originated in North America, and had been used for bacterial infection by the native people. Recent studies revealed that it is effective for preventing refractory urinary infections, and also suggested possible roles in the eradication of *Helicobacter pylori* from human and mice. Besides, the cranberry high-molecular-constituent was shown to have anti-adhesion activity to human gastric cells. The aim of this paper was to investigate the growth inhibitory effects of cranberry on *H. pylori* in vitro and getting the basic backgrounds before possible clinical application.

**Method.** The *H. pylori* strains used were NCTC11637 and 11638 that were cultured in brain–heart infusion (BHI) or on BHI/horse blood plasma plates with or without the cranberry hot-water extract supplied by Kikkoman Co. The concentrations of the bacteria were estimated by measuring OD660, and the bacteria proliferating were titrated using HelicoBlue plates. The shapes of the bacteria were analyzed by a scanning electron microscope.

**Results.** The cranberry extract suppressed the bacterial proliferation in a dose-dependent manner and did almost completely at concentration of 1 mg/ml and 3.3 mg/ml in liquid and on plate culture, respectively. The shapes of the bacteria after incubation with the cranberry extract showed the tendency of more coccoid, shortened, L-shaped appearance, and clustering formation.

**Conclusion.** The cranberry extract inhibits *H. pylori* proliferation and seems inducing unhealthy condition of the bacteria in vitro. Further basic studies on the mechanisms and the effective ingredients of the growth inhibiting function, as well as in vivo studies, will be necessary.

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**Abstract no.: 01.16**

**Optimization of Culture Conditions for Isolation and Recovery of Helicobacter pylori Strains**

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*Helicobacter pylori* is regarded as a highly fastidious organism that typically requires 5 and 10 days for passage and isolation from gastric biopsies, respectively.

The aim of this study was to optimize the isolation and freezing procedures in order to obtain and maintain *H. pylori* strains at their most viable form under most time and cost-effective conditions.

*H. pylori* growth response to different plating media; brucella blood agar and *H. pylori* special peptone agar (HPSPA) supplemented with 10% defibrinated blood was determined, and significant differences were observed. Gastric biopsies cultured on HPSPA medium resulted in larger and separated colonies, leading to easier isolation of single colonies from otherwise contaminated clinical samples.

To identify the optimum gas composition that enhances the growth rate as well as colony size, four different conditions were compared: 1, 5% CO₂; 2, 10% CO₂; 3, 85% N₂, 10% O₂, 5% CO₂; and 4, 85% N₂, 10% CO₂, 5% O₂. Mixed composition of 85% N₂, 10% CO₂, and 5% O₂ yielded the highest rate of strain recovery and larger colony size.

Chances of strain recovery following deep freezing is influenced by enrichment of freezing media and initial load of bacterium. The optimized freezing media in this study was determined as 25% glycerol, 25% fetal bovine serum, and 50% bacterial wash in brain-heart infusion media.

Collectively, the mentioned optimized conditions reduced the required time of incubation by half and doubled the rate of strain recovery.
Abstract no.: 01.17
Performance of Serologic Assays for Helicobacter pylori in European and Asian Populations


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Aims. To investigate the performance of commercial kits in Western (Swedish) and Asian (Vietnamese) populations and to find commercial test alternatives for Asian populations.

Methods. Serum samples were collected from 91 Swedish gastric ulcer patients and 270 Vietnamese peptic ulcer disease patients (PUD); control samples came from 141 Swedish healthy individuals, positive for Helicobacter pylori by urea breath test and 429 Vietnamese population controls of unknown H. pylori status. The assays used were Pyloriset EIA-GIII, HM-CAP, Helicoblot 2.1 and an in-house ELISA with Swedish and Vietnamese strains, respectively.

Results. All four assays had high sensitivities of 96.7–100% in the Swedish populations and in the Vietnamese PUD patients, but HM-CAP was significantly (p < .001) less sensitive. In the Vietnamese population controls, with immunoblot as reference, the in-house ELISA and the Pyloriset EIA-GIII showed good performances, whereas the HM-CAP was both less sensitive and less specific. The performance of the Pyloriset could be further improved in the Vietnamese population controls by lowering the cut-off level to 19, whereas HM-CAP performed poorly even at the indicated optimal cut-off level of 1.8. For the immunoblot, the only difference noted was the response to the 35 kDa antigen, significantly (p < .001) less common in the Vietnamese as compared to the Swedish study populations. The immunoblot performed, however, equally and well in both populations.

Conclusion. Asian populations were found to have lower antibody concentrations to H. pylori strains used in commercial kits than Western populations. The Pyloriset EIA-GIII and Helicoblot 2.1 performed well in both populations.

Abstract no.: 01.18
Is Helicobacter hepaticus Antigen of New Monoclonal Antibodies Related to Strain Diversity of the Bacteria?


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Aims. Several studies have suggested Helicobacter hepaticus was involved in the pathogenesis of chronic liver disease in humans. However, nobody has succeeded in the isolation of H. hepaticus from the human samples and has found out H. hepaticus antigen for ELISA corresponding to the polymerase chain reaction (PCR) result. As one of the reasons, we speculate that the mutagenesis of H. hepaticus may be easily induced. This study investigated whether one strain’s H. hepaticus antigen could be detected in other strains.

Methods. Monoclonal antibodies were prepared from H. hepaticus (A. Lee strain) antigen immunized spleen cells and myeloma cells (P3-X63-Ag8.653), and selected noncross reactivity with other bacteria (Helicobacter five species and five bacteria). The sonicated whole cells of H. hepaticus strains (ATCC, JG Fox strain, A. Lee strain) was estimated by Western blot analysis with the selected monoclonal antibody.

Results. The selected monoclonal antibodies were reacted to H. hepaticus antigen with the molecular mass of 13–15 kDa. The antigen could be detected in A. Lee strain but undetectable in the ATCC strain and GJ Fox strain. The A. Lee strain’s antigen (Lee antigen) was gradually diminished because of the passage culture under the same condition and was shown in an irreversible expression. Several sera of patients with chronic liver disease were reacted to the Lee antigen.

Conclusion. Strain diversity of H. hepaticus may be easily induced with the change of environments. The Lee antigen might be strongly implicated in the strain diversity of H. hepaticus and is diminished easily by the fine change of environment factors.
Abstract no.: 01.20
The Novel Helicobacter pylori Cadmium, Zinc, and Nickel Resistance Determinants CznA (HP0971), CznB (HP0970), and CznC (HP0969) are Required for Urease Modulation and Gastric Colonization

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Maintaining proper metal ion metabolism plays a crucial role in the adaptation of Helicobacter pylori to the changing gastric environment. The nickel-cofactorated urease is essential for survival in the gastric mucosa and it has been demonstrated that iron, copper, and nickel homeostasis is required for H. pylori colonization in animal models. Here we demonstrate that the HP0969-71 gene cluster encodes a novel H. pylori metal resistance determinant, which is essential for gastric colonization and modulation of urease activity. The corresponding genes were designated cznA (hp0971), cznB (hp0970), and cznC (hp0969), because individual hp0971, hp0970, or hp0969 mutants displayed increased sensitivity to cadmium, zinc, and nickel (czn). In the cznA mutant, accumulating nickel ions led to a 8– to 10-fold increase in urease activity, indicating that nickel export by the encoded H. pylori-specific protein is required for urease modulation. Furthermore, the functions of H. pylori cznA, cznB, and cznC in metal export are essential for survival in the gastric mucosa, as the corresponding individual knockout mutants were unable to colonize the gastric mucosa in a Mongolian gerbil-based animal model. In summary, these phenotypes observed indicate that the cznABC genes encode a novel H. pylori metal ion exporter and underline the extraordinary importance of metal ion homeostasis for the survival of H. pylori in the hostile gastric environment.

Molecular Genetics and Genomics

Abstract no.: 01.21
A Novel Copper Regulation System in the Gastric Pathogen Helicobacter pylori

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Ion metabolism plays an important role in the adaptation of Helicobacter pylori to the changing gastric environment and it was demonstrated that maintaining proper metal ion homeostasis is of extraordinary importance for gastric colonization, as mutants defective in maintaining proper iron, nickel, or copper metabolism were unable to colonize in animal models. Here we describe that the H. pylori sensor kinase HP1364 and response regulator HP1365 designated CrdS and CrdR, respectively, are both required for transcriptional copper-induction of the H. pylori copper resistance protein CrdA, which is proposed to sequestrate copper ions in the periplasmic space. H. pylori mutants deficient in CrdR or CrdS production lacked copper-induction of crdA expression and were copper-sensitive. A direct role of CrdR in transcriptional regulation of crdA was confirmed by in vitro binding of CrdR to the crdA upstream DNA region and a 21 nucleotide sequence located nearby the crdA promoter was shown to be required for CrdR binding. We conclude that the first description of a copper regulator in H. pylori will give novel insights in the role of metal homeostasis in the adaptation to the hostile gastric niche.

Abstract no.: 02.01*
HP0906 Controls Flagellar Hook–Filament Transition in Helicobacter pylori

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Helicobacter pylori is dependent on motility for infection. The H. pylori genome encodes a near-complete complement of flagellar proteins compared to model enteric bacteria. One of the few flagellar genes not annotated in H. pylori is that encoding FliK, a hook-length control protein whose absence leads to a polyhook phenotype in Salmonella enterica. We investigated the role of the H. pylori gene HP0906 in flagellar biogenesis because of linkage to other flagellar genes, its transcriptional regulation pattern, and because of the properties of an ortholog in Campylobacter jejuni. A nonpolar mutation of HP0906 in strain CCUG 17874 was generated by insertion of a chloramphenicol resistance marker. Cells of the mutant displayed impaired motility, and produced sheathed, undulating polyhook structures. Expression of HP0906 in a Salmonella fliK mutant restored motility, confirming that
HP0906 is the \emph{H. pylori} \textit{fliK} gene. Mutation of HP0906 caused a dramatic reduction in \emph{H. pylori} flagellin protein production, and a significant increase in production of the hook protein FlgE. The HP0906 mutant showed increased transcription of the \textit{flgE} and \textit{flaB} genes relative to the wild-type, down-regulation of \textit{flaA} transcription, and no significant change in the transcription of the flagellar intermediate class genes \textit{flgM}, \textit{flsD}, and \textit{flsA}. We conclude that the \emph{H. pylori} HP0906 gene product is the hook-length control protein Flik, and that its function is required for turning off the sigma S4 regulon during the progression of the flagellar gene expression cascade.

\begin{abstract}
\textbf{Conclusion.} The NikR and Fur proteins each regulate the expression of the two other copies of \textit{nickel-supplemented medium}, possibly due to the compensatory confirmed for \textit{frpB3}. Mutation of \textit{translational level the nickel- and NikR-dependent regulation was} shows increased transcription of the \textit{flgE} and \textit{flaB} genes. The preliminary public release of the \textit{H. mustelae} genome sequence was screened for genes encoding putative iron-uptake proteins. An isogenic mutant was created in \textit{H. mustelae} ATCC 43772. Strains were grown in iron-restricted and iron-sufficient conditions, and gene expression was monitored by Northern hybridization and SDS-PAGE.
\textbf{Results.} Five genes were identified in the \textit{H. mustelae} genome that encode putative outer membrane receptors for iron uptake (IROMPs). Transcription of the IROMPs FecA, FrpB1B, and CfrA was repressed by iron and Fur, whereas expression of FrpB1a and ChuA was not affected by either iron or Fur. Iron- and Fur-responsive regulation of FecA, FrpB1B, and CfrA was confirmed at the protein level.
\textbf{Discussion.} The \textit{H. mustelae} genome encodes four different families of iron-acquisition genes, which contrasts with the two families encoded by the \emph{H. pylori} genome. The Fur regulatory protein regulates only three out of five \textit{H. mustelae} iron-uptake genes, suggesting either constitutive expression of the other two, or the presence of an additional regulatory system controlling their expression.
\end{abstract}

\begin{abstract}
\textbf{Abstract no.: 02.02} The NikR Protein Mediates Nickel-Responsive Regulation of the \textit{Helicobacter pylori} Iron-Uptake Genes \textit{fecA3} and \textit{frpB3}

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\textbf{Objective.} Intracellular homeostasis of the iron is a necessity for most organisms, as both iron deficiency and iron overload will result in cell death. The gastric pathogen \textit{Helicobacter pylori} has three copies of the \textit{fecA} and \textit{frpB} iron-uptake genes, of which the \textit{fecA1/2} and \textit{frpB1/2} genes are regulated by the Fur protein. Surprisingly, \textit{fecA3} and \textit{frpB3} are not Fur-regulated and thus were thought to be constitutively expressed. \textit{H. pylori} expresses a second metal-regulatory protein, NikR, in this study we have investigated whether the \textit{fecA3} and \textit{frpB3} genes are regulated by NikR.

\textbf{Methods.} \textit{H. pylori} reference strain 26,695 and isogenic mutants were grown in brucella broth supplemented with NiCl\textsubscript{2}. Gene expression was determined by Northern hybridization, primer extension and SDS-PAGE.

\textbf{Results.} Expression of the \textit{fecA3} and \textit{frpB3} genes was nickel-repressed in wild-type \textit{H. pylori}, but constitutively expressed in the \textit{nikR} mutant. However, the genes \textit{fecA1/2} and \textit{frpB1/2} did not display any nickel or NikR-dependent regulation. On the translational level the nickel- and NikR-dependent regulation was confirmed for \textit{frpB3}. Mutation of \textit{frpB3} had no effect on growth in nickel-supplemented medium, possibly due to the compensatory expression of the two other copies of the \textit{fecA} and \textit{frpB} genes.

\textbf{Conclusion.} The NikR and Fur proteins each regulate the expression of a subset of iron-transporter proteins, allowing differential expression of iron-uptake systems depending on the environmental conditions. This may help \textit{H. pylori} to survive the acidic conditions in the gastric mucosa.
\end{abstract}

\begin{abstract}
\textbf{Abstract no.: 02.03} Fur Mediates Regulation of Iron-Uptake Genes in \textit{Helicobacter mustelae}

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\textbf{Objective.} Iron restriction at mucosal surfaces by host is a nonspecific defense mechanism against bacterial pathogens, and hence iron acquisition is considered to be an important virulence factor. \textit{Helicobacter mustelae} is a gastric pathogen of ferrets, and its pathogenesis mimics many aspects of human infection with \textit{Helicobacter pylori}. However, relatively little is known about the virulence factors of \textit{H. mustelae} and their regulation. In this study, we have determined the role of Fur in the regulation of iron acquisition in \textit{H. mustelae}.

\textbf{Methods.} The high-density oligonucleotide microarray analysis gives an opportunity for studying the genetic and gene expression background of the diseases, even in small biopsy specimen.

\textbf{Aims.} Our aims were to identify \textit{Helicobacter pylori} infection and antrum erosion-associated gene expression patterns (GEP) and to compare the gene expression profile of \textit{Hp+} and \textit{Hp–} gastric erosion to explain the possible role and effect of \textit{H. pylori} infection.

\textbf{Materials and Methods.} Total RNA was extracted from frozen gastric biopsy specimens of eight patients with \textit{Hp+} antrum erosion and eight adjacent normal mucosa and eight patients \textit{Hp–} antrum erosion and eight adjacent normal mucosa. The mRNA fraction from the extracted total RNA was amplified by T7 RNA amplification method. Biotin-labeled cRNA probes were synthesized and fragmented. The genome-wide mRNA expression profile was evaluated by GeneChip U133 Plus 2.0 microarrays. Two independent normalization methods (MAS 5.0, RMA), PAM feature selection and hierarchical cluster analysis were performed. The microarray results were confirmed by reverse transcription-polymerase chain reaction (RT-PCR).

\textbf{Results.} Significant overexpression of HLA-DMA, HLA-DQA1 antigen presentation genes, IL7R, ubiquitin D, CCR7 chemokine receptor, killer cell lectin-like receptor, lactoferrin antimicrobial immune response-related genes, and CXCL13 and CCL19 chemokine ligand genes were established in \textit{Hp+} patients compared

\end{abstract}
to H. pylori-infected patients. In antrum erosion increased blood coagulation (F2R, F3), apoptosis (p53-induced protein-3), proliferation (TNFR superfamily 11a), and complement activation (DAF) were found, whereas genes associated with angiogenesis (angiopoietin-like-3), adhesion (alpha2-glycoprotein-1) and transport (Sclf/12, aquaporin-4) were down-regulated.

Conclusions. The presented GEP can be used for multifunctional mRNA-based classification of routine gastric biopsies. These genes can be the targets of further DNA sequence-based studies.

Abstract no.: 02.05
Gastrokine 1 in Helicobacter pylori-Related Gastric Carcinogenesis: A Genomic, Proteomic, and Immunohistochemical Study

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Helicobacter pylori infection is the most important etiological factor responsible for the development of gastric cancer, a leading cause of cancer-related deaths. Prevention is likely to be the most effective strategy for reducing the incidence and mortality from this disease. To be successful, a better understanding of the molecular changes underlying gastric carcinogenesis is mandatory. Proteomic technology is providing a rapid expansion of the basic knowledge, particularly in the discovery of new biomarkers involved in the tumorigenesis process. Our study was aimed to define new molecular markers implicated in H. pylori-related carcinogenesis by a genomic, proteomic, and immunohistochemical approach. The study population comprised of 41 dyspeptic patients and 28 gastric cancer cases. Proteins extracted from gastric biopsies were analyzed by 2D electrophoresis and identified by MALDI-TOF–MS. Protein expression was evaluated by 2D gels and monodimensional Western blot, semiquantitative RNA RT–PCR, and immunohistochemistry. H. pylori infection was detected in 21/41 of the dyspeptic patients. In the H. pylori-positive group, a lower expression of a protein about 18 kDa was observed that corresponded to AMP-18 gastric protein currently referred as Gastrokine-1. The expression of Gastrokine-1 was completely lacking in the gastric cancer cases. Thus, our data suggest that Gastrokine-1 may play a key role in early phase of H. pylori-related gastric carcinogenesis and provide in-depth information for a better understanding of the GC risk of H. pylori-infected individuals with chronic gastritis.

Abstract no.: 02.06
Analysis of Gene Expression Profiles in Gastric Cancer Cells According to Tyrosine Phosphorylation Site of CagA Using Naturally Occurring Isogenic Strains

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Background. Helicobacter pylori CagA protein is injected into cells and undergoes tyrosine phosphorylation. The phosphorylated tyrosine in CagA have various effects on cellular signal transduction, proliferation, apoptosis, and cytoskeletal rearrangement. 147A and 147C are a pair of naturally retrieved isogenic strains which have difference in tyrosine phosphorylation of CagA (J Infect Dis 2003;188:486–496). We analyzed the gene expression profiles in gastric cancer cells stimulated with naturally occurring isogenic strains in order to search for the genes that are affected by the phosphorylated tyrosine of CagA.

Methods. The AGS cells were stimulated for 24 hours by 147A and 147C, respectively. The RNA was extracted and compared by cDNA microarray for human genes in quadruple. The data from microarray were analyzed and searched for the genes showing at least a twofold difference in expression level. Differently expressed genes identified on the microarray were validated by reverse transcriptase-polymerase chain reaction (RT-PCR).

Results. Eighty genes showed significant changes in mRNA expression according to difference in tyrosine phosphorylation of CagA between 147A and 147C. There were nine genes (EGFR, PP12R, etc.) associated to signal transduction, 19 genes (AKAP13, etc.) to cell growth and cytoskeleton, and three genes (FOSL2, etc.) to cell death. Among 80 genes, up-regulations of five genes including AKAP13 and the PTPRA genes were analyzed by RT–PCR. Results. Among at least a twofold difference in expression level. Differently expressed genes identified on the microarray were validated by reverse transcriptase-polymerase chain reaction (RT-PCR).

Conclusion. The differential expression of various genes in relation to tyrosine phosphorylation in this experiment may provide the genetic basis regarding the genofunctional role of cagA gene and could give us the shortcut to the answer for gastric carcinogenesis mediated by H. pylori CagA.

Abstract no.: 02.07
Helicobacter pylori Genotyping in Gastric Low-grade MALT Lymphoma and its Correlation with Clinical Outcome

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Background. Helicobacter pylori has different factors of pathogenicity, which can be associated with diseases like peptic ulcer or gastric adenocarcinoma. Prevalence and influence of H. pylori pathogenic factors in positive low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma and their potential influence
on clinical outcome after eradication of *H. pylori* have not been investigated so far.

**Aim and Methods.** Genotyping of *H. pylori* for *cagA*, *babA2*, *iceAI*, *vacA*M, *vacA*S, and JHP950 has been performed using paraffin wax-embedded tissue from patients with *H. pylori*-positive MALT lymphoma. The different genotypes were correlated with time to complete remission. In addition, the lymphoma cells were analyzed for monoclonality and translocation t (11; 18) to identify potential risk factors for an unfavorable clinical outcome. Patients with only partial remission 12 months after *H. pylori* eradication were irradiated.

**Results.** So far, 24 patients have been included. Twenty-two (92%) were tested positive for *ureA*. During clinical follow-up *n* = 4 were drop outs, *n* = 3 (17%) had to be irradiated because of no complete remission. Fifty percent of MALT lymphomas showed monoclonality, *n* = 2 (1%) had translocation t (11; 18). Genotyping revealed positive results for *cagA*: 64%; *babA2*: 86%; *iceAI*: 50%; and JHP950: 36%.

**Conclusion.** Percentage for *babA2* and *cagA* were identical to former reported results. *IceAI* and the new marker JHP950 (Lehours et al.) were less frequent than already published. Perhaps due to the small number of genotyped *H. pylori*-positive patients there is no correlation between an unfavorable outcome and a special genotype. Still more patients have to be investigated. The genetic markers for JHP162, *oipA*, and *sabA* on-off-status are in work at the moment.

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**Abstract no.: 02.08**

**Effect of Different Clarithromycin Resistance Genotypes on Helicobacter pylori Eradication Different Therapeutic Schemes**

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*Helicobacter pylori* clarithromycin resistance is caused by three-point mutations (A2143G, A2142G, and A2142C), although their association with MIC is still controversial. This study aimed to evaluate the role of these mutations on eradication success and to compare standard triple therapy to sequential regimen according to resistance.

Genetic pattern of clarithromycin resistance at entry was assessed by using a real-time polymerase chain reaction (PCR) on gastric biopsies from *H. pylori*-infected patients (histology, rapid urease test, 13C-urea breath test). Patients were treated with a 7-day triple therapy including rabeprazole, clarithromycin, and amoxycillin (75 patients) or a 10-day sequential regimen including rabeprazole plus amoxycillin for 5 days and rabeprazole, clarithromycin, and tinidazole for the remaining 5 days (81 patients). All drugs were given twice daily. *H. pylori* status was checked 4–6 weeks after treatment as at entry.

Clarithromycin resistance was observed in 38 (24.3%) patients, and A2143G was the most frequently detected point mutation (55.3%). Overall, *H. pylori* eradication was achieved in 47.8% of patients with the A2143G mutate strain and in 94.1% of those with either A2142G or A2142C strains (p = .0019). A significantly higher cure rate in those patients infected with A2143G mutate strains was achieved with the sequential regimen as compared to triple therapy (69.2% versus 20%; p = .024). This is the first study showing that the A2143G mutation confers to *H. pylori* a crucial clarithromycin resistance in vivo. The sequential regimen achieved a significantly higher cure rate as compared to standard therapy even in patients infected with A2143G mutate strains.

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**Abstract no.: 02.09**

**Molecular Detection of Host Cytokine Expression in Cancer and Noncancer Patients via Semiquantitative RT-PCR**

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**Introduction.** The aim of this study was to determine the level of IFN-γ (pro-inflammatory) and IL-4 (anti-inflammatory) cytokine expression as indicators of Th1 and Th2 immune responses in gastric cancer (GC) and nongastric cancer dyspeptic (non-GC) specimens from Iranian patients by gene-specific reverse transcriptase-polymerase chain reaction (RT-PCR).

**Materials and Methods.** Biopsy specimens were collected from three groups of gastric cancer patients (n = 18), non-ulcer dyspepsia (NUD = 38), and ulcer patients (PUD = 20). Total RNA was extracted from gastric tissue under RNase-free condition. Complementary DNA was prepared from total RNA using reverse transcription system, and PCR amplification was performed for HPRT, IFN-γ, and IL-4 cytokines and the intensity of each band was measured by densitometry and normalized against HPRT expression as a housekeeping gene.

**Results and Discussion.** Comparison of results between different groups of patients indicated that gene expression of IFN-γ was similar in non-GC dyspeptic patients (NUD and PUD group; 3.4 ± 1.84, respectively; p < .01). Whereas, in GC cases, the IFN-γ gene expression was significantly higher than that of non-GC individuals (5.46 ± 0.65; p < .0001). This pronounced expression indicates an association between TH1 response and cancer development. There were no considerable differences between NUD and cancer patients with regard to IL-4 gene expression, whereas the expression rate of this cytokine was significantly higher in PUD group (3.7 ± 0.1; p < .05). These data indicate an association between TH2 immune response and ulcer development.

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**Abstract no.: 02.10**

**The Association Between Asp299Gly Polymorphism in TLR-4 Gene and the Risk of Gastric Cancer in Korean Population**


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**Backgrounds/Aims.** Toll-like receptor-4 (TLR-4) is a pattern recognition receptor involved in host defense against Gram-negative
bacteria. It has been suggested to affect lipopolysaccharide responsiveness and expression of pro-inflammatory cytokines. *Helicobacter pylori* infection plays a crucial role in gastric carcinogenesis and *H. pylori* LPS is one of the major virulence factors. The recent report suggested that Asp299Gly polymorphism in TLR-4 gene was associated with impaired LPS signaling and increased susceptibility to Gram-negative bacteria. We evaluated the association between the polymorphism of TLR-4 gene and the risk of peptic ulcer and gastric cancer.

**Methods.** Blood were collected from patients with gastric cancer, peptic ulcer disease, and healthy controls. We performed polymerase chain reaction restriction length fragment polymorphism assay and assessed Asp299Gly allele frequency.

**Results.** A total of 328 subjects (165 men and 117 women) were enrolled. The numbers of enrolled subjects in gastric cancer, gastric ulcer, duodenal ulcer, and control were 122, 58, 52, and 50, respectively. There were no differences in mean age and sex ratio among the groups. *H. pylori* infection rate had no significant difference (66.4% in gastric cancer, 67.2% in gastric ulcer, 65.4% in duodenal ulcer, and 66.0% in control subjects). The Asp299Gly allele of TLR-4 gene was not detected in any patients with gastric cancer, peptic ulcer disease, and controls.

**Conclusions.** The Asp299Gly allele of the TLR-4 gene was not detected in any of the subjects. We suggest that it is very rare in the Korean population and is not involved in the pathogenesis of gastric cancer in the Korean population.

**Abstract no.: 02.11**

**DNA Fingerprinting of Single Colonies Isolates of Helicobacter pylori from a Gastric Cancer Patient by REP-PCR**

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**Introduction.** A large degree of heterogeneity among *Helicobacter pylori* strains suggests that complex consequences of bacterial infection such as ulcer or cancer might be due to infection with specific strains of *H. pylori*. In this regard, DNA fingerprinting methods are recruited as powerful tools to reveal genetic diversity among *H. pylori* population and also to determine genetic changes that *H. pylori* strains might undergo during colonization in the stomach, one consequence of which might be cancer.

**Methods.** Biopsies from corpus and antrum of a cancer patient were cultured on brucella agar and incubated under microaerobic conditions at 37 °C. Twelve single colonies obtained from each biopsy were subcultured and pure cultures were used for DNA extraction. REP-PCR (repetitve extragenic palindromic-polymerase chain reaction) was performed after optimization with designed primers. DNA fingerprints were analyzed on 1.5% agarose gel.

**Results.** DNA fingerprints of single colonies from each corpus and antrum contained similar band profiles. However, genetic fingerprints of single-colony isolates from corpus exhibited only 66% homology with those isolates from antrum.

**Discussion.** The results of this study suggest that *H. pylori* strains colonizing corpus or antrum could have different genetic fingerprinting patterns. This difference might be the result of changes that *H. pylori* strains undergo to be able to adapt to different loci of stomach.

**Abstract no.: 02.12**

**Sequence Diversity of the cagA-3′ Region in Malaysian Helicobacter pylori Strains**

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**Background.** Variation in the cagA-3′ region of *Helicobacter pylori* strains isolated from different geographic regions has been observed.

**Aim.** To investigate the sequence variation in cagA-3′ of *H. pylori* strains and determine their association with clinical outcome and patient’s ethnicity.

**Material and Method.** DNA of 25 strains was randomly selected from the DNA collection of Malaysian *H. pylori* isolates. cagA-3′ region was amplified by polymerase chain reaction (PCR) and cloned using pCR2.1 vector. The recombinant plasmids were sequenced at both strands and sequence analysis was performed.

**Results.** Four subtypes of cagA identified based on the strands of cagA-3′ region were type A1, type A2, type B, and type C. Deduced amino acid sequences showed that there are four different segments present in the region named as α, β, γ, δ. The α segment is more conserved among different genotypes whereas segments β, γ, and δ showed variability in the sequence among strains. The sequence of β1, γ1, and δ1 are similar to the East Asian CagA specific sequence and β2, γ2, and δ2 are similar to the Western CagA. The number of EPIYA motifs detected were three in cagA type A1 and A2, four in cagA type B, and two in cagA type C. cagA type A1 was found to be associated with patients of Chinese origin and also with peptic ulcer disease.

**Conclusion.** It is observed that Malaysian strains of *H. pylori* possess two types of CagA protein: the Western and the East Asian CagA.

**Abstract no.: 02.13**

**Determination of Genetic Diversity Among Iranian and Danish Strains of Helicobacter pylori via RAPD–PCR Fingerprinting**

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Genetically, *Helicobacter pylori* isolates are very diverse and this phenomenon can be used to develop molecular markers for their differentiation. After *H. pylori* single colonies isolation and identification of 50 Iranian and 50 Danish *H. pylori* isolates,
RAPD–PCR was performed according to proposed methods to assess its usefulness in categorizing isolates with geographically diverse origins.

PCR experiments were double-checked to ensure the reproducibility of the technique. Thereafter, dendrograms were created using PHENETREE software and the diversity within and between the two populations was studied accordingly.

The RAPD-generated profiles were composed of two to 10 major bands (average of three bands/isolates). The band size ranges were 0.18–3 kbp. There was no common amplification band among Iranian strains. Amplification bands sized 0.7–0.8 kb were common among Danish strains. Three separate dendrograms for Iranian, Danish, and Iranian Danish strain were obtained. Cluster analysis revealed a high degree of genetic heterogeneity among both H. pylori populations. The mean genetic diversity for the Iranian and Danish strains was calculated to be 0.302 and 0.236, respectively, indicating less diversity for Danish strains. Dendrogram obtained from the collection of Iranian Danish strains revealed no geographic specific clusterization.

Despite the fact that this method was unable to categorize two H. pylori populations into distinct clusters, Danish strains were identified as less diverse.

These data further indicate that the Iranian strain population is a combination from different races and as indicated by the present genotyping study, Iranian H. pylori isolates appear to exhibit nonrestricted diversity as as opposed to the European strains.

The causative role of Helicobacter pylori in the development of gastric carcinoma risk factor is established. Unlike in developing countries, H. pylori multiple strain infection is rare in developed countries. In this study, RAPD–PCR fingerprinting has been used to analyze H. pylori multiple strains to determine the correlation of strain variation and degree of homogeneity of Iranian strains with their disease outcomes. Thus, H. pylori strains were isolated from different gastric sites of 14 cancer and 53 NUD subjects. Following H. pylori identity confirmation, DNA was extracted and genetic heterogeneity was evaluated via RAPD–PCR method using published primers. A second primer was used to confirm the results. Each profile was compared to determine single or multiple strain colonization for each patient.

H. pylori multiple strain infection was found in 42.9% gastric cancer and 26.4% noncancer patients. Among 14 non-GC and six GC patients harboring > 1 H. pylori-infecting strain, 21.4% and 33.3% showed triple strain infection, respectively. The rest had double strain infection.

This study reveals that in Iran, where H. pylori colonization is relatively high, the rate of multiple strain infection is also higher than that of developed countries and even some developing countries. Multiple strain infection may increase the risk of serious clinical implications and decrease chances of antibiotic eradication among the Iranian patients.

The etiology of multiple strain infections requires careful analysis, which has serious implications on eradication regimens and preventive and therapeutic vaccines design and development that should possess polyvalent antigens to ensure lifelong and effective protection.

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**Abstract no.: 02.14**

**Determination of Single versus Multistrain Infection in Gastric Cancer versus Noncancer Patients by Microbiologic Mapping and Fingerprinting**

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The aim of this study was to examine the genotypes of Helicobacter pylori and putative virulence markers in Iranian patients with chronic gastritis (CG) and peptic ulcer (PU).

**Material and methods.** H. pylori strains were isolated from gastric biopsy samples of 81 consecutive patients (37 with PU, and 44 with CG) who underwent endoscopies in Kaunas Medical University Hospital. The different genotypes of H. pylori were analyzed with polymerase chain reaction (PCR).

**Results.** One-third (59.3%) of Lithuanian H. pylori strains carried the cagPAI and 56.8% possessed potentially toxigenic vacA s1 alleles, independent of disease status. PCR type I DNA motifs were present in 15 of 48 (31.2%) strains carrying the cagPAI, where subtype Ia motifs predominated (12 of 15). Eighteen of 48 H. pylori isolates (37.5%) were identified as type II and 12 (25%) as type III. Subtype IIIa was detected in nine of 12 type III strains. vacA s1a alleles were obtained with vacA specific primers. Forty-six of 81 (56.8%) cultures yielded a 259-bp fragment, indicating vacA s1 alleles, and 35 yielded a 286-bp fragment, indicating vacA s2 alleles. Future analysis of the 46 strains with the s1 genotype revealed that 44 (96.2%) were vacA s1a and two (3.8%) were vacA s1b. Strains obtained from patients with PU more often (p < .01) than patient with CG carried cag PAI + (75.7% and 45.5%, respectively) and vacA s1 (75.7% and 40.1%, respectively).

**Conclusions.** In the Lithuanian population, cagPAI- and vacA s1-positive H. pylori strains are potentially ulcerogenic.
Virulence Factors and Pathogenesis

Abstract no.: 03.01*
A Low-Molecular-Weight Protein of Helicobacter pylori Inhibits Human T-cell Proliferation

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We have previously shown that Helicobacter pylori culture supernatants and whole bacteria inhibit proliferation and induce cell cycle arrest of human T cells. This effect was independent of the known virulence factors cagA, vacA, or babA and not due to changes in T-cell activation or T-cell response (Gastroenterology 2005).

Now we have further characterized the nature of the factor responsible for the inhibition of cell proliferation and signal transduction pathways involved in the effect.

Water extracts of H. pylori culture supernatants were analyzed by gel filtration and cation-exchange chromatography. Gel filtration indicated that the factor has a molecular weight (MW) of 30–60 kDa, because fractions corresponding to this MW inhibited proliferation of human T cells. After further purification by cation-exchange chromatography, Jurkat T cells were incubated with inhibiting fractions and analyzed for expression of several cell cycle regulatory proteins by immunoblotting. These fractions induced a significant up-regulation of p27 and a concurrent down-regulation of cyclin D3 and c-myc protein levels. Nuclear translocation of NFAT in Jurkat T cells was not affected by the inhibiting fractions.

In comparison with wild-type mouse embryonic fibroblasts (MEFs), MEFs deficient in p27 (p27–/–) exhibited significantly reduced inhibition of proliferation when incubated with H. pylori water extracts. This suggests a central role of p27 in mediating the cell-cycle arrest induced by H. pylori.

Conclusion. Inhibition of T-cell proliferation is induced by a secreted low-molecular-weight protein of H. pylori that induces up-regulation of the cell-cycle inhibitor p27.

Abstract no.: 03.02*
An Important New Determinant of vacA Toxicity Is Prevalent Among Chinese Helicobacter pylori Strains

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Helicobacter pylori vacA s1m1 strains are more closely associated with gastric adenocarcinoma than s1m2. Paradoxically, in China where gastric cancer is common, most strains are vacA s1m2. vacA s1m2 strains differ in toxicity. We aimed to define genetic differences underlying differential toxicity, determine whether Chinese strains were of the more toxigenic type, and develop a simple polymerase chain reaction (PCR)-based typing system for these more toxigenic strains.

Methods and Results. Comparing vacA between toxigenic (Tox+) and nontoxigenic (Tox–) s1m2 strains revealed a polymorphic region intermediate (i) between signal (s) and mid (m) regions with two types, i1 and i2. We constructed vacA hybrids of strain 93–67 (s1/i1/m2; Tox+) containing either the first or second half of the i-region from strain 93–72 (s1/i2/m2; Tox–); 93–67 i2-i1 and 93–67 i1-i2, respectively. Both hybrids showed reduced vacuolation of RK13 cells compared to 93–67 (93–67 i1-i2 < < 93–67 i2-i1). An i1-i2 hybrid of 93–72 showed a similar vacuolating activity to 93–67 i1-i2. Next we developed an i-specific PCR typing system, confirmed types by nucleotide sequencing, and assessed vacuolating phenotype of strains on RK13 cells. All (8/8) of the Western s1/i1/m2 strains vacuolated RK13 cells compared with 0/12 s1/i2/m2 strains (p < 10−4). All (13/13) of the Chinese s1m2 strains typed as i1 and all vacuolated RK13 cells. All vacA s1m1 strains typed as i1.

Conclusion. We have identified a new region of vacA important for toxigenicity. vacA s1/i1 strains are toxigenic in vitro; s1/i2 are not. All Chinese s1m2 strains tested are type i1, perhaps contributing to the high prevalence of gastric cancer in China.

Abstract no.: 03.03*
Influence of Knockouts in HorB (HP1421) and virB11 (HP0127) on O-chain Synthesis by Lipopolysaccharide of Helicobacter pylori

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A second virB11 homologue (HP1421) occurs at a separate location from that of the Helicobacter pylori cag pathogenicity island and is genetically linked to the flagellar export ATPase, FilH. Production of an outer membrane protein (OMP) HorB (HP0127) is reduced in a virB11 knockout (KO) that influences colonization. As preliminary studies indicated that the lipopolysaccharide (LPS) in HP1421 and HP0127 KOs differed from that of the wild-type strain, we undertook to characterize the LPSs of such mutants of H. pylori CCUG 17874. Electrophoretic (SDS-PAGE) analysis of purified LPS with silver staining showed that while the horBKO expressed only LPS-like CCUG 17874, the amount of O-chain was significantly reduced, whereas virB11KO expressed only rough-LPS lacking an O-chain. Greater heterogeneity of core structures was seen in the horBKO and virB11KO LPS profiles; that of the horBKO was the most heterogeneous. Immunoblotting indicated expression of Lewisα and Lewisβ by horBKO LPS, but to a lesser extent than wild-type LPS, whereas virB11KO LPS did not exhibit reactivity consistent with the absence of an O-chain. A whole-cell ELISA confirmed these findings. Complementation restored production of the O-chains and Lewis expression. Detailed structural analyses confirmed the occurrence, but lower expression of O-chains in horBKO LPS like those of CCUG 17874. Lipid A structures were as expected for H. pylori rough- and smooth-LPS, and the cores of horBKO and virB11KO LPSs exhibited heterogeneity. Although LPS may play a role in OMP

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assembly, this is the first evidence of OMP influence on LPS O-chain assembly in *H. pylori*.

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**Abstract no.: 03.04**

**Reciprocal Regulation of NFAT Transcription Factors by the Two Major* Helicobacter pylori **Virulence Factors, CagA and VacA**

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Infection with cagA-positive *Helicobacter pylori* is associated with the development of atrophic gastritis, peptic ulcers, and gastric adenocarcinoma. The cagA gene product CagA is injected into gastric epithelial cells and disturbs cellular functions by physically interacting with and deregulating intracellular signaling transducers through both tyrosine phosphorylation-dependent and -independent mechanisms. To gain further insights into the pathophysiological activities of CagA, we performed a genome-wide screening of CagA-responsive genes using DNA microarray and identified NFAT transcription factors whose binding sites were over-represented in the promoter regions of CagA-activated genes. Expression of CagA in gastric epithelial cells elicited translocation of NFATc3, a member of the NFAT family, from the cytoplasm to the nucleus and activated NFAT-regulated gene. CagA-mediated NFAT activation was abolished by inhibiting calcineurin or phospholipase Cg activity. Furthermore, treatment of cells with *H. pylori* VacA toxin, which has been shown to inhibit NFAT in T lymphocytes, counteracted the ability of CagA to activate NFAT in gastric epithelial cells. These findings indicate that the two major *H. pylori* virulence factors inversely regulate NFAT activity. Given the pleiotropic activities of NFAT in cell growth and differentiation, deregulation of NFAT, either positively or negatively depending on the relative exposure of cells to CagA and VacA, may contribute to the diverged clinical outcomes caused by *H. pylori* infection.

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**Abstract no.: 03.05**

**Evolution of the* Helicobacter pylori* cag Pathogenicity Island (PaI) by Partial Deletion in Strains from Relatives of Gastric Cancer Patients**

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Background. *Helicobacter pylori* strains possessing the cag pathogenicity island (PaI) and vacuolating forms of VacA are associated with the development of gastroduodenal diseases. The cag PaI induces interleukin-8 secretion from epithelial cells and delivers CagA into the host cell cytosol. We have recently shown that evolution of vacA and cagA occurs commonly within individuals and within families from Scotland (Argent et al. Gut 54 (S2), A25; 2005). We now turn our attention to determining evolution within the cag PaI in this population.

**Methods/Results.** Forty-three out of 45 individuals had single-strain infections by random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) of single colonies. Twenty-nine strains were shared between at least two family members. Two and five individuals had isolates with different vacA and cagA alleles, respectively, despite identical RAPD patterns. Within families with the same strain, two and eight members had different vacA and cagA alleles, respectively. Single colonies isolated for *H. pylori* strain AB9 possessed either s1/m1 or s1/m2 vacA. The s1/m2 form was not expressed. PCR amplification of cagA, cagE, cagT (virB7), and cag10 (virD4) genes revealed that all isolates possessed cagA and cag10. Some isolates possessing s1/m1 vacA and all with s1/m2 vacA, lacked cagE and cagT, indicating partial deletion of the cag PaI. Co-culture of isolates with AGS gastric epithelial cells revealed that only those with all four genes amplified had CagA phosphorylated within, and induced interleukin-8 secretion from AGS cells.

**Conclusions.** Evolution of virulence factors occurs rapidly and within the lifetime of individuals. This may increase or decrease virulence and has important implications for pathogenesis.

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**Abstract no.: 03.06**

**Iron-Repression of Urease Expression in *Helicobacter hepaticus* is Mediated by the Transcriptional Regulator Fur**

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The pathogen *Helicobacter hepaticus* colonizes the enteric and hepatobiliary tract of rodents and causes inflammatory bowel lesions, hepatitis, and hepatic malignancies. Urease is an environmentally regulated key-virulence factor for gastric *Helicobacter* species, but little is known about its role or regulation in nongastric *Helicobacter* species. Low iron is often used by pathogenic bacteria as a signal for entering the host. Here it is reported that urease activity of *H. hepaticus* is iron-repressed, and this regulation is mediated by the transcriptional regulator Fur. Iron-depletion of growth medium resulted in a three-fold increase in urease activity in wild-type *H. hepaticus* strain ATCC 51449. Using Northern hybridization, it was demonstrated that iron-repression of urease expression was mediated at the transcriptional level. Mutation of the fur gene abolished the effect of iron-depletion, suggesting a role for Fur in iron-repression of urease expression. This was confirmed using a gel-shift assay, as recombinant Fur displayed iron-dependent binding to the urease promoter region.

**Conclusion.** Iron and Fur are likely to contribute to *H. hepaticus* virulence as they are both involved in the regulation of the urease virulence factor. Iron-mediated urease regulation has not been reported before and may be important in the colonization of the hepatobiliary tract by *H. hepaticus*.
Abstract no.: 03.07
Effects of Bile on Helicobacter hepaticus Expression of Virulence Factors

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Helicobacter hepaticus causes chronic hepatitis and liver cancer in mice. It colonizes the lower bowel, a habitat where it comes in contact with bile, which has antimicrobial activities. To elucidate habitat adaptation mechanisms of *H. hepaticus*, a study of its response to bile was performed.

Strain ATCC 51449 was grown for 48 hours in media supplemented with ox bile at concentrations 0–5%. Growth–response curves were obtained by determining the CFU/ml at 0 and 48 hours. The protein expression of bacteria grown at a sublethal bile concentration of 0.1% was compared with those grown without bile using 2D-PAGE. Identification of protein spots unique to one growth condition or with differential intensities greater than twofold between both conditions was performed using tandem mass spectrometry.

Bacterial growth declined by an order of magnitude at 0.1% bile concentration and did not grow at 5.0% bile concentration. Proteins down-regulated in the presence of bile included cytolethal distending toxin (Cdt), superoxide dismutase, major flagellin subunit FlaA2, and glutamate dehydrogenase. Translation elongation factor EF-Ts and the conserved hypothetical protein (HH11427) were up-regulated in the presence of bile.

Cytolethal distending toxin is an important virulence factor of *H. hepaticus*. Its down-regulation in the presence of bile opens the possibility of alternative mechanisms through which the bacterium causes hepatitis and liver cancer. However, the down-regulation of superoxide dismutase and glutamate dehydrogenase, which protect bacteria and *Plasmodesma flagellarum* against reactive oxygen species, respectively, suggested that bile may serve to protect *H. hepaticus* in host colonization.

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Abstract no.: 03.08*
Evaluating Proinflammatory and Adherence Properties of the Helicobacter pylori Outer Membrane Protein 13 (Omp13)

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Background and Aims. Helicobacter pylori strains have a functional or nonfunctional *omp13* (opA) gene, depending on the number of CT dinucleotide repeats in the coding region. We investigated the influence of Omp13 on bacterial adherence, colonization, and gastric inflammation.

Methods. A total of 60 antral biopsies from *H. pylori*-infected patients were assessed in regard to the degree of granulocytic and lymphocytic infiltration. The infecting *H. pylori* strain type was characterized regarding *cagA* and *omp13* status by polymerase chain reaction (PCR) or sequencing. Bacterial colonization densities were determined semiquantitatively after immunohistochemical *H. pylori* staining. IL-8 mRNA expression in the gastric mucosa was analyzed by real-time RT-PCR (reverse transcriptase-PCR).

In vitro adherence assays and cytokine secretion assays were performed with *H. pylori* wild-type, *omp13*-mutant, and *omp13*-recomplemented strains on KATO-III and AGS gastric cancer cell lines.

Results. Omp13-on status was strongly associated with presence of the *cagPAI*. In vitro *omp13*-mutant strains showed reduced adherence to epithelial cells compared to wild-type strains. Complementation of *omp13* completely restored the adherence properties. In accordance with these results, bacterial colonization densities were higher in patients infected with *omp13*-positive than in patients harboring *omp13*-negative strains. However, the *omp13* status did not significantly alter IL-8 secretion in vivo or in vitro.

Conclusion. Omp13 ameliorates the adherence properties of *H. pylori* and thereby augments the density of bacterial colonization. There is no direct effect of Omp13 on IL-8 secretion. In contrast to previous reports, it appears that Omp13 is of smaller clinical importance than originally anticipated.

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Abstract no.: 03.09
Proteomic Characterization of an HpaA-Negative Helicobacter pylori Mutant and its Colonization Ability in Mice

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*Helicobacter pylori* Adhesin A (HpaA) is an immunogenic surface-localized protein present in all *H. pylori* strains. Although originally described as an adhesin, no concluding studies on the function of HpaA have been performed. The aim of this study was to study the importance of HpaA for colonization, by introducing a mutation of the *hpaA* gene into the mouse-adapted *H. pylori* strain SS1. To verify that the interruption of the *hpaA* gene did not cause any polar effects of downstream genes or was associated with a second site mutation, the protein expression pattern of the mutant and wild-type strain was characterized by two different proteomic approaches. Two-dimensional dimensional differential in-gel electrophoresis (DIGE) analysis of whole cell extract and subcellular fractionation combined with nano-LC–Fourier transform ion cyclotron resonance (FT-ICR) MS for outer membrane protein profiling revealed that only HpaA was absent in the mutant strain. Therefore, the mutant strain was tested for its colonizing ability in a well-established mouse model. Whereas the wild-type *H. pylori* strain readily infected mice, the HpaA mutant strain was not able to establish colonization. Thus, we conclude that HpaA is essential for colonization of *H. pylori* in mice.

Abstract no.: 03.10
Carcinogenetic Potential of the WSR cagA Region
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Within CagA, a different number of repeats of the EPIYA, the tyrosine phosphorylation site, might be found. Higher phosphorylated CagA is suggested to worsen Helicobacter pylori infection outcome. The aim of this study was to ascertain whether a different number of the first (FR) or of the second (WSR) repeat region of the cagA gene, encompassing the sequence encoding for the EPIYAs, correlates with inflammation and outcome of cagA-positive H. pylori infections. We studied cagA-positive Italian patients with antral (n = 18) or diffuse (n = 16) gastritis, with duodenal ulcer (n = 16), or with noncardia gastric cancer (NCGC, n = 25). The number of repeats in FR and WSR regions were PCR-analyzed from antral isolates. In a subset of 28 isolates, 14 from NCGC, the whole 3′ cagA region was sequenced. In all patients the FR region contained one repeat. The WSR repeats ranged from zero to three. Twenty-two patients were coinfected by strains with a different number of WSR repeats. H. pylori strains with less than two WSR repeats had a more severe corpus activity (χ² = 13.74, p < .005). NCGC was correlated with H. pylori strains with two or more WRS repeats, whereas those with less than two repeats were correlated with duodenal ulcer (χ² = 12.45, p < .05). Although differences in nucleotide sequence of the 3′ cagA region were found, none was associated with disease diagnosis or inflammatory degree. In conclusion, in Italian patients cagA-positive infections with less than two WSR repeats are correlated with duodenal ulcer, those with two or more repeats with NCGC.

Abstract no.: 03.11
Differences in the Prevalence of EPIYA Tyrosine Phosphorylation Motifs in CagA Protein of Helicobacter pylori Clinical Isolates from Children and Adults
E. Panayotopoulou,* K. Papadakos,* A. Kalliaropoulos,* K. Petraki,* S. Karagiannis,† F. Dimopoulos,‡ S. Michopoulos,§ E. Katsiyiannaki,† E. Roma,§ A. Archimandritis,‖ A. Mentis* and D. N. Sgouras‖
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The presence of tyrosine phosphorylation motifs (TPMs) A:EPIYAKVKN, B:EPIYAQQVK, and C:EPIYATIDLGG in CagA protein have been proposed to enhance cagA-dependent pathogenicity. Based on the peptide identity of published CagA protein sequences, we developed degenerate polymerase chain reaction (PCR) primers and subsequently sequenced and mapped the EPIYA motifs in 124 Helicobacter pylori isolates (64 adults, 60 children). Strains were also typed with respect to vacA signal and mid-region. A higher prevalence of EPIYA-negative strains in children (20.0%) versus the adult (9.4%) population was observed, although there was no real difference in H. pylori cagA-negative strains (adults 14.1%, children 11.7%). The majority of strains harbored the ABC (adults 53.1%, children 51.7%) and the ABCC combination of TPMS (adults 15.6%, children 13.3%). Five strains with additional TPM-C repeats (ABCC) were detected exclusively within the adult population (7.9%). A significant trend for a higher number of TPM-C in adults (see table below, χ² test, p = .045) was observed. In addition, the presence of vacA s2m2 isotype was associated with cagA-negative (p < .001), but not EPIYA-negative strains. However, in children a strong association was observed between EPIYA-negative strains (p < .001) and the least vacuolating vacA s2m2 isotype. In conclusion, strains isolated from adults are likely to harbor an increased number of phosphorylation TPM-C repeats within the CagA protein, whereas in children a significant trend for higher prevalence in EPIYA-negative vacA s2m2 H. pylori strains is observed.

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<td>6</td>
<td>12</td>
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<tr>
<td>1</td>
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Abstract no.: 03.12
mtDNA Alterations in Helicobacter pylori Chronic Gastritis and Gastric Carcinoma
C. Figueiredo,‖‡ V. Maximo,* P. Soares,*§ S. Sousa,* F. Carneiro,*‡ R. Seruca* and M. Sobrinho-Simoes*‡
†IPATIMUP – Institute of Molecular Pathology and Immunology and ‡Faculty of Medicine of the University of Porto, Porto, Portugal

Mitochondrial DNA (mtDNA) alterations have been demonstrated in various types of tumors, including gastric carcinoma. Our aim was to elucidate the significance of mtDNA alterations in the context of Helicobacter pylori chronic gastritis (CG) and gastric carcinoma (GC).

A total of 160 H. pylori-infected patients (99 with CG and 61 with GC) were genotyped for vacA and cagA genes, and screened for mtDNA alterations in ND1, ND3, ND4, ND5, COI, and ATPase6 genes, and D-Loop regions D310 and D514.

Overall, mtDNA alterations were more frequent in GC (28/61, 46%) than in CG patients (20/99, 20%) (p < .001). D-loop mutations were more frequently detected in GC (22/61, 36%) than in CG patients (17/99, 17%) (p = .008). Gastric carcinoma patients harbored mutations in both D310 (19/61, 31%) and D514 (9/61, 15%) D-loop regions, whereas CG patients harbored mutations only in D310 (17/99, 17%).

In coding genes, mutations were more frequent in GC (13/61, 21%) than in CG (5/99, 5%) (p = .003). GC patients presented a broader spectrum of mutations than CG patients. In the first group, mutations were detected in all genes, except ND4. ND1 (5/13, 38%) and ND5 (6/13, 46%) were the most frequently mutated genes. In contrast, CG patients harbored mutations only in ND1 gene.
There was a relationship between specific *H. pylori* genotypes and mtDNA mutations.

These results suggest that mtDNA alterations are frequent events in GC, and that these alterations may be mediated by specific *H. pylori* strains.

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**Abstract no.: 03.13**

**Positive Association Between the Presence of CagA Protein EPIYA Motifs in Helicobacter pylori Clinical Strains and the Severity of Histopathologic Lesions**

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We aimed to study the potential association between the presence of EPIYA tyrosine phosphorylation motifs (TPMs) in CagA protein of *Helicobacter pylori* clinical isolates with the clinical manifestation of the disease and the observed histopathologic lesions.

We analyzed 58 cagA-positive *H. pylori* clinical isolates taken from patients with gastroduodenal ulcer (*n* = 39) and nonulcer dyspepsia (*n* = 19). EPIYA motifs were determined by polymerase chain reaction (PCR) amplification and sequencing of the resulting products. cagA status was determined by PCR cagA amplification and serum CagA ELISA. *H. pylori* colonization and the associated gastritis was evaluated by the modified Sydney system and statistical analysis performed by Fischer's exact test. EPIYA-positive *H. pylori* clinical isolates in the antrum were correlated significantly with the presence of gastroduodenal ulcer (*p* = .002). The association was strong with the presence of duodenal (*p* = .016) but not gastric ulcer (*p* = .091). There was significant positive association with the severity of chronic inflammatory infiltration (*p* = .037) and the activity of chronic gastritis (*p* = .091). There was significant positive association with the severity of chronic inflammatory infiltration (*p* = .037) and the activity of chronic gastritis (*p* = .033), but not with higher levels of *H. pylori* colonization (*p* = .126). No statistical significance was observed in the fundus.

In conclusion, the severity of chronic inflammatory infiltration and the activity of chronic gastritis developed in the antrum of *H. pylori*-positive patients seem to be associated with the presence of EPIYA TPMs in CagA protein, irrespective of the levels of *H. pylori* colonization in the gastric mucosa. This association is significant in patients with duodenal but not gastric ulcer.

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**Abstract no.: 03.14**

**UreA2B2: A Second Urease System in the Gastric Pathogen Helicobacter felis**

R. G. J. Pot,* E. J. Kuipers,* P. J. M. Nuijten,† L. A. M. de Haan,‡ P. Loeffen,‡ A. H. M. van Vliet* and J. G. Kusters*

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**Objective.** Urease enzyme is a major virulence factor of *Helicobacter* species. Murine infection with the animal pathogen *Helicobacter felis* is a model for human *H. pylori* infection and has been used frequently to test the efficacy of urease-based vaccines against *Helicobacter* infection. The aim of this study was to investigate the urease system of *H. felis*.

**Results.** Immunoblot analysis of *H. felis* strains with urease-specific antibodies revealed two immunoreactive bands of 67 and 70 kDa. The 67 kDa protein was identified as the urease large subunit UreB, whereas the N-terminal amino acid sequence of the 70 kDa protein displayed 45% identity with the *H. felis* UreB protein and was tentatively named UreB2. The gene encoding the UreB2 protein was subsequently shown to be organized in a gene cluster named *ureA2B2*. This gene cluster was present in the chromosome of all tested *H. felis* strains, even in those strains where UreB2 expression was absent. Insertional inactivation of the *ureB* gene led to complete absence of urease activity, whereas inactivation of the *ureB2* gene did only result in lowered urease activity (*p* = .043).

**Discussion.** The gastric pathogen *H. felis* expresses two sets of urease subunits, a unique feature among bacterial pathogens. The exact function of the UreA2B2 system is currently unknown, as the UreA2B2 proteins do not seem to constitute an active urease enzyme. We hypothesize that UreA2B2 may contribute to the pathogenesis of *H. felis* infection by either antigenic variation or by allowing a switch in urease expression in unfavorable conditions.

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**Abstract no.: 03.15**

**New Pathogenicity Markers for Peptic Ulcer Disease Found in Helicobacter pylori Strains Isolated from Portuguese Children**

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Comparison of two *Helicobacter pylori* strains isolated from a peptic ulcer (PUD) and a gastritis from two young children, by polymerase chain reaction (PCR)-based subtractive hybridization, showed the presence of two genes ulcer-associated and J99-specific (*jhp562* and *jhp870*). We investigated by PCR and sequencing the presence of these genes on *H. pylori* strains isolated from 77 Portuguese children (21 DU, 3 GU, and 53 with gastritis). We also evaluated the association to previously described virulence-associated genes. Both *jhp562* and *jhp870* were significantly associated with PUD (*p* < .01, OR = 8.44 and 6.60, respectively). The presence of *jhp562*-positive genotype was strongly associated with *vacAs1*, *cagA*, *babA2*+ genotypes, and *oipA* “on” status, whereas *jhp870* was associated with *vacAs1*, *cagA*, *babA2*+ genotypes, *hopQ* I allele, and *oipA* “on” status. The risk for PUD increased slightly when *jhp562*-positive genotype was combined with (1) *cagA*+ alone (OR = 9.17 versus OR = 9.56) (2) *vacA3* alone (OR = 13.76 versus OR = 15.36), or (3) both *cagA*+/*vacA3* (OR = 8.13 versus OR = 11.07). Association of *jhp870* with *cagA* or *vacA* did not improve the discrimination between disease outcome.

To support these results, we studied a group of *H. pylori* strains isolated from Brazilian children with DU and gastritis, from which data concerning *cagA* and *vacA* were available. Association of *jhp562* and *jhp870* with *cagA*+ and *vacA*+ was also observed.
Although individually neither jhp562 nor jhp870 were statistically associated with DU, the combination cagA+/vacAs1/jhp562+ presented the higher risk for this disease.

These results suggest that jhp562 and jhp870 genes are candidates as virulence markers of H. pylori.

Abstract no.: 03.16
The Effects of Helicobacter pylori Virulence Factors on the Immune Response in Human Gastric Mucosa

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Helicobacter pylori infects more than half of the world’s population and constitutes a major risk factor for peptic ulcer and gastric cancer development. H. pylori virulence factors are considered to play important roles in determining the clinical outcome of the infection. Intimate contact with host cells, mediated by outer membrane proteins such as BabA, is thought to be involved in the pathogenesis of the histologic changes. However, the underlying mechanism is still largely unknown. We therefore studied the gene expression profiles in human gastric mucosa infected by different strains of H. pylori, using cDNA microarrays. The genotype of H. pylori in infected human gastric biopsies was determined by reverse transcriptase-polymerase chain reaction (RT-PCR). Five triple-positive (CagA+, VacAS1+, BabA2+) and five double-positive (CagA+, VacAS1+, BabA2−) samples were identified, and the corresponding biopsies subsequently analyzed by cDNA inflammatory microarrays. The data show that a number of genes are up-regulated in the triple-positive as compared to the double-positive samples, including interleukin 1 receptor accessory protein (IL1RAP) and teratocarcinoma-derived growth factor 1 (TDFG1), which are both involved in cancer development. In addition, ENA78, a CXC chemokine that potentially attracts and activates neutrophils in the inflammatory process, is down-regulated. Further analysis of these genes and related factors/pathways may help us to better understand the role of BabA in the pathogenesis of H. pylori infection.

Abstract no.: 03.17
Lesser Potency of Helicobacter pylori than Escherichia coli Lipopolysaccharide, but Positive Interaction with Ibuprofen, in Apoptosis Induction

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Helicobacter pylori lipopolysaccharide (LPS) causes apoptosis in the stomach and in cultured gastric mucosal cells, but its reported high potency (10^6 g/ml) contrasts with the low immunologic activity of this LPS. NSAIDs, which have been suggested to interact with H. pylori to promote gastric damage, also induce apoptosis in the stomach and in primary cell cultures. The present study was undertaken to further investigate the apoptosis-inducing action of H. pylori LPS and explore potential interactions between the NSAID, ibuprofen, and LPS on apoptosis in gastric mucosal cells. Apoptotic effects of LPSs from H. pylori NCTC 11637 and Escherichia coli O111:B4 on primary cultures of guinea-pig gastric mucosal cells were investigated in the presence and absence of ibuprofen. Cell loss was estimated by a crystal violet assay, and apoptosis determined from caspase activity and from condensation and fragmentation of nuclei. Exposure to E. coli LPS for 24 hours caused cell loss and enhanced apoptotic activity at 10^6 g/ml, but similar effects were only obtained with H. pylori LPS at higher concentrations (10^8 g/ml). Caspase 3-, 8-, 9-like activities were all significantly increased (p < .05) after 6 hours exposure to H. pylori LPS (10^5 g/ml). The onset of apoptosis in response to ibuprofen was slower than with LPS (24 hours versus 6 hours). Whereas ibuprofen (250 µmol/l) caused cell loss and apoptosis, addition of E. coli or H. pylori LPSs enhanced these effects. Hence, although of lower potency, H. pylori LPS could contribute to apoptosis-related chronic gastric damage that is enhanced by the use of NSAIDs.

Abstract no.: 03.18
Helicobacter pylori Stimulates Cancer Cell Invasion: Molecular Mechanisms Involved


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Helicobacter pylori, a bacterial pathogen that colonizes the human gastrointestinal tract, is associated with the development of gastritis, gastric and duodenal ulcers, and gastric cancer. In the last two decades, several studies have been performed on the characterization of the major bacterial virulence factors and on the molecular interactions established between the bacterium and the host cells. However, how these molecular interactions contribute to gastric cancer development remains to be determined.

Invasion is the hallmark of malignancy either through local spread or through metastasis to distant organs. At the primary site of cancer, invasion occurs when cancer cells are stimulated by extrinsic factors. Bacteria, abundant at the primary site, are putative sources of such factors. We have recently demonstrated that enteric bacteria stimulate colon cancer cell invasion and motility through the production of a soluble motility factor.

Using a similar approach, we investigated the effect of H. pylori on gastric cancer invasion and clarified the molecular mechanisms involved. AGS gastric cancer cells were cocultured with distinct H. pylori strains on top of collagen type I gels or of Matrigel-coated filters. Our results indicate that H. pylori stimulates cancer cell invasion and that direct contact between the bacterium and the cells and a functional bacterial type IV secretion system are required. Stimulation of invasion and motility occurred in a P13K-independent manner but involved the activation/phosphorylation of specific tyrosine kinase receptors and an increased production of matrix metalloproteases.
Abstract no.: 03.19
Relationship Between the Diversity of cagA Gene of Helicobacter pylori and Gastric Cancer in Japan

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Background and Aims. CagA is one of the most studied virulence factors of Helicobacter pylori. We recently showed that there are two different CagA sequence motifs between East Asian strains and Western strains. East Asian CagA has a distinct sequence at the SHP-2-binding site and conveys stronger SHP-2-binding and -transforming activities than those of Western-type CagA. We investigated the diversity of CagA in strains from two different areas in Japan (Fukui and Okinawa), where gastric cancer risk is different, and examined the relationship between CagA sequence diversity and clinical outcome. The death rate of gastric cancer is more than 2.4 times higher in Fukui than in Okinawa.

Methods. A total of 176 strains (97 in Fukui and 79 in Okinawa) was subjected to sequence analysis of the cagA gene.

Results. Prevalence of East Asian CagA was significantly higher in cancer strains than in gastritis strains in Okinawa. Most cancer strains in Okinawa (19/21) carried East Asian CagA as well in Fukui. Two Okinawan cancer strains carried Western-type CagA with two or three repeats of WSS (Western CagA-specific, SHP-2-binding sequence), whereas most Western-type CagA strains from patients with gastritis or peptic ulcer carried only one repeat of WSS.

Conclusion. These findings suggest that East Asian CagA motif and the number of repeats of WSS correlate with the pathogenesis of gastric cancer.

Abstract no.: 03.20
sabA and hopZ are not Pathogenicity Markers for the PUD in a Portuguese Population

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The aim of this work was to study the potential of sabA and hopZ genes as virulence markers for peptic ulcer disease (PUD) in Helicobacter pylori strains isolated from Portuguese patients, 106 isolated from adults (50 with PUD, 56 with gastritis) and 72 from children (19 with PUD, 53 with gastritis). Genotyping was performed by polymerase chain reaction (PCR) and sequencing of the 5′ region of the genes.

In the majority of strains, both genes were on (57.3% for sabA, 59.0% for hopZ). We also observed that sabA was more associated with gastritis only in strains isolated from children (p = .019, OR = 2.56), hopZ on was more prevalent in strains from PUD, however, the difference was not significant. Supporting these results, we found that sabA on was significantly associated with the less-virulent genotypes (cagA−, vacAs2, and oppA off) in the strains isolated from children. On the other hand, there was no association between any functional status of sabA and hopZ with other genes in the adult population. Finally, we compared some genotype combinations. In strains isolated from children, the risk for PUD increased when sabA on was associated with: cagA+ (OR = 11.733 versus OR = 9.17), vacA1 (OR = 15.600 versus OR = 13.76), and cagA+/vacA1 (OR = 13.475 versus OR = 8.125).

We concluded that neither sabA nor hopZ is pathogenicity markers for the PUD in a Portuguese population, however, sabA on genotype can contribute to the distinction between PUD and gastritis when associated with other virulence genes.

Abstract no.: 03.21
The Mongolian Gerbil Model is Useful to Study Helicobacter pylori-Associated Gastrroduodenal Diseases

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The effects of Helicobacter pylori cag-PAI were investigated on the development of chronic atrophic gastritis, peptic ulcer, and chronic pancreatitis in a gerbil model.

Methods. Mongolian gerbils were infected with H. pylori type I strain or an isogenic cagY mutant for 7 months. Paraffin sections of antrum and corpus were stained for metaplastic changes of the gastric mucosa and fibrous changes in the pancreas. Stomach pH, blood glucose and plasma gastrin, lipase, and amylase were measured. Frozen biopsies were used for reverse transcriptase-polymerase chain reaction (RT-PCR) quantifying the expression of H+/K+-ATPase and of cytokines IL-1β, IFN-γ, and KC.

Results. Wild-type infected gerbils showed a severe transmucosal inflammation in the antrum and corpus with an increased IL-1β, IFN-γ, and KC mRNA expression. Atrophy, hyperproliferation, hypergastrinemia, and hypochlorhydria were observed. About 90% of the WT-infected gerbils developed peptic ulcer and metaplastic changes. Transmural inflammation and pancreatitis were developed in 56% and 33% of WT-infected and mutant-infected gerbils, respectively. Typical parameters of a chronic pancreatitis were present.

Conclusion. The cag-PAI of H. pylori is responsible for a severe inflammation that results in corpus-dominant atrophic gastritis, metaplastic changes, and peptic ulceration. Independent of the cag-PAI, we observed the development of pancreatitis, especially in animals that revealed a transmural antral inflammation and peptic ulceration. The chronic pancreatitis revealed fibrous changes as shown in trichrom stained sections. This novel observation demonstrates that Mongolian gerbils are an adequate model to investigate the H. pylori-induced chronic pancreatitis. A deeper understanding of the pathomechanism of a H. pylori-associated pancreatitis might reveal clinical relevance.
Abstract no.: 03.22
The Relationship of *Helicobacter pylori* CagA Status with Preneoplastic Conditions of Gastric Mucosa

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**Background.** Patients with *Helicobacter pylori* infection and abnormalities in the gastric mucosa, such as intestinal metaplasia (IM), severe atrophy, and with the topographic characteristics of pangastritis or corpus-dominant gastritis are at a higher risk to develop gastric cancer. Bacterial and host factors contribute to the development of gastritis pattern at increased risk for gastric cancer development.

**Aim.** Assess the relationship of bacterial CagA status with preneoplastic conditions of gastric mucosa in our population.

**Methods.** One hundred thirty-seven patients with preneoplastic conditions of gastric mucosa of whom 31 suffered from a corpus-dominant gastritis, 82 from a pangastritis, 24 with at least moderate atrophy as well as 49 with IM were included. The control group consists of 68 subjects without dyspepsia or history of peptic ulcer disease but known *H. pylori* infection. Furthermore, 40 patients with gastric cancer and evidence of an *H. pylori* infection were included. CagA status was assessed by an immunoblot (*H. pylori* ViraBlot, ViraMed Biotech AG, Germany).

**Results.** In patients with IM or atrophy, the prevalence of CagA was significantly higher as compared to controls (see table).

<table>
<thead>
<tr>
<th>CagA prevalence (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancer</td>
<td>95.0</td>
<td>11.8</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>91.8</td>
<td>6.96</td>
</tr>
<tr>
<td>Atrophy</td>
<td>82.5</td>
<td>4.33</td>
</tr>
<tr>
<td>Corpus-dominant gastritis</td>
<td>80.6</td>
<td>2.58</td>
</tr>
<tr>
<td>Pangastritis</td>
<td>72.0</td>
<td>1.59</td>
</tr>
<tr>
<td>Controls</td>
<td>61.8</td>
<td>1</td>
</tr>
</tbody>
</table>

**Conclusion.** The presence of CagA is tightly related with preneoplastic conditions of the gastric mucosa. Corpus-dominant or pangastritis, unlike in other reports (Uemura et al. 2001), are not tightly linked to the phenotypic expression of chronic gastritis.

Abstract no.: 03.24
Lewis Expression by *Helicobacter pylori* Isolates of Indigenous Nahuatl Patients from Mexico

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Mimicry of Lewis (Le) antigens, expressed on the O-sidechain of lipopolysaccharide (LPS), have been implicated in *Helicobacter pylori* pathogenesis. Previous studies have demonstrated a tendency for isolates from particular geographic regions to express certain Le antigens. Previous work has shown that although Leα and Leβ occurs, Leα and Leβ expression is high in European isolates but lower in Chinese and Japanese isolates, where high expression of Leα or Leβ was found. In the present study, we analyzed 42 isolates from 29 individuals of Nahuatl origin from Mexico. In nine cases, isolates from multiple gastric sites of individual patients (antrum, corpus, and gastric juice) were taken. The strains were analyzed by dot blotting, but predominantly using a whole-cell ELISA with anti-Leα, anti-Leβ, anti-Leγ, and anti-Leβ monoclonal antibodies. Based on Le differential expression, nine expression accounted for the majority of isolates (15); Leα (1), Leγ (10), and Leβ (1) alone occurred; Leα (2) was only expressed in combination with other Le antigens; and only three strains were untypeable. Leα expression by Mexican isolates (55%) was similar to that of European isolates; Leγ expression (83%) was lower but more comparable to that of the European than Japanese population. Isolates from the antrum (67%) and gastric juice (58%) had similar Leα expression, whereas corpus isolates had lower Leα expression (33%). Leγ expression was identical (89%) in corpus and antrum isolates. Thus, European and Mexican isolates show certain homogeneity in Le expression despite geographically isolated backgrounds.

Abstract no.: 03.23
Effect of *Helicobacter pylori* and Recombinated Protein CagA on the Expression of Heat Shock Protein (HSP70) in MKN-7 Cells


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Heat shock proteins (HSP) are crucial for the cell integrity under normal growth and during pathophysiologic conditions such as colonization of human stomach by *Helicobacter pylori*. The effect of *H. pylori* on mRNA expression for HSP70 in the gastric epithelial cells has been little studied and remains unconclusive. We determined the gene expression for HSP70 in the epithelial MKN7 cells stimulated by two live strains of *H. pylori*: 1, cagA+ and vacA+ and 2, cagA– and vacA– with or without the coincubation with the recombinated protein cagA (OraVax Inc., Cambridge). Culture of MKN7 cells were incubated with *H. pylori* (1 × 10⁹ of per dish) with or without cagA (10 μg/ml of medium). After 3, 24, and 48 hours of incubation, the cells were harvested and total cellular RNA was isolated; the expression for HSP70 mRNA being determined by reverse transcriptase-polymerase chain reaction (RT-PCR). The strain *H. pylori* (cagA + vacA+) inhibited in a time-dependent manner the expression of mRNA for HSP70. When the MKN-7 cells were coincubated with *H. pylori* (cagA+; vacA+) and cagA, the disappearance of the signal for HSP70 mRNA after 48 hours was observed. *H. pylori* (cagA–; vacA–) incubated with MKN-7 cells also attenuated the expression of mRNA for HSP70 but the addition of the recombinated cagA to *H. pylori* (cagA–; vacA–) failed to affect the signal intensity for HSP70 mRNA as compared to that obtained with *H. pylori* alone. We conclude that *H. pylori* suppresses gene expression of HSP70 in MKN-7 human gastric epithelial cells possible because of the presence of bacterial secretory system responsible for the translocation of cagA into the eukaryotic cells.

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Abstract no.: 03.25
Genotyping and Phenotyping Helicobacter pylori Virulence Markers in Iranian Gastric Cancer and Nongastric Cancer Patients

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The epidemiology of Helicobacter pylori infection in Iran creates a public health implication because of its high prevalence and its association with gastric cancer and peptic ulcer disease. Recent studies suggest that genotypes and phenotypes of H. pylori with regard to its virulence markers such as VacA and CagA potentially correlate with the severity of the associated gastroduodenal diseases. vacA s1m1 strains are identified as highly toxic and are associated with increased gastric epithelial damages, enhanced gastric inflammation, and duodenal ulceration. However, in developing countries, type 1 H. pylori strains (cagA+/vacA tox+) are highly prevalent regardless of the clinical manifestations. To evaluate the most prevalent genotype among our highly infected population, H. pylori strains were isolated from 25 gastric cancer (GC) and 61 non-GC patients. polymerase chain reaction (PCR) analysis of vacA and cagA genes, and serum Western blotting were performed.

s1/m2 was found to be the most prevalent vacA genotype in both studied groups. There was no association between cagA gene status and disease outcome as nearly all of the isolated strains were cagA positive. According to serological studies, host responses toward H. pylori virulence markers (mainly CagA and VacA) are similar in GC and non-GC patients. Furthermore, host antibody responses to 35 kDa and 37 kDa antigens were more frequent in non-GC subjects and were inversely associated with GC. This study thereby questions the role of cagA gene status or antibody response thereto as a suitable marker in screening H. pylori-infected population in risk of gastric cancer development and proposes further studies.

Abstract no.: 03.26
Proliferating Activity of Gastric and Duodenal Epithelium in Helicobacter pylori-Associated Duodenal Ulcer Disease

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Introduction. Helicobacter pylori influences both proliferation and apoptosis of gastric epithelial cells.

Aims and Methods. The aim of our work was to evaluate gastric and duodenal epithelial cell proliferation in patients with H. pylori-associated duodenal ulcer disease before and after the course of eradication therapy. Twenty patients were examined. Triple therapy consisting of proton pump inhibitor, amoxicillin, and clarithromycin was used for eradication. H. pylori diagnosing and eradication were proved by biochemical and cytology methods. Eight biopsies were examined in each case: from the margin of ulcer, 0.5 cm near the ulcer, from antral part and body of the stomach before treatment and 6 weeks after the end of eradication therapy. Paraffin sections were stained immunohistochemically with antibodies to proliferative cell nuclear antigen (PCNA).

Results. In all cases we observed increased proliferative activity of epithelial cells. The number of PCNA-positive cells was the largest in biopsies from the margin of ulcer, less – near the ulcer and in antral part, least of all – in biopsies from the body of stomach. The number of positive cells correlated with the activity of inflammation. After the eradication of H. pylori, the number of proliferating cells decreased in all cases although it was still higher in zones of inflammation.

Conclusion. So increased proliferation of epithelial cells is the result of both influence of H. pylori and tissue injury (ulceration). Increased proliferation in zones with inflammation confirms the important role of inflammation in epithelium regeneration.

Abstract no.: 03.27
Virulent Helicobacter pylori Associated with Gastric Intestinal Metaplasia Assessed in Archived Gastric Tissue

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Background. Helicobacter pylori has been recognized as a major gastric pathogen the most severe being gastric cancer. Distal cancer evolves from the sequence of events from normal tissue to gastritis to atrophy to intestinal metaplasia, and finally, dysplasia. The accurate detection of the organism could be essential for proper patient management, and particularly for the eradication of the bacteria following treatment. Both the host and bacterial virulence factors are involved in the clinical outcome.

Aims. To detect and evaluate the association of the H. pylori virulence factors (cagA, vacA, and iceA) in patients with intestinal metaplasia (IM) and gastritis (G).

Methods. Eighty-six patients were confirmed with IM and G by histology finding; DNA was extracted from 10 µm sections of paraffin wax embedded tissues. To purify DNA, Gene Clean III Kit was used. Two µl of DNA was subjected to the nested polymerase chain reaction (PCR) for the analysis of cagA, vacA subtype (s & m), and iceA genes (iceAI, iceA2).

Results. The overall prevalence of cagA in H. pylori strains was 43% (37/86). The prevalence of cagA, vacAs1m1, and iceAI was significantly associated with IM (p = .001, p = .002, p = .001, respectively).

Conclusion. Our results suggest that the nested PCR is a highly valuable method in the detection and genotyping of H. pylori in paraffin wax embedded tissue. Virulent H. pylori is associated with IM.

Table Distribution of cagA, vacA, and iceA according to clinical disease (*p < .05)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>cagA</th>
<th>vacA</th>
<th>iceA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s1m1</td>
<td>s1m2</td>
<td>s2m2</td>
</tr>
<tr>
<td>IM (n = 44)</td>
<td>68%</td>
<td>36%</td>
<td>59%</td>
</tr>
<tr>
<td></td>
<td>(30/44*)</td>
<td>(16/44*)</td>
<td>(26/44*)</td>
</tr>
<tr>
<td>G (n = 42)</td>
<td>17%</td>
<td>7%</td>
<td>7%</td>
</tr>
</tbody>
</table>

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Abstract no.: 03.28
Study of hpaA Patterns in Helicobacter pylori Clinical Isolates from Children or Adults and from Patients with Gastritis and Peptic Ulcer

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Aim. To know the prevalence of the different patterns of hpaA gene in Helicobacter pylori clinical isolates obtained from children or adults.

Materials and Methods. One hundred nine H. pylori clinical isolates were studied: 50 from children and 59 from adults. Isolation of H. pylori was carried out from gastric biopsy specimens by standard culture methods. The hpaA gene was detected by polymerase chain reaction (PCR) (375 bp fragment) and after digestion with Hinfl fragments were separated on agarose gel (3.5%). Pattern 1 showed two fragments of 115 and 260 bp, pattern 2 showed three fragments of 46, 69, and 260 bp, and pattern 3 had the undigested 375 bp fragment.

Results. Sixty-four percent of the strains showed hpaA pattern 1, 17% pattern 2, and 3% pattern 3, whereas 16% were negative for this gene. Only 66% of strains from children were hpaA positive, whereas 98% of strains from adults give a positive signal for this gene (p < .05).

<table>
<thead>
<tr>
<th>Pattern 1</th>
<th>Pattern 2</th>
<th>Pattern 3</th>
<th>hpaA-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>hpaA pattern in strains from children</td>
<td>48%</td>
<td>14%</td>
<td>4%</td>
</tr>
<tr>
<td>hpaA pattern in strains from adults</td>
<td>77%</td>
<td>19%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Conclusion. A high prevalence of strains showing no amplification of the hpaA gene was found in our Spanish H. pylori clinical isolates. The percentage was higher in strains from children than from adults. Pattern 1 was the most prevalent in both groups of patients studied.

Abstract no.: 03.29
Local Immune Response and Virulence Factors of Helicobacter pylori Infection in Chile

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Clinical outcomes of Helicobacter pylori infection are multifactor and most likely depend on either bacterial pathogenic abilities or host and environmental factors. The study investigated in a Chilean high-risk gastric cancer population the linkage between local immunity response and H. pylori virulence factors (vacA, cagA, and iceA). Gastric samples were obtained from 87 adult patients, with (n = 72) and without (n = 15) H. pylori infection. Four samples were taken for histology (n = 2); semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) for IL-4, IL-10, and IFN-gamma (n = 1); and H. pylori genotyping (n = 1). Results show that in 86% of patients infected with H. pylori; it was possible to determine Th1-Th2 immune response, with Th1 in 37/62 patients (60%) and Th2 in 25/62 patients (40%). There was no relationship between cagA, vacA1, and iceA1 genotype and the type of immune response (Th1 or Th2). Finally, the presence of iceA2 allele type was statistically significantly correlated with Th1/Th2 immune response and had been more frequently presented in patients with Th2 than Th1 response (84% and 49%, respectively, x², p = .002).

Discussion. The most frequent immune response in H. pylori-related gastritis was a cell-mediated one, as previously described in the literature. However, the frequency of Th2 immune response was higher than the usually reported in Western countries. On the other hand, a clear correlation was found between iceA2 allele type with Th2 immune response, a genetic marker that would be useful to identify H. pylori species that stimulates a more efficient immune response to H. pylori removal.

Abstract no.: 03.30
Prevalence of Infection with Multiple Helicobacter pylori Strains in Slovenian Pediatric Population

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Background. Helicobacter pylori genome contains only a single copy of vacA (s-region, m-region) and iceA (iceA1, iceA2) gene. Therefore, detection of multiple H. pylori genotypes in a clinical sample indicates the presence of multiple H. pylori strains.

Aims. To determine the prevalence and genetic diversity of vacA and iceA genes, and the frequency of multiple H. pylori strain gastric infections in Slovenian pediatric population; and to analyze the relationship between multiple H. pylori strain infections and the severity of antral inflammation.

Methods. DNA was isolated from 53 H. pylori-positive gastric biopsies. They were also obtained for histologic evaluation according to the Updated Sydney Classification. H. pylori vacA and iceA1 status was determined.

Results. Among 53 children infected with H. pylori, vacA genotypes s1a/m1, s1a/m2, and s2/m2 were found in 32.1%, 28.3%, and 28.3% of the cases, respectively. Multiple vacA genotypes were observed in 11.3% of the cases. Allelic genotypes iceA1 and iceA2 were identified in 31 (58.5%) and 18 (34%) patients. Furthermore, while two biopsy specimens (3.8%) contained both iceA allelic genotypes, they were also undetectable in two cases. Based on the presence of vacA and iceA genotypes in the same tissue sample, 13.2% of the gastric samples contained multiple H. pylori genotypes. No statistically significant differences existed between the severity of antral inflammation and single or multiple strain H. pylori infections (p = .22).

Conclusion. Coinfection with multiple H. pylori strains is rare in Slovenian children. Multiple strain H. pylori infections might not be associated with more severe antral inflammation, as suggested recently in some studies.
Abstract no.: 03.31

Lewis Antigen Expression in Helicobacter pylori Isolates from Spanish Patients: Strains from Children Versus Strains from Adults

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Purpose. To determine the presence of Lewis antigen in Helicobacter pylori strains by using different methods; to compare the Lewis antigen expression in strains isolated from children and adults.

Methods. Ninety-one H. pylori strains were studied: 53 were from children and 38 from adults. Upper endoscopy was performed and biopsy cultured following standard methodology. Strains were conserved at −70 °C until used. Lipopolysaccharide (LPS) was extracted from a 48-hour culture by miniphenol water, and detection of Lewis antigen was determined by different methods: a standard serodot (DB) method using monoclonal anti-LewisX and anti-LewisY and peroxidase-labeled secondary antibodies, Enzimo immuno assay (EIA) using LPS (EIA-LPS), whole cells 48-hour culture or whole cells frozen culture using the same monoclonal antibodies and alkaline phosphatase-labeled secondary antibodies.

Results. EIA performed with whole cells (fresh or frozen cultures) showed the highest positivity for both LewisX and LewisY. Of the 87 strains tested, 33% expressed LewisX and LewisY simultaneously; 7% only LewisX, 31% only LewisY, and 29% no Lewis antigen. Sixty-seven percent of strains from pediatric patients showed one or more Lewis antigen compared with 76% of strains from adult patients.

Conclusions. Whole cells EIA showed the highest detection of LewisX and LewisY. Although Lewis expression were more frequent in strains obtained from adults, no statistical differences were observed compared with strains from children.

Abstract no.: 03.32

In Vitro Investigation of Proinflammatory Properties of Helicobacter pylori Strains Involved in Low-Grade Gastric Mucosa-Associated Tissue (MALT) Lymphoma

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In a previous study, we showed that the main Helicobacter pylori virulence factors including CagA are not associated with strains isolated from patients with MALT lymphoma, suggesting that MALT pathogenesis was not linked to the occurrence of more proinflammatory H. pylori strains. Our aim was to test, in vitro, the proinflammatory properties of H. pylori strains isolated from MALT.

We investigated the ability of 36 H. pylori MALT strains to induce interleukin-8 (IL-8) secretion by AGS cells. Briefly, H. pylori strains were harvested from 2 days plate cultures then resuspended in brucella broth medium. The broth was adjusted to an optical density of 0.6 at 600 nM. The bacterial suspensions (35 µl) were inoculated in each well in triplicate on AGS cells cultured to 60–70% of confluence. After 18 hours of coculture, the supernatants were recovered in order to quantify the proinflammatory cytokine IL-8 (R & D Systems).

Because IL-8 production is mostly dependent on a functional cag secretion system, we studied 16 cagA-positive and 20 cagA-negative MALT strains. High IL-8 production was observed in cagA-positive strains (1379 pg/ml ± 659). All the cagA-negative strains induced low rates of IL-8 (84 pg/ml ± 52), indicating that they do not have proinflammatory potential and probably no other important proinflammatory factor. The results are being currently analyzed according to the presence of other virulence factors.

In conclusion, if the involvement of H. pylori strains in MALT lymphoma is well established, its pathomechanism still remains to be elucidated.

Abstract no.: 03.33

CagA, VacA Helicobacter pylori Strains and Epitheliocyte Apoptosis in Stomach Mucus in Ulcer Disease in Khakassia

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Aim. To study Helicobacter pylori genotypes, the relationship between proliferation and epitheliocyte apoptosis in ulcer disease of duodenum in the Europoid and Mongoloid of Khakassia.

Methods. We examined 27 Europoid and 24 Khakass duodenum ulcer disease patients. Immune histochemical test was carried out in biopsats from three parts of stomach, using streptavidin-biotin method. As initial antibodies we used mice monoclonal antibodies: proliferation antigen Ki-67, representative of caspase effector CPP-32, which regulates apoptosis irreversible stage. We determined proliferation index as percentage share of positively colored epitheliocytes. In all the patients, H. pylori infection was found by morphological and urease methods. Besides, we determined VacA, CagA, IceA, BabA types and subtypes of H. pylori by microbiological and polymerase chain reaction (PCR) methods.

Results. CagA and S1S2 subtypes of H. pylori VacA strains were registered in 95.3% and 70.0% Europoid and in 57.1% (p < 0.01) and 42.9% (p < 0.05) Mongoloid. Proliferation index (PI) in the Khakass was evidently higher than in the Europoid. Apoptosis index (AI) in ulcer diseases was 32.0% in the Khakass and 32.9% in the Europoid and was higher (p < 0.01) than in patients without ulcer disease. PI/AI coefficient in both target populations in ulcer disease was lower than 1 (0.71 in the Khakass; 0.62 in the Europoid), which is caused by apoptosis hyperfunction.

Conclusion. There are definite differences in proportions between proliferation and apoptosis processes in the examined target population, which can be connected with differences between H. pylori genotypes.
In perforated peptic ulcer patients (PPU), *Helicobacter pylori* infection tends to expose clinically as recurrent ulcer. We have investigated PPU patients for the persistent infection or reinfection with *H. pylori*, emerging after surgical intervention and eradication therapy.

**Materials and Methods.** Peptic ulcer patients patients (33) were operated on and *H. pylori* eradication therapy applied. The colonization of antral mucosa by *H. pylori* was graded by histologic evaluation, and DNA of *H. pylori* was isolated initially, 2–5 months and 1 year after treatment. Polymerase chain reaction (PCR) amplification was applied for examination of vacA allelic types, cagA and ureA genes of *H. pylori*. Ten patients were selected for PCR-RFLP analyses of glmM gene products, digested by HhaI.

**Results.** After 1 year, the vacA alleles of *H. pylori* showed the complete eradication in 10/33 (30%) of the PPU patients and the same strain persisted in 19/33 (58%) of noneradicated patients, whereas in 4/33 (12%) the new strain emerged. Persistent infection by the same strain was proven by PCR-RFLP in 8/9 of tested patients. Regardless of the results of eradication, the significant decrease (p < .05) in density of *H. pylori* colonization 2–5 months after therapy was detected.

**Conclusion.** In the majority of PPU patients 1-year after therapy, no eradication of *H. pylori* was obtained because of the persistent infection. After therapy, the short-term decrease of the density of bacteria hints that the reason for eradication failure is not associated with the antibiotic resistance of *H. pylori* strains.

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**Epidemiology and Transmission**

**Abstract no.: 03.34**

In Perforated Peptic Ulcer Patients *Helicobacter pylori* Infection Tends to Persist

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In perforated peptic ulcer patients (PPU), *Helicobacter pylori* infection tends to expose clinically as recurrent ulcer. After therapy, the short-term decrease of the density of bacteria hints that the reason for eradication failure is not associated with infection. After therapy, the short-term decrease of the density of bacteria hints that the reason for eradication failure is not associated with infection.

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**Abstract no.: 03.35**

Virulence of CagA and VacA in *Helicobacter pylori* Adult Infection in our Country

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CagA and VacA are two important factors implicated in *Helicobacter pylori* virulence. The prevalence of these factors is variable according to the geographical location.

The aim is to seek for these factors in subjects infected by *H. pylori* and to evaluate the importance according to the clinical statute.

Two hundred ten *H. pylori*-positive sera (58 ulcers and 152 gastritis) collected between 2000 and 2004 were tested by HelicoBlot 2.1 (Genelabs)

A high diversity of *H. pylori* strains was found with a predominance of CagA+ VacA− Carracter: 32% of 210 were tested CagA+ VacA+, 40% CagA+ VacA−, 8.5% CagA− VacA+, and 19.5% were CagA− VacA− (19.52%).

According to the clinical statute: in 152 sera of gastritis, 28% were CagA+ VacA+, 38% were CagA+ VacA−, 8.5% were CagA− VacA+, 32.5% were CagA− VacA−; about the ulcers, 41% of 58 sera were tested CagA+ VacA+, 44.8% were CagA+ VacA−, 8.6% were CagA− VacA+, and 5% were CagA− VacA−.

So CagA is more implicated in ulcer (86% of cases) than in gastritis (66% of cases).

The *H. pylori* virulence (CagA+ 72%, VacA+ 40.4%) is in correlation with Mediterranean results, but it is less important than in the Asiatic one: Turkey (Absyanik et al.) CagA+ 83%, VacA+ 65%; Japan (Maeda et al.) CagA+ 90%, VacA+ 59%.

Determination of *H. pylori* virulence is very important for diagnostic purposes and also to evaluate *H. pylori* infection gravity.

**Abstract no.: 04.01**

Specific African Genotype among *Helicobacter pylori* Strains

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*Helicobacter pylori* spread within families is the primary mode of transmission in humans. Colonization persists throughout the lifetime of a host. *H. pylori* strains possess specific gene sequences that can identify the geographic origin of its cognate human host, and thus may represent useful biomarkers for human migrations and ancestry. To determine whether *H. pylori* strains isolated from African American patients contain a particular genotype that is shared with strains from African patients but is not present in strains isolated from other ethnic groups, we studied 235 *H. pylori* isolates [African American (14.9%), Caucasian (20.0%), Asian (19.5%), Hispanic (34.0%), and Amerindian (16.2%)] from isolates of African American patients contain a particular genotype that is shared with strains from African patients but is not present in strains isolated from other ethnic groups, we studied 235 *H. pylori* isolates [African American (14.9%), Caucasian (20.0%), Asian (19.5%), Hispanic (34.0%), and Amerindian (16.2%)].
the 180-bp insert correlated with the sequence analysis in 73% of the 26 strains. As expected, the 180-bp insert was rarely found in strains from Asian [1/35 (2.9%)]. Using this as the reference group, the 180-bp insert was found significantly more often in Hispanics, Amerindians, and African-Americans. We can now identify H. pylori strains of African origin from among patients of other geographic origins using a simple method.

Abstract no.: 04.02
Helicobacter pylori: Epidemiological Investigations by a Noninvasive Protocol Using Stool Specimens
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Twenty years after the first successful cultivation of Helicobacter pylori, the source(s) of infection and the route(s) of transmission are not yet clarified. Nearly all protocols, so far, require cultured isolates. Thus, studies including relatives of index patients are extremely restricted.

Therefore, we developed a noninvasive and easy-to-perform protocol, which enables for H. pylori typing using not only cultured isolates but also biopsies and, most importantly, stool samples. The method is based on two H. pylori-specific biprobe real-time polymerase chain reaction (PCR) assays using fragments of the glmM and the recA genes as target sequences. Discrimination between strains results from differences in melting curves after successful amplification. In case of identical melting curves, sequencing of the amplification products is necessary to confirm strain identity.

In comparison to the gold standard of H. pylori diagnosis (by rapid urease test, histology, and culture), the sensitivity of the glmM assay in stool specimens was 98% and that of the recA assay was 92%. The specificity of both assays was 100%. Using unrelated H. pylori isolates and stool samples from unrelated patients, the discriminatory capacity of the typing protocol was nearly 100%. Furthermore, this protocol allows for single-strain identification in mixed infections.

In an ongoing study, the investigation of eight African and 15 Austrian households with an infected index child may contribute to a more precise characterization of intrafamilial transmission patterns.

Abstract no.: 04.03
The Association of Helicobacter pylori CagA Strains Prevalence with Ulcer Diseases in Siberia Mongoloids
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Aim. To study the interconnection of Helicobacter pylori CagA strains and ulcer diseases in the Mongoloids and the Europoids in different regions of Siberia.

Methods. We carried out large-scale epidemiologic research in Tyva, Khakassia, Evenkia, Yakutia, and Eastern Siberia middle latitudes. H. pylori was determined by serologic, morphologic (Gimza coloring), and urease techniques, by polymerase chain reaction (PCR) method in 3494 patients (1365 Mongoloid, 2129 Europoid). IgG CagA was diagnosed by immune-ferment-analysis (IFA) technique in blood serum in 562 subjects in Khakassia and 493 in Evenkia. Esophagogastroendoscopy was carried out for 5215 subjects (2701 Europoid and 2514 Mongoloid).

Results. Total prevalence of ulcer disease in the Europoid made 8.4%, in the Mongoloid 3.5%. H. pylori prevalence made in the Mongoloid 91.3%, in the Europoid 89.6%. H. pylori CagA prevalence in the Mongoloid made 39.4%, in the Europoid 60.3% (p < 0.001). In all the regions, H. pylori dissemination density in mucous layer of the stomach’s antral part was higher in the Europoid than in the Mongoloid by 1.5–3 times. H. pylori strains were associated with ulcer disease in the Mongoloid, but not in the Europoid of Siberia.

Conclusion. H. pylori CagA association with ulcer diseases is opposite to CagA prevalence index in target population.

Abstract no.: 04.04
Prevalence and Spread of Enterohepatic Helicobacter Species in Mice Reared under Specific Pathogen-Free Conditions
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Aim. The aim of this study was to detect prevalence and spread of enterohepatic Helicobacter species (EHS) in mice reared under specific pathogen-free conditions.

Methods. Feces samples of 41 mouse strains were analyzed for Helicobacteraceae using a group-specific polymerase chain reaction (PCR). Species identification in positive samples was performed by DNA-sequencing and restriction fragment length polymorphism (RFLP). Several additional experiments were carried out to evaluate the spread of EHS when mice are harbored in different caging systems.

Results. Helicobacter species were detected in 35/41 (85%) of the tested mouse strains. Altogether five different Helicobacter strains were identified: H. ganiitani, H. hepaticus, H. typhlonicus, Helicobacter sp. “hamster B,” and H. sp. “ulmiensis.” In 26 mouse strains, a single Helicobacter species was detected and nine mouse strains were shown to have coinfections with two or more Helicobacter species. Transmission of EHS occurred in 100% (35/35) when pairs of Helicobacter negative and infected mice shared the same cage. If Helicobacter-free mice were reared in open cages next to cages with EHS-positive animals, de novo infection of mice was observed in 32% (8/26) within 8 weeks. In contrast, when the same experiment was repeated with polycarbonate-filter top equipped cages, all 26 mice remained uninfected in the observation period. Helicobacter infection could also be completely prevented during a 12-month follow-up in mice harbored in individually ventilated cages.

Conclusion. Infection with EHS occurs in mice reared under SPF conditions and can easily spread in animal facilities. This can be prevented if polycarbonate-filter tops or individually ventilated cages are used.
Abstract no.: 04.05

Helicobacter pylori Reinfecion in Insulin-Dependent Diabetes Mellitus Patients: A 5-Year Follow-Up

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Objective. Previous data showed that diabetic patients had low Helicobacter pylori eradication and high reinfection rate.

Aim. To evaluate H. pylori reinfection in insulin-dependent diabetes mellitus (IDDM) patients and in dyspeptic controls 5 years after successful eradication.

Methods. Forty patients affected by IDDM and 50 dyspeptic controls, previously treated for H. pylori infection and eradicated, were submitted to urea breath test (UBT) after 5 years of follow-up. Daily insulin requirement, glycosilated hemoglobin (HbA1c), and development of organ damage were evaluated.

Results. We found a significantly higher incidence of H. pylori reinfection in IDDM patients versus controls. In particular, 27% IDDM patients versus 4% controls resulted reinfected after 5 years (p < .001). In 5-year follow-up in reinfected diabetic patients, an increase in the levels of HbA1c, compared to values at enrolment (8.26 versus 7.48, p < .01) was observed, whereas no significant increase was found in the negative group. Furthermore, the H. pylori-positive group showed increased insulin requirement compared to H. pylori-negative patients (0.69/kg versus 0.61/kg). These data reached not statistical significance. Finally, we found statistically significant higher prevalence of diabetic organ damages in the reinfeected group compared to negative patients: neuropathies, 42% versus 10% (p < .01); nephropathies, 42% versus 15% (p < .01); retinopathies, 28.6% versus 5% (p < .05), respectively.

Conclusions. IDDM patients show higher H. pylori reinfection rates compared to dyspeptic controls. H. pylori reinfection is associated with poorer glycemic control and higher incidence of organ damage. UBT as follow-up screening in eradicated IDDM patients seems a useful tool to detect reinfected patients and optimize glycemic control.

Abstract no.: 04.06

The Mother as Intrafamilial Source of Helicobacter pylori Infection: A Prospective Birth Cohort

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Objective. We investigated the Helicobacter pylori prevalence in a large group of parents and their offspring during a 3-year follow-up to elucidate the intrafamilial source of infection.

Methods. Between November 2000 and November 2001, all mothers and their partners were recruited after delivery of their offspring at the University of Ulm. Infection status of women was determined by 13C-urea breath test. Infection status of the fathers at baseline and of the infants at the age of 1, 2, and 3 years was determined by a monoclonal H. pylori antigen proof in stool.

Results. Of the included 1066 children at baseline, stool samples were available for 890 (84%), 870 (82%), and 834 (78%) at the age of 1, 2, and 3, respectively. Incidence of infection was 1.3%, 1.4%, and 0.6% in the first, second, and third years of life, respectively. The prevalence among children at age 3 was 2.4%. After adjustment for infection status of the spouse and nationality, the odds ratio for H. pylori infection of the child at age 3 was 12.9 (95% CI 3.2–52.5) if the mother was infected and 1.4 (95% CI 0.4–4.6) if the father was infected. The number of older siblings was no risk factor for H. pylori infection of the child.

Conclusions. This study suggests that infected mothers are the main source of H. pylori infection of their children in the population studied and that prevalence of H. pylori infection is decreasing rapidly in recent birth cohorts in Western countries.

Abstract no.: 04.07

Recurrence of Helicobacter pylori Infection After Eradication: Long-Term Follow-Up of 1000 Patients


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Aim. To study the incidence of Helicobacter pylori recurrence and its chronological aspects (the exact moment when it appears), and to evaluate whether it would be recommendable to perform periodic controls (e.g., with 13C-urea breath test [13C-UBT]) to detect H. pylori recurrence after eradication success.

Methods. One thousand patients in whom H. pylori had been eradicated were prospectively studied. Several therapies were used, mainly proton pump inhibitor-based regimens for 7–10 days. Four to eight weeks after completion of therapy, 13C-UBT was performed, and it was repeated each from 12 months to 5 years to study H. pylori recurrences.

Results. Up to now, 1000 patients have been followed up (407 for 1 year, 189 for 2 years, 23 for 3 years, 215 for 4 years, and 166 for 5 years), giving 2.544 patient-years of follow-up. A total of 73 H. pylori recurrences were observed, giving a yearly recurrence of 2.9% per patient-year of follow-up. Respective risk of H. pylori recurrence for each period was 13% at 1 year, 5.3% at 2 years, 4.3% at 3 years, 1.4% at 4 years, and 2.4% at 5 years.

Conclusion. Risk of posteradication H. pylori recurrence is higher during the first year, which suggests that most recurrences during this period are recrudescences and not true reinfections. However, long-term recurrence of H. pylori infection is infrequent in our area. Therefore, after the first year, it seems not necessary to perform periodic controls (e.g., with 13C-UBT) to detect H. pylori recurrence after eradication success.
Abstract no.: 04.08
Natural History of Helicobacter pylori in Childhood and Factors Determining the Epidemiology of Infection
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High seroprevalence rates for Helicobacter pylori have been reported in developing countries, yet few studies exist determining the pattern of change in the epidemiology of H. pylori infection in children. Our aim was to conduct a prospective study to elucidate the outcome, rate of acquisition, and loss of H. pylori infection in a population of healthy children in order to identify the best time to institute strategies that might protect children from infection.

Methods. This study is based on follow-up of 327 healthy Turkish children aged 3–12 years. The follow-up was conducted 6 years after the baseline examination. H. pylori status was determined by 13C-urea breath test (UBT). Children were investigated for sociodemographic variables and several symptoms.

Results. Data from 136 of 327 (41%) children were available. The prevalence of infection increased from 52.9% to 56.6%, which was mainly confined to children younger than 10 years of age. The annual acquisition and loss rates of H. pylori were 2.3% and 0.9%, respectively. Socioeconomic status, household density, and antibiotic use during 6 months were inversely related to H. pylori prevalence. The frequency of headache but not abdominal pain or dyspepsia was higher in H. pylori-infected children.

Conclusions. In this study, the acquisition rate of H. pylori infection was 2.5-fold higher than the loss of infection, and the acquisition mostly occurred before 10 years of age. Data regarding acquisition and loss of H. pylori infection are critical for understanding the epidemiology of infection and development of preventive and treatment strategies.

Abstract no.: 04.09
Helicobacter pylori Infection is No Longer the Major Cause of Peptic Ulcer in Copenhagen County
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Introduction. Peptic ulcer disease is almost always caused by infection with Helicobacter pylori or by ulcerogenic drugs or by a combination of these.

Aims and Methods. We aimed to examine the prevalence of H. pylori infection and the intake of NSAIDs and aspirin (ASA) in patients diagnosed with peptic ulcer at an endoscopic center. Case forms for patients with an endoscopic diagnosis of uncomplicated peptic ulcer during the years 2002 and 2003 were reviewed.

Results. One hundred thirty-four patients (66 males) were investigated. Mean age was 70 years, range 12–94 years. Thirty-eight percent were smokers. Eighty-three patients had duodenal ulcer, and nine patients had combined ulcer. In six patients, H. pylori status could not be determined. A total of 66/134 (49%) peptic ulcer patients were infected with H. pylori. H. pylori was more common in patients with gastric ulcer compared to patients with duodenal ulcer (61% versus 42%, p = .0024). A total of 48/83 gastric ulcer patients used either NSAIDs (11), ASA (30), or both (7). Six of these 83 patients were classified as having idiopathic ulcer disease. A total of 28/48 duodenal ulcer patients had used either NSAIDs (7), ASA (13), or both (8). Six of these 42 patients were classified as having idiopathic ulcer disease.

Conclusion. The frequency of H. pylori infection in patients with peptic ulcer disease is rapidly decreasing. Use of NSAIDs and aspirin is now the major causative factor for duodenal ulcer in Copenhagen County.

Abstract no.: 04.10
Quality of Life of Peptic Ulcer Patients After Eradication Treatment of Helicobacter pylori
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Objectives. The aim of this study was to assess the effectiveness of Helicobacter pylori eradication treatment for the life quality of the patients.

Methods and Patients. Upper gastrointestinal endoscopy, the SF-36 scale, and the gastrointestinal symptoms rating scale (GSRS) were used. Altogether, 91 peptic ulcer patients (mean age 46.7 years, 45 male and 46 female) were included in the study. Follow-up was conducted 5 years after H. pylori eradication treatment. Peptic ulcer was healed in 59 cases and relapsed in 32 cases. The control group was formed of 598 (mean age 49.4 years, 39% male, 61% female) randomly selected persons. Summary statistics were obtained for each of the dimensions of SF-36 and GSRS scales for all performed measurements. Differences between the two peptic ulcer groups and the population group were calculated.

Results. Overall evaluation of health (p = .039) and physical functioning (p = .02) was lower and physical pain disturbed patients more (p = .005) in the cases of recurrent ulcer than in cases of healed ulcer. The peptic ulcer patients irrespectively of recurrences evaluated their emotional functioning (p = .04) lower and it disturbed more their everyday life (p < .0001) and social functioning (p = .02) compared with the population group.

Conclusion. Physical health after peptic ulcer treatment was more disturbed in recurrent ulcer cases. The disturbances in mental component of health occurred even in cases of healed ulcer. It shows that early effective treatment is needed to predict the chronic course of peptic ulcer and to ensure improvement of the patients’ quality of life after treatment.

Abstract no.: 04.11
Time Trend on the Prevalence of Helicobacter pylori Infection and Gastric Mucosal Atrophy in an Urban Area of Japan
K. Yagyu, S. Kikuchi and Y. Obata
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Subjects and Methods. Subjects were healthy workers aged 48–83 years in 1989 (1182 [940 men; 252 women]) and 1998 (2950 [2439 men; 521 women]) who had participated in an annual health check-up program of a company in an urban area of Japan. We measured Helicobacter pylori IgG antibody and pepsinogen I and
II in their sera using J-HM-CAP™ and RIAbeads Pepsinogen I and II™. Subjects were classified by H. pylori status (positive/negative) and pepsinogen values (normal/mild/severe). We observed the changes in the prevalence of H. pylori infection and serologic atrophy of gastric mucosa in each 9-year age group (48–56, 57–65, and 66–83) between 1989 and 1998.

Results. The prevalence of H. pylori infection in each group was 64% (48–56), 69% (57–65), and 64% (66–83) in 1989, 52% (48–56), 60% (57–65), and 65% (66–83) in 1998, respectively. The prevalence of mild atrophy in each group was 20% (48–56), 18% (57–65), and 27% (66–83) respectively. The prevalence of severe atrophy in each group was 14% (48–56), 20% (57–65), and 27% (66–83) in 1989, respectively. The prevalence of mild atrophy in each group was 20% (48–56), 18% (57–65), and 27% (66–83) in 1998, respectively. The prevalence of severe atrophy in each group was 14% (48–56), 20% (57–65), and 27% (66–83) in 1989, 20% (57–65), and 16% (66–83) in 1998, respectively. The prevalence of mild atrophy in each group was 20% (48–56), 18% (57–65), and 27% (66–83) in 1998. The prevalence of severe atrophy in each group was 14% (48–56), 20% (57–65), and 27% (66–83) in 1998. The prevalence of severe atrophy in each group was 14% (48–56), 20% (57–65), and 27% (66–83) in 1998, respectively. The prevalence of severe atrophy in each group was 14% (48–56), 20% (57–65), and 27% (66–83) in 1998, respectively.

Conclusion. The prevalence of H. pylori infection and gastric mucosal atrophy was decreased in 48–56 and 57–65 age groups during the 9-year period. The decline of the prevalence of gastric mucosal atrophy during the 9-year period was smaller than that of H. pylori infection.

Abstract no.: 04.12
Detection of Helicobacter pylori Urease Gene in the Oral Yeast

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Understanding the mode(s) of transmission of Helicobacter pylori comprises one of the headings that are emphasized regarding treatment and control of H. pylori infection. In order to reveal the important role of oral yeast in the transmission of H. pylori, polymerase chain reaction (PCR) was recruited to target H. pylori urease gene in total DNAs extracted from oral yeasts.

PCR was performed on DNAs from five oral yeasts in which H. pylori-specific genes, 16SrDNA, cagA, and vacA were detected in the previous studies. Primers (two forward and one reverse) were designed for amplification of the segments of ureA and ureB genes. PCR conditions were optimized as 2.5 mmol/l MgCl₂, 100 ng of template and annealing temperature of 53 °C. H. pylori and Escherichia coli were used as positive and negative controls, respectively. PCR products were analyzed using 100 bp ladder.

The size of amplified products of five oral yeasts and control H. pylori, obtained from seminested PCR, was 406 bp, which corresponded to H. pylori urease gene. No band was detected from control Escherichia coli.

Several lines of evidence propose fecal–oral and oral–oral routes for transmission of H. pylori. Although isolation of the bacterium from environmental or human oral cavity has not been successful. In this study, H. pylori urease gene has been detected in the oral yeast in which 16SrDNA, vacA, and cagA genes have been detected in the previous studies. It is proposed that ubiquitous yeast plays a crucial role in accommodating H. pylori outside the human stomach and its delivery to GI tract.

Abstract no.: 04.13
Factors Affecting the Adhesion of Water-Stressed Helicobacter pylori to Plumbing Materials

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The most important route(s) of transmission for Helicobacter pylori among the human population has yet to be identified, but water and associated biofilms have been considered as potential environmental reservoirs in several studies. Although molecular techniques have identified H. pylori in water-associated biofilms, there is a lack of studies reporting what factors affect the attachment of the bacterium to plumbing materials. Therefore, the influence of shear stress, temperature, inoculation concentration, and different abiotic substrata on the total counts of attached H. pylori was evaluated using epifluorescence and scanning electron microscopy. Results were statistically significant for adhesion of the bacterium at different shear stress ($p < .001$), with higher numbers of attached H. pylori being obtained at the lowest flow velocities of the water. By contrast, temperature, inoculation concentration, and different substrata appeared to have no effect on attached bacteria ($p > .25$).

The importance of shear stress in the attachment of the microorganism indicates water storage reservoirs or wells, where low shear forces are usually present, as more probable locations for the subsistence of H. pylori attached to the surfaces and consequently embedded in biofilms. This conclusion supports the findings observed by others where the ingestion of well water was correlated with an increased chance of developing an H. pylori infection.

Abstract no.: 04.14
Time Trend on the Prevalence of Helicobacter pylori Infection and Gastric Mucosal Atrophy in a Rural Area of Japan

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Subjects and Methods. Subjects were healthy individuals aged 40–69 years in 1997 (560 [195 men; 365 women]) and 2003 (499 [164 men; 335 women]) who had participated in a health check-up program in a rural area of Japan. We measured Helicobacter pylori IgG antibody and pepsinogen I and II in their sera using J-HM-CAP™ and RIAbeads Pepsinogen I and II™. Subjects were classified by H. pylori status (positive/negative) and pepsinogen values (normal/mild/severe). We observed the changes in the prevalence of H. pylori infection and serologic atrophy of gastric mucosa in 10-year age group (40–49, 50–59, and 60–69) between 1997 and 2003.

Results. The prevalence of H. pylori infection in each group was 52% (40–49), 66% (50–59), and 74% (60–69) in 1997, 34% (40–49), 59% (50–59), and 63% (60–69) in 2003, respectively. The prevalence of mild atrophy in each group was 16% (40–49), 19% (50–59), and 22% (60–69) in 1997, 4% (40–49), 7% (50–59), and 12% (60–69) in 2003, respectively. The prevalence of severe atrophy in each group was 3% (40–49), 13% (50–59), and 18% (60–69) in 1997, 1% (40–49), 7% (50–59), and 13% (60–69) in 2003, respectively.
Conclusion. The prevalence of serologic atrophy of gastric mucosa was decreased in all age groups during the 6-year period, and especially the great decline was observed in younger age groups. The decline of the prevalence of H. pylori infection during the 6-year period was smaller than that of gastric mucosal atrophy.

Abstract no.: 04.15
Seroprevalence of Helicobacter pylori Infection in Tehran University Students from 2001 to 2003

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Objectives. Helicobacter pylori infection is a birth-cohort-related phenomenon, i.e., different cohorts show different rate and prevalence of infection. A seroepidemiological study was conducted in a young population of Tehran University students to investigate the seroprevalence of H. pylori and to detect the relationship with birth-cohort phenomenon.

Subjects and Methods. During the 3 years (from 2001 to 2003), a total of 2000 serum samples collected from Tehran University students, between 18 and 24 years old, were tested for the presence of IgG antibodies against H. pylori by using an in-house ELISA technique. A whole cell sonicated antigen was used for coating the ELISA plates.

Results. In this study, 1434 subjects were seropositive, 566 subjects were seronegative, and the seroprevalence of H. pylori infection was 71.7%.

Conclusions. According to the results of prior studies, the seroprevalence of H. pylori infection in Iran was about 90% but this study indicated the seroprevalence of H. pylori infection decreased to about 71.7%. Decreasing the seroprevalence of H. pylori infection may be related to improvement of socioeconomic status, public hygiene, and traditional living conditions in Iran.

Abstract no.: 04.16
Helicobacter pylori Infection in Havana, Cuba. Prevalence and cagA Status of the Strains

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Almost no studies have been performed to determine the prevalence, antibiotic resistance, or virulence factors of the bacterium. To measure the prevalence of Helicobacter pylori infection among patients attending endoscopy in three clinics in Havana, Cuba, to evaluate clarithromycin resistance, and to determine the cagA status of the strains obtained. Endoscopy was performed and biopsies were obtained from 117 successive patients attending the Institute of Oncology, the Institute of Gastroenterology, and the Calixto Garcia Hospital in Havana, Cuba. Biopsies were maintained at −70 °C before being cultured on three different media (two selective and one nonselective) and incubated for 7 days at 37 °C under a microaerobic atmosphere. The presence of H. pylori was identified by oxidase, catalase, and urease activities. DNA was extracted, and polymerase chain reaction (PCR) was performed with primers H2761676, which amplify a 397 bp fragment of the cagA gene. Clarithromycin susceptibility was measured by the gel diffusion method. The diagnoses of patients were: 1 gastric carcinoma; 19 duodenal ulcers; 8 gastric ulcers; and 89 non-ulcer dyspepsia, including (62) gastritis, (9) hiatal hernia, (2) biliary reflux, (1) gastric polyps, and (15) no abnormality. Among the 117 biopsies tested, 83 were H. pylori-positive (70.9%). The cagA status determined for 35 cases gave a positive result in 31 cases (88.5%). Only 3% of the strains were resistant to clarithromycin. Most strains were cagA-positive and likely harbor the cag pathogenicity island. The low resistance to clarithromycin in the strains studied probably reflects the low degree of use of the antibiotic in this population.

Abstract no.: 04.17
Helicobacter pylori-Negative Gastric Ulcers

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Objective. The prevalence of Helicobacter pylori-negative ulcers is highly variable geographically. In pooled series from the United States, 26% of 315 patients with uncomplicated gastric ulcers (GUs) were H. pylori-negative. In contrast, the reported prevalence of H. pylori-negative ulcers from Europe is much lower. The aim of this study is the determination of the prevalence of H. pylori-negative GU among the southern Greek population.

Methods. One hundred ten patients of a median age of 51 years (21–88) who were not taking NSAIDs and underwent upper GI tract endoscopy because of epigastric discomfort were diagnosed with GUs. The methods of diagnosis used for H. pylori infection were firstly by histologic examination and secondly by rapid urease test.

Results. Seventy-two patients (65.5%) were H. pylori-positive and 38 (34.5%) H. pylori-negative. The percentage of H. pylori-negative GU is higher than that reported internationally.

The majority of these ulcers (after histologic examination) were diagnosed as “idiopathic” except for a small number, that were related to lymphoma, carcinoma, Crohn’s disease, or other uncommon causes of GU.

Abstract no.: 04.18
Risk Factors of Intratrafamilial Transmission of Helicobacter pylori in Thailand

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Background and Aims. Intratrafamilial transmission is considered as a major route of Helicobacter pylori infection. This cross-sectional
study was design to evaluate the factors associated with intrafamilial \( H. pylori \) transmission and seroprevalence of \( H. pylori \) infection among Thai families.

**Patients and Methods.** Clinical information, structured questionnaires, and blood samples were obtained between June 2002 and May 2003. The structured questionnaire was completed for all family members by the same researcher. \( H. pylori \) status assessed by anti-\( H. pylori \) serology (HM-CAP; Enteric Product Inc., Westbury, NY and HB 2.1; Genelabs Diagnostics, Singapore).

**Results.** Total of 140 family members including 71 \( H. pylori \)-positive family members and 69 \( H. pylori \)-negative family members were evaluated in this study. The seroprevalence of \( H. pylori \) in \( H. pylori \)-positive family members was significantly higher than family members of \( H. pylori \)-negative (74.7% versus 39.1%; \( p < .001 \)). The multiple logistic analysis demonstrated that age (OR = 6.3; 95% CI = 2.1–19.2, \( p < .01 \)), share drinking cup behavior (OR = 10.5; 95% CI = 2.7–40.5, \( p < .001 \)), and belong to family member of \( H. pylori \)-positive (OR = 10.8; 95% CI = 4.1–28.5, \( p < .001 \)) are the independent risk factors of \( H. pylori \) infection.

**Conclusion.** This study supported the role of intrafamilial transmission of \( H. pylori \) infection among Thai families. Improvement of personal and family hygiene should be emphasized for prevention of \( H. pylori \) infection.

**Abstract no.: 04.19**

**The Influence of Helicobacter pylori Infection and Surgical Procedures on the Development of Gastric Ulcers in Symptomatic Patients after Bariatric Surgery**

M. Wu, C. Yang, W. Lee, H. Wang, and J. Lin

**Background.** There are few data relating to the role of \( H. pylori \) infection and surgical procedures in gastric ulcer following bariatric surgery.

**Methods.** Subjects with upper gastrointestinal symptoms postbariatric surgery and receiving gastroscopic examinations were prospectively enrolled. All clinical data including age, sex, body mass index (BMI) before surgery, and surgical method were recorded. IgG antibodies against \( H. pylori \) were measured in preoperative serum by enzyme-linked immunosorbent assay.

**Results.** A cohort of 636 patients undergoing laparoscopic vertical banded gastroplasty (L VBG) or gastric bypass (LGBP) was recruited. The seropositivity of \( H. pylori \) in symptomatic and asymptomatic patients after surgery was 39% (32/82) and 39.7% (220/554), respectively. Endoscopic examinations revealed 22 (26.8%) of 82 symptomatic patients had gastric ulcer. Comparison of demographic characteristics between patients with ulcer (n = 22) and patients without ulcer (n = 60) showed no difference in distribution of gender, age, BMI, and seroprevalence of \( H. pylori \) (27.3%, 6/22 versus 43.3%, 26/60, \( p = .212 \)). Patients undergoing LGBP showed a higher rate of gastric ulcer (45.5%, 10/22) when compared with patients undergoing LVNG (20%, 12/60; \( p = .027 \)).

**Conclusion.** Gastric ulcers in symptomatic patients following laparoscopic bariatric surgery are related to surgical procedures rather than exposure to \( H. pylori \) infection.

**Abstract no.: 04.20**

**The Association Between Helicobacter pylori, Betel Chewing, and Oral Cancer**

G. Jayakumar, N. Perera, N. Fernando, I. Amarasingha, and J. Holton

**Introduction.** Betel chewing predisposes to periodontal disease and oral cancer. Studies show that people with gum disease are more likely to test positive for \( H. pylori \). It is not known if the lesions produced by betel predispose to colonization by \( H. pylori \). Further the role of this organism in oral cancer is not known. We determined the presence of antibodies to \( H. pylori \) in oral cancer patients, betel chewers, and nonbetel chewers to determine the association \( H. pylori \), betel chewing, and oral cancer.

**Method.** Ninety subjects (66 men) of which 30 were patients with oral cancer presenting to Cancer Institute Maharagama, 30 betel chewer, and 30 nonbetel chewers from Religious and Welfare Service Center Maharagama were tested for \( H. pylori \) by commercial ELISA (Novum Diagnostica, Dietzenbach, Germany). Oral biopsies from patients were cultured for \( H. pylori \).

**Results.** Three (10%) of oral cancer patients tested positive for \( H. pylori \) by serology, two cultures were also positive. Sixty-seven percent of the oral cancer patients were betel chewers using tobacco and areca nut. Three (10%) of betel chewers also tested positive for \( H. pylori \). However, none of the nonbetel chewers tested positive for \( H. pylori \). There is a significant difference in the presence of \( H. pylori \) between betel chewers and nonbetel chewers at presence of \( \alpha \) equal to 0.2 (\( p = .118 \), which is < 0.2); Fisher exact test.). Same association was found between oral cancer and nonbetel chewers (\( p = .118 \)). No significant difference was seen between presence of \( H. pylori \) in betel chewers and oral cancer patients.

**Conclusion.** Betel chewing predisposes to colonization with \( H. pylori \).

**Abstract no.: 04.21**

**Epidemy of Consumption of Proton Pump Inhibitors?**

K. Seppälä, L. Veijola, P. Sipponen, P. E. T. Arkkila, and T. U. Kosunen

**Background.** After eradication of \( H. pylori \) infection gastric mucosa and ulcers are healed and PPI are rarely indicated.

**Aim.** To study the use of PPI (A) nationally and (B) in patients with peptic ulcers after healed \( H. pylori \) infection.

**Material and Methods.** 1, National consumption of PPIs as daily doses per 1000 inhabitants (DDD) in 1998–2004 and DDD of combinations of drugs for eradication of \( H. pylori \) in 2000–04 was obtained from the National Agency for Medicines. 2, We selected in alphabetic order 1000 of 4465 patients receiving a mailed questionnaire. All had gastroscopy at our hospital within 1989–95.
therapy; consumption of PPI could be analyzed in 220/271 patients who returned the questionnaire.

Results. 1, The national DDD of PPIs increased gradually from 5.95 in 1998 to 21.87 in 2004. The DDD of packages for eradication decreased from 0.09 in 2000 to 0.07 in 2004. 2, One hundred seven of the 220 patients reported no further endoscopies and 113 reported at least one additional endoscopy after the initial study. No ulcer recidives were reported.

<table>
<thead>
<tr>
<th>Number of subjects according to PPI used PPI and later endoscopies</th>
<th>PPI</th>
<th>Additional endoscopies</th>
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<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>No</td>
<td>17</td>
<td>90</td>
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<tr>
<td>Yes</td>
<td>92</td>
<td>21</td>
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<tr>
<td>Total</td>
<td>109</td>
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p = .0001.

Conclusions. The national consumption of PPIs associated with eradication of _H. pylori_ infection decreased but increased strongly with other indications. Many patients with healed peptic ulcer disease seemed to receive PPIs for symptoms that could have been treated with cheaper antacids.

Abstract no.: 04.22

Prevalence of _Helicobacter pylori_ Infection in Santo Domingo

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Santo Domingo is one of the few areas where the prevalence of _Helicobacter pylori_ has not been studied despite the frequent occurrence of associated diseases. Our aim was to determine the prevalence of the infection in a group of 100 consecutive patients submitted to upper digestive tract endoscopy in a capital city hospital.

Information concerning the age, gender, and main symptoms were collected and a serum sample obtained. _H. pylori_ serology (IgG) was performed using Pyloriset EIA-IIIG from Orion Diagnostic (Finland).

The reason for endoscopy was epigastralgia in more that 90% of the cases, the mean age was 43 years, with 54 men and 46 women. Peptic ulcer disease was diagnostic in 20 cases, normal mucosa in 12 cases, and endoscoped gastritis was found in the other cases.

Among the 100 serums tested, 84 were positive (84%). The prevalence of _H. pylori_ infection in Santo Domingo is high and is typical of that reported in other developing countries. Further studies are needed to get further insight on _H. pylori_ infection in the Caribbean.

Abstract no.: 04.23

Sero prevalence of _Helicobacter pylori_ Infection Among Patients Attending Lagos University Teaching Hospital Dental Clinic, Lagos, Nigeria

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Background and Aim. One of the modes of transmission of _Helicobacter pylori_ is oral–oral route, and the organism has been isolated from plaque of dental caries patients in Western, Nigeria. The level of dental hygiene and dental education is still very low in Nigeria making this route more predisposed to _H. pylori_ infection. This study attempts to determine the seropositivity to _H. pylori_ among patients who ordinarily come for dental check-ups.

Material and Methods. One hundred twenty serum samples were collected from 120 patients that visited the Lagos University Teaching Hospital dental Clinic within the study period (January to July 2004). The sera were screened for _H. pylori_ antibody using the enzyme-linked immunosorbent assay method.

Results. All the samples were positive for _H. pylori_ antibody after the ELISA serologic screening comprising of 19 (15.8%) men and 101 (84.2%) women. The average age of males and females screened was 44.5 years and 41.2 years, respectively. Most of the patients belong to the low-income educated class.

Conclusion. This result indicates that the rate of _H. pylori_ infection is very high in this environment and that most people are asymptomatic carriers, therefore signifying the need for general screening of the populace for possible _H. pylori_ infection to reduce the rate of transmission. As some of the patients were diagnosed as having dental infection, it buttresses the fact that _H. pylori_ could be transmitted through oral route and also the act of premastication of food for infants be discouraged to further limit the spread of the infection.

Abstract no.: 04.24

Possible Predisposing Factors to _Helicobacter pylori_ Infections among Dyspeptic Nigerians

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Background and Aim. Most epidemiologist believe the primary route of _Helicobacter pylori_ infection in developing countries is through fecal–oral route. However, no work has been carried out to determine the route of _H. pylori_ transmission in our environment despite its high prevalence (77.1%). This is the first study to document the possible predisposing factors to _H. pylori_ infection among dyspeptic Nigerians by the use of questionnaires on respondents.

Materials and Methods. One hundred thirty-eight respondents who had complaints of dyspepsia and were positive to the diagnostic
methods for *H. pylori* positivity in this environment were involved in the study. Structured questionnaires were administered on the subjects. All questionnaire data were entered and analyzed using epiinfo software (version 6.04: Center for Disease Control and Prevention, Atlanta, GA, USA).

**Results.** Majority of the respondents were traders and had post-secondary education. The commonest sign experienced by the respondents during ulcer episode was acute peppery pain (31.9%), whereas 5.1% experienced dizziness.

Majority of the respondents (31.9%) attributed the cause of the *H. pylori* infection to the source of drinking water. Twenty-two percent (22.5%) attributed it to inadequate sanitation or hygiene, whereas 18.1% responded that it may be because of their poor living condition.

**Conclusion.** The possible predisposing factors to *H. pylori* infection can be attributed to the source of drinking water, inadequate sanitation and hygiene, poor living condition, and overcrowding. However, habit or type of food does not have much influence on the acquisition of the organism in this environment.

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**Inflammation and Host Response**

**Abstract no.: 05.01**

**Complement-Mediated Phagocytosis of Helicobacter pylori-infected Gastric Epithelial Cells: A Novel Mechanism for Sensing Luminal Bacterial Infection**

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*Helicobacter pylori* resides in the mucous layer of the gastric lumen, yet elicits a robust immune response in the lamina propria. It is known that gastric epithelial cells undergo apoptosis in response to *H. pylori* infection, and that phagocytes engulf apoptotic cells. As the phagocytosis of infected epithelial cells could provide a mechanism for luminal antigen detection, we assessed the ability of macrophages to bind and engulf *H. pylori*-infected gastric epithelial cells in vitro.

Control or *H. pylori*-infected AGS gastric epithelial cells were cocultured with peripheral blood-derived macrophages or PMA-differentiated THP-1 cells and fluorescent microscopy was used to assess binding of infected cells to phagocytes. Macrophages preferentially bound and engulfed AGS cells undergoing apoptosis after exposure to *H. pylori* or camptothecin and phagocytosis of apoptotic cells were confirmed by confocal microscopy. Pretreatment of *H. pylori*-infected AGS cells with Annexin V diminished binding by 90%, indicating that apoptosis was necessary for recognition by the macrophage. Engulfment of gastric epithelial cells by macrophages was abolished by removal or heat-inactivation of media serum, whereas reconstitution with C1q complement protein restored binding of the macrophage to the apoptotic epithelial cells. Macrophages and THP-1 cells, but not the epithelium, expressed the receptors for PS, C1q, and C3bi as assessed by flow cytometry and reverse transcriptase-polymerase chain reaction (RT-PCR). Internalization of *H. pylori* following engulfment of apoptotic, infected epithelial cells in a complement-mediated process may be a novel mechanism whereby phagocytes contribute to the inflammatory response to *H. pylori* infection.

**Abstract no.: 05.02**

**Influence of Endotoxic Activity of Helicobacter pylori Lipopolysaccharide on Physicochemical Parameters**


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Lipopolysaccharide (LPS) is an important component of the outer membrane of *Helicobacter pylori* and induces significantly lower endotoxic and immunologic responses than enterobacterial LPS. This property may contribute to the ability of *H. pylori* to produce persistent infection compared to more aggressive pathogens. To understand the physical basis within LPS for these lower bioactivities, we performed a physico-chemical analysis of rough- and smooth-form *H. pylori* LPSs, their lipid A components, and the dephosphorylated forms of these LPSs and lipids A. Biophysical analysis included determination of 1, the acyl chain-melting behavior of the different samples; 2, the inclination angle of the lipid A backbone plane against the membrane plane; 3, the aggregate structure of samples; and 4, the LPS binding protein (LBP)-induced intercalation of the samples into a phospholipid membrane (corresponding to the composition of the macrophage membrane). Results were correlated with those obtained in bioassay of endotoxin-induced production of cytokines in human mononuclear cells and in a CHO cell system for testing receptor (i.e., TLR-2 and -4) reactivity. *H. pylori* preparations showed similar behavior to those of enterobacterial endotoxins, such as LBP-induced intercalation and tendency to produce an inverted cubic aggregate structure, but also deviations, such as phase transition behavior and lower TLR-reactivity. Furthermore, a low inclination angle of the lipid A diglucosamine backbone with respect to the membrane plane was found, which has been correlated previously with low bioactivity. Hence, these investigations give a biophysical basis for understanding the reduced bioactivities of *H. pylori* LPS.
Abstract no.: 05.03*
The Secreted Peptidylprolyl cis-, trans-Isomerase HP0175 of Helicobacter pylori Induces Vascular Endothelial Growth Factor (VEGF) Expression in Gastric Epithelial Cells

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Helicobacter pylori causes gastric damage and is involved in gastric carcinogenesis. Vascular endothelial growth factor (VEGF) is an angiogenic factor that plays a major role in the reconstruction of normal mucosa architecture by stimulating angiogenesis. It is overexpressed in human gastric carcinomas. H. pylori up-regulates VEGF expression in gastric epithelial cells. We have previously reported that the secreted peptidylprolyl cis-, trans-isomerase, HP0175 of H. pylori signals through Toll-like receptor 4 and induces apoptosis of gastric epithelial cells. In this study, we have evaluated the role of HP0175 in VEGF expression, another contributor to remodeling of the gastric mucosa.

We addressed this question by analyzing whether HP0175 modulates VEGF expression in the gastric epithelial cell line AGS. AGS cells treated with HP0175 showed increased levels of VEGF mRNA as assessed by reverse transcriptase-polymerase chain reaction (RT-PCR) as well as VEGF protein as assessed by ELISA. Activation of the MAP kinase pathway is known to regulate VEGF expression. Transfections with dominant-negative constructs, as well as use of pharmacologic inhibitors suggested that HP0175-mediated VEGF expression was dependent on the MEK/ERK pathway and on phosphatidylinositol 3-kinase. In addition, HP0175 activated the transcription factor Sp1, which regulates VEGF expression and is widely associated with carcinoma. In conclusion, HP0175 emerges as one of the newly identified secreted antigens of H. pylori involved in VEGF expression and remodeling of the gastric epithelium. The role of epidermal growth factor receptor- and cyclooxygenase-2-dependent pathways, both of which stimulate VEGF expression, in the HP0175-stimulated process, are being investigated.

Abstract no.: 05.04*
Function and Recruitment of Mucosal Regulatory T Cells in Human Chronic Helicobacter pylori Infection and Gastric Adenocarcinoma

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The presence of CD4+CD25high natural regulatory T cells (Treg) may limit the magnitude of effector responses, which may result in failure to sufficiently control infections and tumors. However, the suppressive function and antigen specificity of CD4+CD25high Treg in Helicobacter pylori-infected gastric mucosa is largely unknown. Therefore, we characterized the CD4+CD25high cells in the gastric mucosa of H. pylori-infected patients suffering from gastric adenocarcinoma with regard to function and homing phenotype. This was performed in both nontumor and tumor mucosa to also identify potential differences in Treg phenotype between the two tissues. CD4+CD25high cells from both tissue types were shown to have an increased expression of CTLA-4 compared to CD4+CD25low/– cells, and to exclusively express mRNA for the Treg-specific gene FOXP3. CD4+CD25high Treg from nontumor mucosa were shown to suppress H. pylori-induced proliferation and IFN-γ production. Furthermore, preliminary results indicated that Treg with comparable activity can be found also in the tumor mucosa. CD4+CD25high Treg expressed significantly higher levels of 1-selectin and CCR4 in both tissues, compared to CD4+CD25low/– cells, and the CCR4-ligand CCL22 was produced in the nontumor and tumor mucosa. In conclusion, we now show that CD4+CD25high Treg from both nontumor and tumor mucosa express CTLA-4 and FOXP3 and can suppress CD4+CD25low/– cells in an antigen-specific manner. Increased expression of 1-selectin and CCR4 on mucosal Treg suggests that these molecules are important for Treg recruitment to both nontumor and tumor gastric mucosa.

Abstract no.: 05.05*
Helicobacter pylori-Induced Gastritis and Carditis, but not Reflux-Induced Carditis, is Associated with an Infiltration of CD4+CD25+ Regulatory T Cells into the Inflamed Mucosa

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Background. Helicobacter pylori infection and abnormal gastroesophageal reflux are the two main etiologic factors of chronic inflammation at the cardia. CD4+CD25+ regulatory T cells (Treg cells) are characterized by the expression of the transcription factor FOXP3.

Aim. To study the role of Treg cells in carditis with respect to H. pylori and gastroesophageal reflux disease (GERD).

Methods. The study included 73 patients, 32 patients were H. pylori-positive, and 17 had GERD. From the 41 H. pylori-negative patients, 19 had GERD. Biopsies were taken from the antrum and cardia. Histologic assessment was performed according to an analogy to the updated Sydney classification. FOXP3, TGF-β1, and IL-10 were analyzed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and statistically evaluated by nonparametric Mann-Whitney U-test.

Results. FOXP3 transcript levels were induced 15- and 14-fold in antrum and cardia mucosa of H. pylori-positive compared to H. pylori-negative patients (p < .003). Furthermore, the gene expression of Treg cell-derived cytokines TGF-β1 and IL-10 were similarly up-regulated in antrum (TGF-β1: 3-fold, p = .014; IL-10: 2.8-fold, p = .019) and cardia (TGF-β1: 2.7-fold, p = .05; IL-10: 1.5-fold, p = .28). The expression levels of all three genes positively correlated with the degree of gastritis. Furthermore, positive correlations were identified between FOXP3 and TGF-β1 and IL-10 (r = .361, p < .0001) and IL-10 (r = .307, p < .0001) demonstrating the functional activity of the infiltrating regulatory T cells. The presence of GERD did neither affect the gene expression of FOXP3, TGF-β1 nor IL-10 in cardia mucosa.

Conclusion. The H. pylori-induced gastritis in antrum and cardia, but not the chemically induced carditis is associated with an activation of Treg cells.
Abstract no.: 05.06
**Helicobacter pylori-Induced FOXP3 + Regulatory T cells in the Mouse and Human Gastric Mucosa Favor Chronic Bacterial Persistence**

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Background/Aims. *Helicobacter pylori* chronically infects more than half of the world’s population, thereby causing peptic ulcers, lymphoma, and carcinoma. The reasons for its lifelong persistence in the human host are poorly understood.

Methods/Results. We found that *H. pylori* induces the gastric influx of regulatory T (Treg) cells, which are potent suppressors of antimicrobial immune responses. After infection of C57Bl/6 mice with *H. pylori* SS1, large numbers of CD4+/CD25+ Treg cells infiltrated the gastric mucosa. In parallel, expression of the Treg cell marker FOXP3 increased in the infected mouse stomach. To analyze the functional relevance of this Treg cell response, CD25+ T cells were depleted systemically in vivo before infection (using anti-CD25 antibodies). Six weeks later, these mice developed dramatically increased gastric inflammatory responses compared to nontreated mice. This resulted in markedly reduced bacterial colonization. Analyzing gastric biopsies from 108 patients, FOXP3 expression was absent or very low in non-infected, but high in infected persons. Of note, patients with a pronounced gastric Treg cell response were colonized more densely, suggesting that Treg cells favor bacterial survival. Virulent *H. pylori* strains bearing a CagPai1 induced stronger Treg cell responses, which may explain their high prevalence.

Conclusion. Treg cells contribute to the inability of the immune system to eradicate the bacterium, resulting in chronic persistence of the microbe. Immune evasion by induction of a Treg cell response, with a strain-type-dependent modulation of the extent of this response, reflects the excellent adaptation of *H. pylori* to humans over thousands of years of coevolution.

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Abstract no.: 05.07
**Lactobacillus johnsonii La1 Attenuates Helicobacter pylori-Associated Gastritis and Reduces the Levels of Proinflammatory Cytokines in the Gastric Epithelium of C57BL/6 Mice**

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The effect of *Lactobacillus johnsonii* La1 administration to experimental *Helicobacter pylori* infection was studied. We administered continuously over a period of 3 months, through the water supply, live La1 to *H. pylori*-infected C57BL/6 mice and followed colonization, and development of *H. pylori*-associated gastritis in the lamina propria. We also determined the levels of pro-inflammatory chemokines macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived cytokine (KC) in the serum and gastric tissue. We documented a significant attenuation both in lymphocytic (*p = .038*) and neutrophil inflammatory infiltration (*p = .003*) in the lamina propria as well as in the circulating levels of anti-*H. pylori* IgG antibodies (*p = .003*), although we did not observe a suppressive effect of La1 on *H. pylori* colonization numbers. Other phylogenetic-related lactobacilli did not attenuate *H. pylori*-associated gastritis to the same extent. MIP-2 serum levels were distinctly reduced during the early stages of *H. pylori* infection in the La1-treated animals and gastric mucosal levels of MIP-2 and KC were also found depressed. Accordingly, *H. pylori*-induced IL-8 secretion by human adenocarcinoma AGS cells in vitro was also significantly reduced (*p = .046*) in the presence of La1 spent culture supernatants, when neutralized to pH 6.8 without concomitant loss of *H. pylori* viability. These observations point out that during the early infection stages, administration of La1 can attenuate *H. pylori*-induced gastritis in vivo possibly, by reducing proinflammatory chemotactic signals responsible for the recruitment of lymphocytes and neutrophils in the lamina propria.

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Abstract no.: 05.08
**Helicobacter pylori Activates Human Monocyte-Derived Dendritic Cells (MoDC) Followed by NK-Cell Activation In Vitro and In Vivo**

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*Helicobacter pylori* infection leads to chronic gastric inflammation. We investigated the response of human monocyte-derived dendritic cells (MoDCs) and their ability to activate NK cells in vitro. MoDCs were generated from isolated monocytes by culture in the presence of IL-4/G-CSF. MoDCs were incubated with *H. pylori* and expression of MHC-class II, CD80, and CD83 as well as cytokine release was measured by FACS and ELISA. CD56+ NK cells were isolated from allogenic donors and cocultured with *H. pylori* pulsed MoDCs. Cytokine release from cocultures was determined by ELISA and cytotoxicity of NK cells was assessed by cytotoxicity assays. CD56+ NK cells were stained in sections from gastric biopsies of infected and non-infected individuals.

Results. *H. pylori* strongly stimulated the expression of MHC-class II, CD80, and CD83 molecules in MoDCs and induced a 50-fold increase in IL-12 secretion. Immature MoDCs were further incubated with different proteasome inhibitors (CytoschalinA, PSI, MG132) before pulse with *H. pylori* leading to complete inhibition of IL-12 secretion but not IL-8 secretion. Coculture of mature MoDCs with NK cells strongly potentiated IFN-γ and TNF-α release from NK cells and expression of CD69, indicating their activation. We found no induction of cytotoxic NK cells in the cytotoxicity assay. Large numbers of CD56+ cells were found in the *H. pylori*-infected mucosa but not in non-infected controls.

Conclusion. *H. pylori* stimulates human MoDCs and induces their maturation. MoDCs in turn activate NK cells of the secretory but not the cytotoxic type. Thus, *H. pylori*-induced NK cells may function as NK- “helper” cells that potentiate the innate and adaptive immune responses toward *H. pylori*.
Abstract no.: 05.09

Innate Immune Response of Human Neutrophils to Helicobacter pylori is Toll-Like Receptors 2- and 4-dependent

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Introduction. Helicobacter pylori is a Gram-negative bacterium that colonizes the human gastric mucosa. Infection is associated with a marked infiltration of gastric epithelium and lamina propria by neutrophils. It is well established that neutrophil density correlates with H. pylori-induced tissue damage. Initiation of the innate immune response to H. pylori is just beginning to be unraveled and recent works support the role of Toll-like receptors (TLRs). We investigated the role of TLR2 and TLR4 in the response to H. pylori.

Methods. Peripheral blood neutrophils were isolated from healthy H. pylori-negative volunteers and were cocultured with H. pylori strain 26,695. After 0.5, 1, 3, 6, 24, and 48 hours the release of IL-8, TNF-α, and IL-10 into the culture supernatants was measured by ELISA. Total RNA was extracted from cells and TLR2 and TLR4 mRNA were quantified by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). The role of TLR2 and TLR4 were investigated by blocking assay with specific monoclonal Abs anti-TLRs.

Results. At 3 hours after stimulation, the neutrophils produced a significant increase in IL-8 production. TNF-α and IL-10 secretion was significantly increased since 6 and 24 hours, respectively. The mRNA expression levels of TLR2 and TLR4 was down-regulated by H. pylori at 6 hours after stimulation, and blocking experiments revealed that TLR2 and TLR4 participate in IL-8 and IL-10 secretion in response to H. pylori.

Conclusion. In this study, we have demonstrated for the first time the role of TLR2 and TLR4 in the inflammatory response of human neutrophils to H. pylori.

Abstract no.: 05.10

Interferon Gamma (IFNG) and Interleukin-10 (IL-10) Haplotypes Differently Influence Helicobacter pylori Infection Outcome

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Interferon gamma (IFNG) is associated with the development of Th1-like, cell-mediated immune responses and plays a pivotal role in H. pylori-infected mucosa, as does the anti-inflammatory cytokine IL-10. Our aims were to ascertain whether the haplotypes made of 1, intron 1 IFNG +874TA SNP and CA repeat polymorphism or 2, IL-10−819CT and −592AC SNPs, influence H. pylori infection outcome and mucosal inflammation. H. pylori-infected patients were studied: 100 noncardia gastric cancer (NCGC), 28 duodenal ulcer (DU), and 71 chronic gastritis (CG). The haplotypes were estimated using the ARLEQUIN software version 2.000. Nine possible IFNG haplotypes were found, the most frequent being A-13 and T-12. DU was less frequent in T-12 haplotype with respect to NCGC (\( \chi^2 = 23.6, p < .01 \)) or CG (\( \chi^2 = 17.0, p < .01 \)). Three possible IL-10 haplotypes (−1082, −819, −592) were identified, ACC, ATA, GCC, none being correlated with disease diagnosis. None IFNG or IL-10 haplotypes were correlated with gastritis grade or intestinal metaplasia. Six possible IL-10 genotypes were inferred: ACC/ACC, ACC/ATA, ATA/ATA, ACC/GCC, ATA/GGC, GCC/GCC. The ATA/ATA was more frequent in NCGC with respect to CG (\( \chi^2 = 4.04, p < .05 \)). The ATA/ATA was more frequent, whereas GCC/GCC was less frequent in CG patients than in those without intestinal metaplasia (\( \chi^2 = 11.04, p < .005 \)). In conclusion, the IFNG high producer haplotype T-12 was protective for DU, probably because of the inhibitory effect on acid secretion of this cytokine. The attenuated IL-10 response of ATA/ATA low producers is probably implicated in the development of precancerous (intestinal metaplasia) and cancerous H. pylori-associated lesions.

Abstract no.: 05.11

Heligmosoides polygyrus Parasitism Attenuates the Progression of Typhlocolitis, Epithelial Hyperplasia, and Dysplasia in the IL-10−/− Mouse Model of Helicobacter hepaticus-Associated Inflammatory Bowel Disease

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Prior studies demonstrated that Th1-promoted gastric atrophy secondary to Helicobacter felis infection in C57BL/6 mice was ameliorated by concurrent Th2 responses to Heligmosoides polygyrus, a murine intestinal nematode. To determine if H. polygyrus infection would reduce Th1-mediated typhlocolitis, we examined the effect of coinfection with H. polygyrus and Helicobacter hepaticus on progression of disease in the IL-10−/− mouse model of inflammatory bowel disease. IL-10−/− mice on the C57BL/6 background were infected with H. polygyrus, H. hepaticus, or sequentially with H. polygyrus followed by H. hepaticus and evaluated at 4, 8, and 16 weeks post-infection. H. hepaticus infection caused significant typhlocolitis by 4 weeks that was characterized by mononuclear infiltration of the lamina propria, epithelial hyperplasia, and epithelial dysplasia. H. polygyrus alone did not cause pathology but coinfection with H. hepaticus significantly reduced the severity of inflammation (\( p < .01 \)), hyperplasia (\( p < .03 \)), and dysplasia (\( p < .05 \)) associated with H. hepaticus infection of the lower bowel. The parasitic infection was also associated with elevated systemic levels of eosinophils, Th2-associated IgG1 and total IgE levels, and the Th1-associated IgG2c response to H. hepaticus antigens was reduced early in the course of infection. The ameliorating effect of parasitism on the mucosal damage associated with H. hepaticus in the IL-10−/− mouse IBD model supports prior findings that parasitism can reduce Helicobacter-associated gastrointestinal tissue damage.
Inflammation and Host Response

Abstract no.: 05.12

Helicobacter pylori Vacuolating Cytotoxin Activates Expression of Eotaxin in Gastric Epithelial Cells via STAT6 Pathway

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Background and Aims. Helicobacter pylori-infected gastric mucosa from patients with active chronic inflammation is characterized by infiltration of various inflammatory cells such as neutrophils and eosinophils. Although many papers have demonstrated several mechanisms for neutrophil infiltration, there has been no report regarding eotaxin, which is known to be a potent chemoattractant to recruit eosinophils. The present study was to investigate the mechanisms of eotaxin expression in H. pylori vacuolating cytotoxin (Vac)-stimulated gastric epithelial cells.

Methods and Results. The combined stimulation with Vac purified from H. pylori and IL-4 increased the expression of eotaxin in MKN-45 gastric epithelial cell lines and primary human gastric epithelial cells, as assessed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and ELISA. In the gastric epithelial cells transfected with an eotaxin promoter-luciferase reporter plasmid, costimulation with purified Vac and IL-4 induced more luciferase activity than either purified Vac alone or IL-4 alone did. However, such up-regulation was significantly decreased in MKN-45 cells transfected with luciferase reporter plasmid bearing an eotaxin promoter that has a mutation at binding site for STAT6. In MKN-45 cells transfected with luciferase reporter plasmid bearing an eotaxin promoter-luciferase reporter plasmid, costimulation with purified Vac and IL-4 induced more luciferase activity than either purified Vac alone or IL-4 alone did. However, such up-regulation was significantly decreased in MKN-45 cells transfected with luciferase reporter plasmid bearing an eotaxin promoter that has a mutation at binding site for STAT6. In MKN-45 cells transfected with STAT6 antisense oligonucleotide (ODN), eotaxin promoter activity was also suppressed compared with cells transfected with sense ODN. Furthermore, tyrosine phosphorylation of STAT6 was increased in both MKN-45 and primary gastric epithelial cells stimulated with purified Vac and IL-4, as assessed by immunoblot assay.

Conclusions. These results suggest that Vac-mediated up-regulation of eotaxin expression in human gastric epithelial cells may be facilitated by IL-4 via a STAT6-dependent mechanism.

Abstract no.: 05.13

The Neutrophil Activating Protein (HP-NAP) of Helicobacter pylori Plays a Role in Adherence to Gastric Epithelial Cells

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Objectives. Adherence of Helicobacter pylori to the gastric epithelium is believed to be an important step in the induction of active inflammation of the mucosal layer. Several specific adhesins, like SabA, BabA2, AlpA, and AlpB, have already been identified, but other factors may well be involved.

Aim. To assess the role of the neutrophil activating protein (HP-NAP) in adherence of H. pylori to epithelial cells.

Methods. The napA gene of H. pylori strain ATCC43504 was disrupted by insertion of a kanamycin cassette. Strain ATCC43504 and napA mutant were tested for their ability to adhere to Hela cells and gastric tissue sections. In addition, the binding of HP-NAP to mucin and sulfated oligosaccharides was studied by ELISA.

Results. Wild-type H. pylori ATCC43504 displayed strong binding to Hela cells, but mutation of the napA gene resulted in a 10-fold reduction of this binding. The difference in adherence between the wild-type strain and napA mutant was also observed when using gastric tissue sections. Adhesion of the wild-type strain was primarily towards the mucus layer covering the epithelial cells. When compared to the wild-type strain, the napA mutant displayed strongly reduced binding to purified mucin and sulfated oligosaccharides, suggesting that HP-NAP is indeed involved in binding to sulfated mucins.

Conclusion. HP-NAP is a multifunctional protein that plays an important role in the chronicity of H. pylori infection through binding to the gastric epithelium. The cell surface receptor involved in the interaction with HP-NAP is still unknown, but probably contains sulfated oligosaccharides.

Abstract no.: 05.14

Modulation of the Oxidative Stress and Mitochondrial Dysfunction by Antioxidants in AGS Cell Line Infected by Helicobacter pylori

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Introduction. Variations of intracellular redox status may either trigger or block apoptotic death program, depending on oxidative stress’ severity through mitochondrial transmembrane potential (ΔΨm)’s alteration.

Therefore, ROS and apoptosis have been implicated in Helicobacter pylori-mediated gastric disorders whereas diets high in antioxidants are thought to be protective.

Objectives. Analysis of ROS content and ΔΨm in AGS cells infected by H. pylori using membrane-permeable lipophilic cationic fluorochromes and the role of supplementation with antioxidants (vitamin E).

Materials and Methods.
• AGS human gastric epithelial cells (ATCC-CRL1739) were cocultured with H. pylori (ATCC-49503, cagA+/vacA+), for 24 hours (10^7–10^8 cfu/ml), without or with vitamin E (10^-4 M).
• (a) ROS production was assessed by oxidation of 2,7’-dichlorodihydrofluorescein diacetate (H2-DCF-DA) and MitoSOX Red reagent.
• (b) Mitochondrial function was indirectly assessed by ΔΨm measured by 5,5’6,6’-tetrachloro-1,1,3,3’-tetraethylbenzimidazolcarbocyanine (JC-1) and Mitotracker® Orange/Green.

Assays were performed by flow cytometry (FC) (FACScan) and confocal microscopy (CM) (Olympus IX81).

Results. H2-DCF-DA (FC): approximately 2.3-fold increased was observed following incubation of AGS with H. pylori. The addition of vitamin E avoided this effect. These data were confirmed by CM using MitoSOX. JC-1 red/green mean of fluorescence (CM) decreased in cocultures with regard to control (1.3–0.9), and showed intermediate values with the antioxidant (1.1). Mitotracker assays by CM indicated that green/red fluorescence increased with the coinfection (0.71–1.31) and with vitamin E reverted to initial data (0.69).

Conclusions. Treatment with radical scavengers confirm that a link exists between both processes (oxidative stress and apoptosis)
Lipopolysaccharide (LPS) and innate pattern-recognition receptors are important for the outcome of infections diseases. LPS-binding protein (LBP) and soluble CD14 (sCD14) facilitate transfer of LPS to a cell surface protein CD14 (mCD14) and Toll-like receptor 4, triggering proinflammatory response.

In this study, the CD14-159C/T polymorphism of the gene encoding the CD14 receptor has been estimated together with plasma concentration of sCD14 and LBP.

The Helicobacter pylori infection was detected by serology (anti- H. pylori IgG/IgA). sCD14 and LBP were measured by ELISA, and CD14 genotyping was performed with polymerase chain reaction (PCR). The H. pylori-infected (Hp+) or -uninfected (Hp−) dyspeptic patients and patients with coronary heart disease (CHD): unstable angina pectoris (UAP) or myocardial infarction (MI) as well as healthy subjects were included in the study.

In the patients with H. pylori-related or -unrelated dyspepsia, there was no difference in the LBP and sCD14 concentration. However, there was a correlation between the level of anti-H. pylori IgG and sCD14. In Hp+ group, the CC genotype was linked with active gastritis whereas CT genotype with non-active gastritis. However, there was no correlation between the genotype and sCD14 concentration. In the CHD group the LBP and sCD14 levels were significantly higher as compared with healthy donors. These proteins were at a higher concentration in H. pylori-seropositive than in H. pylori-seronegative patients. There was a correlation between CT genotype and sCD14 concentration in MI patients.

The results show that an individual susceptibility to H. pylori LPS may influence the course of infection.

**Abstract no.: 05.15**

**Response to LPS and Helicobacter pylori Disease Diversity**


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Lipopolysaccharide (LPS) and innate pattern-recognition receptors are important for the outcome of infections diseases. LPS-binding protein (LBP) and soluble CD14 (sCD14) facilitate transfer of LPS to a cell surface protein CD14 (mCD14) and Toll-like receptor 4, triggering proinflammatory response.

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The results show that an individual susceptibility to H. pylori LPS may influence the course of infection.

**Abstract no.: 05.16**

**Modulation of Gastric Epithelial Human β-Defensins (HBD)-2 and -3 during Helicobacter pylori Infection**


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**Background.** Human β-defensins are potent bactericidal agents against Helicobacter pylori. In the present study, we have investigated the contribution of MAPK and NFκB pathways in regulating defenin gene expression.

**Methods.** AGS gastric epithelial cells were pretreated with MAPK inhibitors prior to infection with a cytotoxic H. pylori strain (60, 190), and hBD-2 and hBD-3 gene expression was quantified by reverse transcriptase-polymerase chain reaction (RT-PCR). Transient transfection studies were conducted utilizing HBD-2 and -3 promoter luciferase constructs followed by bacterial stimulation. Activation of stably transfected conditional MAP kinase mutants in HEK-293 cells allowed further delineation of the role of individual MAPK pathways in defenin gene regulation. The potential role of bacterial virulence factors in host innate defense was established by the use of isogenic mutant strains.

**Results.** Wild-type H. pylori-induced hBD-2 and -3 gene expression in AGS cells in a time and dose-dependent manner. Two to fourfold increase of hBD-2 and hBD-3 was observed by promoter-luciferase assays. Marked induction of β-defensin (hBD-3 > hBD-2) genes was observed during specific activation of the JNK and p38 pathways. A conditional kinase mutant stimulating only the Raf-MEK1/2 ERK1/2 pathway was able to induce hBD-3. Among the isogenic mutants tested CagPAI-negative strain was unable to up-regulate defenin expression.

**Conclusions.** An intact CagPAI is required for full activation of hBD-2 and hBD-3. Selective activation of p38 and JNK MAPK pathways increased both hBD-2 and hBD-3 gene expression in contrast, the ERK pathway alone was sufficient for hBD-3 expression.
Abstract no.: 05.17
The Down-Regulation of Secretory Leukocyte Protease Inhibitor (SLPI) Expression in Antral Mucosa is a General Phenomenon in Helicobacter pylori-Related Gastroduodenal Diseases

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Background. The secretory leukocyte protease inhibitor (SLPI) represents a multifunctional protein of the gastric mucosa exerting antimicrobial and anti-inflammatory effects. Recently, a down-regulation of antral SLPI expression in Helicobacter pylori-infected healthy volunteers was demonstrated.

Aim. To analyze mucosal SLPI expression in patients with different gastroduodenal disorders.

Methods. The prospective study included 90 patients with the following diseases: gastric cancer (GC, n = 22), duodenal ulcer (DU, n = 17), H. pylori-positive dyspeptic patients (NUD, n = 31), and H. pylori-negative dyspeptic patients (n = 20). During gastroduodenoscopy, biopsies were taken each from antrum, corpus, and tumor tissue if suspected. SLPI expression was analyzed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and ELISA.

Results. Antral SLPI levels were reduced by 75% in H. pylori-infected patients (1494–1826 pg/50 µg protein) regardless the underlying disease (NUD, DU, GC) compared to H. pylori-negative dyspeptic patients (6563 pg/50 µg protein, p < .001, ANOVA). Tumor tissue had twofold higher SLPI levels than the surrounding normal gastric mucosa (3900 pg/50 µg protein versus 1826 pg/50 µg protein, p = .013, ANOVA). SLPI levels of the tumor tissue were significantly reduced compared to SLPI levels of antral mucosa in H. pylori-negative dyspeptic patients (p < .001, ANOVA). This reduction was slightly stronger in the intestinal type than diffuse gastric cancer, but without reaching statistical significance. The SLPI levels of the corpus mucosa were unchanged among all groups. SLPI protein levels did not correlate with SLPI-mRNA amounts suggesting that the SLPI down-regulation is not primarily regulated at the transcriptional level.

Conclusion. The down-regulation of SLPI in antral mucosa is a general phenomenon of H. pylori infection and is therefore unlikely to be involved in the disease outcome.

Abstract no.: 05.18
The Sialic Acid-Binding SabA Adhesin of Helicobacter pylori is Essential for Non-Opsonic Activation of Human Neutrophils

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Infiltration of neutrophils and monocytes into the gastric mucosa is a hallmark of chronic gastritis caused by Helicobacter pylori. Certain H. pylori strains non-opsonized stimulate neutrophils to production of reactive oxygen species causing oxidative damage of the gastric epithelium. Here, the contribution of some H. pylori virulence factors, the blood group antigen-binding adhesin BabA, the sialic acid-binding adhesin SabA, the neutrophil-activating protein HP-NAP, and the vacuolating cytotoxin VacA, to the activation of human neutrophils in terms of adherence, phagocytosis, and oxidative burst, was investigated. Neutrophils were challenged with wild-type bacteria and isogenic mutants lacking BabA, SabA, HP-NAP, or VacA. Mutant and wild-type strains lacking SabA had no neutrophil-activating capacity, demonstrating that binding of H. pylori to sialylated neutrophil receptors plays a pivotal initial role in the adherence and phagocytosis of the bacteria and the induction of the oxidative burst. The link between receptor binding and oxidative burst involves a G-protein-linked signaling pathway and downstream activation of PI3-kinase as shown by experiments using signal transduction inhibitors. Collectively our data suggest that the sialic acid-binding SabA adhesin is a prerequisite for the non-opsonic activation of human neutrophils, and thus is a virulence factor important for the pathogenesis of H. pylori infection.

Abstract no.: 05.19
Helicobacter pylori Infection Induced Cardiovascular Changes in Rats

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Background. Helicobacter pylori infection has been known to affect local gastrointestinal tract and systemic functions.

Objective. To investigate the effect of H. pylori infection on cardiovascular system.

Methods. Twelve male Sprague–Dawley rats pretreated with streptomycin were inoculated with 1 ml of H. pylori suspension (10^9–10^10 cfu/ml) by gavage for three consecutive days. Another 12 rats serving as controls received sterile normal saline. Two weeks after inoculation, six rats in each group were sacrificed, whereas the rest of the animals were sacrificed at week 3. Intra-arterial blood pressure, heart rate (HR), and serum TNF-alpha were measured. H. pylori infection in gastric tissues was identified by positive rapid urease test and histopathology.

Results. At 2 weeks, there were no differences between the two groups on systolic blood pressure (SBP), diastolic blood pressure (DBP), and HR. At week 3, H. pylori-infected rats showed a significantly higher SBP (123.8 ± 4.4 versus 108.2 ± 4.2 mmHg,
Sugar Turns Benefit of Cholesterol into Immune Evasion in Microbial Infection

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_Helicobacter pylori_ represents a most successful bacterial parasite that colonizes the stomach since early human evolution. Following the initial demonstration of _H. pylori_'s pathogenic potential, evidence has been accumulated that _H. pylori_ is the leading cause of gastric ulcers, carcinoma, and lymphoma. Cholesterol is a physiologic constituent of membranes critical for their biologic function, but is stigmatized as mediating detrimental effects in obesity and cardiovascular disease. Since _H. pylori_ is auxotrophic for cholesterol, we explored the assimilation of cholesterol by _H. pylori_ upon infection. Here we show that _H. pylori_ follows a cholesterol gradient and extracts the lipid from plasma membranes of epithelial cells for subsequent glycosylation. Cholesterol promotes phagocytosis of _H. pylori_ by antigen-presenting cells and enhances an antigen-specific T-cell response. Consistently, cholesterol-rich diet during bacterial challenge leads to a reduction of the _H. pylori_ burden in the stomach. Intrinsic α-glycosylation of cholesterol, however, abrogates phagocytosis of _H. pylori_ and subsequent T-cell activation. Hence, we propose a novel mechanism regulating host–pathogen interaction which describes glycosylation of a lipid tipping the scales towards immune evasion or response.

Effects of Chronic _Helicobacter pylori_ Infection on Changes of Gastric Microcirculation, TNF-alpha, and IL-10 Levels in Rats

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Background. _Helicobacter pylori_ infection causes gastric inflammation and the release of cytokines.

Aims. To investigate the effects of chronic _H. pylori_ infection on leukocyte adhesion, serum TNF-alpha, and IL-10 levels.

Methods. The 12 male Sprague–Dawley rats were inoculated with _H. pylori_ suspension (about 10^10–10^11 cfu/ml; 1 ml/rat) by gavaging twice daily, with an interval of 4 hours, for three consecutive days. Three weeks after _H. pylori_ inoculation, defined infection by positive urease test and histopathology, the animals were performed intravital fluorescent microscopy to examine leukocyte adhesion on post-capillary venules by using acridine orange to label leukocyte. Serum TNF-alpha and IL-10 levels were analyzed using ELISA technique.

Results. In _H. pylori_ infection groups, the leukocyte adhesion were significantly increased when compared with the control groups (13.40 ± 1.00 cells/field versus 2.47 ± 0.62 cells/field). TNF-alpha levels were higher in _H. pylori_ infection groups (76.76 ± 23.18 pg/ml versus 9.91 ± 2.62 pg/ml). IL-10 levels were also higher in _H. pylori_ infection groups (663.60 ± 105.33 pg/ml versus 383.99 ± 62.58 pg/ml, respectively) (p < .05).

Conclusion. Chronic _H. pylori_ infection could induce infiltration of inflammatory cells and enhance the release of proinflammatory cytokine such as TNF-alpha, IL-10. By means of these mediators, they induced the expression of leukocyte–endothelium interaction.
Abstract no.: 05.23
Cox-2-Dependent Water Influx Through Aquaporin 5 in Gastric Inflammation

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Helicobacter pylori infection causes chronic gastric inflammation leading to a variety of gastroduodenal disorders ranging from peptic ulcer disease to gastric carcinoma. H. pylori infection also causes expression of the prostaglandin synthase cyclooxygenase-2, which has been implicated in tumorigenesis. We investigated the mechanisms underlying the observation that H. pylori infection leads to Cox-2-dependent swelling of gastric epithelial cells in vitro. We show that H. pylori-induced swelling is facilitated by the translocation of aquaporin 5 (Aqp5) to the plasma membrane and is initiated by the influx of chloride. Chloride channels become activated due to Ca²⁺ influx triggered by inflammatory mediators such as prostaglandin E2 (PGE2). Furthermore translocation of Aqp5 to the cell membrane is mediated by the tight junction adaptor protein zona occludens-1 (Zo-1). This process may represent a general mechanism underlying the development of edema formation during acute inflammatory events.

Abstract no.: 05.24
Relationship Between Ghrelin and Helicobacter pylori Infection in Polish Adult Shepherds and Their Children

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Ghrelin is a natural endogenous ligand for the growth hormone secretagogue receptor, known to stimulate food intake and body weight gain, but the relationship between ghrelin release and Helicobacter pylori infection is controversial. We assessed plasma and gastric contents of ghrelin and leptin by RIA in 150 H. pylori-positive and H. pylori-negative adult shepherds and their children before and after H. pylori eradication. Shepherds with full contact with sheep showed the highest 100% H. pylori prevalence compared to other mountain residents without contact with sheep (80%) or urban age-matched controls (64%). Shepherd children with full sheep contact were H. pylori-infected at about three times higher rate than those from the same area, but without contact with sheep or those from the urban area. The gastric ghrelin content in corpus mucosa was several folds higher than in antral mucosa and it was significantly higher in H. pylori-infected mucosa. Serum levels of ghrelin were greatly increased, while levels of gastrin significantly decreased in H. pylori-negative as compared to H. pylori-positive subjects. In mountain children, serum levels of ghrelin and leptin were about twice higher in H. pylori-negative than in H. pylori-positive children, whereas gastrin levels were significantly reduced in H. pylori-negative children. We conclude that H. pylori infection is extremely high in shepherds and their children and this may contribute to the fall in serum levels of ghrelin and the rise in levels of gastrin being responsible for the decrease in appetite and dyspeptic symptoms observed in H. pylori-infected mountain children, the effects that could be ameliorated by H. pylori eradication.

Abstract no.: 05.25
Helicobacter pylori IgA and IgG Antibodies as Indicators of the Risk of Gastric Cancer and Peptic Ulcer Disease in Comparison to Gastritis

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Background. Helicobacter pylori infection is the most important risk factor for peptic ulcer disease and distal gastric cancer.

Aim. To analyze H. pylori IgG and IgA antibodies as indicators of the risk of gastric ulcer (GU), duodenal ulcer (DU), and subsequent gastric cancer (CA) in comparison to chronic gastritis (CHR-G).

Materials and Methods. The risk analysis was based on the data of H. pylori antibodies of IgG and IgA classes reported in part earlier (Gut 2002; 51, Suppl.11:A22). The original data, obtained using enzyme immunoassay, showed high prevalence of IgA and IgG antibodies in all 20-year-age cohorts in all four groups. The risk analysis was carried out in a one year study involving cohorts aged 15 to 94 years in a logistic regression model.

Results. The prevalences of IgG antibodies were similar (89–97%) in all age cohorts in all groups. The prevalence of IgA antibodies showed only minor variation by age. It was higher in the CA (84–91%) and GU groups (78–91%) than among DU (68–77%) and CG patients (55–75%); OR 2.49, 95% CI 1.86–3.34 between the GU and DU groups, OR 2.57, 95% CI 1.95–3.39 between the GU and CG groups.

Conclusion. Association of H. pylori IgA and IgG antibodies with the risk of CA, DU, or GU in comparison to CHR-G.
Abstract no.: 05.26

T- and B-cell Responses in Helicobacter pylori-Infected Duodenal Ulcer Patients and Asymptomatic Subjects in Bangladesh

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Available evidence indicates that the prevalence of Helicobacter pylori infection in the Bangladeshi population is over 80%, although the majority of infected individuals remain asymptomatic with only about 15% developing peptic ulcers and a smaller proportion with gastric malignancies. Although H. pylori induces a vigorous immune response, it is not protective and the host fails to clear the infection.

In order to better understand factors that predispose to symptomatic illness, we analyzed the B- and T-cell responses in blood and gastric and duodenal mucosa of duodenal ulcer (DU) patients (n = 10) and asymptomatic carriers of H. pylori infection (AS) (n = 10) using flow cytometry.

Lower frequencies of CD19+ B cells were found in the blood of DU patients than in the AS individuals. The numbers of CD4+CD25+ regulatory T cells were decreased in the antrum as well as the duodenum in DU patients compared to AS individuals, although the differences were not significant. CD4+ and CD8+ lamina propria T cells from the antrum and the duodenum of DU patients expressed decreased levels of the chemokine receptors CXCR3 and CCR4 compared to cells from AS individuals. Antral T cells from DU patients also expressed the homing receptor α4β7-selectin to a lower extent than T cells from AS individuals.

These preliminary results suggest that different T- and B-cell populations may be recruited to and accumulate in the gastrointestinal mucosa of DU patients compared to in AS individuals and that this may be of importance for development of duodenal ulcer disease.

Abstract no.: 05.27

Helicobacter pylori Infection of the Patients with Ischemic Heart Disease

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The purpose of the given work was examination of the patients with atherosclerotic lesions of vessels for Helicobacter pylori infection comparing with Chlamydia pneumoniae.

Thirty-four male patients with ischemic heart disease (IHD) of ages ranging from 45 to 65 years (a basic group) were investigated. Twenty-three practically healthy men formed a control group. We determined IgG and IgM antibodies to H. pylori and C. pneumoniae in their blood serum by immune-enzyme method (“Human,” Germany) before treatment by traditional methods and by specialized liposomes (SL) with antimicrobial and antioxidant components.

In the basic group, the index of seropositive results made 53%, whereas in the control group, it made only 17.3% (p < 0.01). Influence of conservative and surgical treatments on amount of antibodies to H. pylori was not found, but in the group of the patients using at treatment SL, the authentic reduction of titer of antibodies (p < 0.05) was revealed.

Seropositivity to C. pneumoniae at determination of IgG antibodies was more expressed in the basic group and made 33.3%, whereas it was 12.6% (p < 0.05) in the control group. IgM antibodies to C. pneumoniae in both groups were not revealed that speaks for availability of the chronic infectious process at the patients. The realization of treatment by SL promoted authentic reduction of titer of antibodies to C. pneumoniae.

These data confirm an essential role of the infectious factor, particularly H. pylori, in atherosclerosis etiology and its complications, requiring changes of the approaches to their diagnostics, treatment, and prevention.

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gastric mucosa. The GBF in \textit{H. pylori}-infected gerbils treated with gastrin-17 or omeprazole was significantly lower than that in \textit{H. pylori}-infected treated with vehicle, and this fall in GBF remained constant until the end of the observation period. We conclude that gastrin shows antiapoptotic activity and promotes \textit{H. pylori}-induced precancerosis due to increased expression of Bcl-2 and survivin.

Abstract no.: 06.02*
\textbf{NH}_{4}^{+} Inhibition of SLC26A9: A Possible Mechanism Behind \textit{Helicobacter pylori}-Induced Reduction in Gastric Juxtamucosal pH}

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\textbf{Background.} The gastric mucosa is covered by a mucus layer wherein a pH gradient can be formed. This mucus-bicarbonate barrier is the first line of defense against acid, and it can maintain the epithelial surface neutral when challenged with luminal acid. SLC26A9, an apical Cl\textsuperscript{−}/HCO_{3}^{−} exchanger expressed in gastric surface epithelial cells, is unlike other anion exchangers or transport proteins reported to date, in that it is inhibited by \textit{NH}_{4}^{+}. The gastric pathogen \textit{Helicobacter pylori} produces \textit{NH}_{4}^{+} through its urease activity, and could thus inhibit SLC26A9 and the bicarbonate transport. We have previously shown that both \textit{NH}_{4}^{+} and a chronic infection with \textit{H. pylori} reduce the ability to maintain the epithelial surface pH neutral. In this study, we investigated if a chronic infection with \textit{H. pylori} alters the expression of SLC26A9.

\textbf{Methods.} FVB/N mice expressing human α-1,3/4-fucosyl transferase (producing Leb epitopes) were inoculated with \textit{H. pylori}. mRNA levels were measured in total stomach preparation using Northern blot analysis and real-time polymerase chain reaction (PCR) in surface epithelial cells obtained by gentle scraping of the mucosa.

\textbf{Results.} The expression of SLC26A9 increased by 45\% in total stomach (\(n = 3\)) and by 80\% in surface cells (\(n = 3\)) in infected mice.

\textbf{Conclusions.} The reduction in gastric juxtamucosal pH by \textit{H. pylori} most probably reflects a reduced bicarbonate transport across the epithelium, e.g., through inhibition of the Cl\textsuperscript{−}/HCO_{3}^{−} exchanger SLC26A9. The up-regulation of SLC26A9 in \textit{H. pylori}-infected mice might be an attempt to overcome the effect of \textit{NH}_{4}^{+} inhibition.

Abstract no.: 06.03*
\textbf{Apoptosis in \textit{Helicobacter pylori} Gastritis is Related to cagA Status}

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\textbf{Background.} Apoptosis has been reported in \textit{Helicobacter pylori}-associated gastritis and it has been related to atrophy development. Infection by \textit{cagA+} strains is associated with atrophy.

\textbf{Aims.} To investigate if \textit{cagA} status is related to apoptosis in \textit{H. pylori}-associated gastritis.

\textbf{Patients and Methods.} Fifty patients (22 men, 28 women, median age 40 ± 13.8 years, range 17–75 years) presenting with \textit{H. pylori} gastritis were studied. Immunoperoxidase (sABC) for antiapoptotic (bcl2 and bclx) and proapoptotic proteins (bax and bak) was scored (0–4) in gastric biopsies from the antrum (lesser and greater curvatures), incisura, and corpus (greater curvature). \textit{H. pylori} and \textit{cagA} status were determined by polymerase chain reaction (PCR).

\textbf{Results.} All proteins were expressed in the cytoplasm of foveolar cells, frequently in a granular pattern; bcl2 expression was weak in most of the cases; bax and bak expression was higher than bcl2 and bclx in most cases. In intestinal metaplasia (IM), bax and bak were strongly expressed at the bottom of the lesion; bax and bak expression was significantly higher in patients infected by \textit{cagA+} strains than by negative ones (\(p = 10^{-9}\)). Anti-apoptotic proteins were significantly more expressed in the antral lesser curvature than in the other regions of the stomach, irrespective of IM (bcl2: \(p = .02\); bclx: \(p < .001\)); bak expression was higher in the lesser curvature (antrum and incisura), than in the other regions (\(p = .002\)).

\textbf{Conclusions.} Infection by \textit{cagA+} strains is significantly associated with apoptosis, mainly in the lesser curvature, which may have a role on atrophy development.

\textbf{Financial Support.} CNPq and Fapemig.

Abstract no.: 06.04*
\textbf{Effect of \textit{Helicobacter pylori} Infection on Hepatitis C Virus Infection}

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\textbf{Background.} Hepatitis C virus (HCV) is a major cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. A possible explanation for differences observed in patient’s response to antiviral treatment is the confounding effect of concurrent infections such as \textit{Helicobacter pylori}.

\textbf{Aim.} To compare response to antiviral treatment in \textit{H. pylori}-positive versus \textit{H. pylori}-negative patients.

\textbf{Methods.} HCV viral load was determined at baseline, after 24 and 48 weeks of treatment, and at 6 months after stopping antiviral therapy. Sustained virologic response (SVR) was defined as negative hepatitis C virus-polymerase chain reaction (HCV-PCR) 6 months post-therapy. \textit{H. pylori} status was assessed by serology.

\textbf{Results.} One hundred seven patients with HCV infection were included. Forty-two (39\%) were \textit{H. pylori} antibody-positive. The
SVR rate was lower among *H. pylori*-positive patients compared to *H. pylori*-negative patients (19% versus 43%) \((p = .01).\) By ordered logistic regression analysis, other factors associated with a lower SVR rate were genotype 1 and being African American. The baseline viral load was also lower in *H. pylori*-negative patients compared to *H. pylori*-positive patients \((p < .001).\) After treatment, the mean viral load in the nonresponder patients \((27 H. pylori\text{-negative and 32 } H. pylori\text{-positive})\) was higher in *H. pylori*-positive patients \((2.32 \times 10^6 \pm 3.2 \text{ versus } 1.51 \times 10^6 \pm 4.8 \text{ copies} (p = .056).\) **Conclusion.** *H. pylori* infection is associated with lower sustained virologic response rate and a higher HCV viral load compared to patients without *H. pylori* infection. Eradication of *H. pylori* infection might result in a significant improvement in the outcome of HCV treatment.

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**Abstract no.: 06.06**

**Improved Diagnoses of Corpus Gastric Atrophy**

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**Background.** The current hypothesis is that gastric adenocarcinoma develops through a cascade of events that involves multifocal atrophic gastritis. We recently confirmed corpus atrophy is a continuous process that progresses proximally and towards the greater curve.

**Aim.** To compare a sampling and histopathologic approach for the evaluation of corpus atrophy with the Sydney System.

**Methods.** Patients had eight gastric biopsy specimens obtained from defined locations (four corpus, four antral). Sites were designed to capture the atrophic border as it expands proximally; two corpus sites correspond to Sydney System recommendations. Atrophy was defined as loss of normal glandular components with or without its replacement with intestinal metaplasia and/or pseudo-pyloric metaplasia. The overall grade of atrophy was then scored on a scale of 0–3 based on number and location of corpus biopsies with atrophy.

**Results.** One hundred eighty patients were examined (139 *Helicobacter pylori*-positive). Corpus atrophy was present in 73 patients. Atrophy in patients with grade 1 and grade 2 was present in distal corpus biopsies located proximal to the normal antrum–corpus junction (lesser curve > greater curve), which are not included in the Sydney System recommendations. More severe atrophy was present in more proximal biopsies, which corresponds to those recommended by the Sydney System. Atrophy was never present in the proximal biopsies with sparing of distal corpus biopsies. Corpus atrophy was significantly under diagnosed using the Sydney System \((p < .001).\)

**Conclusion.** The system used is more suitable for clinical evaluation and research related to identifying the presence and extent of precancerous progressive atrophic gastritis.

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**Abstract no.: 06.07**

**Different Mechanisms are Involved in the Control of Gastrin Synthesis and Secretion in Helicobacter pylori-Infected Patients with Duodenal Ulcer and Functional Dyspepsia**

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**Background.** Gastric *Helicobacter pylori* infection leads to chronic gastritis which is characterized by the presence of inflammatory cells as well as changes in the cytokine and hormone pattern. The aim of our study was to analyze gastrin expression in relation to other cytokines in *H. pylori*-positive patients with duodenal ulcer (DU) and functional dyspepsia (FD).

**Methods.** The study included 47 *H. pylori*-infected patients (17 DU, 30 FD). During endoscopy, antral mucosa biopsies were taken for histologic examination, protein, and transcript analyses as well as for organ culture. Expression levels of IL-1β, IL-8, TNF-α...
and gastrin were determined by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and in part by ELISA. Serum gastrin concentration was determined using ELISA, whereas antral G cells were identified using immunohistochemistry and expressed as number of cells/mm² mucosa.

**Results.** Antral gastric mucosa of *H. pylori*-positive patients revealed similar levels of gastrin in both DU and FD that were not significantly increased in comparison with *H. pylori*-negative group. Groups did not differ in antral G cells number, nor in respect of gastrin mRNA contents. Gastrin serum levels were significantly lower in DU (45 ± 9 pg/ml) versus FD (91 ± 12 pg/ml) patients. Gastrin mRNA levels in FD patients (p < .05) and antral gastrin protein concentration in DU patients correlated with antral IL-8 protein concentration (p < .05). Correlation of serum gastrin levels and antral TNF-α concentration was observed only in FD patients.

**Conclusions.** It appears that different mechanisms are involved in the control of gastrin synthesis and secretion in *H. pylori*-infected individuals with and without duodenal ulcer.

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**Abstract no.: 06.08**

**Study of the Prevalence of Helicobacter pylori in Accordance with Biopsy Sites in Asian Populations**

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**Background.** Japanese gastric mucosa is atrophic compared with other Asian populations (Matsuhisa T., *Helicobacter* 8:29–35, 2003). Although the type of gastritis in Japanese changes from antrum-predominant to corpus-predominant in older people, other Asian populations is antrum-predominant in every age (Matsuhisa T., *J Gastroenterol* 39:324–328, 2004). Histologic prevalence of *Helicobacter pylori* was studied in accordance with biopsy sites among Asian populations.

**Materials and Methods.** In all, 1950 Japanese, 561 Chinese, 473 Vietnamese, 434 Thai, and 243 Nepalese were included in this study. Histologic diagnosis of *H. pylori* infection was performed according to triple-site gastric biopsy method. The first site is taken from the greater curvature of the upper antrum, the second is taken from the greater curvature of the upper body, and the third is taken from the lesser curvature of the lower body.

**Results.** Histologic diagnosis of Japanese showed significantly high in the second compared with in the first and the third (first: 64.4%; second: 72.5%; and third: 65.5%). Chinese, Vietnamese, Thai, and Nepalese prevalence showed similar value in each specimen (50.1–51.3%, 45.9–49.4%, 64.2–70.9%, and 48.1–49.8%, respectively). There were no differences in prevalence among biopsy sites.

**Conclusion.** There was a difference in histologic diagnosis of *H. pylori* among biopsy sites in Japanese and no difference in other Asian populations. These results depend on the difference of gastric mucosa and the type of gastritis.

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**Abstract no.: 06.09**

**Helicobacter pylori Infection Induces a Shift in the Proliferation–Apoptosis Distribution Pattern of the Gastric Epithelium**

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**Background.** In the normal gastric mucosa, the proliferative zone is localized to istmus whereas apoptotic shedding dominates in superficial epithelial. An altered architecture in *Helicobacter pylori*-positive gastritis may result from a changed balance in apoptosis/proliferation kinetics. Therefore, kinetics and its association to *H. pylori* status were examined.

**Design.** Antral biopsies from 55 dyspeptic patients were previously evaluated for *H. pylori* status (FEMS 36:175, 2003). Additional sections were immunostained for the proliferation marker Ki-67 and for apoptosis using the TUNEL method. Immunopositive epithelial cells were graded (0–++++) regardless of the microanatomic distribution, and in the following compartments: surface (f1), upper half (f2), and lower half of foveolae (f3). Cases with reverse pattern were recorded, i.e., TUNEL-positive cells in f3 exceeding that of f2 and f1 and Ki-67-positive cells in f1 exceeding that of f2 and f3.

**Results.** Reversed apoptotic pattern was correlated to *H. pylori* density (p = .048) and to the extent of apoptosis regardless of compartments (p = .006). Reversed proliferative pattern was not recorded; however, 20% of the study population showed Ki-67-positive cells in f1 and the density was correlated positively to the extent of apoptosis (p = .003) and marginally to *H. pylori* positivity.

**Conclusion.** This disruption of normal apoptosis/proliferation distribution pattern deserves attention, considering comparable dynamic alterations characterizing dysplasia in other parts of the GI-tract (Am J Clin Pathol 119:723, 2003). This switch, particularly in apoptosis, to a lesser degree in proliferation, may signify an early event in the *H. pylori*-gastritis-dysplasia sequence.

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**Abstract no.: 06.10**

**Local Infection by Helicobacter pylori has no Effect on the Number of Serotonin-Producing Endocrine Cells of the Cardiac Mucosa**


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It has been proposed that *Helicobacter pylori*-associated gastritis may have an effect on the endocrine cell density in gastric mucosa. Serotonin is produced by the enterochromaffin (EC) cells found along the gastrointestinal mucosa. The aim of the present study was
to examine the effect of *H. pylori*-associated carditis on the local number of EC cells. Forty-eight dyspeptic patients (24 men, 24 women, mean age 42 years, range 7–79 years) underwent an upper gastroduodenal endoscopy with gastric biopsies from the cardiac mucosa. Sections of the biopsies were stained for H&E and Giemsa and used for histology and *H. pylori* detection, respectively. Staining of argyrophil cells and EC cells was performed by the Grimelius and immunoperoxidase methods, respectively. The quantitative evaluation of EC cells in cardiac mucosa was classified according to the number of these endocrine cells in the mucous-secreting or mixed glands, which are typical of the cardiac mucosa, as: 1, absent; 2, scattered; and 3, numerous. Among the 48 patients studied, 35 were *H. pylori*-negative, and 13 were *H. pylori*-positive, i.e., presenting active carditis with local *H. pylori* infection. Most of the argyrophil cell population was composed by EC cells. The Table shows that the number of EC cells of the cardiac mucosa does not seem to be affected by *H. pylori* infection (*p > .05*).

Distribution of 48 patients according to the number of EC cells in the cardiac mucosa

<table>
<thead>
<tr>
<th>Patients</th>
<th>Numerous EC cells n (%)</th>
<th>Scattered EC cells n (%)</th>
<th>Absent EC cells n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em>-positive (n = 13)</td>
<td>09 (69)</td>
<td>04 (31)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>H. pylori</em>-negative (n = 35)</td>
<td>16 (46)</td>
<td>15 (43)</td>
<td>04 (11)</td>
</tr>
</tbody>
</table>

**Abstract no.: 06.11**

**Bacterial Load after Helicobacter pylori Eradication Predicts the Changes of Gastric Antral Mucosa, Serum Gastrin-17, Pepsinogen-I, and Pepsinogen-II Levels**

S. I. Pimanov and A. V. Makarenka
State Medical University, Vitebsk, Belarus

**Aim.** To investigate the changes of gastric mucosa morphology, levels of serum gastrin-17, and pepsinogen I and II after eradication of *Helicobacter pylori*.

**Patients and Methods.** Eighty patients with *H. pylori*-associated duodenal ulcer before 2 months and 1 year after eradication were studied. Among patients with unsuccessful eradication, two groups were distinguished: the group with negative rapid urease test, positive results of polymerase chain reaction (PCR), and decreased bacterial density according to morphologic score (partial eradication group, 21 patients); the group with positive RUT (rapid urease test) and high bacterial density (failed eradication group, 37 patients).

**Results.** Before eradication and 1 year after its serum, levels of gastrin-17 (pmol/l) were 2.91 ± 1.83 and 1.55 ± 1.14 (*p = .005*) in the group with successful eradication; 2.56 ± 1.61 and 1.48 ± 1.15 (*p = .021*) in the group with partial eradication; 2.69 ± 2.16 and 2.21 ± 2.68 (*p = .10*) in the group with failed eradication. Serum levels of pepsinogen-I (mg/l) constituted 110.19 ± 34.85 and 76.53 ± 28.02 (*p = .001*); 116.08 ± 29.64 and 85.45 ± 38.50 (*p = .001*); 108.20 ± 32.84 and 100.17 ± 47.38 (*p = .070*), respectively. Serum levels of pepsinogen-II (pmol/l) were 12.17 ± 8.77 and 7.68 ± 5.35 (*p = .006*); 16.66 ± 11.92 and 7.17 ± 4.56 (*p = .001*); 13.55 ± 9.37, and 11.27 ± 9.28 (*p = .10*), respectively. In the group with successful eradication inflammation, activity, atrophy, and lymphoid follicles in the antral mucosa fell. In the group with partial eradication, antral mucosa activity changed from 1.86 ± 0.85 to 0.98 ± 0.33 (*p = .003*) and *H. pylori* score reduced from 2.26 ± 1.04 to 0.75 ± 0.60 (*p = .0004*). Other morphologic changes in antrum and body mucosa in different groups were statistically not significant.

**Conclusion.** Patients with duodenal ulcer after successful and partial eradication have positive morphologic and functional changes of gastric mucosa.

**Abstract no.: 06.12**

**Non-invasive Testing for Gastric Atrophy in North America**

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**Background.** Gastric cancer risk is directly correlated with the severity and extent of mucosal atrophy.

**Aim.** To evaluate non-invasive testing for antral and/or corpus atrophy in North America.

**Methods.** Gastric biopsies were obtained from four defined locations in the antrum and four in the corpus. Sections were scored for the presence of *Helicobacter pylori* and gastric atrophy on a visual analog scale (0–5). Atrophy was defined as loss of normal glandular components with and without its replacement with intestinal metaplasia and/or pseudo-pyloric metaplasia. Corpus atrophy was then scored as 0–3 based the number of biopsies with atrophy. Patients sera were also examined for pepsinogen 1, pepsinogen 2, gastrin 17 (fasting and stimulated) (Biohit Diagnostics, Finland).

**Results.** Forty-three of 179 patients were *H. pylori* positive. Corpus atrophy was present in 9.6% of study subjects (n = 135) (grade 1 = 8, grade 2 = 4, and grade 3 = 1) and antral atrophy was present in 30.8%. There was a significant inverse relationship between the grade of corpus atrophy and the PGI/II ratio (*r = 0.31, p < .01*). The PGI/II means were 9.3, 8.3, and 3.9 for superficial gastritis, mild atrophy, and moderate to severe corpus atrophy, respectively. Stage 2 or 3 was significantly different from those without atrophy. There was also a significant negative correlation between fasting serum gastrin and PGI/II ratio (*r = 0.33*) (*p = .01*).

**Conclusion.** Non-invasive testing in North American patients is both possible and practical using G17 and pepsinogen assays for the diagnosis of the precancerous condition of moderate to severe corpus atrophy.
**Abstract no.: 06.13**  
**Accuracy of “Serologic Gastric Biopsy” in a Cohort of Dyspeptic Patients**

L. G. Cavallaro,* R. Merli,* S. Liatopoulou,* I. Franzê,* M. Maino,* V. Iori,* G. Cavestro,* A. Gnocchi,† P. Caruana,‡ A. Franzê‡ and F. Di Mario*  
*University of Parma/Department of Clinical Sciences, Section of Gastroenterology, Parma, Italy; †Gastroenterology and Endoscopic Unit, Az. Ospedaliera, Parma, Italy; ‡University of Parma/Pathologic Institute, Parma, Italy

**Background.** Serum pepsinogens (sPGI and sPGII), gastrin-17 (G-17), and antibodies anti-*Helicobacter pylori* have been proposed as a “serologic gastric biopsy.” These markers could be important in a pre-endoscopic management of dyspeptic patients.  

**Aim.** To assess the accuracy of sPGI, sPGII, G-17, and anti-*H. pylori* to discriminate normality, inflammation, and atrophy of gastric mucosa in dyspeptic patients.  

**Methods.** One hundred seventy-six consecutive patients (49 years ± 17 SD, 107 women, 69 men) with dyspeptic symptoms and not in therapy with antisecretory gastric drugs were studied. All the patients underwent gastroscopy with biopsies and blood test for *H. pylori* infection. Biopsies were classified in N (normal gastric mucosa), NACG (non-atrophic chronic gastritis), and ACG (with atrophic chronic gastritis moderate/severe) according to histologic findings and separately by means of serologic tests, in a masked way.  

**Results.** According to the histologic findings, patients resulted: 76 N, 79 NACG, and 21 ACG (7 predominantly in antrum, 8 in corpus, and 6 diffused). By means of serologic analysis, the same patients were classified in: 77 N, 82 NACG, and 17 ACG (4 predominant in antrum, 10 in corpus, and 3 diffused). Accuracy, sensitivity, specificity, positive and negative predictive values of serologic diagnosis in comparison to histology were, respectively, 81, 79, 83, 78, 81% to detect normality; 80, 80, 80, 77, 83% to detect inflammation; 96, 78, 98, 82, 98% to detect atrophy.  

**Conclusion.** Serum pepsinogens, gastrin-17, and anti-*H. pylori* are useful markers to screen normal gastric mucosa from non-atrophic and atrophic chronic gastritis.

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**Abstract no.: 06.14**  
**Atrophy with Helicobacter pylori Gastritis in Adults**

K. Kalem, H. Guedouar, N. Djennas, Z. Lamari, F. Souilah, A. Bouhadef, A. Berrah, F. Mouffok and Z. Guechi  
Pathology UHC Parnet, L.A.R.H., Algiers, Algeria

**Background.** The atrophy of chronic gastritis (GC) with *Helicobacter pylori* in Algerian children has not been studied yet. The score atrophy determines the groups of the young patients presenting a risk for neoplasia.  

**Aim.** To determine the prevalence of *H. pylori* and to evaluate atrophy according to the Sydney System.  

**Methods.** The study was prospective, led in coobservation by five pathologists using gastroscopic biopsy from children presenting recurrent abdominal pains or an intolerance to gluten. The patients were submitted to other tests (test fast to the urease, serology, culture, HpSA).

**Results.** Two-hundred fifty-four (sex ratio = 0.85). The mean age was of 8.25 years (11 months–16 years). *H. pylori* was histologically diagnosed in 242 (95% antral and in 66% fundal) and the load is, respectively, minimal, moderate, or important in 32%, 61%, and 17%. The GC was observed in 248 (97%).  

In antrum, mucosa was not modified in 6 (2%) and presenting gastritis without atrophy in 18 (7%). The atrophy was noted in 230 (90%) with 121 (52%) mild, 109 (47%) moderate.  

In fundus, mucosa was not modified in 80 (31%) and presenting gastritis without atrophy in 31 (13%). The atrophy was observed in 143 (56%) with 129 (90%) mild, 14 (9%) moderate.  

There was no severe atrophy or intestinal metaplasia or epithelial dysplasia in children in this study.  

**Conclusion.** Chronic gastritis with *H. pylori* was frequent in Algerian children.  

In antrum, the atrophy was high, important, and associated to mild atrophy of fundus in half of the cases.

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**Abstract no.: 06.15**  
**Prevalence and Age-Related Distribution of Intestinal Metaplasia of the Gastric Mucosa**

N. Rossolimos,* C. Vassiliou,* P. Aggelis,* S. Balas,* M. Theofilopoulou,* V. Xiromeritou,* G. Karatzikos,* A. Bousiotou,* G. Kafiri† and K. Papadimitriou†  
*Ippokration General Hospital, Department of Gastroenterology, Athens, Greece; †Ippokration General Hospital, Department of Histopathology, Athens, Greece

**Objective.** The excessive consumption of antibiotics and antisecretory drugs during recent years principally by older ages and the systematic eradication of *Helicobacter pylori* worldwide (according to the criteria of Maastricht) may have caused remarkable changes in the *H. pylori* population, which is implicated in the provocation of gastric intestinal metaplasia (IM). The aim of this study is the determination of the prevalence and of the age related distribution, of gastric IM as it has developed recently.  

**Methods.** A total of 751 patients of a median age of 54 years (22–90) who underwent gastroscopy were included in the study. None of them had history of upper GI tract surgical procedure. Biopsies were taken from the antrum, the angularis, and the body of the stomach for the diagnosis of IM according to the Houston modification of Sydney’s classification.  

**Results.** Of all the patients, 238 (31.7%) were diagnosed with IM of all types. The higher and the lower percentages of prevalence of IM were 36.0% and 13.0% in the eighth and third decades of age, respectively. The analytical results are cited below.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Patients with IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>22</td>
</tr>
<tr>
<td>30–39</td>
<td>50</td>
</tr>
<tr>
<td>40–49</td>
<td>125</td>
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<tr>
<td>50–59</td>
<td>180</td>
</tr>
<tr>
<td>60–69</td>
<td>222</td>
</tr>
<tr>
<td>70–79</td>
<td>69</td>
</tr>
</tbody>
</table>

**Conclusion.** Instead of wide eradication of *H. pylori* in the general population, gastric IM is prevalent in a high percentage of them.
Abstract no.: 06.16
Atrophy with Helicobacter pylori Chronic Gastritis in Adults

Pathology UHC Parnet, L.A.R.H., Algiers, Algeria

Background. Chronic gastritis (GC) with Helicobacter pylori involves gastric atrophy. Now, atrophy is defined better but remains variably appreciated according to gastric regions, examined specimens, and used histologic techniques.

Aim. To determine the prevalence of H. pylori gastritis and to evaluate atrophy according to the Sydney system.

Methods. Study was prospective, led in coobservation by five pathologists using gastroscopic biopsy from adults with epigastric pain. Patients were submitted to other tests: urease test, serology, culture, HpSA, Cag, and VacA.

Results. Five hundred twenty-four patients (sex ratio = 0.6), 98 presenting duodenal ulcer. The mean age was 36.5 years (18–75).

The serology, urease test, culture, HpSA, Cag, VacA were positive, respectively, in 92%, 87%, 64%, 54%, 72%, and 40%. The histology showed H. pylori in 497 (94.8%) and GC in 524 (100%).

In antrum, chronic gastritis was diagnosed in all patients and without atrophy in 30 (6%). Atrophy was detected in 494 (94%) with 19 (39%) mild, 287 (58%) moderate, and 2 (1%) severe. Intestinal antral metaplasia was observed in 30 (6%) with 7 (1%) severe dysplasia.

In fundus, mucosa was not modified in 57 (10%) and presenting gastritis without atrophy in 121 (23%). The atrophy was observed in 347 (66%) with 268 (77%) mild, 77 (22%) moderate, and 2 (1%) severe. Intestinal fundal metaplasia was noted in six (1%) with one severe dysplasia.

Conclusion. In adults presenting epigastritic pain, chronic gastritis with H. pylori was quasi-constant. Atrophy was frequent, predominant in antrum with 60% moderate and rarely severe. Intestinal metaplasia and severe epithelial dysplasia were rare and always associated to severe atrophy.

Abstract no.: 06.17
A Simple Rat Model of Chronic Helicobacter pylori Infection for Research Study

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Chulalongkorn University Hospital, Bangkok, Thailand.

Background. Helicobacter pylori is accepted as a human pathogen for the development of gastritis and gastroduodenal ulcer diseases.

Aim. To develop a simple rat model of chronic H. pylori infection for research study in the future.

Materials and Methods. Eighty-five Sprague-Dawley rats were divided into three groups. The first group of 63 rats was pretreated with streptomycin and then was inoculated with H. pylori. The second group of 10 rats was pretreated with omeprazole and then was inoculated with H. pylori. The third group of 12 saline-inoculated rats served as control. Two weeks after inoculation, rats were sacrificed and the stomachs were removed. Antral biopsies were performed for urease test and the stomachs were taken for histopathology. The success of H. pylori inoculation is defined as positive both in the urease test and histopathology.

Results. There were 44/63 (69.84%) in group 1 and 6/10 (60.00%) in group 2 success of H. pylori inoculation, respectively. Histopathology detected organism along mucus lining the surface epithelium and crypt lumen and demonstrated mild to moderate gastric inflammation in the successfully inoculated rats. There were normal histopathology and no organism in the control group and the group that represented failure of H. pylori inoculation. The results of the urease test and pathology are all in concordance with one another.

Conclusion. In this study, we purposed the simple model of chronic H. pylori infection in rats. There was a favorable successful rate and was accompanied by a mild to moderate mucosal inflammation. This animal model could be used for research studies in the future.

Abstract no.: 06.18
Evaluation of Elementary Histologic Lesions of Chronic Gastritis After Eradication of Helicobacter pylori

F. Souilah
Pathology UHC Parnet, L.A.R.H., Algiers, Algeria

Aims. In Algeria, gastric pathology related to Helicobacter pylori is very frequent and begins in early childhood and rare studies about it are published. The purpose of this study is to appreciate in short term the evolution of the histologic elementary lesions and to evaluate their scores after eradication.

Materials and Methods. This is a prospective study of gastric biopsies of 58 adult patients, suffering from gastritis caused by H. pylori, of which the positive diagnosis has been established by the cultivation and/or histology and another test. The biopsies had been taken before treatment and after H. pylori’s eradication, and were controlled after a period of at least 6 months (6–24 months). The usual histochemical and immunohistochemical techniques (hematoxylin–eosine, giemsa, PAS, antibody to H. pylori) have been used. The classification according to the Sydney System had been adopted, the parameters had been scored from 0 to 3 before and after eradication, and the fibrosis had also been evaluated.

### Table

<table>
<thead>
<tr>
<th></th>
<th>Antrum</th>
<th>Body</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After eradication</td>
</tr>
<tr>
<td>Chronic Inflammation</td>
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<td></td>
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<tr>
<td>Activity</td>
<td>1.70</td>
<td>3.44</td>
</tr>
<tr>
<td>Atrophy</td>
<td>3.44</td>
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<tr>
<td>Lymphoid follicles</td>
<td>3.60</td>
<td>3.46</td>
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<tr>
<td>Activity</td>
<td>5.17</td>
<td>6.55</td>
</tr>
<tr>
<td>Atrophy</td>
<td>3.44</td>
<td>4.00</td>
</tr>
<tr>
<td>Lymphoid follicles</td>
<td>3.60</td>
<td>3.46</td>
</tr>
</tbody>
</table>
Results. Four cases of intestinal metaplasia had been observed before treatment and just two after eradication. The fibrosis increased and surrounded some lymphoid follicles.

Conclusion. We can say that 6 months after the eradication of *H. pylori*, we observed a persistence of the elementary lesions, but there is a regression of the intensity of the scores.

### Preneoplastic and Neoplastic Diseases

**Abstract no.: 07.01**

**Oxidative DNA Damage in Patients with Gastritis and Gastric Cancer Infected by Helicobacter pylori**


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Abstracts

Background. Oxidative DNA damage is a consequence of DNA repair enzymes. The aim of the present study was to evaluate the relationship among oxidative DNA damage, efficiency of DNA repair, density of *H. pylori* and host polymorphisms of *GSTP1*, *GSTT1*, *GSTM1*, and *CYP2E1*.

Methods. DNA damage and efficiency of DNA repair were analyzed by the Comet assay, bacterial density was measured by quantitative real-time polymerase chain reaction (PCR), and allelic variants of *GSTP1*, *GSTT1*, *CYP2E1*, and *IL-1β* in 24 non-infected patients, 41 infected patients with gastritis, and 61 with gastric cancer. Oxidative DNA damage and the efficiency of DNA repair were analyzed by the Comet assay.

Results. Oxidative DNA damage and efficiency of DNA repair were analyzed by the Comet assay.

Conjunction with High Salt Diet Does not Result in Gastric Cancer – Revisiting the Model

I. T. Padol, M. Sagar, R. H. Riddell and R. H. Hunt.

Background. The model of gastric adenocarcinoma induced by longstanding *Helicobacter pylori* infection in Mongolian gerbils was documented in Japan in the late 1990s. High consumption of salt has also been shown to be an independent carcinogenic factor for gastric cancer. We postulated that augmenting *H. pylori* infection with a high salt diet would exaggerate gastric cancer in this animal model.

Methods. Outbred Mongolian gerbils (Crl:MON [Tum] Charles River Laboratories, Wilmington, MA) were infected with *H. pylori* Sydney strain (SS1) and maintained on either normal or high salt (8%) diet and water ad libitum for up to 2 years (49 in the normal diet group and 31 in the high salt diet group, 21 and 22 in non-infected controls, respectively) and sacrificed in equal groups at 12, 62, and 104 weeks postinfection. The entire stomach was sectioned and stained with hematoxylin and eosin (H&E) and Warthin–Starry stain and then examined.

Results. *H. pylori* colonization of gastric mucosa did not result in gastric cancer or in dysplasia at any time point during the study, up to 2 years post-infection in either normal or high salt groups.

Conclusion. High salt consumption in conjunction with *H. pylori* infection is not enough to trigger malignant changes in the gastric mucosa of this animal model over 104 weeks. Our findings also question the suitability of the Mongolian gerbil model in studies of *H. pylori*-associated gastric cancer.
Abstract no.: 07.03*  
C/EBP-Beta is Expressed in Gastric Carcinoma and Leads to Overexpression of Cyclooxygenase-2

G. Regalo,†‡ P. Canedo,‡ M. L. Campos,‡ M. B. Loncar,‡ C. Figueiredo,†‡ F. Carneiro†‡ and J. C. Machado†‡  
*IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal, †Faculty of Medicine, University of Porto, Porto, Portugal, ‡Division of Molecular Genetics, Institute of Anthropology and Human Genetics, University of Tubingen, Tubingen, Germany

Chronic inflammation plays a key role in progression towards Helicobacter pylori-related gastric carcinoma (GC). Interleukin-6 receptor (IL-6R) signal transduction pathway was shown to be potentially involved in gastric carcinogenesis. One of the key molecular effectors of this pathway is the C/EBP-beta transcription factor. Additionally, the putative tumorigenic cyclooxygenase-2 (COX-2), effectors of this pathway is the C/EBP-beta transcription factor. Helicobacter pylori Chronic inflammation plays a key role in progression towards -related gastric carcinoma (GC). Interleukin-6 receptor (IL-6R) signal transduction pathway was shown to be potentially involved in gastric carcinogenesis. One of the key molecular effectors of this pathway is the C/EBP-beta transcription factor. Additionally, the putative tumorigenic cyclooxygenase-2 (COX-2), effectors of this pathway is the C/EBP-beta transcription factor.

Methods. A431 epithelial cells preincubated with the EGFR inhibitor EKB-569 (0.001–1 µmol/l) were cocultured with H. pylori (G27 cagPAI +, H12-5 A cagM–isogenic mutant). Total ERK and pERK status was simultaneously quantified in situ using two-color “in-cell Western blot” analysis.

Results. Both G27 and H12-5 A significantly increased pERK in A431 cells compared to unstimulated controls. Maximum pERK was observed at 1.5 hours (G27, mean ± SEM units, 205.1 ± 10.9 versus 100.0 control, n = 12, p < .001; H12-5 A, 210.6 ± 13.6, n = 12, p < .001). EKB-569 dose-dependently inhibited pERK induced by both strains. Inhibition of H12-5 A induced pERK was observed at 100 nM EKB-569 (139.4 ± 14.4 versus 225.9 ± 22.2 untreated control, n = 4 paired t-test, p < .02) and inhibition increased at 1000 nM EKB-569 (133.2 ± 9.4, p < .01). EKB-569 similarly inhibited H. pylori G27-stimulated ERK phosphorylation.

Conclusion. ERK activation was induced by H. pylori strains could be partially blocked by the specific EGFR inhibitor EKB-569. The two-color “in-cell Western blot” analysis represents a useful tool to evaluate inhibitors of H. pylori cell signaling pathways.

Abstract no.: 07.05*  
Significant Differences in the Distribution of Polymorphisms in IL-1β-511, IL-1β-1473, and IL1RN Between Ethnic Groups Resident in Malaysia

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*School of Microbiology & Immunology, University of New South Wales, Sydney, Australia, †Department of Medicine, University of Malaya, Kuala Lumpur, Malaysia, ‡Changi General Hospital, Singapore, Singapore, §Cancer Registry, Leeds, UK

Background. Although early studies showed that specific polymorphisms in the IL-1β promoter and IL-1RN were associated with an increased risk of gastric cancer, this finding has not been upheld in all populations. This may relate to the background prevalence of specific polymorphisms in different populations.

Aims. To determine the prevalence of polymorphisms in IL-1β-511, IL-1β-1473, and IL1RN in Chinese, Malay, and Indian subjects diagnosed with functional dyspepsia (FD) and to compare this with that previously reported in East Asian and Western populations.

Methods. DNA was extracted from whole blood and polymorphisms in IL-1β-511 and IL-1β-1473 determined by PCR–RFLP (polymerase chain reaction–restriction fragment length polymorphism) using Ava I and Sty I, respectively. IL-1RN polymorphisms were determined by PCR. Genotype and allele frequencies of IL-1 polymorphisms in East Asian and Western countries were determined using MEDLINE.

Results. One hundred eighty-four residents in Malaysia (75 Chinese, 63 Indians, 46 Malays) were examined. Significant differences in genotype frequencies of IL-1β-511 (p = .002), IL-1β-1473 (p < .006), IL-1RN (p < .001) existed between ethnic groups (χ² test). In each ethnic group, genotype frequencies did not deviate significantly from Hardy–Weinberg equilibrium, except in Malaysia for polymorphism IL1β-1473C/G (χ² = 4.98 > 3.84 = χ²; d.f. = 1 (0.05). For each ethnic group, strong linkage disequilibrium existed between IL1β-511T and IL1β-1473C (D’ = 0.5–1). Comparison of East Asian and Western populations showed the T allele frequency of IL1β-511 and the IL1RN T allele frequency was significantly different (T-test, p = .001, p < .0005).

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Preneoplastic and Neoplastic Diseases

Abstract no.: 07.04  
Helicobacter pylori-Stimulated Epithelial ERK Phosphorylation is Reduced by the Specific EGFR Inhibitor EKB-569

Molecular Medicine Unit, University of Leeds, Leeds, UK

Introduction. Helicobacter pylori activates multiple signaling pathways in gastric epithelial cells, including transactivation of the EGFR receptor (EGFR) and activation of MAP kinases, extracellular-signal related kinases 1 (ERK1), and ERK2. H. pylori activation of the EGFR signaling pathway may be relevant to the epithelial hyperproliferation and increased risk of gastric carcinogenesis. The aim of this study was to evaluate whether H. pylori-induced phosphorylation of ERK (pERK) is via the EGFR receptor.
Conclusion. Differences in the prevalence of IL-1 gene polymorphisms in different populations may explain the lack of association between IL-1 polymorphisms and GC in some populations.

Abstract no.: 07.06
Epigenetic Alterations in Patients with Helicobacter pylori-Associated Advanced Changes in Gastric Mucosa and Patients with Gastric Cancer – Risk Assessment by Determination of Global Hypomethylation of CpG Sites

A. Leodolter, S. Alonso, M. Ebert, C. Roecken, T. Wex, U. Peitz, P. Malfertheiner and M. Perucho
“The Burnham Institute, La Jolla, CA, USA, †Department of Gastroenterology, Magdeburg, Germany, ‡Institute of Pathology, Magdeburg, Germany

Hypomethylation of genome-wide CpG-sites is observed in tumor tissues from cancer patients. Global hypomethylation is associated with hypo- and hypermethylation of promoter regions. It is well known that those with atrophy, intestinal metaplasia (IM), and corpus predominant pangastritis (CPG) are at a higher risk for development of gastric cancer (GC). The aim of this study was to evaluate the methylation status of CpG-sites in GC patients and to compare these with Helicobacter pylori-positive patients with advanced gastritis to identify if genome-wide hypomethylation event occurs in precancerous lesions.

Methods. Gastric DNA was analyzed from patients with GC (n = 95, tumor/nontumor tissues), CPG (n = 19, H. pylori-positive, 9 with additional IM, 2 with atrophy), and controls (n = 17) without H. pylori infection. In all patients, an M.SssI-enzyme-assay for methylation of genome-wide CpG-sites with s-adenosyl-L-[methyl-3H]methionine was performed. For standardization of DNA amount and to calculate the percentage of methylated CpG sites, a dam-enzyme assay was performed.

Results. The median value of methylation of CpG-sites (82.7%) differed significantly in patients with GC compared to all other groups. The non-tumor tissue revealed a lower amount of methylated CpG-sites (85.8%) compared to tissue of controls. No differences were observed between risk gastritis (88.8%) and controls (89.0%). More importantly, 6 out of 19 patients (32%) with advanced gastritis have lower levels of global methylation than gastric cancer patients.

Conclusions. A substantial part of patients with H. pylori infection and advanced changes in gastric mucosa have highly decreased levels of global methylation of CpG-sites indicating that in those patients frequent epigenetic alterations have been already accumulated.

Abstract no.: 07.07
Cytokine-Mediated Regulation of Oncogene Pim1 in Helicobacter hepaticus-Induced Colon Carcinoma in Mice

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Chronic gut inflammation predisposes to colon cancer in humans and mice. Mouse models of colon cancer are invaluable to test novel therapies and more importantly to elucidate underlying mechanisms of carcinogenesis. Previous studies from our laboratory have established that: 1, Helicobacter hepaticus-infected 129/SvEv Rag2−/− mice develop severe colitis and cancer; 2, IL-10-competent CD4+ CD45RB+CD25+ regulatory lymphocytes disrupt the progression from colitis to cancer; and 3, treating mice with established cancer with IL-10 was sufficient to abolish epithelial tumors and fully restore gut homeostasis. However, the mechanisms underlying regression of severe colitis and associated carcinoma are complex and poorly understood. Here we identify a cytokine-mediated signaling pathway that regulates homeostasis in the colonic epithelium. In 129/SvEv Rag2−/− mice infected with H. hepaticus, severe colitis and invasive adenocarcinoma develop by 16 weeks postinfection. Analysis of colon tissue samples collected at necropsy has consistently revealed up-regulation of IL6 and Pim1, an oncogene previously implicated in the pathogenesis of human prostate cancer. Colonic epithelium from mice treated with IL-10-competent CD4+CD45RB+CD25+ regulatory lymphocytes, or with IL-10-Ig fusion protein alone, has displayed normalized expression of IL6 and Pim1 and reversion to normal morphology. This study implicates cytokine-mediated modulation of epithelial oncogenes as an important mechanism during cancer progression and suggests that identifying such mechanisms might provide valuable targets in designing future strategies aimed at cancer treatment and prevention.

Abstract no.: 07.08
MDR1 P-Glycoprotein Expression in Human Gastric Tissue: Not Only Multidrug Resistance

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P-glycoprotein (P-gp), one of the main factors implicated in cancer multidrug resistance, can act as a primary antiapoptotic agent. P-gp, undetectable in normal gastric mucosa, is overexpressed in up to 50% of gastric cancers. Tumorigenesis can be imagined to mirror organogenesis. In both processes apoptosis plays a key role.

To evaluate P-gp expression in human gastric mucosa we studied 40 Helicobacter pylori− normal gastric mucosa samples, 134 H. pylori+ chronic gastritis with (36) and without (98) intestinal metaplasia, 69 gastric cancers (45 intestinal type and 24 diffuse
type), and 10 abortive human fetuses between the 17th and 36th week of gestational age. P-gp expression was investigated by Western blot and immunohistochemistry (anti-MDR1-glycoprotein p, dilution 1:150).

Results. Western blot found that P-gp level progressively increased from normal mucosa to H. pylori+ cases, gastric cancer, and human fetal mucosa. By immunohistochemistry, P-gp was undetectable in all normal gastric mucosa samples, whereas it was detected in 41/77 H. pylori+ chronic gastritis without metaplasia and in all 36/36 H. pylori+ chronic gastritis with metaplasia. In gastric cancer, an intense P-gp immunostaining was found in 40/45 of intestinal type and in 4/24 of diffuse type. In the human fetal stomach, P-gp was expressed in the glandular cells progressively stronger from earlier stage (17th–19th week) to later stage (24th–36th week). Further, P-gp was found expressed in blood vessel wall but not in the stromal cells.

Conclusion. Our data suggest that P-gp plays a key role during organogenesis and in the early phase of gastric tumorigenesis.

Abstract no.: 07.09

iNOS Expression in Gastric Carcinogenesis: A Molecular and Immunohistochemical Study in Tissue Microarrays (TMAs) – Correlation with Helicobacter pylori Infection

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Aim. To examine the role of iNOS and Helicobacter pylori infection in gastric carcinogenesis.

Material and Methods. Forty-five (25 H. pylori-positive [Hp+] and 20 H. pylori-negative [Hp−]) gastric carcinoma cases were studied. Twelve Hp+ and 10 Hp− carcinomas were classified as diffuse and 13 Hp+ and 10 Hp− as intestinal type. Intestinal metaplasia (IM) type I were observed in 5 Hp+ cases, IM II in 8, and IM III in 12 cases. Controls included 5 Hp− IM I, 6 IM II, and 10 IM III cases. The TMArrayer apparatus (Chemicon, USA) was used for the construction of TMAs. iNOS expression was determined by immunohistochemistry and differential polymerase chain reaction (PCR) in microcore samples taken by TMArrayer and properly analyzed with an Image Analysis System (DIS-200, Digital Image Systems, Heelas).

Results. Twelve of 13 of intestinal and 10/12 of diffuse Hp+ gastric carcinomas overexpressed iNOS. Two of 5 IM I, 8/12 IM II, and 7/8 IM III Hp+ cases also expressed increased iNOS levels as well as 5 diffuse and 5 intestinal-type H. pylori carcinomas. In addition, 0/5 Hp− IM I, 1/6 Hp− IM II, and 2/10 Hp− IM III cases expressed high iNOS levels. Statistically significant differences, concerning the expression of iNOS, were observed between Hp+ Ca and Hp− Ca (&lt;.01), between IM II and III (&lt;.05), as well as between Hp+ and Hp− IM II and III (&lt;.01).

Conclusions. High iNOS expression is significant for the initiation and promotion of gastric carcinogenesis. High iNOS levels in IM type II and III correlated well with H. pylori infection and this may be a strong evidence for long-term follow-up.

Abstract no.: 07.10

Helicobacter pylori – A Prognostic Indicator After Curative Resection of Gastric Carcinoma


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The influence of Helicobacter pylori on survival after curative resection for gastric adenocarcinoma is unknown. We report follow-up of H. pylori-positive (Hp+) and H. pylori-negative (Hp−) gastric cancer patients who underwent curative resection for gastric adenocarcinoma between 1992 and 2004.

Preoperative H. pylori status of 166 curatively resected patients with gastric adenocarcinoma was examined by means of bacterial culture, histology (H&E and Warthin–Starry stain), and serology. We investigated associations with various prognostic factors as well as the effect of H. pylori status on relapse-free and overall survival.

At median follow-up of 53.0 (mean 73.9) months, disease-free and overall 5-year survival were 56.7% and 61.9%, respectively, in Hp+ patients, and 19.2% and 19.2%, respectively, in Hp− patients (p = .002). For multivariate analysis of various clinicopathologic features, the impact of H. pylori status on relapse-free (hazard ratio 1.94 CI [1.20–3.13]) and overall survival rates was found to be a new independent beneficial prognostic factor (hazard ratio 1.83 CI [1.13–2.99]). Other established prognostic factors (depth of invasion, lymph node metastases, and preoperatively elevated levels of carcinoembryonic antigen) were also significantly linked with survival in our study.

Interpretation. Hp+ patients’ status is a new independent prognostic factor for better relapse-free and overall survival after curative resection for gastric adenocarcinoma. Our findings should lead to more careful follow-up of Hp− patients due to their dismal prognosis. If the mechanisms that lead to the reported differences in survival are elucidated, our findings could contribute to new strategies in the treatment of gastric cancer.

Abstract no.: 07.11

Comparison of Biopsy Sampling Methods for Detecting Stomach Mucosal Precancerous Changes

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Aim. To compare the results of different schemes of stomach mucosal biopsy sampling by evaluation of serum pepsinogen-1 (PG-1) and gastrin-17 (G-17) levels in patients with Helicobacter pylori-associated chronic atrophic gastritis, with reference to endoscopic Kimura–Takemoto’s staging, chromoendoscopic and histologic features.

Materials and Methods. Two hundred sixty-seven dyspeptic H. pylori-infected patients were examined by chromoendoscopy
with random biopsy sampling according to the Sydney System and according Kimura–Takemoto’s scale. Simultaneous assessment of serum PG-1 and G-17 levels by enzyme immunoassay was performed as a screening method for atrophic gastritis. The serologic and morphologic results were compared with correlation analysis.

**Results.** There was strong reverse correlation between the histologically detected stomach mucosal atrophy (antral or corpus) and the serum levels of the proper marker (respectively, G17 or PG1) when gastric biopsies taken according to the Sydney System were assessed. The use of Kimura–Takemoto’s scale revealed the decreasing of serum PG-1 levels at O-2 and O-3 grades of corpus atrophy, whereas serum G-17 levels were depressed in parallel with the development of antral atrophy. There were not any advantages in sampling biopsies for detecting intestinal metaplasia (IM) by the Sydney System, or by Kimura–Takemoto’s scheme. The obvious concordance was revealed between histologically detected extent of IM and the number of foci of IM detected by chromoendoscopy.

**Conclusions.** Biopsy sampling for detecting stomach mucosal precancerous changes after non-invasive screening of atrophic gastritis (e.g., by means of EIA) should be based preferably on the visual signs acquired via chromoendoscopy

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**Abstract no.: 07.12**

**Immunoproteome of Helicobacter pylori Strains with Serum Samples from Patients with Gastric Carcinoma (GC), Duodenal Ulcer (DU), and Chronic Gastritis Only (CGO)**

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**Background.** Different Helicobacter pylori antigens may determine the intensity of the inflammatory responses and the degrees of gastric mucosa damage. To better define these antigens, we carried out a comparative proteomic and immunoproteomic analysis of different H. pylori strains, using different serum samples collected from patients and controls.

**Methods.** We examined two H. pylori strains from two patients with gastric carcinoma (GC), one strain from a DU (duodenal ulcer) case, and one strain from a CGO (chronic gastritis only) case. All strains were cagA and s1/m1 vacA subtype positive. Proteins were submitted to 2D-PAGE, electrotransferred onto nitrocellulose sheets, and were reacted with serum samples, diluted 1:600, from patients with GC, DU, and CGO. Identification of protein spots was performed by N-terminal microsequencing, gel matching, and immunoblotting.

**Results.** The number of protein spots found ranged from 1500 to 1650. We observed a high variability in the expression of proteins and sharing of virulence determinants, such as UreB, Cag26, CagA, catalase, UreA, TagD, 26K antigen, HSPs, etc. We also detected pathology-related potential markers: 1, CGO: tagD, 3R; 2, DU: ppsae, Ef-Tu; 3, GC: UreB accessory protein, HP0697, napA, and 12 unidentified proteins.

**Discussion.** The high degree of protein polymorphism and the anti-H. pylori antibody distinct patterns observed could possibly be related to the variable clinical outcome of the infection. Some proteins, reacting exclusively with GC sera, might be considered potential markers of GC.

**Acknowledgements.** This study was funded by the grant of the Siena University PAR 2004, “Helicobacter pylori infection, host’s apolotypes of inflammatory cytokines and risk of ischaemic heart disease.”

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**Abstract no.: 07.13**

**Relationships Between Gastric Cancer Among Young Adults and Helicobacter pylori – Comparison Over a Decade in the Tokyo Area, Japan**

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**Background.** Since 1986, incidence of gastric cancer among young adults (less than 40 years old) (yGC) has been rapidly decreasing in Japan.

**Aim.** To assess recent relationships between yGC and Helicobacter pylori and to compare them with our previous study whose sera were collected in 1988–1990.

**Subjects and Methods.** For a case–control study, plasma of incident 54 yGC patients and 54 screenees (matched for age and gender with patients) was collected in 1997–2002. H. pylori IgG antibody using J-HM-CAP™ and pepsinogen I and II using RIABeads Pepsinogen I and II in the plasma were measured. Subjects were classified by H. pylori status (positive/negative) and pepsinogen values (normal/mild/severe). Cutoff for H. pylori antibody was defined at 2.3. It was defined as severe serological atrophy when pepsinogen I is less than 50 ng/ml and I to II ratio is less than 2.0, as mild atrophy when less than 70 ng/ml and less than 3.0, respectively, and as normal in the other cases.

**Results.** Twenty (37%) controls and 44 (81%) patients were H. pylori seropositive. In the H. pylori-seropositive, numbers of subjects with normal, mild, and severe atrophy were 13, 5, and 2 among controls, and 28, 12, and 4 among patients, respectively. In the seronegative, they were 33, 1, and 0 among controls and 9, 1, and 5 among patients, respectively.

**Conclusion.** Compared with our previous study, H. pylori seroprevalence was lower (81% versus 88%) and serologic atrophy was less severe among patients, whereas no remarkable difference was observed among controls.
**Abstract no.: 07.14**

**Helicobacter pylori as Inhibitor of Neoplastic Processes**

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For the last years, the researches of various microorganisms’ role in malignant neoplasm regression have been sharply activated in the world. The study of microbial substances, analogues to “Coley’s vaccine” is one of the most prospective directions among them. We have studied the ability of toxic substances of Helicobacter pylori to induce regression of neoplastic processes.

We tested the preparation made of H. pylori clinic cultures (HPP) and the preparation analogous to a classic “Coley’s vaccine” (CV). We studied efficiency of these preparations on white mice (line Balb/c) with carcinoma of small intestine. The mice (36 pieces) were divided into three groups: 1, control (placebo); 2, injections of CV into tumor; 3, injections of HPP into tumor. Injections were made every second day for at least 2 weeks with increasing doses. All animals of the first group perished within 3 weeks. For the second and third groups of mice, 66.7% and 83.3% survived, respectively. The morphologic researches confirmed absence of atypical cells at the survived mice.

Thus, the preliminary results prove high efficiency of elaborated anticancer drugs based on microbial toxic H. pylori substances. These medicines are standard on the structure and are stable during long storage. They do not contain living microorganisms, and they are easily prepared and low in toxicity. Moreover, they can play a certain role in organism immunization against H. pylori.

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**Abstract no.: 07.15**

**Helicobacter pylori Infection and Gastric Cancer in Thailand**

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**Background and Aims.** Helicobacter pylori infection is considered to be one of the major causes of gastric cancer. This cross-sectional study was designed to evaluate the clinico-pathologic features and prevalence of H. pylori infection in gastric cancer in Thailand.

**Methods.** Clinical information, endoscopic findings, histologic features, and H. pylori status were collected from gastric cancer patients between January 2000 and May 2002. H. pylori infection assessed by the combination of rapid urease test, histology, culture, and serology. Patients were regarded as H. pylori-positive if at least one of the tests gave a positive result.

**Results.** A total of 59 gastric cancer patients were enrolled in this study (31 men and 28 women, mean age 59.8 years [range 33–86 years]). The common presenting symptoms were dyspepsia (71.2%), weight loss (69.5%), and anorexia (42.4%). Overall prevalence of H. pylori infection was 83.1% and there was no difference between male and female (87.1% versus 78.6%; p > .05). The histologic staging had no significant difference between H. pylori-positive and H. pylori-negative group. In addition, there was no different between prevalence of H. pylori infection in diffuse type and intestinal type gastric cancer (82% versus 85%; p > .05). However, the prevalence of H. pylori infection was significantly higher in nonproximal than proximal gastric cancer (89% versus 6%; p < .01).

**Conclusion.** H. pylori infection was commonly found in nonproximal gastric cancer. There was no difference of clinical symptoms, histology type, and staging between H. pylori-positive and H. pylori-negative gastric cancer in Thai patients.

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**Oesophageal and Extradigestive Diseases**

**Abstract no.: 08.01**

**Gastrooesophageal Reflux and Helicobacter pylori: A Population-Based Study**

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**Introduction.** Gastrooesophageal reflux disease and its sequelae are increasing in incidence in Western countries over the past few decades as the prevalence of Helicobacter pylori is falling. Moreover, carriage of CagA-producing strains of H. pylori may have a protective effect against Barrett’s esophagus and esophageal adenocarcinoma.

**Aims and Methods.** The aim of the study was to estimate the prevalence of symptoms of gastrooesophageal reflux (GER) in the general population of Novosibirsk, Russia, and to assess H. pylori and CagA positivity.

A representative sample of 816 adults (368 males, 448 females aged 45–70 years) completed a bowel disease questionnaire, and GER symptoms (heartburn and acid regurgitation) were registered. Sera were tested for antibodies against H. pylori and CagA protein (HelicoBest, Vector-Best, Russia) using ELISA.

**Results.** Both symptoms were reported with the same frequency by males and females. GER was reported at least once a month by 30.0% (29.1% in males and 30.8% in females, p = .59). Weekly GER was found in 17.3% (17.1% in males and 17.3% in females, p = .91). The prevalence of H. pylori infection was 84.4%, among seropositive subjects antibodies against CagA protein were detected in 55.4%. No significant difference was found in GER symptoms according to H. pylori and CagA status.

**Conclusion.** The prevalence of GER symptoms in Russian adult population appeared to be similar as compared to Western
Abstract no.: 08.02

**Helicobacter pylori CagA-positive Strains Are Associated with Microalbuminuria in Type 2 Diabetes**

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**Background.** Microalbuminuria among patients with type 2 diabetes is associated with an increased risk of atherosclerosis. The nature of this association is unclear. Infection with cytotoxin-associated geneA (CagA) strains of Helicobacter pylori may induce atherosclerosis probably through cross-reaction between anti-CagA antibodies and endothelial antigens, damaging endothelial cells, and inducing local inflammation. In infected diabetic patients, endothelial damage at the renal level may produce microalbuminuria.

**Methods.** The seroprevalence of infection by *H. pylori* and by strains bearing CagA was assessed by ELISA. Microalbuminuria was defined as a 24-hour urinary albumin excretion between 30 and 299 mg and was measured by nephelometry.

**Results.** *H. pylori* was found in 64% of patients with microalbuminuria, 59% of patients without microalbuminuria, and 58% of control group (p = NS), whereas the prevalence of CagA-positive strains was significantly higher in patients with microalbuminuria than in patients without microalbuminuria (55% vs 24%; OR 5.12, 95% CI 1.92–8.15) or in controls (55% vs 21%, OR 6.33, 95% CI 2.74–11.64), after adjusting for age, sex, social status, history of peptic ulcer disease, diabetes duration, glycosylated hemoglobin, hypertension, and renal function.

**Conclusions.** Patients with diabetes and microalbuminuria have an increased prevalence of infection with *H. pylori* CagA-positive strains. This finding might explain the association between microalbuminuria and atherosclerosis in these patients.

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**Abstract no.: 08.03**

**Influence of Helicobacter pylori on Symptoms of Mild GERD During Long-Term Management with Pantoprazole**

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**Introduction.** The results of the ORION TRIAL (published elsewhere) showed that on-demand treatment with pantoprazole is an effective treatment strategy in patients with mild GERD after preceding healing from heartburn.

**Objective.** To point out the influence of *Helicobacter pylori* status on symptoms of mild GERD during long-term on-demand therapy with pantoprazole.

**Methods.** In this randomized, double-blind, placebo-controlled study, patients with GERD 0/1 were treated with pantoprazole 20 mg for 4 weeks. At baseline endoscopy, four biopsies were taken for the determination of *H. pylori* by CLO® Test. Following this, patients were assigned to a long-term phase of 6 months with either pantoprazole 20 mg or 40 mg or placebo on demand if they were free of heartburn.

**Results.** Six hundred thirty-four patients were enrolled in this study (ITT). Five hundred forty-three of them (ITT) were free of heartburn. Other factors than *H. pylori* status on the weighted GERD symptom load in the three treatment groups of evaluable patients is shown in the table:

<table>
<thead>
<tr>
<th></th>
<th>P40 group</th>
<th>P20 group</th>
<th>PLA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n Mean SD</td>
<td>n Mean SD</td>
<td>n Mean SD</td>
<td></td>
</tr>
<tr>
<td><em>H. pylori-positive</em></td>
<td>37 2.86 1.68</td>
<td>45 3.18 2.74</td>
<td>24 3.93 2.57</td>
</tr>
<tr>
<td><em>H. pylori-negative</em></td>
<td>178 2.68 1.79</td>
<td>168 2.85 1.96</td>
<td>82 3.93 3.13</td>
</tr>
</tbody>
</table>

**Conclusion.** The results of this study indicate no influence of *H. pylori* on symptoms of patients with mild GERD during long-term management with pantoprazole on demand.

This trial was funded by an unrestricted grant of ALTANA Pharma AG.

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**Abstract no.: 08.04**

**Does Helicobacter pylori Infection Affect the Occurrence of Reflux-Like Symptoms in General Population? Multicenter Study**

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**Background.** The role of *Helicobacter pylori* infection in gastroesophageal reflux disease remain controversial.

**Aim.** To assess the incidence of reflux-like symptoms in *H. pylori*-infected representative group of Polish population (multicenter study).

**Methods.** The *H. pylori* infection incidence was assessed in 3307 adults subjects selected randomly from the population of big cities,
small towns, and villages in the proportion to age, basing on the anti-\textit{H. pylori} IgG antibody titers determined by ELISA. Every subject was interviewed using questionnaire regarding reflux-like complaints (pyrosis, belching, casting).

**Results.** The incidence of the infection in observed group was 2784/3307 (84.2%). It was more frequent in pyrosis (+) group (86.5%) versus pyrosis (−) group (82.7%), \( p < .01 \). It also prevailed in subjects with other reflux-like symptoms for 4–6% (\( p < .05 \)–\( p < .001 \); see table).

**Conclusion.** \textit{H. pylori} infection seems to have small but significant impact on the occurrence of reflux-like symptoms in the general population.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>( n )</th>
<th>( \text{H. pylori} ) positive (%)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrosis (+)</td>
<td>1119</td>
<td>86.5</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Pyrosis (−)</td>
<td>1665</td>
<td>82.7</td>
<td></td>
</tr>
<tr>
<td>Belching (+)</td>
<td>841</td>
<td>86.2</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Belching (−)</td>
<td>1943</td>
<td>83.3</td>
<td></td>
</tr>
<tr>
<td>Casting (+)</td>
<td>411</td>
<td>89.7</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Casting (−)</td>
<td>2372</td>
<td>83.3</td>
<td></td>
</tr>
</tbody>
</table>

*Project granted by Polish Ministry of Health # PCZ-08–19 and Polish Committee for Research, #C 007/P05/2000.

**Abstract no.: 08.06**

**Helicobacter pylori Infection Protects Against Pollen Allergy**

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**Background and Aims.** The prevalence of atopic diseases is recently increasing especially in the developed countries, whereas the prevalence of \textit{Helicobacter pylori} infection is decreasing. Infectious diseases during childhood are often thought to protect atopic diseases and \textit{H. pylori} infection acquired mostly in childhood. Overall, we hypothesized that \textit{H. pylori} infection might protect atopic diseases such as pollinosis, defined as paroxysmal ocular and nasal symptoms upon contact with pollens.

**Materials and Methods.** We collected urine samples from 94 volunteers (25–45 years old) and blood samples from 133 consecutive patients (17–99 years old) in the season of cedar pollen. The presence of urine \textit{H. pylori} IgG antibodies (URINELISA, Otsuka Pharmaceutical, Tokyo, Japan) was compared with typical pollinosis symptoms. The presence of serum \textit{H. pylori}-IgG antibody was compared with serum pollen (cedar)-specific IgE antibodies.

**Result.** Among the urine samples, 26 (28%) were \textit{H. pylori}-positive and 39 (42%) cases had typical pollinosis. Pollinosis was present only in 4 of 26 (15%) cases with \textit{H. pylori} infection compared to 35 of 68 (51%) without infection (\( p = .002 \)). In the serum samples, 57 (43%) samples were \textit{H. pylori}-positive and 67 (50%) were pollen IgE positive. Pollen IgE was present in 16 of 57 (28%) with \textit{H. pylori} infection compared to 51 of 76 (67%) without infection (\( p < .0001 \)).

**Conclusions.** These data strongly suggest that \textit{H. pylori} infection reduces the risk of atopic disorders such as pollinosis.

**Abstract no.: 08.07**

**Role of Helicobacter pylori in Protection from Intestinal Candidosis**

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**Objective.** \textit{Candida} spp. are classical representatives of transitional flora. Acid environment and enzymes of stomach represent barrier for their transit to intestine. The products of vital activity of \textit{Helicobacter pylori} compound another barrier. The peptide HP (2–20), derived from ribosomal protein L1, displayed strong fungicidal activity. The isolation of \textit{Candida} from gastric
juice after course of standard triple therapy at *H. pylori*-positive patients enlarged by 2.5 times and during antisecretory monotherapy in *H. pylori*-negative patients by 3.5 times.

**Aim.** To study mutual relations of *H. pylori* and *Candida* in gastric mucus in acid-related diseases and factors influencing coexistence of these microorganisms.

**Methods.** Samples of antral mucosa obtained from 679 patients with acid-related diseases before and after course of standard triple therapy. We used histology and crush-cytology for revealing *H. pylori* and *Candida*.

**Results.** *Candida* in gastric mucus at primary inspection revealed at 112 patients mainly as single cells. In different groups of patients, occurrence of *Candida* varied: it was maximal at chronic gastritis – 21% (4% coexistence with *H. pylori*), and minimal at oesophagitis – 5%. In duodenal ulcer, *Candida* were found in 13% (10% coexistence). At gastric ulcer, in 16% and 4%, respectively. After treatment, *Candida* were found in the form of large colonies and pseudomycelium in 33%.

**Conclusion.** The elevation of intragastric pH as a result of antisecretory therapy and the elimination of *H. pylori* with its fungicidal component from gastric mucus create optimal conditions for development of *Candida* in the stomach and their passage into an intestine with an early invasive growth.

**Acknowledgements.** Partly funded by the Siena University grant PAR 2004, “*Helicobacter pylori* infection, host’s apolotypes of inflammatory cytokines and risk of ischaemic heart disease.”

**Abstract no.: 08.09**

**Virulent Strains of Helicobacter pylori in Patients with Stable and Unstable Angina Pectoris**

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**Background.** Previous studies have shown a potential role of CagA-positive strains in the destabilization of atherosclerotic plaques in patients with ischemic heart disease (IHD). In order to verify this hypothesis, we have designed a study aimed at determining either the prevalence of CagA-positive strains or the antibody titer in patients with stable (SA) and unstable angina (UA).

**Methods.** Thirty-eight (30 males, mean age 64 ± 11 years) patients with angiographically documented UA, 25 patients with angiographically documented SA (21 males, mean age 62 ± 10 years), and 50 healthy volunteers (38 males, mean age 62 ± 10 years) were enrolled. The prevalence of *Helicobacter pylori* infection and CagA-positive strains and the antibody titers were evaluated in all subjects through ELISA.

**Results.** Prevalence of *H. pylori* infection was significantly higher in patients with SA and UA compared to controls (60% in SA, 61% in UA, and 38% in controls; *p < 0.04*). Prevalence of CagA-positive strains was significantly higher in patients with SA and UA compared to controls (44% in SA, 50% in UA, and 18% in controls; *p < 0.001*). Prevalence of CagA-positive strains was higher in patients with UA compared to those with SA, although was not statistically significant. Interestingly, the titer of anti-CagA antibodies was significantly higher in patients with UA compared to those with SA (161 ± 120 RU/ml versus 78.7 ± 63.1 RU/ml; *p < 0.04*).

**Conclusions.** The anti-CagA antibody titer is significantly higher in patients with UA compared to those with SA. This finding may be consistent with antibodies anti-CagA playing a role in the destabilization of atherosclerotic lesions.
Factors can influence a good response to standard therapy. The role of \textit{Helicobacter pylori} on gastroesophageal reflux symptoms is still a matter of debate.

**Aim.** To evaluate symptomatologic response to PPI-treatment (esomeprazole) in GERD patients and its relationship to \textit{H. pylori}.

**Materials and Methods.** One hundred fifteen consecutive outpatients (43 males, 72 females; mean age 55 years, range), referring to our Gastroenterology Unit with GERD symptoms, underwent upper GI endoscopy with biopsies and were divided into two groups accordingly to \textit{H. pylori} status: 80 \textit{H. pylori} positive (group A) and 35 \textit{H. pylori} negative (group B).

Patients were asked about typical GERD symptoms (i.e., heartburn and acid regurgitation) that were scored as follows: 0 = no symptoms; 1 = one symptom (heartburn or acid regurgitation); and 2 = both symptoms (heartburn and acid regurgitation). All patients started a therapy with esomeprazole 40 mg once daily for 2 months then reduced to 20 mg daily for the following 2 months. Clinical evaluation and symptoms recording were performed at baseline and at second and fourth months.

**Results.** At baseline, there were no differences in the two groups regarding age, sex, and severity of GERD symptoms. Each group showed a significant decrease in GERD-symptoms severity after 2 and 4 months with no statistically significant difference in symptoms relief between the two groups.

**Conclusion.** \textit{H. pylori} seems not to influence symptomatologic response to esomeprazole in patients with GERD.

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**Abstract no.: 08.11**

**Significance of \textit{Helicobacter pylori} Eradication in the Treatment of Gastroesophageal Reflux Disease**

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**Aim.** To determine the effect of eradication of \textit{Helicobacter pylori} infection on subjective symptoms in patients with GERD.

**Material and Methods.** A total of 110 patients with diagnosed GERD were included in the study. Of 65 patients with \textit{H. pylori} infection, eradication therapy was performed in 35 (the success rate 90%; 7-day therapy with pantoprazole, amoxicillin, clarithromycin) and 45 patients tested negative for \textit{H. pylori}. All patients received pantoprazole for 4 weeks. Severity of subjective symptoms was evaluated with the Nepean Dyspepsia Index at baseline and after 1 month of treatment. Statistical analysis was performed using analysis of variance.

**Results.** In all three groups of patients with GERD (\textit{H. pylori}-positive eradicated, \textit{H. pylori}-positive non-eradicated, and \textit{H. pylori}-negative), a statistically significant decrease in dyspeptic symptoms was reported \((p < .001)\). No statistically significant differences were discovered in decrease in dyspeptic symptoms between the groups \((p > .05)\).

**Conclusions.** 1. Eradication of \textit{H. pylori} infection in patients with GERD was not found to statistically significantly influence the decrease of subjective symptoms, the patients with eradicated and non-eradicated \textit{H. pylori} infection experience a similar decrease; 2. \textit{H. pylori} infection does not have a statistically significant influence on symptoms decrease in patients with GERD, the patients with non-eradicated \textit{H. pylori} infection and \textit{H. pylori}-negative patients have decreased to an approximately same extent; 3, statistically significant decrease in symptoms in all three groups of patients is the result of PPI therapy.

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**Abstract no.: 08.12**

**An Evaluation of Immunological Cross-Reactivity Between VacA of \textit{Helicobacter pylori} and Myelin P0 Protein Using the Cerebrospinal Fluid from Patients with Guillain–Barre Syndrome**

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**Objective.** We have previously reported that antibodies against recombinant vacuolating cytotoxin (VacA) of \textit{Helicobacter pylori} were detected in the cerebrospinal fluid (CSF) from patients with Guillain–Barre syndrome (GBS). This study was carried out to evaluate the immunologic cross-reactivity between VacA and myelin P0 protein.

**Materials and Methods.** CSF samples positive for antibodies to VacA were donated from 16 patients with GBS (13 male, 3 female; mean age, 45.5 years). The recombinant fusion protein consisting of P0 and GST protein (P0-GST) was harvested from cultured recombinant \textit{Escherichia coli}, which were transfected the P0-GST expression plasmid. P0-GST was obtained as inclusion bodies due to its hydrophobicity. Crude antigens including P0-GST were separated by SDS-PAGE. Western blotting analyses were performed according to the standard protocol; however, 0.05% SDS had to be added to the transfer buffer due to hydrophobicity of P0-GST.

**Results.** No specific IgG antibody against P0 protein was detected in any of the CSF samples. The present study could not elucidate the immunologic cross-reactivity between VacA and P0 protein.

**Discussion.** As regards the pathophysiologic role of CSF antibodies to VacA in patients with GBS, our previous study indicated a sequence homology between VacA and P0 protein together with human (Na$^+$ + K$^+$)-ATPase $\alpha$ subunit, as the possible target molecules of these antibodies. The present study failed to find the molecular mimicry of VacA and myelin P0 protein. Further study, including the evaluation of the molecular homology between VacA and human (Na$^+$ + K$^+$)-ATPase $\alpha$ subunit, is necessary because the exact mechanism of GBS remains unresolved.
Abstract no.: 08.13
Role of Helicobacter in Etiology and Pathogenesis of Idiopathic Parkinsonism: Biologic Gradients of Its Facets, Progression, and Global Severity Based on Helicobacter pylori Antibody Profile


Abstract
Background. A double-blind, placebo-controlled RCT of the effects of Helicobacter pylori eradication on the time-course of idiopathic parkinsonism (IP) indicates a direct, or surrogate, but not necessarily unique, pathogenic role (Bjarnason et al. Helicobacter 2005; in press).

A discriminant index for IP was based on the Western blot pattern of serum H. pylori antibodies. The predicted probability of having diagnosed IP was greatest when retaining an anti-CagA band, but not anti-VacA, with age, and being antiurease B seronegative irrespective of age. By age 80, this circumstance increases the odds of IP fivefold (Dobbs et al. Gut 2002; 51 [Suppl 11]:A77). Antiurease ELISA antibody status did not complement the model.

A unifying explanation would be that the immune/inflammatory pathogenic process continues, albeit more quietly, after Helicobacter is undetectable by screening methods. Biologic gradients between index and putative consequences would strengthen the hypothesis.

Methods. Association of index with neurologic disease load and progression over 4 years was assessed and in 124 subjects with 196 without parkinsonism.

Results. Clinically relevant gradients were found between index and disease burden, despite any confounding effect of antiparkinsonian medication. The more archetypal the antibody profile, the worse was posture, as gauged by forward displacement of occiput (p = .04); the shorter mean stride-length (= .003), longer reaction time (= .002), and lesser cognitive efficiency (= .03), and the greater their deterioration (= .006, .002 and .03); and the greater overall disease severity (< .001).

Conclusion. The apparent importance of H. pylori in the etiology/pathogenesis of IP is not confined to those with overt evidence of infection.

Abstract no.: 08.14
Portal Hypertensive Gastropathy: Participation of Helicobacter pylori in Pathogenic Mechanisms

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Abstract
Objective. Portal hypertensive gastropathy (PHG) was recently discovered in 65% of patients with portal hypertension caused by liver cirrhosis. Pathogenesis of this lesion is not cleared up, but Helicobacter pylori could be important factor in its development.

Aim. To evaluate state of gastroesophageal mucosa, prevalence and diagnostic methods for Helicobacter pylori infection in CRI patients after kidney transplantation.

Introduction. The states of gastroesophageal mucosa and prevalence of Helicobacter pylori infection in chronic renal insufficiency (CRI) patients after kidney transplantation are not well known.

Methods. Thirty CRI patients after kidney transplantation (26 male and 4 female, median age 32.4 years) were included in clinical research, endoscoped, and tested for H. pylori. The presence and severity of gastritis were graded according to a modified updated Sydney classification. The grade of reflux esophagitis (RE) was assessed according to Los Angeles classification, H. pylori status – by serology, histology, and CLO test.

Results. The presence of H. pylori infection defined serologically in 9 (30%), histologically in 26 (86.7%), and by CLO test in 25 (83.3%) of the 30 researched patients. Of 25 H. pylori-positive patients, 21 (84%) had gastroesophageal reflux disease (GERD): non-erosive GERD in 9 (36%), RE grade A in 8 (32%), and RE grade B in 3 (12%) patients. Antrum and/or corpus gastritis revealed in 18 (72%) H. pylori-positive patients. Gastric erosions and ulcers occurred in 10 (40%) and 1 (4%) H. pylori-positive patients, respectively.

Conclusions. The prevalence H. pylori infection was high in CRI patients after kidney transplantation. The most reliable tests for H. pylori infection in researched patients were the CLO test and gastric histology. Whereas gastroesophageal lesions were defined frequently in H. pylori-positive patients after kidney transplantation, their severity was not high.
Paediatric Issues

Abstract no.: 09.01
Polymorphisms of TLR-4/TLR-2 and Risk of Helicobacter pylori Infection and Duodenal Ulcer Disease in Children

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Although host factors linked to the outcome of Helicobacter pylori infection have been studied, no study has evaluated yet the role of single nucleotide polymorphisms (SNP) in genes that encode mediators of the innate immune response such as Toll-like receptor (TLR)-4 and TLR-2 in the risk of H. pylori infection and H. pylori-associated diseases in children. The SNPs of genes encoding TLR-2 and TLR-4 are thought to impair the efficiency of bacterial ligand signaling and the capacity to elicit the host’s defense against microorganisms. Considering that the infection is mainly acquired in childhood, we evaluated 432 children (193 boys, mean age 9.4 ± 3.8 years, range 1–18). Among them, 207 were H. pylori-positive (Hp+) (72 with DU). cagA status was investigated by polymerase chain reaction (PCR). TLR-4 (Asp299Gly) and TLR-2 (Arg753Gln, Arg677Trp) polymorphisms were investigated by PCR–RFLP and confirmed by sequencing. The allele Arg677Trp was not seen in this population. The other alleles were in Hardy–Weinberg equilibrium. No association was seen between the polymorphic alleles and DU (p = .71, OR = 0.78, 95% CI = 0.53–1.19 for TLR-4 and p = .32, OR = 1.68, 95% CI = 0.45–6.65 for TLR-2). In addition, these polymorphisms did not associate with H. pylori status (p = .34, OR = 0.63, 95% CI = 0.26–1.49 for TLR-4 and p = .69, OR = 0.75, 95% CI = 0.27–2.02 for TLR-2). However, the polymorphic G allele of TLR-4 was significantly associated with infection with cagA+ strains (p = .02, OR = 9.08, 95% CI = 1.21–68.8). Our results show that SNPs in TLR-2 and TLR-4 were neither associated with the susceptibility to H. pylori infection nor with the outcome of the infection in childhood. However, TLR-4 SNP was associated with susceptibility to more virulent H. pylori strains.

Financial Support. CNPq/FAPEMIG.

Abstract no.: 09.03*
Gastric Epithelial Proliferation in Helicobacter pylori-Infected Children

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Background. Epithelial proliferation stimulated by Helicobacter pylori may have a role on gastric carcinogenesis. Most studies on gastric epithelial proliferation have been performed in adults.

Aims. To analyze gastric epithelial proliferation in children infected by cagA+ strains.

Materials and Methods. We studied 54 children: 28 boys, 26 girls, age 10 ± 3.7 years, 45 H. pylori-positive (Hp+) (34 cagA+), 15 with duodenal ulcer (DU), and 30 with H. pylori gastritis. H. pylori and cagA status were determined by polymerase chain reaction (PCR). Epithelial proliferation was studied by immunohistochemistry (sABC) by counting Ki67+ nuclei in the antral neck zone; the percentage of Ki67+ nuclei was higher in DU (p = .02); LeX foveolar expression was associated with DU in Hp+ children (p = .04), but not in adults (p = .2). LeY foveolar expression (LeYf) was higher in adults with DU than those with H. pylori gastritis (p = .01); in children, no difference was found in LeY expression between these groups (p = .8).

Conclusions. Le expression is different in the gastric mucosa of children and adults. Comparative studies addressing Le expression in these groups can contribute to understand the acquisition and pathogenesis of H. pylori infection.

Financial Support. CNPq, Fapemig.

Abstract no.: 09.02
Lewis Expression in Helicobacter pylori Infection: A Comparative Study Between Children and Adults

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Background. Lewis antigens (Le) act as Helicobacter pylori adhesin receptors. Differences in LeA and LeB expression between children and adults have been observed.

Aims. To compare Le expression in the gastric mucosa of children and adults, with and without H. pylori infection.

Patients and Methods. Gastric immunoexpression (sABC) for Lewis A (LeA), LeB, X (LeX), and Y (LeY) was studied in children (n = 70, range 1–18 years; 46 H. pylori-positive (Hp+), 17 DU) and adults (n = 46, range 23–81 years; 36 Hp+, 20 DU). H. pylori was detected by urease, histology, and culture. Le expression was scored as: negative, 5%, 5–25%, 25–50%, 50–75%, and > 75% of stained epithelium.

Results. LeA was expressed in 45/70 children and in 28/46 adults (p = .9). LeB expression was more frequent in adults (42/46; 91%) than in children (31/70; 44%) (p = .003), regardless of H. pylori infection. Among children, LeB expression was higher in H. pylori gastritis than in DU (p = .04); in adults LeB expression was higher than in Hp+ (p = .04). LeX foveolar expression (LeXf) was more frequent in Hp+ children (43%) than in adults (17%) (p = .02); LeXf expression was associated with DU in Hp+ children (p = .04), but not in adults (p = .2). LeY foveolar expression (LeYf) was higher in adults with DU than those with H. pylori gastritis (p = .01); in children, no difference was found in LeY expression between these groups (p = .8).

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nuclei/neck zone was significantly higher in DU than in gastritis \( (p = .001) \).  

**Conclusion.** In children, infection by cagA-positive strains is significantly associated with high epithelial proliferation. Differently from adults, higher proliferative activity was observed in DU compared to patients with non-ulcer gastritis.

**Financial Support.** CNPq, Capes, Fapemig.

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**Abstract no.: 09.04**  
**Validation of Non-invasive Tests for Helicobacter pylori Infection in Very Young Children**

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Non-invasive tests in very young children are particularly useful for epidemiologic studies on risk factors and transmission of *Helicobacter pylori*. Two multicenter studies (Megraud, *Pediatrics* 2005, Oderda, *Gut* 2001) showed low accuracy of both 13C-urea breath test (UBT) and *H. pylori* stool antigen (HpSA) possibly for inadequate specimen storage.

**Aim.** To evaluate accuracy of 13C-UBT and HpSA in children < 6 years by performing the tests locally.

**Patients and Methods.** In 7 years, we enrolled 169 consecutive young children (median 2.2 years, range 0.2–5.9) undergoing endoscopy with gastric biopsy for histology (Giemsa) and urease test (RUT). Children underwent 13C-UBT (100 ml orange juice plus 50 mg 13C-Urea) breath analyzed at baseline and after 30 minutes. Same-day stools were collected, stored at \(-20\, ^\circ\text{C}\) and locally analyzed within a few days. Cutoff for 13C-UBT was 5%, for HpSA 0.160 OD. Children with two or more positive tests were considered *H. pylori*-positive, with all tests negative taken as *H. pylori*-negative.

**Results.** Thirty children were *H. pylori*-positive (17.5%), 124 were *H. pylori*-negative, 15 equivocal (only one test positive was considered false positive). Sensitivity and specificity of histology were 100% and 96%, of RUT 96% and 100%, of HpSA 100% and 98%, of UBT 93% and 94%. Twenty-two percent of positive UBT were false positive.

**Conclusion.** Accuracy of non-invasive tests (13C-UBT and HpSA) in very young children is quite satisfactory, provided samples are locally analyzed, because storage and transportation may impair results.

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**Abstract no.: 09.05**  
**The Prevalence of Helicobacter pylori Infection in Childhood, Demographic, and Socioeconomic Factors: Results of a Population-Based Study by Monoclonal Stool Antigen Test from the Czech Republic**

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**Background.** Prevalence of *Helicobacter pylori* varies between developing and developed countries. Some evidence indicates a more precipitous decline in *H. pylori* transmission in children than in adults in industrialized nations. A better understanding of the epidemiology of any particular region’s population is crucial. *H. pylori* prevalence in asymptomatic children in the Czech Republic currently remains inconclusive.


**Methods.** *H. pylori* status was evaluated using non-invasive monoclonal stool antigen test (HpSTAR DAKO Cytomation). A structured questionnaire was completed and three-step statistical analysis undertaken (pc alienware).

**Results.** Overall, 1600 of 1737 eligible healthy, mostly Czech subjects, were randomly selected through stratified sampling (response rate 92.1%). A total 0.3% of infection acquisition occurred before 4 years of age. Overall, *H. pylori* prevalence was 6.1% (98/1600), increasing significantly with age \((p < .001)\). Risk factors included socioeconomic variables, overcrowding, and mother’s education. Non-risk factors included sex, day-care center attendance before 6 years of age, breastfeeding, antimicrobial agents, geographic differences, Z scores for weight-for-age, weight-for-height, height-for-age, dietary habits, water, pets, and any gastric complaints of family members.

**Conclusions.** Taking cohort effect into account, current prevalence of *H. pylori* from birth to 15 years of age appears to be changing. The low infection prevalence (6.1%) reflects data from other European countries demonstrating a decreasing trend in *H. pylori*. However, socioeconomically disadvantaged children are still at risk of this preventable infection.

Abstract no.: 09.06
Serum Ferritin, Hemoglobin, Soluble Transferrin Receiver, and Helicobacter pylori Infection in Peri-Urban Children in Bangladesh

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Background. Both iron-deficiency anemia (IDA) and Helicobacter pylori infection are common in developing countries. Few studies suggested a link between H. pylori infection and IDA in adults.

Objective. To investigate the association between H. pylori infection and iron status in asymptomatic children of a peri-urban community.

Methods. H. pylori infection was determined in 1086 children aged 2–5 years by 13C-urea breath tests (UBT). The Hb level was quantified by cyanmethemoglobin method; serum ferritin (SF) and soluble transferrin receptor (sTfR) were assessed by ELISA (Ramco, Houston, TX). Informed consents were obtained from parents or legal guardian.

Results. Of the 1086 children, 74% were infected with H. pylori. Compared to the children non-infected, SF and hemoglobin (Hb) levels were significantly lower in H. pylori-infected children (mean ± SD, 10.6 ± 1.5 versus 10.3 ± 1.2%, p = .01 for Hb; 23.1 ± 20.2 versus 19.1 ± 16.1, µg/l for SF, p = .003). There was, however, no difference in sTfR between infected and non-infected children (mg/l: 8.8 ± 4.4 versus 8.6 ± 4.8, respectively, p = .28). The prevalence of iron deficiency (ID) (SF < 30 µg/l) and IDA (Hb < 11% plus SF < 30 µg/l or sTfR > 8.5 mg/l) in H. pylori-infected children was significantly higher than in noninfected children (52% versus 61%, p = .007, OR 1.45, 95% CI 1.90–1.93). The difference in prevalence also existed when ID was defined by SF < 30 µg/l or sTfR > 8.5 mg/l.

Conclusion. A significantly lower serum ferritin and Hb levels and higher prevalence of ID and IDA, indicate an association between H. pylori infection and ID in this community.

Abstract no.: 09.07
Comparison of Two Nitrofuran-Based Second-Line Quadruple Therapies in Childhood: Benefit of Helicobacter pylori Eradication After Failure of Initial Treatment

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Background. Metronidazole-based triple therapy is the standard first-line anti-Helicobacter pylori treatment regimen. Unfortunately, the number of pediatric patients with treatment failure is increasing. Aim. The aim of this study was to evaluate the efficacy of nitufuratel compared to furazolidone in non-metronidazole containing second-line combinations consisting of amoxycillin, bismuth subcitrate, and omeprazole for H. pylori eradication in children with failure of initial treatment.

Materials and Methods. Seventy-six H. pylori-positive pediatric outpatients (male 40; mean age 13.7 ± 1.4 years; age range 12–16 years), suffering from chronic abdominal complaints, were enrolled in this study. All had failed one initial attempt of eradication of H. pylori metronidazole-based 1- to 2-week triple therapy as defined by a positive histology.

Patients were randomized to receive a 2-week course of omeprazole (0.5 mg/kg/once daily), bismuth subcitrate (8 mg/kg/day/q.i.d.), amoxycillin (50 mg/kg/day/q.i.d.), with either nifuratel (15 mg/kg/day/q.i.d.) (group A) or furazolidone (10 mg/kg/day/q.i.d.) (group B). intention-to-treat eradication rates were 89.2% and 87.2% (CI 95%) and per protocol eradication rates were 89.2% and 91.9% in groups A and B, respectively. The compliance was excellent in the majority of the patients. Frequency of severe side effects was higher with furazolidone (20.5%) than with nifuratel (2.7%) (p = .0289).

Conclusions. In the pediatric patients, who failed first-line eradication treatment, nitrofuran-based quadruple therapies containing either nifuratel or furazolidone, PPI, amoxycillin, and bismuth salt are successful second-line therapeutic approach for H. pylori eradication. Nifuratel is preferred because of the lower frequency of side effects.

Abstract no.: 09.08
Paediatric Helicobacter pylori Infection: Presentation and Outcome Over Two Decades in a Single Center

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Introduction. Although Helicobacter pylori remains a significant health issue worldwide, in many developed countries rates of infection are decreasing. This retrospective review aimed to describe the experience of H. pylori disease in an Australian tertiary pediatric center for over almost two decades.

Methods. Pathology and hospital records were used to identify all children diagnosed with H. pylori infection at the Sydney Children’s Hospital during the period 1987–2005. The details of presenting symptoms, family history, endoscopic and histologic findings, management, and outcomes were ascertained. Changes in these variables over time were noted.

Results. Of 122 cases of H. pylori infection identified, the pathology records and medical files from 94 patients (aged between 18 months and 19 years) were available for review. The annual frequency of diagnosis fell over time, especially within the last 9 years. More than half of the group originated outside Australia. The most common presenting symptom was epigastric pain (62%). Family history of H. pylori or associated disease was present in 35%. Ulceration was noted in 20% of cases and just one quarter of these presented with acute bleeding. Varied treatment options were used and eradication was successful in 77% of children. Twenty-six children had no symptom improvement despite successful eradication.

Conclusion. The frequency of H. pylori infection at this tertiary pediatric center has decreased substantially over the last 18 years. Presentation patterns did not reflect infection-associated disease or response to treatment. Although of reduced frequency in
Abstract no.: 09.09  
**Serum Pepsinogens in the Diagnosis of Acute *Helicobacter pylori* Infection**  

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Background. Detection of acute *Helicobacter pylori* infection remains problematic. The non-invasive definition includes a positive UBT or stool antigen test and negative serology. However, the possibility of false-positive tests is high and confirmation with a second test would allow a definite diagnosis.

**Aim.** To determine whether serum PG levels can be used to confirm acute *H. pylori* infection.

**Methods.** We tested serum collected from 18 volunteers who became infected after oral *H. pylori* challenge. PG1 and PGII were measured by ELISA (Bioshit, Helsinki, Finland). The cut-off values to define PG-positive samples were defined as the mean + 2SD of the baseline values: PG1 = 144 mg/l and 26 mg/l for PG2.

**Results.** At 1 week postinoculation, only 3 (17%) of *H. pylori*-infected individuals had elevated PG1 levels; 28% had elevated PG2 levels and 33% had either PG1 or PG2 elevations. Serum samples were available for 14 subjects at 2 weeks, and 12 subjects (86%) were PG1 positive; 5 (35%) were PG2 positive. All PG2 positive were also PG1 positive. By 4 weeks, 17 of 18 (95%) were PG1 positive.

**Conclusions.** Because of the relatively low posttest probability of a single positive test (e.g., UBT or stool antigen) a confirmatory test is needed to diagnose acute *H. pylori* infection. A positive PG1 or PG2 would confirm the UBT or stool antigen results. Repeat testing 2–4 weeks after onset of symptoms would increase the likelihood of confirmation of which a recent *H. pylori* infection was responsible for the symptoms.

Abstract no.: 09.10  
**Antral Nodularity, Severe Gastritis, and Positive Cytotoxin-Associated Protein (CagA) Serology in Children Infected with *Helicobacter pylori***  
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*Helicobacter pylori* infection is associated with gastritis and peptic ulcer in children and adults. The endoscopic pattern of antral nodularity is a peculiar finding in children.

To determine whether the antral nodularity and positive CagA serology are associated to severe gastritis in children.

Twenty-five patients (15 male; age range 3–18 years; mean age 9 years) underwent esophagogastroduodenoscopy with antral biopsy (histologic examination and urease rapid test) for a suspicious upper gastrointestinal disease. In all of them, serum sample were assayed for IgG antibodies to CagA.

Results show that nineteen children (76%) were *H. pylori*-positive by histopathology and urease rapid test. 15 of these 19 (79%) *H. pylori*-positive patients were positive for CagA serology, too. At endoscopic examination of the 19 infected children, hyperemia and friability of the gastric antrum was observed in seven (37%) patients and antral nodularity appearance in 12 (63%) children. The histologic examination of all infected patients showed an active microvesicular gastritis and chronic gastritis (lymphoplasmacytic infiltration). The CagA-positive children presented an endoscopic finding of more intense hyperemia of gastric antrum, associated to an important lymphoplasmacellular infiltrate and degenerative and vacuolar lesions of gastric epithelium. The six (24%) non-infected patients, also negative for CagA serology, had a normal gastric finding but they presented cardial hyperemia and distal esophageal erosions. In non-infected children, there were no histologic signs of gastric inflammation.

Endoscopic finding of antral nodularity and positive CagA serology in children suggest the presence of *H. pylori* infection and are important markers of severe gastritis.

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Abstract no.: 09.11  
**Helicobacter pylori and Recurrent Abdominal Pain in Children**  
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Introduction. *Helicobacter pylori* infection causes lesions in the esophagus, stomach, and duodenum that produce recurrent abdominal pain.

**Material and Methods.** We have studied 48 children that visited our paediatric gastroenterology unit. Every child fulfilled Apley’s criteria for recurrent abdominal pain. The children and their parents were studied following a protocol and were required to answer a questionnaire.

**Results.** Out of the 48 studied patients, 19 were boys (39.6%) and 29 were girls (60.4%). They were from 4 to 14 years old. Thirty-four children underwent a 13C-urea breath test (70.8% out of the total); it was negative in 24 children (70.6%), positive in 10 children (29.4%), these 10 children were treated with omeprazole, amoxycilin, and clarithromycin for 14 days. All this was shown that they suffered from chronic gastritis and a positive culture of gastric mucosa. Eighty percent of the children with *H. pylori* infection progressed favorably from the disease.

**Conclusions.** The recurrent abdominal pain occurs in children older than 4 years old, being more frequent in the group between 7 and 14 years old. We consider that 13C-urea breath test is a non-invasive method and useful in determining the etiology of the recurrent abdominal pain and should take part of the initial diagnosis. The upper endoscopy, when indicated, is fundamental for the diagnosis. In this study, we confirm that there is a relationship between *H. pylori* infection and gastritis. The eradication of *H. pylori* infection reduces the symptoms of dyspepsia in infants. This strategy turns out to be economical.
Diagnosis

Abstract no.: 10.01*
Candidate Biomarkers of Helicobacter pylori for Discrimination of Clinical Isolates Associated with Duodenal Ulcer or Gastric Cancer

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Helicobacter pylori infection is closely associated with the development of severe pathologies such as gastroduodenal ulcers and gastric cancer. With regard to cancer, the predictive value of most studied H. pylori virulent genotypes (e.g., cagA, vacA s1m1, babA2) remains limited, which is mainly due to their wide distribution among strains. In the search of other H. pylori markers predictive for the evolution to gastric cancer, we compared the protein profiles of H. pylori strains associated with either duodenal ulcer (n = 12) or gastric cancer (n = 12). To that purpose, protein extracts of the strains were analyzed by SELDI-TOF-MS through using two types of ProteinChip array, CM10 and Q10. In this way, 13 statistically significant potential biomarkers (p < .001) were selected, which all have a mass/charge ratio below 20,000. Our results imply that discrimination between strains associated with different clinical outcome is therefore possible using protein profile biomarkers. Structural identification of the most relevant biomarkers is under way.

Results. One out of five Helicobacter genus probes was specific for Helicobacter. Only one of the four published H. pylori probes was found to be specific for H. pylori. Both probes gave a positive signal with Campylobacter-like organism (CLO)-positive biopsies but not CLO-negative biopsies. Assessment of 100 infectious diarrhea samples showed that non-pylori Helicobacter species were not present, with H. pylori detected in one sample.

Conclusions. We have developed a FISH assay for the assessment of Helicobacter species that has been successfully applied to a variety of GI samples. We will continue to investigate patient material where Helicobacter are implicated as etiological agents of disease.

Abstract no.: 10.02*
Development of a Helicobacter Genus DNA Probe for Fluorescent In Situ Hybridization in Gastrointestinal Clinical Samples

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Background. Fluorescence in situ hybridization (FISH) can be used for the rapid detection of bacteria, determining their location, spatial distribution, and enumeration within a complex community, such as the human gastrointestinal (GI) tract.

Aims. To develop a Helicobacter genus (FISH) probe to investigate Helicobacter prevalence in clinical GI samples. To assess published Helicobacter pylori probes for specificity and suitability for use in a subtractive assay with the Helicobacter genus probe.

Materials and Methods. Published 16S rRNA sequences were collected for Helicobacter species and FISH probes designed using PRIMROSE computer software. Helicobacter genus probes were selected, tested, and optimized against a panel of reference strains (9 Helicobacter and 44 non-Helicobacter species) for specificity. Four published H. pylori probes were also tested. Once optimized, the FISH protocol was used to assess Helicobacter prevalence in a variety of GI samples including gastric biopsies and fecal samples.

Results. Detection of Clarithromycin-Resistant Helicobacter pylori from Human Feces

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Backgrounds. Gastric infection of clarithromycin (CAM)-resistant Helicobacter pylori is one of the major causes of failure to eradicate this organism. A non-invasive and useful method for the detection of CAM-resistant H. pylori from human feces was developed in this study.

Methods. Feces taken from 33 patients were used in this study. H. pylori DNA was extracted from feces by physical crushing using the Fast-Prep System. The 23S rRNA gene of H. pylori containing mutations that confer CAM resistance was amplified by nested polymerase chain reaction (PCR). The mutations in the 23S rRNA gene were detected by restriction fragment length polymorphism (RFLP) method. H. pylori infection was tested by an enzyme-linked immunosorbent assay of H. pylori in feces (HpSA ELISA) from all patients and gastric biopsy specimens were taken for H. pylori cultures from 18 patients.

Results. The 23S rRNA gene of H. pylori was amplified from feces in 25 patients in whom both HpSA ELISA and culture results were positive. Furthermore, the 23S rRNA gene of H. pylori was detected in four patients in whom either HpSA ELISA (n = 1) or culture (n = 3) results were negative. H. pylori DNA was not detected by nested PCR in feces from patients who were H. pylori-negative in both HpSA ELISA and culture. The results of mutation analysis of the H. pylori 23S rRNA gene amplified from feces completely correlated with the susceptibilities of H. pylori isolates to CAM. This nested-PCR/RFLP method can detect CAM-resistant H. pylori within 8.5 hours.

Conclusion. This nested-PCR/RFLP method is useful for the accurate diagnosis of CAM-resistant H. pylori infection from feces.
Abstract no.: 10.04
Western Blotting for the Diagnosis of Helicobacter pylori Infection in Patients with Atrophic Body Gastritis

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Background. Helicobacter pylori may disappear from the stomach of patients with atrophic body gastritis (ABG), decreasing the reliability of histology and standard ELISA to diagnose an infection.

Patients and Methods. We examined by Western blotting (WB) 143 serum samples from patients with ABG: 31 with histology and serology negative for H. pylori (H/-S-), 80 with H-/S+ and 32 with H+/S-. CagA+, s1/m1, VacA subtype H. pylori strains 10K and 4Cb were used as antigens, polyclonal rabbit sera anti-CagA, -58 kDa VacA subunit, -UreB, -UreA, and -HspB as positive control and sera from subjects with histology, serology, and culture negative for H. pylori as negative control. Patients whose sera reacted with CagA and/or VacA, and/or two or more other H. pylori antigens were considered infected.

Results. One hundred thirty-eight serum samples (96.5%) were positive for H. pylori infection independently of the antigen used. One sample in the group H-/S- and four in the group H-/S+ were negative at WB. VacA was the most frequently reacting antigen (87.1% and 96.7% with strains 10K and 4Cb, respectively); CagA reacted with c. 90% of sera. The mean number of antigens recognized by the three groups of sera was similar with the two strains and ranged from 3.0 ± 0.8 to 3.4 ± 0.9 (NS).

Conclusions. Western blotting may be superior to other techniques to diagnose H. pylori infections in patients with ABG. Utilization of two strains as an antigen may increase the sensitivity of serologic tests.

Acknowledgements. Partly funded by the Siena University grant “Helicobacter pylori infection, host’s aplotypes of inflammatory cytokines and risk of ischemic heart disease.”

Abstract no.: 10.05
Development of a Novel Real-Time Polymerase Chain Reaction Assay for the Detection of Helicobacter pylori in Wastewater Samples

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Introduction. Helicobacter pylori is the causative agent of gastritis and duodenal ulcer and has been related to gastric cancer. The transmission of H. pylori remains unclear but fecal-oral pathway has been suggested. Epidemiologic studies suggest that environmental factors are important. It has been hypothesized that water supplies contaminated by sewage are potential routes of H. pylori transmission.

Objectives. We have developed a real-time polymerase chain reaction (PCR) assay to detect H. pylori in environmental samples and compared its effectiveness with traditional PCR method.

Methods. Thirty wastewater samples were collected from several secondary wastewater treatment plants located in Valencia, Spain, and analyzed directly and after enrichment in nutrient broth with dent-selective supplement. Samples were incubated under microaerophilic conditions at 37 °C for 48 hours and submitted to both real-time PCR and traditional PCR assay. Two specific primers that amplify a 382-bp fragment from H. pylori 16S gene were used.

Results. Without enrichment, we were not able to detect H. pylori neither by traditional PCR nor by real-time PCR in any sample. After enrichment, traditional PCR was not able to detect H. pylori in any sample. However, H. pylori was detected in three wastewater samples using real-time PCR assay.

Conclusions. We have developed an accurate, specific, and sensitive method, capable of detecting H. pylori cells when traditional PCR is not capable. Results demonstrate the presence of H. pylori in wastewater samples and that real-time PCR is an excellent tool to detect this bacteria in poorly contaminated environments.

Abstract no.: 10.06
Significantly Increased Helicobacter pylori Concentration in Erosions, Ulcers as Compared to Normal Antrum and Corpus, Determined by UreaseA (ureA) Real-Time or Quantitative PCR

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Background and Aim. Rapid DNA-based quantitative and qualitative assays for Helicobacter pylori determinations in gastric or duodenal mucosa are missing. Our aim was to develop such assays using real-time polymerase chain reaction (PCR).

Methods. Real-time PCR procedures were developed for quantitative detection of H. pylori ureA generally by fluorescence resonance energy transfer (FRET) and for cagA, vacA genotypes of H. pylori by using SYBR Green I dye (SG). Calibration curve of the H. pylori ureA was determined with serial dilutions of H. pylori DNA (from 16 to 0.016 mg/µl). We compared the data with different diagnostic methods: histology, 13C-urea breath test (UBT), and Western blot in 45 patients. The bacterial density was determined in the erosion, antrum, and corpus biopsies from 20 patients.

Results. All DNA preparations from H. pylori-infected patients were amplified and detected, whereas none of the non-infected patients’ DNA were found. Quantitative PCR detection of ureA had the greatest sensitivity (98%) and specificity (100%). PCR-detected genotypes of H. pylori correlated with results of serology. Results obtained with UBT (94% sensitivity, 90% specificity) were equivalent to detection of H. pylori genes by Western blot (95% sensitivity, 93% specificity). In erosions, the bacterial amount as determined by the ureaseA concentration was 2835 ± 536 bacteria as compared to the 785 ± 323 in the control antrum biopsies and 221 ± 76 in the corpus.

Conclusion. Results of this work indicate that real-time PCR FRET method of ureA is the most reliable for qualitative–quantitative detection of H. pylori in gastric biopsy specimens. The erosions show significantly increased H. pylori density.
Abstract no.: 10.07
Immunoblot for Detection of Helicobacter Infections in Laboratory Mice
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Mouse models for various human diseases are widely used in biomedical research. This is a first systematic attempt to develop a specific serology to analyze the immune response in naturally colonized and infected mouse colonies.

Aim. To evaluate the antibody responses to antigens of Helicobacter bilis, Helicobacter hepaticus, and Helicobacter ganmani in mice sera obtained from six animal houses.

Material and Methods. Sera from 106 mice (BalbC, C57BL/6, C3H, IL-10-deficient, ICOS+/−, CD8+/−, IgA−/−/IgG2a, IL-18−/−, SPF) were analyzed by immunoblot (IB) using the cell surface proteins of H. bilis (CCUG 38995) and H. hepaticus (CCUG 33637). For the H. ganmani (CCUG 47872), a whole cell lysate was employed. For interpretation, stained band patterns were compared to the sera of experimentally infected mice of each species. From 62 of these mice, genetic material were analyzed by PCR–DGGE (denaturing gradient gel electrophoresis) for comparison with IB results.

Results. A specific antibody reactivity to H. bilis was found in 20%, to H. hepaticus in 18% and to H. ganmani in 16% of tested mice sera and agreement between IB and PCR–DGGE was found in 84%, 95%, and 63%, respectively.

Conclusions. Infection with enteric Helicobacter seems to be common in mice kept for animal experiments, independent of mouse strain. Intestinal and hepatic naturally infected laboratory mice may represent confounding factors in the interpretation of animal bioassays. A serologic test with high specificity could be of great value in health controls of laboratory mice and killing of animals will not be necessary. Suggested antigens for future new immuno-based tests are Helicobacter m Reidarum, Helicobacter typhlonius, and Helicobacter rodentium.

Abstract no.: 10.08
Diagnosis of Helicobacter pylori Infection in Stool: 23S rDNA Real-Time PCR Versus Antigen Test
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During recent years, non-invasive methods to detect Helicobacter pylori infection gained importance especially for pediatric patients. In a previous study, a 23S rDNA real-time polymerase chain reaction (PCR) assay based on biprobe technology was developed, allowing for the accurate detection of H. pylori and clarithromycin-susceptibility testing both in biopsy and stool specimens. In the present study, the 23S rDNA real-time assay shall be compared to the Amplified IDEIA H. pylori StAR stool antigen test.

Stool specimens of 46 European pediatric patients with abdominal pain (0.5–19 years of age, average 9.8 years) and of 81 African individuals (0.5–70 years of age, average 12.4 years) were tested by both assays.

Of the 46 stool specimens of the European pediatric patients, 10 were positive and 35 negative by both tests. One specimen (2.2%) was positive by PCR but negative by the antigen test. Of the 81 specimens of the African individuals, 62 were positive and 13 negative by both tests. The results of the assays were divergent in six specimens (three positive by PCR and negative by antigen test and three vice versa, 7.4%). Considering the long transportation under suboptimal conditions of the African specimens, the overall agreement between the two tests of 94.5% may be satisfactory.

In an ongoing study, the comparison of both tests to the gold standard of H. pylori diagnosis (by rapid urease test, histology, and culture) shall reveal whether the 23S rDNA real-time PCR, also allowing for clarithromycin-susceptibility testing, might be the better alternative to the antigen test.
**Abstract no.: 10.10**

**Development of a Real-Time or Quantitative PCR Specific to Helicobacter pullorum**

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Helicobacter pullorum is now considered as an emerging human pathogen. Indeed, it has been detected in several enteritis cases and it has also been found in serious illnesses of the liver. However, the real incidence of *H. pullorum* in human diseases as well as the sources of possible food contamination are not known because these bacteria are difficult to isolate and identify. The goal of this study was to develop a real-time PCR allowing both a detection and a quantification of this bacterium.

The gene coding for subunit A of the DNA gyrase was selected as the PCR target because this gene is both present in all bacteria and displays a great interspecies variability in nucleic sequences, thus ensuring a good specificity. A 1300-bp fragment of this gene whose sequence was hitherto unknown was amplified and sequenced for five strains of *H. pullorum* and one strain of *Helicobacter canadensis*, using degenerated primers. A couple of primers and a TaqMan probe were designed, and a plasmid for calibration and an internal PCR inhibition control plasmid were constructed.

The sensitivity of the method is one equivalent genome by reaction and the specificity is high as 10 different *H. pullorum* strains lead to a positive PCR result, whereas *H. canadensis* strains as well as diverse bacteria species were not detected. The method was applied to 10 chicken cecal specimens that were all positive as well as diverse bacteria species were not detected. The method strains lead to a positive PCR result, whereas

**Abstract no.: 10.11**

**Point-of-Care Detection of Helicobacter pylori Infection Using a Rapid Urine Antibody Detection Device**

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Background. Point-of-care (POC) serology tests for *Helicobacter pylori* IgG antibody generally have lower specificity and sensitivity than send-out tests.

**Aim.** To evaluate POC test for detection of anti-*H. pylori* IgG antibodies in urine. We compared the RAPIRUN® urine antibody test, the HM-CAP® serum IgG antibody test and the 13C-urea breath test (UBT) among outpatients in a primary care setting.

To exclude prior *H. pylori* infections, active *H. pylori* infection was defined as both HM-CAP- and UBT-positive, *H. pylori*-negative status was defined as both HM-CAP- and UBT-negative.

**Methods.** Each patient provided a blood sample, a urine sample, and completed the UBT. The rapid urine test and UBT tests were analyzed on site.

**Results.** One hundred eighty-nine adults (62 men, 127 women, age range: 18–76 years; 83 White Hispanic, 71 Asian American, 20 Black people, 7 White people, and 8 other races). Seventy-eight patients had active *H. pylori* infections (positive HM-CAP and UBT) and 86 were *H. pylori* negative; 25 had discordant HM-CAP/UBT results. Sixty-nine of the 78 with active *H. pylori* infection had positive urine antibody tests and all 86 without *H. pylori* infection had negative results (sensitivity = 88.5%, specificity = 100%, PPV 100%, NPV 90.5%). The sensitivity was 85% and 100% when the rapid urine test was compared to the UBT results.

**Conclusion.** The rapid urine antibody test is suitable for POC detection of anti-*H. pylori* antibodies in urine and appears to have better sensitivity and specificity than most POC serum tests and appears suitable for use in Western populations.
Usefulness of RAPID HpStAR™, a New Immunochromatographic Monoclonal Antigen-Based Fecal Test for Diagnosing Helicobacter pylori Infection in Dyspeptic Patients

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Abstract no.: 10.13

Background. Helicobacter pylori, a common human pathogen, causes a chronic inflammatory process, which may ultimately lead to the development of peptic ulcer disease or gastric carcinoma. H. pylori antigens can be measured in human stools, which is potentially a valuable non-invasive diagnostic tool.

Objective. To evaluate the usefulness of two new monoclonal tests for detecting H. pylori antigens in dyspeptic patients’ feces (Amplified IDEIA™ HpStAR, DakoCytomation, Denmark, and a rapid immunochromatographic test RAPID HpStAR™, DakoCytomation, Denmark).

Methods. H. pylori infection was determined in 68 dyspeptic patients who underwent endoscopy by concordance of urease test and histology. Fecal tests were performed according to the specifications of the manufacturer. Sensitivity, specificity, and positive and negative predictive values (PPV and PNV, respectively) were calculated for both Amplified IDEIA™ HpStAR and RAPID HpStAR™. Concordance between different measurements was estimated by Kappa statistics.

Results. The sensitivity of the RAPID HpStAR™ ranged from 96% to 98% and its specificity was 67%. PPV was 92% and PNV 80–90%. Corresponding Amplified IDEIA™ HpStAR values were 98%, 83%, 94%, and 94%, respectively.

Conclusions. Both Amplified IDEIA™ HpStAR and RAPID HpStAR™ were very sensitive, specific, and easy-to-perform diagnostic tools for the diagnosis of H. pylori infection.

Applicability of a Helicobacter pylori Stool Antigen Test to Determine the Results of Eradication Therapy

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Abstract no.: 10.15

Background and Aims. 13C-urea breath test (UBT) is usually used to test the results of eradication therapy of Helicobacter pylori. However, in most hospitals, results cannot be obtained immediately because this test requires particular equipment. H. pylori stool antigen tests are non-invasive and results are obtained within 10 minutes. The aim of this study was to examine whether stool antigen test is applicable to determine the results of eradication therapy.

Methods. Thirty-six patients infected with H. pylori (20 male patients, 16 female patients, mean age 57.6) received eradication therapy consisted of lansoprazole, amoxicillin, and either clarithromycin or metronidazol. At least 5 weeks after finishing the treatment, results were evaluated by 13C-UBT. On the same day, Testmate rapid pylori antigen, which detects H. pylori-native catalase in feces, was also performed and compared with 13C-UBT.

Results. In all the six patients with positive 13C-UBT, Testmate rapid pylori antigen was also positive. In 29 13C-UBT-negative patients 28 were also negative by Testmate rapid pylori antigen. A patient, however, could not be tested because his stool was watery. One patient who had gray zone result by 13C-UBT was negative by Testmate rapid pylori antigen and was tested by 13C-UBT again 1 month later and the result was negative. Overall accuracy of Testmate rapid pylori antigen to determine H. pylori eradication was 97.2% when compared with 13C-UBT.

Conclusions. Testmate rapid pylori antigen is a useful diagnostic test for immediate and accurate evaluation of H. pylori eradication therapy.

Characteristics of Non-Ulcer Dyspepsia in Algeria

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Abstract no.: 10.14

Aim. To evaluate the prevalence of non-ulcer dyspepsia (NUD) in the Algiers area and to appreciate its features and the eradication rate of Helicobacter pylori infection.

Patients and Methods. From 2000 to 2005, on 445 consecutive endoscopies carried out on adult patients suffering from dyspepsia, 336 (75.5%) cases (88 males; mean age: 33 years) of NUD were found. Gastric biopsies were performed for histology, rapid urease test (RUT), and culture with bacterial susceptibility to antibiotics. Antibodies IgG anti-H. pylori, CagA, VacA, and stool antigens (HpSA) were practiced. H. pylori infection was defined if culture and/or histology and a third test were positive. Patients were treated by five different triple therapies: H. pylori eradication were assessed on the negativity of all tests.

Results. H. pylori infection was established in 292 patients (87%). In these infected patients, H. pylori diagnostic tests were positive for histology (93%), serology (97.6%), RUT (96%), culture (78%), and HpSA (67%). H. pylori resistance rates to antibiotics were: metronidazole = 47%, clarithromycin = 11.5%, amoxicillin, and tetracycline = 9%. A double resistance was found in nine patients. CagA antibody was positive, 61%, and for VacA is 32%. Histology has shown: chronic gastritis interesting antrum + fundus = 79%, only antrum = 20%, and only fundus = 1%. One hundred four patients were treated and controlled: H. pylori was eradicated in 74 (71%).

Conclusion. In the Algiers area, H. pylori infection is almost constant in adult patients with NUD. H. pylori strains mostly are CagA(+). Resistance is high to metronidazole and important to clarithromycin.
Abstract no.: 10.16
14C-Urea Breath Test: A Reliable and Practical Test in Office-Based Diagnosis of Helicobacter pylori Infection

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14C-urea breath test (UBT) is cumbersome and needs infrastructure, whereas HpSA test is not acceptable to patients. 14C-UBT is claimed to be reliable and easy to perform diagnostic test for Helicobacter pylori.

Aims. To validate and compare cost of 14C-UBT with histopathology and rapid urease test (RUT) for diagnosis of H. pylori.

Methods. Sixty consecutive adult males and non-pregnant females with dyspepsia were enrolled. During gastroscopy, two biopsies were taken for histopathology and two for RUT. UBT was performed after gastroscopy by 14C-urea-labeled capsules and labeled CO2 was measured by a scintillation counter. Sensitivity and specificity of UBT were calculated by comparing with results of histopathology and RUT. Cost comparison of these three tests was also performed.

Results. There were 35 (58%) males with a mean age 42.4 ± 13 years. H. pylori was diagnosed in 37 (61.6%) patients by RUT. Histopathology has diagnosed H. pylori in 42 (71%) patients, whereas UBT was positive in 36 (60%) patients. UBT has a sensitivity of 83% and specificity of 94%; see Table 1. The cost of gastroscopy and histopathology or RUT was $110 and $90, respectively, whereas the cost of UBT was $15.

Table 1 Sensitivity and specificity of UBT and RUT against histopathology

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBT</td>
<td>83% (75–86)</td>
<td>94% (75–99)</td>
<td>97% (87–99)</td>
<td>71% (56–75)</td>
<td>87%</td>
</tr>
<tr>
<td>Rapid urea test</td>
<td>87% (80–88)</td>
<td>97% (80–99)</td>
<td>98% (90–99)</td>
<td>77% (63–79)</td>
<td>90%</td>
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</tbody>
</table>

Conclusions. 14C-UBT is a reliable and economical method for the diagnosis of active H. pylori infection in office-based setting.

Abstract no.: 10.17
Diagnosis of Helicobacter pylori Infections in Dyspeptic Nigerians: Comparison of Five Detection Techniques

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Background and Aim. There is considerable interest in diagnostic methods for Helicobacter pylori infection both before and after treatment. The diagnosis of H. pylori infection is very difficult in our region. Most of the available tests are costly and not readily available. This study attempts to address these problems.

Material and Methods. Biopsies and blood samples were taken from 532 patients presenting with varying degrees of gastroduodenal pathology. The biopsies were screened for the presence of H. pylori using culture technique, Campylobacter-like organism test (CLO) (urease test), Gram reaction, serology, and polymerase chain reactions (PCR).

Results. Of the 532 patients, 453 (85%) were seropositive for H. pylori IgG, 327 (62%) were positive by direct Gram’s stain, and PCR detected 245 (46.1%) of the subjects. Urease (CLO) positivity was found in 224 (42%), whereas H. pylori was isolated by culture in 186 (35%) patients. The differences in the results was tested statistically and found to be significant. In this study, 410 (77.1%) patients were H. pylori-positive and 122 (22.9%) patients were H. pylori-negative. Majority (25.0%) of the patients had Gram reaction, serology, and were PCR positive. No test was positive in 79 (14.8%) patients.

Conclusion. From the study, the best detection methods were Gram reaction, serology, and PCR. This means a patient can be defined as positive for H. pylori in this environment even if the culture was negative, but Gram reaction, serology, and PCR tests were positive.

Abstract no.: 10.18
Detection of Helicobacter pylori Infection in Patients on Proton Pump Inhibitors

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Aim. To determine the effect of proton pump inhibitor (PPI) on rapid urease test (Pronto Dry) and compare it with polymerase chain reaction (PCR) for the Helicobacter pylori urease C (ureC) gene.

Methods. Forty-seven patients with dyspepsia attending the endoscopy from January–May 2005 were enrolled. Four antrum biopsy specimens were collected, two specimens each for Pronto Dry and PCR. Pronto Dry results were read in 30 minutes and 1 hour after sampling. The color changed from yellow to pink when positive. PCR amplified a homogeneous DNA fragment of the expected size of 820 bp for ureC gene. The PCR products were digested with restriction enzyme HbaI and analyzed by agarose electrophoresis. PCR was used as the gold standard. Sensitivity and specificity of Pronto Dry was compared against PCR.

Results. Fifty-seven percent (57%) (27/47) were males, mean age 44 ± 11.5 years; 83% (39/47) were using PPI at presentation. Eighty-one percent (38/47) presented with abdominal pain and 19% (9/47) with dyspepsia. Endoscopy showed gastritis 75% (35/47), gastritis with gastroesophageal reflux disease (GERD) in 15% (7/47), and GERD alone in 11% (5/47). Pronto Dry was positive in 28% (13/47) and negative in 72% (34/47), whereas PCR was positive in 51% (24/47) and negative in 49% (23/47). In the presence of PPI, Pronto Dry had a sensitivity of 85% and specificity of 62%.

Conclusion. The sensitivity and specificity of Pronto Dry was reduced on PPI. PCR may be considered in patients on recent acid-reducing medications.
Abstract no.: 10.19
Application of Quantitative or Real-Time PCR Approach for Helicobacter pylori Detection

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Accurate diagnosis of Helicobacter pylori infection is very important in both clinical practice and research purposes. The sensitivity and specificity of the currently available tests for H. pylori detection may be affected by several reasons. The aim of the present study was to evaluate the sensitivity of real-time PCR on H. pylori detection and quantification in 81 patients considered to be negative by means of “gold standard” diagnostic methods rapid urease test, culture, and histology. Our results showed that the sensitivity of the real-time PCR assay is higher than those from standard methods. Sixteen patients from the 81 considered to be uninfected according to the references methods were H. pylori-positive, from which six were untreated patients, and 10 were patients considered to be given eradication therapy. Thus, based on these findings we recommend the real-time PCR should be used in clinical studies to monitor treatment results in addition to the regular methods.

Abstract no.: 10.20
Specific Antibodies to Helicobacter pylori and Cytokine in Patients with Polyps of the Stomach

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Aim. Study of the specific antibodies to Helicobacter pylori (ab-HP) and cytokine levels in patients with polyps of the stomach (PS).

Material and Methods. ELISA was used to examine in the peripheral blood of the ab-HP (IgG, IgA, IgM + IgG + IgA) and cytokine levels in 52 patients with PS. Results. Increase of concentration of serum antibodies (IgG + IgA + IgM) to CagA-HP (1 : 40–1 : 640, median = 1 : 180, in control –1 : 10) were detected in 84% of patients with PS. In the patients with polyps of antrum were found the maximal levels of ab-HP (median = 1 : 240). Increase of concentration of serum IgG-HP (median = 1 : 1060, in the control –1 : 110) were detected in 80% of patients with PS. Increase of concentration of serum IgA-HP (median = 1 : 660, in the control –1 : 140) were detected in 46% of patients with PS. Increase of concentration of serum ab-HP (IgG, IgA, IgM + IgG + IgA) is one of the indications of the intensiveness of infiltration, atrophy, and proliferation in the stomach in patients with PS.

In the patients with PS were found increase of cytokine levels (IL-1β, IL-8, IL-12, IFNγ, TNF-α) – 190–510 pg/ml; in control –1 : 40.

Conclusion. Increase of serum ab-HP levels may be useful as one of the diagnostic criteria of polyps of stomach, associated with H. pylori, indications for anti-Helicobacter therapy in the patients with PS.

Abstract no.: 10.21
Diagnosis of Helicobacter pylori Infection Among Dental Caries Patients by Stool Antigen Test

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Background and Aims. The route of transmission of Helicobacter pylori especially in developing countries is probably fecal–oral and oral–oral. As the oral cavity can be regarded as a possible reservoir of the organism, it provides a possible tool for the rapid and non-invasive diagnosis of infection especially in Nigeria where power outages are constant and culture of H. pylori is difficult. This study aims to detect H. pylori in dental plaques and stomas of patients who reported with dental problems using stool antigen test.

Methods. Forty patients presenting with various dental problems had their stool samples screened for H. pylori infection, using the stool antigen test by DakoCytomation, and biopsies taken from them after informed consent for culture. All patients had not been on any prior medication.

Results. Fourteen patients (35%) had peptic ulcer disease, whereas 26 (65%) had marginal gingivitis and were either normal or had mild gastritis. All the patients (100%), irrespective of their disease status, were found to have H. pylori by stool antigen test, whereas culture showed only 2.5% of the patients with H. pylori infection.

Conclusion. The results show the significance of the stool antigen test as a diagnostic tool in the absence of culture of the organism and its relative affordability. This is because in Nigeria, power outages occur frequently and has made the culture of H. pylori in Nigeria absolutely difficult apart from the costs associated with endoscopy. From earlier studies, culture had always been found to be of very low sensitivity.

Abstract no.: 10.22
The Difference in the Incidence of Helicobacter pylori Infection Between Old (more than 65 years) and Young Patients with Upper Gastrointestinal Bleeding due to Peptic Ulcer

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Recent reports show that Helicobacter pylori infection accompany 90% of duodenal ulcers and 75% of benign gastric ulcers. There is no study of the relationship between H. pylori infection and upper gastrointestinal bleeding that may be lethal to the old patients.

We examined the incidence of H. pylori infection for upper GI bleeding due to peptic ulcer according to age.

The enrolled patients were diagnosed as duodenal ulcer (DU) and/or benign gastric ulcer (BGU) by gastroscopy on first visit, and Campylobacter-like organism (CLO) test was performed. They were divided into two groups, those patients older than 65 years, and those younger than 65 years. We calculated the incidence of H. pylori infection and the odds ratio.
In the group of less than 65 years of age, 68.6% were infected. But in the older age group, 40% were infected. This result was significant (odds ratio: 0.30). If we considered the location of the ulcer in each group, the incidences of *H. pylori* infection in gastric ulcer were 40.9% in the older group and 61.9% in the younger group, and in duodenal ulcer, 50.0% and 72.7%, respectively, in each group. The trend of lower infection incidence was shown in the older group than in the younger group. On the other hand, in the cases of combined ulcers the older group had no infected patient, the younger group had 75.0% infection of enrolled cases.

We think that this result in the combined ulcer case may be caused by other reasons, for example, NSAIDS or the weakness of defense mechanism.

**Abstract no.: 10.23**

**Specific Antibodies to Helicobacter pylori in Diseases of Stomach**

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**Aim.** Comparative study of the specific antibodies to *Helicobacter pylori* (ab-HP) levels in patients with diseases of stomach (DS).

**Material and Methods.** ELISA were examined in the peripheral blood of the ab-HP (IgG, IgA, IgM + IgG + IgA) levels of 112 DS patients with chronic gastritis (CG), gastroesophageal reflux disease (GERD), stomach peptic ulcer (SPU), and gastric cancer (GC).

**Results.** Increase of concentration of serum IgG-HP (median = 1:602) were detected in 78% of patients with DS. In the patients with CG, GERD were found in maximal levels of IgG-HP (1:420–1:1060); in the patients with SPU, GC were in minimal levels (1:220–1:340); in the control, 1:110. Increase of concentration of serum IgA-HP (median = 1:442) were detected in 39% of patients with DS. Serum IgA-HP were maximally increased (1:640–1:980) in the patients with SPU, GC; minimal (1:180; in control, 1:140) in the patients with GERD, CG. Concentrations of antibodies (IgM + IgG + IgA) to CagA-HP were significantly increased (1:40–1:640, median = 1:180; in control, 1:10) in the patients with DS in 82%. Basic therapy in patients with SPU led to a statistically significant decrease of concentration of serum ab-HP.

**Conclusion.** Increase of serum ab-HP levels is one of the tests of *Helicobacter pylori* therapy in the patients with DS, as well as additional criterion for the estimation of its efficiency and prognosis of diseases.

**Abstract no.: 10.25**

**Diagnosis of Helicobacter pylori Infection and Determination of Clarithromycin Resistance in Stool Specimens by Real-Time PCR**

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Macrolide resistance in *Helicobacter pylori* infections is becoming an increasing therapeutic problem. *E*-test and agar dilution tests have been used as in vitro phenotypic methods for the detection of macrolide resistance in *H. pylori*. When bacterial culture cannot be used routinely, the patient should benefit from the determination of macrolide resistance using noninvasive genotype-based methods. In clinical *H. pylori* isolates, macrolide resistance is due to point mutations in the 23S rRNA gene of *H. pylori*. In our study, a real-time polymerase chain reaction (PCR) hybridization assay was used to determine *H. pylori* infection and clarithromycin resistance in stool specimens of 54 adult dyspeptic patients. Of 54 patients who had an upper endoscopy, 46 (85.2%) were found to be *H. pylori*-positive by histopathology and/or rapid urease test. Thirty-six patients (78.3%) were found positive by real-time PCR in stools; 17 out of 36 (47.2%) were wild-type *H. pylori*, 13 (36.1%) were mutant A enchantment, 4 (11.1%) were mutant A–C genotype, 1 (2.8%) showed an unknown mutant genotype, and one (2.8%) patient carried two strains. The high number of clarithromycin-resistant isolates could be explained by the clarithromycin-based eradication therapy that the patients received in the past. Real-time PCR in stool specimens is an easy and useful noninvasive method to detect *H. pylori* infection that allows clarithromycin-susceptibility testing.
**Clinical Trials and Novel Treatments**

**Abstract no.: 11.01**
Levofloxacin-Based Triple Therapy in First-Line Treatment for *Helicobacter pylori* Eradication: Update

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**Introduction.** Therapies based on clarithromycin and amoxicillin or metronidazole are the treatment of choice to eradicate *Helicobacter pylori*. Levofloxacin seems to be a new option for *H. pylori* eradication.

**Aims and Methods.** To compare two 7-day standard triple therapies versus 7-day levofloxacin-based triple therapy in first-line treatment. Three-hundred consecutive *H. pylori*-positive patients (132 male patients, 18–65 years of age) were randomized to receive clarithromycin 500 mg b.i.d., amoxicillin 1 g b.i.d., esomeprazole 20 mg b.i.d. (group A: 100 patients); clarithromycin 500 mg b.i.d., metronidazole 500 mg b.i.d., esomeprazole 20 mg b.i.d. (group B: 100 patients) and clarithromycin 500 mg b.i.d., levofloxacin 500 mg od, esomeprazole 20 mg b.i.d. (group C: 100 patients). *H. pylori* status was checked by 13C-urea breath test 6 weeks after the end of the treatment. A questionnaire on side-effects was also administered.

**Results.** Two-hundred eighty-four patients completed the efficacy analysis per protocol; eradication rate was 75% (79/100 patients) and 79% (75/95) in group A, 72% (72/100 patients) and 77.4% (72/93) in group B, 87% (87/100 patients) and 90.6% (87/96) in group C in ITT and PP analysis, respectively. Eradication rate was significantly higher using levofloxacin-based therapy than using standard therapies in either ITT (87% versus 75%; p < .05; 87% versus 72%; p < .01) or PP (92.6% versus 79%; p < .05; 90.6 versus 77.4; p < .05) analysis. Prevalence of side-effects was similar among groups.

**Conclusions.** A 7-day levofloxacin-based therapy showed a higher eradication rate than standard regimens in first-line scheme and could be considered the best treatment, at least in the Italian population.

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**Abstract no.: 11.02**
Long-Term Treatment Outcome of Primary Gastric Low-Grade B-Cell Mucosa-Associated Lymphoid Tissue Lymphoma

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**Objectives.** Primary gastric low-grade mucosa-associated lymphoid tissue (MALT) lymphoma is known to be successfully treated with anti-*Helicobacter pylori* therapy alone. Aims of this study were to analyze duration to complete remission (CR), recurrence rate, and to assess factors associated with recurrence.

**Methods.** Between 1996 and 2003, 97 *H. pylori*-infected patients with MALT lymphoma were treated with anti-*H. pylori* therapy. Endoscopy, endoscopic ultrasonography, abdominal CT scans, bone marrow examination, and histologic examination of biopsy samples were analyzed at initial diagnosis. Eradication of *H. pylori* and tumoral response were assessed by follow-up endoscopy. Mean follow-up period after CR was 29 months (range 3–97).

**Results.** Among 97 treated patients, 92 (94.8%) reached CR. Median duration to CR was 3 months. Seventy (76.9%) patients were in CR at 6 months, and 82 (90.1%) were in CR at 12 months. Among 83 patients who were followed up after CR, MALT lymphoma recurred in nine (10.8%). Kaplan–Meier survival analysis showed 4.0% and 12.7% recurrence rate at 12 months and 24 months. *H. pylori* reinfection was only a significant risk factor of recurrence, whereas endoscopic features, depth of invasion, and BM involvement were not associated with recurrence.

**Conclusions.** Most of low-grade MALT lymphoma showed CR with *H. pylori* treatment alone, and most patients were in CR at 12 months. However, survival analysis showed 12.7% of recurrence rate at 24 months, and *H. pylori* reinfection was only risk factor of recurrence. Hence, endoscopy with evaluation of *H. pylori* status should be needed during follow-up of low-grade MALT lymphoma.

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**Abstract no.: 11.03**
Levofloxacin-Based Triple Therapy in Second-Line Treatment for *Helicobacter pylori* Eradication: Update

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**Introduction.** Compliance is the limit of the recommended second-line treatment for *Helicobacter pylori* infection. Levofloxacin is a new good option for *Helicobacter eradication.**

**Aims and Methods.** To compare a 10-day or 7-day levofloxacin-based therapy versus quadruple therapy in second-line treatment. One hundred forty-six consecutive patients (67 male patients) who had failed standard triple therapy were randomized to receive levofloxacin 500 mg od, PPI b.i.d. and amoxicillin 1 g b.i.d. for 10 days (group A: 46 patients); or for 7 days (group B: 50 patients); tetracycline 500 mg q.i.d., metronidazole 500 mg t.i.d., PPI b.i.d. and bismuth salt 120 mg q.i.d. (group C: 50 patients) for 7 days. *H. pylori* status was checked by 13C-urea breath test (UBT) 6 weeks after the treatment.

**Results.** Four dropouts occurred in group C due to side-effects. Eradication rate in group A was 91.3% and in group B was 74% in either ITT and PP analysis. In group C was 68% and 73.9% in ITT and PP analysis, respectively. Eradication rate of 10 days of levofloxacin-based therapy was significantly higher than that observed using a 7-day levofloxacin-based therapy or quadruple therapy in either ITT (91.3% versus 74%; p < .05; 91.3% versus 68%; p < .005) or PP (91.3% versus 73.9%; p < .05) analysis. Prevalence of side-effects was significantly lower in groups A and B than in group C.

**Conclusions.** A 10-day levofloxacin-based triple therapy showed a higher eradication rate and provides a better compliance than quadruple regimen and seems the best second-line option for *Helicobacter pylori* infection.
Abstract no.: I I.04*
Comparison of the Efficacy of Pre-Operative Versus Post-Operative Proton Pump Inhibitor-Based Triple Therapy for Helicobacter pylori Eradication in Patients Undergoing Subtotal Gastrectomy

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Background. The efficacy of proton pump inhibitor (PPI)-based triple therapy for Helicobacter pylori eradication is well established in patients with intact stomach. However, it has not been adequately evaluated in patients with subtotal gastrectomy that might affect the outcome of eradication therapy due to altered gastrointestinal motility or antibiotic activity.

Aim. This study is aimed to evaluate the efficacy of postoperative PPI-based triple therapy in patients undergoing subtotal gastrectomy.

Methods. One hundred fifteen patients with distal gastric cancer were randomly assigned to either preop group (treatment before surgery, 58 subjects) or postop group (treatment 2–4 weeks after surgery, 57 subjects). The regimen was a 7-day triple therapy based on PPI (rabeprazole 10 mg b.i.d.), clarithromycin (500 mg b.i.d.), and amoxicillin (1000 mg b.i.d.). H. pylori eradication was evaluated by histology, rapid urease test, and urea breath test at 12 weeks after surgery.

Results. By intention-to-treat (ITT) analysis, eradication was successful in 82.8% (48/58) in the preop group and 75.4% (43/57) in the postop group (p = .33). By per protocol (PP) analysis, H. pylori eradication rate was 84.2% (48/56) and 79.2% (42/53) in each group (p = .50). In the postop group, there was no difference in eradication rates according to the Billroth I or II procedure by ITT (72.5% versus 82.4%, respectively; p = .52) or PP analysis (77.8% versus 82.4%, respectively; p = 1.00).

Conclusions. The postoperative PPI-based triple therapy was as effective as the preoperative therapy in patients with distal gastric cancer. In addition, the efficacy was not affected by gastrointestinal reconstruction methods.

Abstract no.: I I.05
HELYX Study Parts I and II: Treatment of Low-Grade Gastric Non-Hodgkin’s Lymphoma of Mucosa-Associated Lymphoid Tissue Type Stages I and II, an Interim Analysis

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Background. Eradication of Helicobacter pylori is an accepted treatment for H. pylori-positive, low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma. However, about 20% show refractory disease or H. pylori-negative lymphoma.

Aim and Methods. A prospective, randomized study has been designed to investigate occurrence of complete lymphoma remission (CR) in H. pylori-positive gastric low-grade MALT lymphoma stages I and II (part I) after eradication therapy. Refractory disease or H. pylori-negative patients will receive radiotherapy (25.2 Gy versus 36 Gy, part II). Correlation of clinical outcome with t(11; 18), and B-cell monoclonality is also performed.

Results. So far, 65 patients have been included. Sixty-two received eradication therapy. Fifteen patients were randomized for radiation (2 being H. pylori-negative, 13 patients with refractory disease). Forty-four patients in HELYX part I already had three endoscopic controls (30% (68%) of those have shown CR. From 15 patients having received radiation therapy (part II), 8 were radiated with 36 Gy, and 7 with 25.2 Gy. All patients with three or more endoscopic controls (n = 5) achieved stable CR after radiation irrespective of the dose applied. In part II t(11; 18) occurs in 22% compared to 4% in part I. Seventy-eight percent of the irradiated patients show an initial monoclonal lymphocyte population versus 50% of patients with CR after eradication.

Conclusion. Most patients with H. pylori-positive low-grade gastric MALT lymphoma stages I and II respond with stable CR after eradication therapy. Nonresponders, t(11; 18) + or H. pylori-negative patients may benefit from a radiation therapy. Low-dose radiation with 25.2 Gy seems to be as effective as standard dose. However, a larger patient number is needed.

Abstract no.: I I.06
Systematic Review and Meta-Analysis: Proton Pump Inhibitor Versus Ranitidine Bismuth Citrate Plus Two Antibiotics in Helicobacter pylori Eradication

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Objective. To systematically review the Helicobacter pylori eradication efficacy with ranitidine bismuth citrate (RBC) and two antibiotics, and to conduct a meta-analysis of randomized clinical trials comparing the efficacy of proton pump inhibitor (PPI) versus RBC with two antibiotics for 1 week.

Methods. Selection of studies: Studies evaluating RBC plus two antibiotics were considered. For the meta-analysis, randomized controlled trials comparing PPI versus RBC plus two antibiotics for 1 week were included. Search strategy: Electronic and manual bibliographical searches. Assessment of study quality and data extraction: Independently performed by two reviewers. Data synthesis: “intention-to-treat” eradication rate. Meta-analysis was performed combining the odds ratios (OR) of the individual studies. Subanalysis: Depending on the type of antibiotics and the quality of the studies.

Results. Mean H. pylori eradication with 7-day RBC–clarithromycin–amoxicillin, RBC–clarithromycin–nitromidazole, and RBC–amoxicillin–nitromidazole was 83%, 86%, and 71%, respectively. The meta-analysis showed comparable efficacy with RBC and PPI when they were combined with clarithromycin and amoxicillin (OR = 1.11; 95% CI = 0.88–1.40), or with amoxicillin and metronidazole (OR = 0.92; 95% CI = 0.60–1.41). However, when comparing PPI versus RBC plus clarithromycin and a nitromidazole, higher cure rates with RBC than with PPI were demonstrated (OR = 1.65; 95% CI = 1.15–2.37).
Conclusion. The efficacy of RBC and PPI-based triple regimens were comparable when using the clarithromycin–amoxicillin or the amoxicillin–metronidazole combination. However, RBC seems to have a higher efficacy than PPI when clarithromycin and a nitroimidazole are the antibiotics prescribed. Therefore, if one prefers to use the clarithromycin–nitroimidazole regimen, RBC should be used instead of a PPI.

Abstract no.: II.07
Esomeprazole-Based Therapy in Helicobacter pylori Eradication: Any Effect by Increasing the Dose of Esomeprazole or Prolonging the Treatment?

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Aim. To study the efficacy of esomeprazole-based triple therapy in Helicobacter pylori eradication and to evaluate, by a randomized trial, the effect of increasing the dose of esomeprazole or prolonging the treatment.

Methods. Four-hundred and fifty duodenal ulcer patients were randomized to receive: 1, esomeprazole (20 mg b.i.d.), clarithromycin (500 mg b.i.d.), and amoxicillin (1 g b.i.d.), for 7 days (E20–7d); 2, esomeprazole (40 mg b.i.d.) with the same antibiotics, also for 7 days (E40–7d), and 3, esomeprazole (40 mg b.i.d.) with the same antibiotics, for 10 days (E40–10d). Cure rates were evaluated by 13C-urea breath test.

Results. One hundred fifty patients received each treatment. Groups were comparable in terms of demographic variables. Eight percent of the patients did not return for follow-up. Compliance (98%) and side-effects (only mild to moderate) in the two groups were comparable. Per-protocol cure rates were 83.5% (E20–7d), 84.8% (E40–7d), and 88.2% (E40–10d). Intention-to-treat cure rates were, respectively, 74%, 78%, and 80% (nonstatistically significant differences).

Conclusions. Esomeprazole-based triple therapies offer comparable efficacy to omeprazole-based therapies used in previous studies. Increasing the dose of esomeprazole or prolonging the treatment does not improve the results. Therefore, if esomeprazole-based triple therapy is used in duodenal ulcer patients, a regimen with only 20 mg twice daily of esomeprazole and for only 7 days may be sufficient.

Abstract no.: II.08
Ranitidine Bismuth Citrate Rescue Therapy after Helicobacter pylori Treatment Failure

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Aim. Quadruple rescue therapy requires a complex scheme with four drugs. Our aim was to evaluate the efficacy of ranitidine bismuth citrate (RBC)–tetracycline–metronidazole rescue regimen, and to compare two different metronidazole dose schemes.

Methods. Prospective multicenter study including proton pump inhibitor + clarithromycin + amoxicillin failures. Rescue regimen included two 7-day treatments: 1, RBC (400 mg q.i.d.)–tetracycline (500 mg q.i.d.)–metronidazole (500 mg t.i.d.) (RTM1); or 2, the same regimen but with metronidazole 250 mg q.i.d. (RTM2). Eradication was confirmed with 13C-UBT breath test.

Results. One hundred fifty patients were included (58 RTM1, 92 RTM2). All patients but two (one in each group) returned after treatment. Eighty-six percent (86%) in group RTM1 and 95% in RTM2 took correctly all the medications (p = .576). Per-protocol eradication with RTM1 and RTM2 was 74% (95% CI = 60–84%) and 69% (59–78%), Intention-to-treat eradication rate was 64% (51–75%) and 70% (59–78%) (p > .05). Type of regimen was not associated with eradication success in the multivariate analysis. Adverse effects were more frequent with RTM1 (41%) than with RTM2 (30%) (p > .05).

Conclusion. Seven-day triple rescue therapy with RBC–tetracycline–metronidazole is effective for H. pylori eradication, and represents an encouraging alternative to quadruple therapy, with the advantage of simplicity. The administration of metronidazole every 6 hours (together with tetracycline), and at a low dose (250 mg), achieves similar efficacy and is probably associated with a better compliance and a lower incidence of adverse effects.

Abstract no.: II.09
Higher Reinfection Rate in Celiac Disease Patients After Successful Helicobacter Eradication for Peptic Ulcer Disease

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Eradication regimes for Helicobacter pylori infection have been proven particularly effective and with low reinfection (or recrudescence) rate that is estimated ~1–2% annually.

Aim of the study was to assess maintenance of the eradication in patients with celiac disease. In 29 patients (16 male, 13 female, age range 19–67 years) with celiac disease, all in clinical and histologic remission on a gluten-free diet, benign peptic ulceration (20 duodenal, 9 gastric) was diagnosed endoscopically. None was on long-term NSAIDs.

Of the 29 patients, 23 were Hp+ by histology and Campylobacter-like organism (CLO) test and received for both healing and H. pylori eradication first-line triple schemes (PPI, clarithromycin, amoxicillin) and those who failed (7/23) to become Hp– second-line triple schemes (PPI, bismuth compounds, amoxicillin, metronidazole), both at the recommended dose and duration. All had their ulcers healed, 20 of 23 became Hp– by histology/CLO test and/or 13C-UBT test, whereas three of 23 remained Hp+. Hp– patients were reevaluated for their Hp+ status with 13C-UBT after a mean observation period of 55 months (range 22–68 months). Twelve of twenty (60%) remained Hp–, whereas 8/20 (40%) became Hp+. Reinfection rate for matched nonceliac patients and for similar observation period was ~10%. Nine of twenty patients, four Hp– and five Hp+, were re-endoscoped because of dyspeptic symptoms and in three of nine, all Hp+, recurrence of duodenal ulcer was diagnosed.

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Even if the number of patients studied is small, it seems that patients with celiac disease have higher than expected reinfection rate. Genetic factors influencing susceptibility to *H. pylori* infection by modulating the host immune response might be implicated to explain this observation.

**Abstract no.: 11.10**

**Presence of Duodenal Pseudodiverticulae Is Predictive of Recurrence of Helicobacter pylori and Recurrence of Ulcer Following Eradication Therapy in Patients with Duodenal Ulcer**

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A high rate of recurrence of *Helicobacter pylori* following eradication therapy in patients with peptic ulcer disease is seen in developing countries. Factors implicated are antibiotic resistance, compliance, and treatment efficacy. Presence of pseudodiverticula or duodenal deformity has not been evaluated as a risk factor for recurrence.

**Aim.** To determine if presence of duodenal pseudodiverticula or deformity is a factor predicting recurrence of ulcer and *H. pylori*.

**Methods.** Two hundred fifty-seven patients who had presence of active peptic ulcer and *H. pylori* were given standard triple regime (lansoprazole, amoxycillin, clarithromycin/secnidazole) and were followed up to determine the recurrence of peptic ulcer or *H. pylori*. Urea breath test, rapid urease test, and histology were performed. Presence of duodenal pseudodiverticula (PD) and/or duodenal bulb (DD) deformity was noted on endoscopy.

**Results.** Two hundred fifty-seven patients (222 males) with a mean age of 38.3 (±13.1) years were included in the study. *H. pylori* eradication attempts were: 1–169; 2–59; 3–21; 4–6; 5–1; 6–1 (no. of attempts: no. of patients). In patients with presence of PD and/or DD (n = 172) as compared to patients without PD/DD (n = 85), primary failure of eradication (16.3% versus 18.9%), recurrence of *H. pylori* (30.4% versus 31.6%), and recurrence of ulcer (36.4% versus 18.8%, p = .06) was not significantly different. However, in patients with presence of PD (n = 90) and without PD (n = 167), there was a significantly increased rate of recurrence of *H. pylori* (45.9% versus 31.6%, p = .05) and recurrence of ulcer (36.4% versus 21.2%, p = .01).

**Conclusion.** Presence of duodenal pseudodiverticulae is an important factor predicting recurrence of *H. pylori* and ulcer in patients with peptic ulcer disease.

**Abstract no.: 11.11**

**Is 1-week Proton Pump Inhibitor-Based Triple Therapy Sufficient to Heal Peptic Ulcer?**

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**Aims.** To systematically review the efficacy on ulcer healing of 1-week combination of a proton pump inhibitor (PPI) plus two antibiotics and to perform a meta-analysis of randomized clinical trials to evaluate whether 7 days of PPI-based triple therapy is sufficient to heal peptic ulcer.

**Methods.** Studies where 1-week PPI-based triple therapy was administered to heal peptic ulcer were included. Randomized clinical trials comparing the efficacy on ulcer healing of 7-day PPI-based triple therapy versus this same regimen but prolonging the PPI for several more weeks were included in the meta-analysis. Electronic and manual bibliographical searches were conducted. Meta-analysis was performed combining the odds ratios (OR) of the individual studies.

**Results.** Twenty-four studies (2342 patients) assessed ulcer healing with 1-week PPI-based triple therapy. Mean healing rate was 86%, and 95% in *Helicobacter pylori* eradicated patients. Six studies (862 patients) were included in the meta-analysis. Mean ulcer healing rate with a 7-day treatment was 91% versus 92% when PPI was prolonged for 2–4 more weeks (OR = 1.11; 95% CI = 0.71–1.74).

**Conclusion.** In patients with peptic ulcer and *H. pylori* infection, prolonging therapy with PPI after triple therapy for 7 days with a PPI and two antibiotics is not necessary to induce ulcer healing.

**Abstract no.: 11.12**

**Rescue Therapy with Levofloxacin after Multiple Helicobacter pylori Treatment Failures**

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**Aim.** Eradication therapy with proton pump inhibitor, clarithromycin, and amoxicillin is extensively used, although it fails in a considerable number of cases. A rescue therapy still fails in more that 20% of the cases. Our aim was to evaluate the efficacy and tolerability of a levofloxacin-based regimen in patients with two consecutive *Helicobacter pylori* eradication failures.

**Patients and Methods.** Design: Prospective multicenter study including nine Spanish centers. Patients: In whom a first eradication trial with omeprazole–clarithromycin–amoxicillin and a second trial with omeprazole–bismuth–tetracycline–metronidazole (44 patients) or ranitidine bismuth citrate with these same antibiotics (11 patients) had failed. Intervention: A third eradication regimen with levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.), and omeprazole (20 mg b.i.d.) was prescribed for 10 days. Compliance with therapy was determined from interrogatory and recovery of empty envelopes of medications. Outcome: *H. pylori* eradication was defined as a negative 13C-urea breath test 8 weeks after completing therapy.

**Results.** Fifty-five patients, mean age 52 years, 45% males, 55% peptic ulcer, 45% functional dyspepsia. All patients but four (93%) took all the medications correctly. Per-protocol and intention-to-treat eradication rates were both 67% (95% CI = 54–78%). Adverse effects were reported in five (9%) patients, including metallic taste, abdominal pain, nausea/vomiting, diarrhea, vaginal candidiasis, arthralgia/myalgia, and tendinitis, but none of them were severe. One patient abandoned the treatment due to adverse effects (tendinitis), which were resolved spontaneously after finalizing treatment.
Conclusion. Levofloxacin-based rescue therapy constitutes an encouraging strategy after multiple previous eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole, and tetracycline.

Abstract no.: 11.13
Eradication of Helicobacter pylori for the Prevention of Ulcer Bleeding Recurrence

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Aim. Eradication of Helicobacter pylori is associated with a very low rate of ulcer recurrence. Our purpose was to verify the effect of H. pylori eradication on ulcer bleeding recurrence secondary to peptic ulcer disease.

Methods. Patients with acute hemorrhage secondary to gastroduodenal ulcer were prospectively included. NSAID use was not considered an exclusion criteria. H. pylori infection was confirmed by rapid urease test, histology, or 13C-urea breath test. Several therapies were used, mainly omeprazole or ranitidine bismuth citrate-based regimens. Afterwards, an H2-antagonist (ranitidine 150 mg o.d.) was administered until eradication was confirmed by 13C-urea breath test 8 weeks after completing eradication therapy. Patients with therapy failure received a second or third course of therapy. Patients with eradication success did not receive maintenance anti-ulcer therapy, and were controlled yearly up to 5 years with a 13C-urea breath test. NSAID use was not permitted during follow-up.

Results. Up to now, 163 patients have been followed up for at least 12 months, with a total of 245 patient-years of follow-up. Mean age was 62 years, 71% were males, and 46% were previous NSAID users. Sixty-eight percent (68%) had duodenal ulcer, 25% gastric ulcer, and 7% pyloric ulcer. Recurrence of bleeding was demonstrated in one patient at 1 year (incidence: 0.4% per patient-year of follow-up), which occurred after NSAID use.

Conclusion. Rebleeding does not occur in patients with complicated ulcers after H. pylori eradication. Maintenance antulcer (antisecretory) therapy is not necessary if eradication is achieved. However, NSAID intake may cause rebleeding in H. pylori-eradicated patients.

Abstract no.: 11.14
Reflux Esophagitis After Eradication of Helicobacter pylori in Peptic Ulcer Patients: Impact of Initial Corpus and Antral Gastritis

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Helicobacter pylori infection usually causes antral gastritis with increased acid secretion and risk of duodenal ulcer, or pangastritis with risk of ventricular ulcer and suppression of acid secretion. Eradication of the infection might therefore result in variable effects on acid-related symptoms.

Aim. To investigate the prevalence of new GERD symptoms (heartburn and regurgitation) in gastric and duodenal ulcer patients after H. pylori eradication and consider its association with initial antral and corpus gastritis.

Methods. Four hundred twenty-nine successfully eradicated H. pylori-positive ulcers patients (GU 189; 90 women, 99 men; age 58.7 years; DU 240; 103 women, 137 men; age 51.0 years), were followed up prospectively. After achieving eradication, all patients underwent endoscopy for at least 1-year intervals (range, 1–8 years). H. pylori status was checked by endoscopy with histology (two from antrum and two from corpus), and GERD status using questionnaire. The presence of gastritis was classified according to the updated Sydney classification, modified for the purpose of this study: Gastritis score (GS): activity (1–3) + chronic gastritis (1–3) + atrophy (1–3) for two specimens (corpus, antrum).

Results. Forty-seven (25%) of 189 GU patients and 72 (30%) of 240 DU patients worsened their GERD symptoms after H. pylori eradication. Among these patients initial corpus, GS was significantly higher than in patients without new GERD symptoms (GU 4.45 versus 3.52; DU 3.78 versus 2.78). Antrum GS did not show any significant difference (GU 5.34 versus 5.21; DU 5.42 versus 5.29).

Conclusion. H. pylori eradication in peptic ulcer patients with corpus gastritis, which is associated with an increase in gastric acid secretion, may be an important factor determining the higher prevalence of post-H. pylori eradication GERD.

Abstract no.: 11.15
Rescue Therapy with Rifabutin after Multiple Helicobacter pylori Treatment Failures

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Aim. Eradication therapy with proton pump inhibitor, clarithromycin and amoxicillin is extensively used, although it fails in a considerable number of cases. A rescue therapy still fails in more than 20% of the cases. Our aim was to evaluate the efficacy and tolerability of rifabutin-based regimen in patients with two consecutive Helicobacter pylori eradication failures.

Patients and Methods. Design: Prospective multicenter study. Patients: In whom a first eradication trial with omeprazole–clarithromycin–amoxicillin and a second trial with omeprazole–bismuth–tetracycline–metronidazole (17 patients) or ranitidine bismuth citrate with these same antibiotics (43 patients) had failed.
Intervention: A third eradication regimen with rifabutin (150 mg b.i.d.), amoxicillin (1 g b.i.d.) and omeprazole (20 mg b.i.d.) was prescribed for 10–14 days. Compliance with therapy was determined from interrogatory and recovery of empty envelopes of medications. Outcome: H. pylori eradication was defined as a negative 13C-urea breath test 8 weeks after completing therapy.

Results. Fifty-eight patients, mean age is 55 years, 38% males, 38% peptic ulcer, 62% functional dyspepsia. All patients but one took correctly all the medications. Per-protocol and intention-to-treat eradication rates were 65% (95% CI = 52–76%) and 64% (51–75%). Adverse effects were reported in 23 (38%) patients, including abdominal pain, nausea/vomiting, diarrhea, stomatitis, fever/myalgia, and oral candidiasis. One patient abandoned the treatment due to adverse effects (vomiting). Six patients (10%) had neutropenia (< 1500) and/or thrombopenia (< 150,000), which resolved spontaneously after finalizing treatment (although granulocyte growth factor was prescribed in one case with < 500 neutrophils).

Conclusion. Rifabutin-based rescue therapy constitutes an encouraging strategy after multiple previous eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole, and tetracycline.

Abstract no.: II.16
7-Day Ranitidine Bismuth Citrate-Versus Levofoxacin-Based Triple-Rescue Therapy after Helicobacter pylori Treatment Failure

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Aim. Ranitidine bismuth citrate (RBC)-based rescue regimen has been demonstrated to be an alternative to quadruple rescue therapy after Helicobacter pylori eradication failure. On the other hand, levofoxacin has, in vitro, remarkable activity against H. pylori. Our aim was to compare, by a randomized trial, two different 7-day triple rescue regimens based on RBC or levofoxacin.

Methods. Patients in whom a first eradication trial with omeprazole–clarithromycin–amoxicillin had failed were randomized, in this single-center study, to receive 7-day treatment with: 1, RBC (400 mg b.i.d.), tetracycline (500 mg q.i.d.), and metronidazole (250 mg q.i.d.); or 2, levofoxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.), and omeprazole (20 mg b.i.d.). Cure rates were evaluated by 13C-urea breath test.

Results. At present, 67 patients have been included (22% peptic ulcer, and 78% functional dyspepsia): 36 received the RBC regimen, and 31 the levofoxacin one. Groups were comparable in terms of demographic variables. Three percent of the patients (one in each group) did not return for follow-up. Compliance was slightly worse with the RBC regimen than with the levofoxacin regimen (89% versus 97%; nonstatistically significant differences). Side-effects (only mild/moderate) in the two groups were comparable (about 40%). Tendinitis occurred in three (10%) patients treated with levofoxacin. Per-protocol cure rates were 68% (95% CI, 50–81%) in the RBC group and 69% (51–83%) in the levofoxacin group. Intention-to-treat cure rates were, respectively, 67% (50–80%) and 68% (50–81%) (nonstatistically significant differences).

Conclusions. Both RBC- and levofoxacin-based rescue regimens represent effective alternatives to quadruple therapy in patients with omeprazole–clarithromycin–amoxicillin failure.

Abstract no.: II.17
Telithromycin-Based 7-Day Triple Therapy for the Treatment of Helicobacter pylori Infection

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Background. Failed primary anti-Helicobacter pylori therapy results in a high rate of antimicrobial resistance. This necessitates a search for new regimens to cure H. pylori infection. The aim of this study was to evaluate the efficacy and tolerability of new telithromycin containing 7-day triple therapy and to compare it with standard French triple therapy in patients with known H. pylori susceptibility to metronidazole and clarithromycin.

Methods. Fifty-five patients with documented antibiotic sensitivity (E-test) and indication for anti-H. pylori treatment based on 2/2000 Maastricht guidelines were randomized to receive either esomeprazole 2 × 40 mg, telithromycin 2 × 400 mg, and amoxicillin 2 × 1 g for 7 days (ETA, n = 28), or esomeprazole 2 × 20 mg, clarithromycin 2 × 500 mg, and amoxicillin 2 × 1 g for 7 days (ECA, n = 27). Cure check was performed 4–6 weeks after conclusion of therapy.

Results. All 55 patients completed treatment and returned to the examination after treatment. All 55 patients were available for per-protocol analysis. Twenty-seven of 28 patients of the ETA group (96.4%, CI: 86–99%) became H. pylori-negative compared with 24 of the 27 patients of the ECA group (88.9%, CI: 76–93%, difference between groups statistically not significant). Both regimens were generally well tolerated with minor adverse events being seen in 8 patients (28.6%) of the ETA group and in 11 (40.7%) of the ECA group. None of the patients discontinued treatment prematurely due to adverse events.

Conclusion. The data of this pilot study suggest a better than 80% efficacy of the new 7-day telithromycin triple therapy, which is within the range of the French triple therapy in patients with MET- and CLA-susceptible strains.

Abstract no.: II.18
Eradication of Helicobacter pylori Infection in a Large Population: 1-Day Quadruple Therapy Compared with 7-Day Triple Therapy

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Aims. To compare the efficacies of 1-day and 7-day therapies, we conducted a large-scale trial of Helicobacter pylori eradication with two therapies.

Methods. A total of 401 healthy residents aged 25–49 years from Linqu, China were invited to participate in this study. The status of H. pylori infection of each participant was determined by a 13C-urea breath test. The participants were assigned into two aims: 158 participants received a 7-day triple treatment (four 250-mg capsule of amoxicillin, b.i.d.; two 250-mg tablets of clarithromycin, b.i.d.,
and one 30-mg capsule of lansoprazole, b.i.d.), and 243 participants received a 1-day quadruple therapy (2 g amoxicillin, q.i.d.; 500 mg metronidazole, q.i.d.; three 300-mg capsules of bismuth citrate, q.i.d.; two 30-mg capsules of lansoprazole once daily). Six weeks after the treatment, all the participants underwent a 13C-UBT to assess the eradication of *H. pylori* infection.

**Results.** Of the participants, 229 completed the 1-day therapy (94.3%) and 148 participants completed the 7-day therapy (93.7%). Sixty-four of 229 participants (27.95%) had negative results of the secondary 13C-UBT in the 1-day therapy. One hundred three of 148 participants (69.59%) had negative results in the 7-day therapy.

**Conclusion.** The 7-day triple therapy was efficient for *H. pylori* eradication, and the 1-day therapy had a significantly lower cure rate.

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**Abstract no.: 11.19**

**Helicobacter pylori Infection in HCV-Related Chronic Liver Disease and Thrombocytopenia**

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**Introduction.** *Helicobacter pylori* and hepatitis C virus (HCV) have been related to thrombocytopenia. Aim of this study was to evaluate the effect of *H. pylori* eradication on platelet count in patients with chronic liver disease affected by thrombocytopenia.

**Methods.** Fifty patients (10 women and 40 men; mean age, 65.8 ± 9.1) with liver disease (40 chronic hepatitis, 10 cirrhosis) were enrolled by the Internal Medicine Department of Gemelli Hospital in Rome. All patients with a platelet count < 100,000/cmm performed a 13C-urea breath test and an evaluation of antiplatelet associated antibodies. Patients with thrombocytopenia and positive for *H. pylori* were eradicated with a standard therapy. Eradication of *H. pylori* was evaluated 6 weeks after by UBT. A platelet count was performed 1 and 3 months after *H. pylori* eradication. A Mann–Whitney U-test was used to evaluate differences between groups.

**Results.** Four patients with hepatitis and one with cirrhosis presented low platelets count (mean values: 72,000 ± 17,000/cmm) and were positive for *H. pylori* infection. Three patients with hepatitis and one with cirrhosis were positive for antiplatelet associated antibodies. A significant improvement in platelet count was observed in patients eradicated and with positive platelets autoantibodies at one (70,000 ± 17,000/cmm versus 101,000 ± 12,000/cmm; p < .001) and at 3 months (70,000 ± 17,000/cmm versus 99,000 ± 13,000/cmm). No improvement was observed after eradication in the patient with negative platelet auto-antibodies.

**Conclusions.** Thrombocytopenia is common in HCV chronic liver disease and related to *H. pylori* gastric infection by means of autoimmune mechanisms. *H. pylori* eradication improves platelets count. Further larger studies are needed to confirm these observations.
about that issue. The aim of this study was to evaluate the difference of eradication rates of *H. pylori* between patients with PUD and patients with NUD in Korea.

**Methods.** Two hundred ninety-seven patients who underwent upper endoscopy and treated with 7-day triple therapy (proton pump inhibitor + amoxicillin + clarithromycin) and follow-up urea breath test were reviewed retrospectively.

**Results.** Two hundred thirty-seven of 297 patients were PUD (98 gastric ulcers, 167 duodenal ulcers, 28 both ulcers), and 60 of 297 patients were NUD. The eradication rates of each group were 85.7% (95% CI 80.6–89.6%) and 73.3% (95% CI 61.0–82.9%). The eradication rate of the NUD group was lower than that of PUD group (p = 0.032).

**Conclusion.** The 7-day triple therapy with proton pump inhibitor showed rather lower eradication rate in patients with NUD than patients with PUD. Therefore, extension of treatment duration or a more potent regimen may be needed for eradication of *H. pylori* in patients with NUD.

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### Abstract no.: 11.22

**Eradication Rate After Randomized Treatment in a Population with High Prevalence of Helicobacter pylori Infection**

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The aim of this study was to estimate the efficacy of eradication in the region with high prevalence of the infection.

**Patients and Methods.** A total of 220 patients with *Helicobacter pylori*-associated peptic ulcer were randomized to receive omeprazole 20 mg, clarithromycin 500 mg, and amoxicillin 1000 b.i.d. for 7 days (OCA7, n = 34), for 14 days (OCA14, n = 33), the same but also omeprazole 40 mg b.i.d. for 7 days (OOCA7, n = 33); the same but also omeprazole 20 mg b.i.d. (RCA7, n = 32), colloidal bismuth subcitrate 240 mg b.i.d., amoxicillin 1000 b.i.d., and furazolidone 200 mg b.i.d. for 7 days (BAF7, n = 20; this protocol was stopped prematurely); the same but also with omeprazole 20 mg b.i.d. (OBAF7, n = 33); the same but also for 14 days (OBAF14, n = 35). The results of eradication were estimated in accordance with RUT, data of polymerase chain reaction (PCR) method, and/or histology examinations in 2 months after treatment.

**Results.** According to the results of RUT and RUT + histology/PCR per protocol the eradication rates were 88.9 and 22.2% for the OCA7; 96.2 and 38.4% for the OCA14; 92.9 and 39.3% for the OOCA7; 83.3 and 30.0% for the RCA7; 12.5 and 6.2% for the BAF7; 35.7 and 17.9% for the OBAF7; 81.3 and 34.4% for the OBAF14 groups, respectively.

**Conclusion.** According to RUT data, the efficacy of triple eradication therapy in the population with high prevalence of *H. pylori* infection makes up about 83.3–96.2%, but if histology/PCR methods are used additionally, these results are worse.

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### Abstract no.: 11.23

**Inhibition of Helicobacter pylori by Whole Cells and Cellular Fractions of Lactobacillus Plantarum**

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**Background.** Antibiotic resistance is a growing problem in the treatment of *Helicobacter* infection. Further, it is not recommended to treat all asymptomatic people with antibiotics. A number of strains of lactic acid bacteria used in food fermentation have been shown to inhibit the growth of *Helicobacter pylori*. The aim of this study was to test the anti-*Helicobacter* activity of a *Lactobacillus plantarum* MLBPL1 isolated from sauerkraut.

**Methods.** *L. plantarum* MLBPL1 was cultivated in MRS broth. Cell-free culture supernatant was obtained by centrifugation. Harvested cells were washed with HEPES. Washed cells were mixed with glass beads and disrupted in a homogenizer. Cell wall fragments and the intracellular fraction were separated from the whole cell lysate by centrifugation. *H. pylori* NCTC 11637 suspension was distributed on brucella agar plates. Wells cut with sterile straw were filled with sample of MLBPL1. Plates were incubated under microaerophilic conditions for 3 days, and the diameters of inhibitory zones were measured.

**Results.** The anti-*Helicobacter* activity was present in washed MLBPL1 cells with an average diameter of inhibition zones 22 mm. The inhibitory diameters of culture supernatant, whole-cell lysate and cell wall fragments were 10, 15, and 12 mm, respectively. The intracellular fraction did not inhibit the growth of *Helicobacter*.

**Conclusion.** In addition to the culture supernatant, the whole cells as well as cell lysate of *L. plantarum* MLBPL1 contained anti-*Helicobacter* activity. This suggests that the main activity is primarily located in the cell wall from where it is probably extracted into the culture supernatant.

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### Abstract no.: 11.24

**How Does Vitamin C Administration Decrease the Risk of Gastric Cancer in the Helicobacter pylori-Positive Patients with Atrophic Gastritis?**

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**Introduction.** A significant decrease in intestinal metaplasia of gastric mucosa was achieved by coadministration of ascorbic acid with *Helicobacter pylori* eradication in an Italian study.

**Aims and Methods.** This study investigates the usefulness of vitamin C administration with or without *H. pylori* eradication to prevent the development of gastric cancer in *H. pylori* carriers with atrophic gastritis. Fifteen *H. pylori*-positive patients with moderate to severe atrophic gastritis were divided into two groups, eight patients successfully eradicated and seven patients nontreated. We administrated ascorbic acid (1200 mg/day) orally for 4 months and analyzed gastric juice pH, nitrite, and total vitamin C concentrations in gastric juice and plasma, serum gastrin concentrations, and the intensity of neutrophil infiltration in gastric mucosa before and after ascorbic acid treatment.
Results. In *H. pylori*-eradicated patients, acid output recovered and the ratio of gastric juice to plasma vitamin C concentration increased. Intragastric nitrite concentration decreased by increment of intragastric vitamin C. Administration of vitamin C to *H. pylori* carriers changed neither the concentrations of intragastric vitamin C nor intragastric nitrite.

Conclusion. Administration of vitamin C with successful *H. pylori* eradication can decrease the risk of gastric cancer in *H. pylori*-positive patients before the development of stable mutation.

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**Abstract no.: 11.25**

**Clinical Predictors Affecting the Eradication of *Helicobacter pylori***

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Background and Aims. Antibiotic resistance and compliance are regarded as the more important factors to affect the eradication. However, it is not easy to apply the antibiotic resistance test to clinical field. We investigated other clinical predictors affecting the eradication of *Helicobacter pylori*.

Methods. We retrospectively investigated the patients with documented *H. pylori* infection between January 2004 and March 2005. All received a 1-week proton pump inhibitor (PPI)-based triple therapy and examined the underlying chronic illnesses, smoking, alcohol, therapeutic indication, and compliance scoring. Eradication was assessed by 13C-urea breath test, rapid urease test, or endoscopy at 4–6 weeks after therapy.

Results. Including 195 patients, the intention-to-treat (ITT) eradication rates was 72.31%. The per protocol (PP) analysis on the 169 patient gives an initial eradication rate of 83.43%. The eradication rates according to the endoscopic indication were 81.96% (50/61) in GUs, 86.04% (37/43) in DUs, 81.28% (22/27) in gastritis, 84.21% (16/19) in GU + DU, 85.71% (6/7) in polyps, 50% (1/2) in EGCa, and 100% (6/6) in the familial cancer history. The rates according to the underlying chronic illnesses were 82.60% (19/23) in the diabetes, 70.37% (19/27) in the hypertension, 66.7% (2/3) in the renal disease, 100% (2/2) in the liver disease, 72.72% (8/11) in the cardiovascular disease, and 75% (9/12) in the NSAIDs-related disease. There were no statistically significant differences in the eradication rates according to the endoscopic therapeutic indication, underlying diseases, sex, age, smoking, alcohol, and PPI. The eradication rate was significantly higher in compliant patients than in noncompliant patients (*p < 0.05*).

Conclusions. Patient’s compliance for regimens should be the most important factor that affects *H. pylori* eradication in the clinical practice.

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**Abstract no.: 11.26**

**Difference in *Helicobacter pylori* Eradication Rates of the Second-Line Therapy in Patients with Peptic Ulcer Disease and Non-Ulcer Dyspepsia**

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Purpose. Proton pump inhibitor (PPI)-based triple therapy for *Helicobacter pylori* eradication is widely used with considerable failure rate (10–20%). Physicians should try to achieve an optimal cure rate with their second-line therapy for *H. pylori* eradication. There has been several reports that initial PPI-based triple therapy is more effective in patients with peptic ulcer disease (PUD) than patients with non-ulcer dyspepsia (NUD). But there is no report in second-line therapy for *H. pylori* eradication in that issue. Our aim was to evaluate the difference in *H. pylori* eradication rates of the second-line therapy between patients with PUD and patients with NUD.

Methods. The subjects consisted of 65 patients infected with *H. pylori*, who underwent endoscopy and failed eradication with initial PPI-based triple therapy. They retreated with bismuth-based quadruple therapy (PPI + bismuth + metronidazole + tetracycline) and follow-up urea breath test were reviewed retrospectively.

Results. Forty-six of 65 patients were PUD (19 gastric ulcers, 26 duodenal ulcers, 1 both ulcers), and 19 of 65 patients were NUD. The *H. pylori* eradication rates of the second-line therapy in PUD group and NUD group were 93.1% (90% CI 85.4–100%) and 76.4% (90% CI 55.1–97.9%). The eradication rate of NUD group was lower than that of PUD group (*p < 0.05*).

Conclusions. Bismuth-based second-line therapy for *H. pylori* eradication showed rather lower eradication rate in patients with NUD than patients with PUD. Therefore, extension of treatment duration or more potent regimen may be needed for second-line therapy in patients with NUD.

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**Abstract no.: 11.27**

**Therapeutic Effect of Lactobacillus gasseri and Pluonotol on *Helicobacter pylori* Infection**

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Proton pump inhibitor (PPI)-based triple therapy has been widely applied to patients with *Helicobacter pylori* infection. However, rate of treatment failure due to antibiotic resistance is increasing. Probiotics are microorganisms with beneficial properties for the host. We have demonstrated that *Lactobacillus gasseri* has an inhibitory effect on *H. pylori*. Furthermore, pluonotol, an acyclic diterpene alcohol extracted from leaves of the plu-ноi tree in Thailand, has been used in Japan as a unique anti-ulcer agent for patients with gastric ulcer. To determine the clinical usefulness of *Lactobacillus* strains on *H. pylori* infection, we conducted a pilot study in patients with chronic gastritis.

The 25 subjects were divided into two groups: a group receiving of 120 g yogurt containing *L. gasseri* twice daily for 8 weeks and a
control group receiving yogurt without \textit{L. gasseri}. Gastric biopsy specimens were obtained from both the antrum and the body. Gastric mucosal IL-8 levels were determined by ELISA. Histologic findings were assessed using the updated Sydney classification. IL-8 levels were significantly decreased after 8 weeks of ingesting \textit{L. gasseri} (\(F = 0.0003\)). In contrast, IL-8 levels were not significantly decreased in the control group. However, the histologic gastritis scores did not differ between the two groups. After this study, 11 subjects continued to consume yogurts with \textit{L. gasseri} twice a day and were treated with gastroprotective agents, plaunotol 240 mg for up to 6 months. Thereafter, we found that combination therapy with plaunotol and \textit{L. gasseri} improved histologic findings of gastritis (\(p = 0.0416\)). These findings suggest that plaunotol and \textit{L. gasseri} is effective for patients with \textit{H. pylori}.

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### Abstract no.: 11.29

**Antibacterial Effect of Crude Drugs on Helicobacter pylori Infection**

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**Aim.** The eradication of \textit{Helicobacter pylori} using antibiotics is effective for the treatment of gastroduodenal diseases. However, it has recently been reported that resistance to these antibiotics is developing. In the present study, the antibacterial effect of crude drugs against \textit{H. pylori} was examined in vitro and in vivo.

**Method.** Crude drug: \textit{Rhei rhizoma}, \textit{Coptidis rhizoma}, \textit{Artemisiae capillaris flos}, \textit{Caryophylli flos}, \textit{Glycyrrhizae radix}, etc., were used.

In vitro: 100 µl of water extract solution (1 mg/ml) of each crude drug was added to 900 µl of suspension of \textit{H. pylori} (1 × 10^7 cfu/ml), and shaken in microaerobic round-bottomed tube under 37 °C and then 10 µl of them was applied to blood agar, cultivated for 7 days under microaerobic conditions, and the number of colonies was counted.

In vivo: The water extract solution (1 mg/ml) of each crude drugs was administered orally to C57BL/6 mice for 2 weeks after inoculation with \textit{H. pylori}, and then the number of \textit{H. pylori} in the stomach was measured 4 weeks later.

**Result.** \textit{R. rhizoma}, \textit{A. capillaris flos}, and \textit{C. flos} inhibited the growth of \textit{H. pylori} at a dose of 1 mg/ml and 1 × 10^1 mg/ml in contact for 6 hours or more in vitro. \textit{H. pylori} in the stomach was significantly reduced in the \textit{R. rhizoma}, \textit{A. capillaris flos}, \textit{C. flos}, and \textit{G. radix} treatment groups compared with the control group in vivo.

**Consideration.** These results suggest that \textit{C. rhizoma}, \textit{A. capillaris flos}, \textit{C. flos}, and \textit{G. radix} may be clinically useful for treatment of \textit{H. pylori} infection.

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### Abstract no.: 11.30

**14-day Quadruple Therapy with Ranitidine Bismuth Citrate After Helicobacter pylori Treatment Failure**

D. Thong-Ngam, V. Mahachai and Chulalongkorn Helicobacter Study Group

Chulalongkorn University Hospital, Bangkok, Thailand

**Background.** As a second-line therapy after \textit{Helicobacter pylori} treatment failure, a quadruple therapy with a proton pump inhibitor, bismuth, metronidazole, and tetracycline is recommended.

**Aims.** To evaluate the efficacy of 14-day ranitidine bismuth citrate (RBC)-based quadruple therapy.

**Methods and Materials.** Between June 2003 and May 2005, 34 patients who were \textit{H. pylori}-positive after first-line (omeprazole, amoxicillin, clarithromycin, or metronidazole) treatment failure received 14-day quadruple therapy with RBC (400 mg b.i.d.), rabeprazole (20 mg b.i.d.), metronidazole (500 mg t.i.d.), and tetracycline (500 mg q.i.d.). Four weeks after completion of treatment, eradication was confirmed with ^13C-urea breath test.

**Results.** There are 18 men (52.9%) and 16 women (47.1%) with mean age 47.34 ± 14.62 years. Per protocol eradication rate was...
Abstract no.: 11.31
Antihelicobacterial Effect of Electrochemically Activated Solutions and Prospects of Their Application for Eradication of Helicobacter pylori

L. G. Bajenov,† E. V. Rizaeva* and S. S. Bajenova‡
*Republican Specialized Centre of Surgery, Tashkent, Uzbekistan,
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Antihelicobacterial activity of the electrochemically activated solutions (ECAS), in particular neutral anolyt (NA) and prospects of its use for eradication of Helicobacter pylori were studied. NA was produced with the help of the device “STEL.” The preparation represents a colorless transparent liquid with a slight chlorine odor, contains high-activity oxygenous combinations of chlorine, free radicals, ozone – adding antimicrobial and washing properties. NA is estimated on concentration of active chlorine, in the initial solution it makes 350 ± 50 mg/l, pH 6.0 ± 1.0, redox potential 700 ± 100 mV. NA antimicrobial effect was studied in relation to the H. pylori clinical strains (7) and members of a normal intestinal microflora (Escherichia coli, Lactobacillus plantarum, Bifidobacterium bifidum, Bacteroides fragilis, etc.) by serial dilution method in a fluid medium.

NA inhibited the growth of all examined microorganisms. At the same time, the H. pylori cultures manifested the greatest sensitivity to the researched agent. Even at dilution of the initial active chlorine solution up to 6 mg/l, H. pylori practically instantaneously perished. This sensitivity considerably exceeded that one of members of a normal microflora, that allows to make a selective H. pylori-decontamination at sparing influence on normal microflora, thereby reducing danger of development of disbacteriosis aggravating a basic disease course.

The obtained results can become a basis for elaboration of effective methods of treatment of helicobacteriosis applying ECAS. Moreover, it is important that alongside expressed antihelicobacterial activity and economic accessibility, these preparations also have immunostimulating, anti-inflammatory, and regeneration-accelerating effects. Besides, microorganisms practically do not acquire resistance to them.

Abstract no.: 11.32
Gatifloxacin-Containing Sequential Therapy for Helicobacter pylori Infection

D. Y. Graham,† S. Abudayyeh,* H. M. T. El-Zimaity,*, J. Hoffman,† R. Reddy* and A. R. Opekun*
*Baylor College of Medicine, Houston, TX, USA, †Michael E. DeBakey VAMC, Houston, TX, USA

Background. The success rate of anti-Helicobacter pylori therapy has continued to fall in part due to increasing resistance.

Aim. To test the sequential combination of high dose proton pump inhibitor (PPI)-amoxicillin followed by the addition of gatifloxacin for H. pylori infection.

Methods. This was a pilot study where patients with active H. pylori infection received sequential therapy consisting of 40 mg of esomeprazole and 1 g amoxicillin t.i.d., for 12 days. Both naive and treatment failures were eligible. On days 6 through 12 gatifloxacin (400 mg in the morning) was added to produce a triple therapy. Outcome was accessed by urea breath test (UBT) or endoscopy with histology and culture performed 4–6 weeks after ending antibiotic therapy.

Results. To date, the cure rate has been 100% among those who received all three drugs. One patient stopped therapy after receiving only the PPI plus amoxicillin, and therapy was unsuccessful. Side effects include mild diarrhea/loose stools in 25%. Another patient in whom gatifloxacin was contraindicated received 40 mg of esomeprazole and 1 g amoxicillin t.i.d., for 12 days and then metronidazole 500 mg t.i.d. on days 6 through 12 and the infection was treated successfully.

Conclusion. Sequential therapy using the combination of high-dose PPI and amoxicillin (the German therapy) followed by the addition of gatifloxacin appears to be an excellent first- or second-line therapy for H. pylori infections.

Abstract no.: 11.33
Sensitivity of the Clinical Strains of Helicobacter pylori to Aspirinum

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*Republican Specialized Centre of Surgery, Tashkent, Uzbekistan,
†Uzbek Helicobacter Study Group, Tashkent, Uzbekistan

The aspirinum antimicrobial activity in relation to Helicobacter pylori comparing with other conditionally pathogenic microorganisms was investigated in vitro.

The H. pylori clinical cultures (eight strains), gastric juice sample inoculated with H. pylori (six samples), and Staphylococcus aureus, Escherichia coli, and Candida albicans were tested. Aspirinum antimicrobial activity was studied at its influence on testing culture suspension in a saline. The aspirinum powder was added to microbial suspensions and gastric juice samples from 10% to 0.001% concentrations. Microbial suspensions and gastric juice without aspirinum addition were used as a control. Inclusions from these solutions were made on optimal mediums for each microorganism after different exposures. Changes of pH of solutions were insignificant and only at high aspirinum concentrations (3% and above).

Aspirinum had a certain antimicrobial activity depending on drug concentration, its influence duration, and examined microorganism species. The most sensitive one was H. pylori, for which
the minimum inhibiting aspirinum concentration made 10 µg/ml, that was much lower than aspirinum concentration, created in a stomach at an average therapeutic drug dosage.

Thus, alongside known adverse aspirinum effect on a stomach mucous membrane, its positive effect also can take place that in particular consists in H. pylori inhibition. This phenomenon can be used in the creation of specialized drug forms and in the elaboration of new effective schemes of eradication therapy and prevention of helicobacteriosis with aspirinum application. Besides, it would be expedient to study antihelicobacterial aspirinum effect in cardiovascular patients, who are often prescribed with this drug.

Abstract no.: 11.34
The Efficiency of Helicobacter pylori Eradication in Eastern Siberia Inhabitants

V.V. Tsukanov and E.A. Sugonyako
State Scientific Medical Research Institute for Northern Problems of Siberian Division of Russian Academy of Medical Sciences, Krasnoyarsk, Russian Federation

Aim. To study the influence of Helicobacter pylori eradication on the frequency of ulcer disease relapse.

Methods. We examined 140 patients with duodenal ulcer disease (93 men, 47 women). One hundred one subjects (group I, 57 subjects with uncomplicated cause; group II, 44 subjects with ulcer hemorrhages) were provided with H. pylori eradication by 7-day schedule: omeprazol (20 mg × 2 times) + clarithromycin (500 mg × 2 times) + amoxicillin 1 g × 2 times). H. pylori was diagnosed by morphological and urease techniques. Thirty-nine subjects (group III, 24 subjects with ulcer hemorrhages; group IV, 15 subjects with uncomplicated cause) were made the control group in which H. pylori eradication was not carried out. Monitoring was made during 18 months.

Results. The efficiency of H. pylori eradication made 88.6% in group I and 89.5% in group II. Acute ulcer disease during 18 months was marked in 27% subjects in group I and in 17.5% subjects in group II, in 83.3% subjects in group III (p < .01) in 73.3% subjects in group IV (p < .01). During the reported period, ulcer hemorrhages were not marked in group I and group II, their frequency in group III and IV was 10.3% (p < .05).

Conclusion. H. pylori eradication is an effective means of ulcer disease prophylaxis, with its complications in Eastern Siberia inhabitants.

Abstract no.: 11.35
The Development Frequency of Duodenal and Gastric Ulcer Recurrence Associated with Helicobacter pylori after Eradication Therapy

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Object. The development frequency of duodenal and gastric ulcer recurrence in depending on Helicobacter pylori eradication quality were studied.

Abstract no.: 11.36
Anti-Helicobacter Activity of Novel Chalcones

M. Chen,* L. Zhai,* J. Blom,* L.P. Andersen† and A. Khazrani‡
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Previously, we have shown that licochalcone A exhibits potent anti-Helicobacter activity in vitro. In order to find new anti-Helicobacter agents, a large number of chalcones has been synthesized and their activity in vitro anti-Helicobacter activity has been tested.

Effect of more than 60 different novel chalcones on the in vitro Helicobacter pylori has been tested against 6 different strains including strains resistant to metronidazole (MIC > 32 µg/ml) in an agar dilution assay. Some of the tested novel chalcones exhibit potent inhibitory effect on the in vitro growth of H. pylori at 12.5 to 25 µmol/l and two of the novel chalcones at MIC of 9.4 µmol/l.

The mechanism of antibacterial activity of the novel chalcones has also been investigated. Electron microscopic studies showed that the bacteria swelled and its shape changed to round (coccoid) from curved or spiral when they were incubated with licochalcone A at 16 µg/ml. Licochalcone A and several chalcones inhibited the activity of NADH-fumarate reductase, succinate-cytochrome c reductase and succinate dehydrogenase of the bacterial electron transport chain. The IC₅₀ values of licochalcone A on the three enzymes were 10, 80 and 800 µmol/l, respectively.

These results reveal that some novel chalcones exhibit potent in vitro anti-Helicobacter activity and might provide the basis for the development of new class of antibacterial agents, and suggest that their anti-Helicobacter effect might be due to the inhibition of the electron transport chain and the activity of fumarate reductase of the bacteria.
NSAIDs, COXIBs, ASA and Helicobacter pylori Infection

Abstract no.: 12.01*
Serum Pepsinogen II and Gastrin-17 as Markers of NSAIDs-Gastropathy Independently of Helicobacter pylori Infection

L. Cavallaro,* N. Stefani,* N. Merli,* E. Morana,* M. Maino,* V. Iori,* G. Cavestro,* A. Bertelè† A. Franzè† and F. Di Mario*

"Department of Clinical Sciences, Section of Gastroenterology, Parma, Italy, †Institute of Applied Statistics and Systems Analysis, Joanneum Research, Graz, Austria

Background and Aim. Serum pepsinogen II (sPGII) is a reliable marker of PMN cells infiltration of gastric mucosa during Helicobacter pylori infection. Neutrophils within the gastric microcirculation and cytokines release are critical events in NSAIDs-gastropathy. Inflammation can deregulate somatostatin–gastrin axis in the gastric antrum.

Methods. Four hundred eighty-eight H. pylori isolates originating from different Belgian centers were tested for susceptibility to ciprofloxacin (C) and levofloxacin (L) by E-test. MIC values ≥ 1 µg/ml were classified as resistant. Strain resistant were evaluated for mutations in the quinolone-resistance determining region (QRDR) of gyrA by DNA sequencing.

Results. Eighty-two (16.8%) of 488 strains were resistant to C and L. Of these, 76 were further evaluated; 56 strains (74%) presented a homogeneous resistant phenotype, and 20 (26%), a heterogeneous susceptibility to fluoroquinolones. QRDR-sequencing revealed various types of mutations at position 87 and /or 91 in all homogeneous resistant isolates. In the 20 heterogeneous resistant isolates, a susceptible wild-type genotype was found in 17 and resistance mutations in only 3. Colonies found in the inhibition zone were subcultured and showed a mutated resistant genotype in all cases. Type and location of the mutations did not match with the level of resistance to fluoroquinolone.

Conclusions. Primary resistance fluoroquinolones do frequently occur in H. pylori isolates in Belgium. Resistance is always associated with one or more mutations at position 87 and 91 in the QRDR of gyrA. The high proportion (26%) of heterogeneous susceptibility in H. pylori underlines the risk of missing fluoroquinolone resistance by genotypic methods as well as the importance of a careful inspection of the antibiogram.

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Drug Resistance

Abstract no.: 13.01*
Mechanism of Resistance to Fluoroquinolones among Helicobacter pylori Isolates from Belgian Patients

P. Bogaerts, C. Berhin, H. Nizet and Y. Glupczynski

UCL Mont-Godinne, Mont-Godinne, Belgium

Background and Aim. We previously showed that 18% of clinical Helicobacter pylori strains isolated in Belgium were resistant to ciprofloxacin (EHSG 17th International Workshop, 2004). In this study, we aimed to analyze the mechanisms of resistance of H. pylori to fluoroquinolones.

Methods. Two hundred seventeen dyspeptic patients (105 women, mean age 50.3 ± 18 years) of whom 51 with chronic assumption of ASA 100 mg/die (age: 63 years ± 15, group 1), and 166 without (age: 46 years ± 18, group 2) were enrolled. A blood sample to evaluate sPGI, sPGII, G-17, and IgG-Hp levels was performed.

Results. There was no significant difference as regards to IgG-Hp between group 1 (45 ± 29 U/l) and group 2 (43 ± 34 U/l, p = .9). SPGI levels were not different in group 1 (121 ± 77 µg/l) and group 2 (101 ± 57 µg/l, p = .13). SPGII and G-17 were significantly higher in group 1 (sPGII = 13 ± 4 µg/l, G-17 = 15 ± 9 pg/l) than in group 2 (sPGII = 10 ± 8 µg/l, p = .02; G-17 = 14 ± 1 pg/l, p = .02).

Conclusion. Independently of H. pylori infection, sPGII and G-17 select patients at major risk for NSAIDs gastric damage that could benefit for proton pump inhibitor (PPI) protection.

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Abstract no.: 13.02
ResiNet – A Nationwide Sentinel Study on Helicobacter pylori Resistance – Why We do Sensitivity Testing Already After the First Treatment Failure

M. Kist,* E. Glocker,* B. Hobmaier* and H. P. Stüger†

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†Institute of Applied Statistics and Systems Analysis, Joanneum Research, Graz, Austria

The treatment of Helicobacter pylori infections using antibiotic triple regimens is significantly impaired if H. pylori is resistant against metronidazole (MZ) and clarithromycin (CLA). The aim of this ongoing study is to investigate risk factors for the development of antimicrobial resistance in H. pylori.

For this purpose, the German National Reference Centre (NRZ) for H. pylori in 2001 launched the nationwide sentinel study ResiNet. The NRZ is intercalated with 16 microbiological centers (MC) and about 50 gastroenterologists (GE) in clinical practice. During “study weeks,” GE enrol consecutive patients into the
study, sending gastric biopsies for microbiological investigation and completing a questionnaire. All MC use identical culture media lots and standardized operation procedures.

At the end of March 2005, a total of 506 patients were investigated. Overall, 34.5% and 15.5% of isolates were resistant to MZ or CLA, respectively, and 10.9% showed double resistance against both drugs. The frequencies of primary resistance ($n = 365$) were 26.8% (MZ), 5.5% (CLA), and 2.5% (MZ and CLA), compared to 53.1% (MZ), 50.0% (CLA), and 34.4% (MZ and CLA) in patients pretreated once ($n = 32$). Repeated pretreatment ($n = 38$) was associated with an increase of double resistances of up to 73.7%. These results clearly indicate that a significant increase in resistance to MZ and CLA already occurs after the first treatment failure, reaching dramatically high resistances after repeated empirical therapies.

In conclusion, the data clearly show that empirical treatment of $H. pylori$ infection is only justified in patients not pretreated before, and culture and sensitivity testing are already mandatory after the first treatment failure.

**Abstract no.: 13.03**

**Antimicrobial Susceptibility of Helicobacter pylori in a Swedish Random Adult Population**


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**Background.** Antimicrobial resistance in *Helicobacter pylori* has become an important factor leading to eradication failures. Information on antimicrobial susceptibility is important for selection of treatment regimens and such data are lacking for nonpatients. In this study, we determined the resistance rates for *H. pylori* against commonly used antibiotics in a random adult Swedish population setting.

**Methods.** A random Swedish population sample ($n = 3000$, age 20–81 years) was surveyed using a validated questionnaire assessing GI symptoms with a response rate of 74%; 1000 of the responders were invited in random order and accepted a gastroscopy with two biopsies from antrum and corpus, respectively, for *H. pylori* culture. MIC for metronidazole, clarithromycin, amoxicillin, and tetracycline was determined by *E*-test. Patient gender was recorded for 944 patients (48.7% male; 51.3% female).

Overall MTZ and CLA resistance rates were, respectively, 29.6% and 8.6% in Bangor and 33.6% and 12.6% in Chelmsford. Three isolates were TET resistant, whereas none was AMX resistant. MTZ resistance rates in Bangor increased during 2000–2004 (16.6% to 31%), whereas CLA resistance fluctuated. Similar trends were observed in Chelmsford isolates for MTZ, whereas CLA resistance increased (6.3% to 14.8%). Statistically significantly higher resistance rates were observed in females compared with males (35.3% versus 27.0% for MTZ and 12.8% versus 7.0% for CLA).

In conclusion, CLA resistance rates were higher in *H. pylori* recovered from urban (Chelmsford) compared with rural (Bangor) areas of UK, with higher rates of resistance to both antibiotics observed in females. In both areas, a temporal trend for increased resistance in MTZ was observed. The findings highlight the importance of *H. pylori* antibiotic resistance surveillance to inform local testing and treatment strategies.

**Results.** Three hundred thirty-six of the 1000 participants were *H. pylori*-positive by culture (49.7% women), 16.2% were resistant to metronidazole (74% women), 1.5% to clarithromycin (40% women), 0% to amoxicillin, and 0.3% to tetracycline. The consumption of macrolides in the region was 0.8 DDD (1997).

**Conclusion.** The resistance to clarithromycin in this population was low, probably caused by the low consumption of macrolides in this region (Sweden in total 1.1 DDD) compared to other European countries (Spain 5.8 DDD). The resistance rate to metronidazole was 16.1% compared to 26.6% in a European multicenter study. The results from Sweden show that resistance rates are low, which may reflect the restricted use of antibiotics in Sweden.

|------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| R. J. Owen and S. A. Chisholm | Centre for Infections-Health Protection Agency, London, UK  

Antibiotic resistance is key in *Helicobacter pylori* eradication failure, yet no sentinel scheme exists to monitor trends in resistance in UK. We monitored *H. pylori* resistance in two centers over 5 years to the four antibiotics used in eradication. In total, 1021 isolates, 583 from Bangor in north Wales and 438 from Chelmsford in south-east England, were collected from 2000 to 2004. Susceptibilities to metronidazole (MTZ), clarithromycin (CLA), amoxicillin (AMX), and tetracycline (TET), were determined by disc diffusion and by *E*-test for MTZ and CLA. Patient gender was recorded for 944 patients (48.7% male; 51.3% female).

Overall MTZ and CLA resistance rates were, respectively, 29.6% and 8.6% in Bangor and 33.6% and 12.6% in Chelmsford. Three isolates were TET resistant, whereas none was AMX resistant. MTZ resistance rates in Bangor increased during 2000–2004 (16.6% to 31%), whereas CLA resistance fluctuated. Similar trends were observed in Chelmsford isolates for MTZ, whereas CLA resistance increased (6.3% to 14.8%). Statistically significantly higher resistance rates were observed in females compared with males (35.3% versus 27.0% for MTZ and 12.8% versus 7.0% for CLA).

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<table>
<thead>
<tr>
<th>No. of strains</th>
<th>Codon</th>
<th>Modal MIC (mg/l) Nalidixic acid</th>
<th>Pefloxacin</th>
<th>Ciprofloxacin</th>
<th>Moxifloxacin</th>
<th>Gatifloxacin</th>
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<td>Ciprofloxacin-susceptible (Cip-S) strains (MIC ≤ 0.5 mg/l))</td>
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<td>Thr</td>
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<td>0.5</td>
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<tr>
<td>Ciprofloxacin-resistant (Cip-R) strains (MIC &gt; 1 mg/l))</td>
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Abstract no.: 13.05
Evidence of Polymorphism in Helicobacter pylori GyrA at Codons 83 and 87 Leads to Distinguishing Subpopulations with Regard to the Susceptibility to Classic and New Fluoroquinolones

V. Cattoir, J. Nectoux, C. Lascols, C. Soussy and É. Cambau
Bacteriology, CHU Henri Mondor, APHP, University of Paris 12, Creteil, France

Fluoroquinolones (FQ) are alternate drugs for the treatment of Helicobacter pylori infection. Acquired resistance is known to result from gyrA mutation at the 87 and the 83 codons (numbering system in Escherichia coli) (85% and 15% of the strains described, respectively). MICs of five quinolones were determined by agar dilution for 128 clinical isolates of H. pylori and related to the sequence of the quinolone-resistance determining region (QRDR) in gyrA.

In the 113 (88%) Cip-S isolates, a polymorphism at 83 was observed with 17.7% of strains harboring Thr83 and 82.3% Asn83, the subpopulation harboring Thr83 being more susceptible to quinolones. In the 15 Cip-R isolates (12%), a 32- to 256-fold increase in MICs was associated to substitutions at 83 (60%) or 87 (40%). Determination of gyrA QRDR can predict for quinolone susceptibility, and may help in the therapeutical decision.

Abstract no.: 13.06
Prevalence of Helicobacter pylori Antibiotic Resistance in a Cohort of Italian Children with RAP

G. L. de’Angelis, L. G. Cavallo, B. Bizzarri, S. Liatopoulou, N. de’Angelis, V. Carappolo, A. Franzè and F. Di Mario
†Pediatrics Department, University of Parma, Parma, Italy. †Department of Clinical Science, Section of Gastroenterology, Parma, Italy. †Department of Gastroenterology and Endoscopic Unit, Az. Ospedaliera, Parma, Italy

Background. The primary Helicobacter pylori resistance to standard antibiotic is higher in children than adults because of an increased prescription of these drugs for respiratory tract infections. This could affect the efficacy of the standard antibiotic used in a proton pump inhibitor (PPI)-triple therapy.

Aim. To assess H. pylori antibiotic prevalence in H. pylori positive children with recurrent abdominal pain (RAP).

Methods. Fifty-six H. pylori-positive consecutive children with RAP were evaluated. All children were submitted to upper gastrointestinal endoscopy with biopsies. H. pylori antibiotic susceptibility testing on blood agar medium was performed.

Results. All the children (28 female, mean age: 9 ± 3 years, range 2–14) had the following endoscopic features: 44 patients with gastric hyperemia, 4 with nodular gastropathy, 4 with erosive duodenitis, and 3 with esophagitis. All the patients had H. pylori-related non-atrophic chronic gastritis, 10 of them had lymphoid hyperplasia.

Susceptibility testing showed the following prevalence resistance rate: Ampicillin 6%, ciprofloxacin 6%, metronidazole 15%, tetracycline 3%, and clarithromycin 50%. Three percent of the patients had both resistance to metronidazole and to clarithromycin and another 3% showed resistance to all the antibiotics.

Conclusions. H. pylori resistance to clarithromycin presented the highest rate in these Italian children. A different management of H. pylori infection in this cohort of patients may be necessary.

Abstract no.: 13.07

*Hospital de La Princesa, Madrid, Spain. †Hospital del Niño Jesús, Madrid, Spain

The aim of this study was to determine the resistance to clarithromycin and metronidazole in Helicobacter pylori clinical isolates obtained from pediatric patients (2000–2005).


The in vitro activity of clarithromycin and metronidazole was determined by an agar dilution method using Mueller–Hinton agar supplemented with 7% horse blood containing each antibiotic at twofold dilutions from 128 to 0.008 mg/l. Plates were inoculated with 10° cfu/drop and incubated at 37°C during 2 days in a CO2-increased atmosphere. MIC was determined as the lowest concentration of the drug inhibiting visible growth. Strains were considered resistant to clarithromycin when MIC > 0.5 mg/l, intermediate when MIC = 0.5 mg/l, and susceptible when MIC < 0.5 mg/l. Metronidazole MIC > 8 mg/l was considered resistant and ≤ 8 mg/l susceptible.

Results. Metronidazole resistance was 31.9% and clarithromycin resistance was 50%. Data according to the different periods are shown in the table.

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<tr>
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<tbody>
<tr>
<td>MTZ-R</td>
<td>8.2%</td>
<td>51.4%</td>
<td>36%</td>
</tr>
<tr>
<td>MTZ-S</td>
<td>91.2%</td>
<td>48.6%</td>
<td>64%</td>
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<tr>
<td>CLR-R</td>
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<td>51.4%</td>
<td>64%</td>
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</tr>
<tr>
<td>CLR-S</td>
<td>61.8%</td>
<td>40%</td>
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</table>

Conclusions. Resistance to clarithromycin was very high in the H. pylori strains obtained from Spanish pediatric patients. The resistance was higher in the last period studied.
Abstract no.: 13.08
Primary and Secondary Antibiotic Resistance Rates of Helicobacter pylori Strains Isolated from Korean Patients

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Since antibiotic resistance of Helicobacter pylori is the most common reason for failure in its eradication, we tested the primary and secondary antibiotic resistance rates of Helicobacter pylori strains isolated from Korean patients. H. pylori strains were isolated from antral biopsies in 65 patients with no antibiotic therapy during the preceding 3 months, and in 67 patients who had already undergone proton pump inhibitor (PPI)-based triple therapy consisting of amoxicillin and clarithromycin for 1 week. Antibiotic susceptibility test was performed using agar dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Resistance breakpoints for amoxicillin, clarithromycin, metronidazole, tetracycline, ciprofloxacin, levofloxacin, and moxifloxacin were set at > 1.0 µg/ml.

Results. Overall primary resistance to amoxicillin, clarithromycin, metronidazole, tetracycline, azithromycin, ciprofloxacin, levofloxacin, and moxifloxacin was 18.5, 13.8, 66.2, 12.3, 32.3, 33.8, 21.5, and 21.5%, respectively. The rates of eradication were 96% for the clarithromycin-resistant strains, but none of the patients with clarithromycin-resistant strains could eradicate the bacteria. Secondary resistance rates in triple PPI-based treatment failure patients were: amoxicillin 26.9%, clarithromycin 85.1%, metronidazole 70.1%, tetracycline 0%, azithromycin 89.6%, ciprofloxacin 35.8%, levofloxacin 32.8%, and moxifloxacin 32.8%. Among the clarithromycin-resistant strains studied, more than 95% showed cross-resistance to azithromycin.

Conclusions. These results suggest that the primary treatment failure not only increased the resistance rates of amoxicillin, clarithromycin, and azithromycin, but also a high prevalence of clarithromycin-resistant strains may be associated with eradication failure.

Abstract no.: 13.09
Relevance of Clarithromycin Resistance in Clinical Practice to Cure Helicobacter pylori

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Aim of the study. To study the relevance of primary clarithromycin (C) resistance on cure rates and the emergence of C-resistance in case of treatment failure.

Method. During the last 10 years, nearly all consecutive patients attending the out-patients endoscopy clinic were systematically screened for H. pylori infection and tested for primary C and metronidazole (M) resistance. Most naïve patients received their eradication regimen after the endoscopy according to the result of the rapid biopsy urease test. Eradication regimens consisted in proton pump inhibitor (PPI)- or RBC-triple therapies for 7–12 days.

Post-TX assessment (OGD + B or 13C-urea breath test) was performed 4–10 weeks after therapy. Susceptibility testing to M and C was performed by disc diffusion method. In case of treatment failure documented by a positive UBT, the patients were offered to undergo another OGD to obtain biopsy specimens for post-TX antimicrobial susceptibility testing in order to guide rescue therapy.

Results. Results are reported in the next table. Post-TX antimicrobial susceptibility testing was available for 58/71 (82%) post-TX failures.

<table>
<thead>
<tr>
<th>Treatment regimen</th>
<th>Nb treated and Pre-TX</th>
<th>Hp cure rate (%)</th>
<th>Nb of T_fails</th>
<th>Acquired C-resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC14</td>
<td>19 C⁵, 0 C⁰</td>
<td>11/19 (58%)</td>
<td>8</td>
<td>3/8 (37%)</td>
</tr>
<tr>
<td>OAC7-8</td>
<td>245 C⁵, 0 C⁰</td>
<td>225/245 (92%)</td>
<td>20</td>
<td>10/17 (59%)</td>
</tr>
<tr>
<td>OAC12</td>
<td>17 C⁰, 0 C⁰</td>
<td>6/17 (35%)</td>
<td>11</td>
<td>0/1 (–)</td>
</tr>
<tr>
<td>LAC7-8</td>
<td>147 C⁵, 0 C⁰</td>
<td>135/147 (92%)</td>
<td>12</td>
<td>1/11 (9%)</td>
</tr>
<tr>
<td>LAC10</td>
<td>114 C⁵, 0 C⁰</td>
<td>111/114 (97%)</td>
<td>3</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>OCT8</td>
<td>93 M⁰, 0 C⁰</td>
<td>86/93 (97%)</td>
<td>7</td>
<td>0/4 (–)</td>
</tr>
<tr>
<td>RCM7</td>
<td>56 M⁰, 0 C⁰</td>
<td>54/56 (96%)</td>
<td>2</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>15 M⁰, 0 C⁰</td>
<td>9/15 (60%)</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 M⁰, 0 C⁰</td>
<td>8/15 (53%)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 M⁰, 0 C⁰</td>
<td>0/1 (–)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 M⁰, 0 C⁰</td>
<td>54/56 (96%)</td>
<td>2</td>
<td>1/2 (50%)</td>
<td></td>
</tr>
<tr>
<td>26 M⁰, 0 C⁰</td>
<td>20/26 (80%)</td>
<td>6</td>
<td>1/4 (25%)</td>
<td></td>
</tr>
<tr>
<td>20 M⁰, 0 C⁰</td>
<td>16/20 (80%)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 M⁰, 0 C⁰</td>
<td>2/14 (14%)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion. Primary resistance to clarithromycin affects negatively the cure rate of PPI- and RBC-based triple therapies by, respectively, 35–60% and 15–40%. Acquired resistance to C was observed in 10–60% and 25–50% of treatment failures after PPI- or RBC-triple therapies.

Abstract no.: 13.10
Relationship Between Multidrug Resistance and Outer Membrane Proteins in Helicobacter pylori Strains Isolated from Children

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Background. In Helicobacter pylori, the contribution of outer membrane proteins (OMPs) to antibiotic resistance and or multidrug resistance is not well established.

Objective. To evaluate the relationship between multidrug resistance to amoxicillin, ampicillin, and tetracycline and OMPs profile in pediatric H. pylori strains.

Materials and Methods. Sixteen H. pylori strains isolated from children were characterized with respect to the antibiotic resistance pattern to amoxicillin (AMX), ampicillin (AMP), and tetracycline (TET), using disc diffusion and screening agar methods. Outer membrane proteins were extracted from envelopes of sonicated cells by a sarcosyl method and characterized using SDS-PAGE.
Abstract no.: 13.11

In Vitro Activity of Fluoroquinolones and Rifampicin in Metronidazole and Clarithromycin-Susceptible or -Resistant Helicobacter pylori Spanish Clinical Isolates

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Objective. The aim of our study was to determine the in vitro activity of levofloxacin, ciprofloxacin, and rifampicin in metronidazole- and clarithromycin-resistant and -susceptible Helicobacter pylori strains.

Material and Methods. Thirty-eight isolates of H. pylori were obtained following standard methodology from biopsies of dyspeptic patients. In vitro activity was determined by E-test using 5% sheep blood agar and incubated at 37 °C during 3–5 days in a CO2 atmosphere. MIC was determined as the point of complete inhibition of growth. Breakpoint of the National Committee for Clinical Laboratory Standards (NCCLS) for other microorganisms were considered: susceptible if MIC < 1 mg/l for levofloxacin, ciprofloxacin, and rifampicin; and resistant if MIC ≥ 4 mg/l for rifampicin.

Results. Forty-three percent of the strains were resistant to metronidazole and 51% to clarithromycin. MIC90 and range (mg/l) was: 0.064, 0.125, and 0.012–0.25 for levofloxacin; 0.064, 0.19, and 0.006–0.25 for ciprofloxacin; and 0.75, 1.5, and < 0.002–4 for rifampicin. All the strains were susceptible to fluoroquinolones. For rifampicin, 81.5% of strains were susceptible, 16% intermediate, and 5.2% resistant.

Conclusions. The fluoroquinolones tested showed an excellent in vitro activity against H. pylori, despite the high resistance rate to metronidazole and clarithromycin. A high percentage was sensible to rifampicin but in vitro susceptibility test should be performed before the use in clinical practice.
**Abstract no.: I4.01**

**CD4+ Cells but not Mast Cells are Critical Players in Immune Responses Leading to the Eradication of Gastric *Helicobacter* Infection in the IL-10−/− Mice**

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Compared to wild-type mice, IL-10−/− mice clear has natural *H. felis* infection. We previously showed that the *H. felis* infection clearance in wild-type mice vaccinated intranasally with urease+ cholera toxin infection is dependent on the presence of both CD4+ cells and mast cells in vaccinated mice. In this study, we tested whether the immune responses leading to the *Helicobacter* clearance in IL-10−/− mice depend also on CD4+ cells and mast cells. We depleted CD4+ cells from *H. felis* infected IL-10−/− mice. The depletion of CD4+ cells prevented the *H. felis* eradication, demonstrating that the CD4+ cells are critical players in the bacterial eradication of IL-10−/− mice.

To look for a role of mast cells, we constructed mast cell-deficient IL-10−/− mice: the IL-10−/− Wv/W double mutant mice. Surprisingly, 2 weeks after *H. felis* infection, both IL-10−/− mice and IL-10−/− Wv/W double mutant eradicated *H. felis* infection. This result shows that the natural *Helicobacter* eradication of IL-10−/− mice is not dependent on mast cells.

Altogether, we showed that the immune clearance of *Helicobacter* from the stomach in vaccinated wild-type and IL-10−/− mice both depends on the CD4+ cell population. However, we found that the mast cells are not critical players in immune responses leading to *Helicobacter* eradication in IL-10−/− mice. These results showed the complexity of the immune responses leading to *Helicobacter* eradication from the stomach, and highlighted the need to complete our understanding of the immune mechanisms leading to *Helicobacter* clearance from the stomach.

**Abstract no.: I4.02**

**Immunization of Mice with BabA Protects Against *Helicobacter pylori* Infection**

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**Objectives.**  Investigation of the protection against *Helicobacter pylori* infection in mice intranasally immunized with purified BabA and CTA1-DD.

**Methods.** Both prophylactic and therapeutic studies were performed with 10–12 male *Leb* transgenic mice in each group. In the prophylactic study the mice were immunized intranasally once a week for 4 weeks with 10 g purified BabA and 1 µg CTA1-DD. Four weeks post-immunization they were challenged with 1.5 × 10^9 cfu/ml of a low passage streptomycin-resistant J99 *H. pylori* strain (*J99StrR*) cultured for 24 hours, two times a week for 2 weeks. After 4 weeks, the mice were sacrificed, the stomachs were collected, and the amount of *H. pylori* *J99StrR* was determined by culturing.

In the therapeutic study, 10–12 male *Leb* transgenic mice in each group were infected with *J99StrR* as in the prophylactic study. After 4 weeks the mice were immunized intranasally as in the prophylactic study and after 4 weeks, the study was terminated. Stomachs were collected and the amount of *H. pylori* J99*StrR* was determined by culturing.

**Blood samples were taken before and during the studies to follow the immune response and analyze the titers of antibodies specific for BabA. Gastric secretions were collected from the stomachs to investigate the sIgA titters.**

**Results.** Both the prophylactic and therapeutic intranasal immunizations with BabA and CTA1-DD gave a significant protection against *H. pylori* J99*StrR* infection as compared to the controls. The titers of sIgA in the gastric secretions were significantly higher in the immunized groups as compared to the control groups.

**Abstract no.: I4.03**

**Effect of Rebamipide on Colonic Epithelial Barrier and Immune Response in IL-10-Deficient Mice Infected with *Helicobacter hepaticus***

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**Background.** The aim was to study the effect of rebamipide, a mucosal protective agent, on the colonic epithelial barrier and the immune responses in the experimental model of colitis in interleukin-10 (IL-10)-deficient C57BL/6 mice infected with *Helicobacter hepaticus*.

**Methods.** Four groups of mice were studied: control, infected with *H. hepaticus*, infected with *H. hepaticus* and treated daily for 9 weeks with rebamipide enema (300 µg/day), infected with *H. hepaticus* and treated with placebo enema. At sacrifice, mesenteric lymph node (MLN) cells reactivity (proliferation and cytokine secretion) was studied in vitro in basal conditions and after stimulation with *Escherichia coli* or *H. hepaticus* extract. Colonic samples were used for histology and assessment of intestinal permeability in Ussing chambers with evaluation of electrical resistance (R), and of horseradish peroxidase (HRP) and mannitol (Jman) fluxes.

**Results.** A very mild colitis (score < 2) was observed in all mice without difference among the groups. Reinforcement of colonic barrier (increase of R, decrease of Jman, and HRP transcytosis), was observed in rebamipide-treated mice as compared to other groups (p < .05). Basal- and *H. hepaticus*-stimulated proliferation of MLN cells, as well as basal- and *E. coli*- and *H. hepaticus*-stimulated IFNγ and IL-12 secretion, were increased in rebamipide-treated group as compared to other groups.

**Conclusions.** In this slight inflammatory conditions, rectal rebamipide reinforced integrity of the colonic barrier and revealed a Th1 stimulatory effect on MLN cells. This beneficial local effect
of rebamipide together with its Th1 immuno-stimulatory properties, may be helpful in the management of Th2 inflammatory bowel diseases like ulcerative colitis.

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**Abstract no.: 14.04**  
High Cholesterol/Cholic Acid/Dairy Fat (Lithogenic) Diet Exacerbates Helicobacter pylori Gastritis in the Glandular and Non glandular Stomach of C57L Mice

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To examine whether cholesterol plays a role in Helicobacter pylori-induced gastritis, 4-week-old male C57L mice were infected with H. pylori strain SS1 (n = 15) and another group was sham dosed (n = 10). Mice (n = 10 infected and 10 uninfected) were either fed a lithogenic diet (1% cholesterol, 0.5% cholic acid) for 8 weeks beginning at 8 weeks or fed a standard mouse chow (n = 5). Infected mice developed gastric disease more rapidly and severely than is typical in other permissive strains. Glandular stomachs of H. pylori-infected mice fed either diet demonstrated moderate lymphocytic inflammation often extending into the submucosa with marked glandular inflammation. Uninfected mice rarely had histological signs of inflammation on chow or lithogenic diet had been fed a lithogenic diet (p < 0.2, 0.7, 0.8 ± 0.6 and 0.75 ± 0.8, respectively). Glandular atrophy and mucous metaplasia was pronounced in infected mice; it was common to see complete loss of chief cells and > 50% loss of parietal cells. Only mice that were both infected and fed a lithogenic diet demonstrated a significantly higher metaplasia score than uninfected mice fed a similar diet (p < 0.1, 1.65 ± 0.5 and 0.5 ± 0.8, respectively). Animals that were fed the lithogenic diet developed hyperkeratosis and inflammation of the squamous stomach which lesions were exacerbated by H. pylori infection. The squamous stomach of the mouse is histologically similar to the esophagus of humans. Perhaps this model could be used to study the ability of H. pylori to contribute to esophageal diseases.

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**Abstract no.: 14.05**  
Protective Effect of 17-β-Estradiol and Progesterone on G Cells in Helicobacter pylori-Infected Female Mongolian Gerbils

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Estradiol (E2) and progesterone (P4) have been suggested as regulators of gastric mucosa.  
**Aim:** To evaluate the effect of E2 and P4 on the number of antral mucosa gastrin-producing cells (GC) during early and subacute infection with Helicobacter pylori.

**Methods:** Thirty-four 15-week-old intact or ovariectomized (OVH) female Mongolian gerbils were allocated to six groups: CTL (intact + H. pylori), VEH (OVH + H. pylori + vehicle), E2L (OVH + H. pylori + E2 50 µg/60 days), E2H (OVH + H. pylori + E2 250 µg/60 days), P4L (OVH + H. pylori + P4 15 mg/60 days) and P4H (OVH + H. pylori + P4 50 mg/60 days). Intragastrical H. pylori infection was performed with 1 x 10^6 UFC SS1 strain. One week post-infection (WPI), E2 and P4 were supplemented with subcutaneous 60-day release pellets. Gerbils were euthanized after 6 and 18 WPI. Gastric tissue was obtained and immunohistochemistry for gastrin performed. GC were counted in 10 fields at 40x in antrum.  
**Results:** At 6 WPI, GC were similar in groups. P4L showed the highest (nonsignificant), whereas P4H showed the lowest (significant versus VEH) GC. At 18 WPI, there was a significant GC decrease in CTL and VEH, the latter presenting the lowest value. E2H presented a significant increase of GC. P4L presented the highest GC. E2H almost achieved the same GC number than P4L, E2L, P4L, and P4H were not different in both study periods.  
**Comments:** E2 administration prevents the decrease of GC and P4 increases the GC only at low dose. These results suggest E2 and P4 effects on G cells and H. pylori-induced gastritis (previously evaluated) are related with dose and time of hormonal treatment.

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**Abstract no.: 14.06**  
Interaction of Helicobacter pylori Infection and IL-4 Genetics in the Immunopathogenesis of Atopy

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*University of Helsinki, Helsinki, Finland, †University of Tampere, Tampere, Finland

**Background:** Both genetic and environmental factors, e.g., early childhood infections, have a role in the pathogenesis of atopic diseases.

**Aim:** To examine simultaneously the strength and possible interactions of two known factors, Helicobacter pylori infection and IL-4 genetics, on the risk of atopy and asthma.

**Methods:** H. pylori infection was verified by detecting anti-H. pylori IgG antibodies (Pyloriset EIA-G III, Orion Diagnostica, Espoo, Finland) in 245 adult asthmatics and 405 non-asthmatic controls presenting a population-based case-control study. IL-4–590 genotyping was performed by polymerase chain reaction (PCR)-RFLP method. At least one positive skin prick tests (SPT) was used as an indicator of atopy.

**Results:** A significant negative association was seen between the presence of H. pylori antibodies and SPT positivity in both asthmatics and controls (p = 0.002 and p = 0.025, respectively) but the effect of IL-4 polymorphism (SNP–590C/T) was nonsignificant in both groups (p = 0.71 and p = 0.72, respectively). However, IL-4 genetics had an effect on susceptibility to H. pylori; asthmatics carrying the IL-4–590 allele T had a diminished risk to be H. pylori infected (OR 0.485 95%CI 0.287–0.819). This effect was not seen in controls. Logistic regression analysis indicated that H. pylori and IL-4 effects on atopy risk are not interdependent.

**Conclusions:** The effect of H. pylori infection on atopy risk was stronger than that of IL-4 genetics. There was no interaction between these factors on the pathogenesis of atopy suggesting that these factors have distinct immunopathogenetic mechanisms. However, the genetic effect may modify the role of infective agents via susceptibility to disease.
CpG oligodeoxynucleotides (ODNs) are potent Th1-polarizing adjuvants and activate the innate immune system to produce pro-inflammatory cytokines. In contrast CpG ODN (a C has been replaced by G) can inhibit the activation of Th1 T cells in mice. We examined the role of both ODNs in the Helicobacter pylori-infection model Mongolian gerbil. The animals received four weekly intraperitoneal injections with CpG or GpG ODNs, starting 3 days before infection with H. pylori. After 4 weeks the stomachs were removed and examined histologically. Cytokine levels were evaluated with quantitative polymerase chain reaction (PCR) (TaqMan). All infected gerbils showed inflammation in the antrum, ranging from mild gastritis to the development of large lymph follicles. Significantly elevated expression levels could be observed in all infected gerbils compared to uninfected controls for IFN-gamma, IL-1 beta, IL-6, and iNOS. There were no significant differences between CpG and GpG ODN treated groups, all expression levels were similarly elevated. TNF was only increased in infected animals who received ODNs. No changes were revealed for IL-10, IL-12p35, and IL-12p40 expression. In situ hybridization showed elevated production of IFN-gamma and IL-6 in all infected gerbils. In contrast to TaqMan analysis, the expression of IL-10 was heavily increased in infected animals. The production of IFN-gamma and IL-10 was slightly higher in the group who received CpG instead of GpG ODN. Although immunostimulatory CpG- and immunosuppressive GpG-effects could be observed in several mice models, they had no impact on the expression levels of pro-inflammatory cytokines in H. pylori infection of Mongolian gerbils.

Immunization would be a welcome complementation to fight Helicobacter pylori infection. Preclinical studies have demonstrated the feasibility of vaccination. What may be learned from these preclinical data for the development of a human vaccine will be summarized. In addition, the global analysis of immunogenic proteins, i.e., the immunoproteome, will be presented and how this data set has been used to define criteria that not only confirmed all experimentally tested vaccine antigens but led to the identification of new vaccine antigens such as HP0231 and HP0410. The translation of these results into clinical trials combining vaccination with the recently developed human challenge model will be presented. We expressed H. pylori urease or HP231 in the common live typhoid vaccine Ty21a. This vaccine was promising because three of nine vaccinees in an initial trial cleared H. pylori after experimental infection. The effect on H. pylori infection correlated with increased vaccine antigen-specific T-helper cell responses, suggesting that, as shown before in mice, T-helper cells may be required for protection. Transcriptional profiling of the mucosa suggests that experimental infection recapitulates many features of natural chronic infection and thus vaccination may also be feasible against natural infection. Field studies would greatly benefit from a tractable biomarker correlated with protection and a strategy how such a marker may be identified will be discussed.
Other Helicobacters

Abstract no.: 15.01*
Identification of Helicobacter spp. by Multiple PCR-DGGE Analysis of the 16S rDNA

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Background. The number of species in the genus Helicobacter is rapidly increasing, to include more than 26 species today that colonize the gastrointestinal tract of humans and animals, which are difficult to diagnose because of the close relatedness of these species. The PCR–DGGE (polymerase chain reaction–denaturing gradient gel electrophoresis) technique was developed by us to identify of Helicobacter gut colonization; however, some closely related Helicobacter spp. were difficult to separate. The aim of this study was to increase the diagnostic efficiency of PCR–DGGE by analyzing three variable regions of the 16S rDNA.

Methods. DNA was extracted from 40 Helicobacter strains. Amplification of the 16S rDNA, was performed using Helicobacter genus specific primers. The PCR product was used as a template to amplify three fragments of the 16S rDNA that cover the V1-2, V3, and V6-7 regions. Amplified PCR products were analyzed by DGGE.

Results. DGGE analysis of the three regions showed mobility patterns that were discriminatory for almost all Helicobacter spp. including closely related species such as H. ganmani and H. rodentium that were difficult to separate by other methods.

Conclusions. The PCR–DGGE technique has proven to be an easy, inexpensive, and efficient tool to identify Helicobacter spp. and to detect gut and stomach colonization by more than one Helicobacter spp., without the need for species-specific PCR assays. In addition, the diagnostic efficiency of this technique was increased by analysis of multiple regions of the 16S rDNA allowing discrimination of almost all Helicobacter species.

Abstract no.: 15.02*
PerR is a Regulator of Oxidative Stress Defense in Helicobacter hepaticus

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Infection with Helicobacter hepaticus is associated with an active cellular immune response accompanied by the production of oxygen radicals. Iron also potentiates the formation of reactive oxygen species. Therefore, pathogens are forced to maintain intracellular iron homeostasis and cope with oxidative stresses. The H. hepaticus genome sequence contains genes encoding homologs of bacterial oxidative stress defense proteins, and also a homolog of the iron-responsive regulatory protein PerR, which mediates regulation of peroxide stress defense in several other bacteria. In this study we have investigated the expression and regulation of oxidative stress defense systems of H. hepaticus.

Growth of H. hepaticus in iron-restricted conditions resulted in altered expression levels of six proteins. Three of these proteins displayed iron-repressed expression, whereas three other proteins displayed iron-induced expression. Two of the iron-repressed proteins were identified as AhpC (25 kDa) and KatA (55 kDa). Both proteins are involved in the degradation of peroxide compounds, and are known to contribute to the bacterial oxidative stress defense. Mutation of the perR gene resulted in high-level, iron-independent, expression of both AhpC and KatA.

Conclusion. In H. hepaticus, iron metabolism and oxidative stress defense are intimately connected via the PerR regulatory protein. This regulatory pattern resembles that seen in the enteric pathogen Campylobacter jejuni, but contrasts with the regulatory patterns observed in the human gastric pathogen H. pylori. Therefore, iron-dependent regulation of peroxide stress defense may be an adaptation advantageous for enteric colonization.

Abstract no.: 15.03*
Detection of Enterohepatic Helicobacter Species and Wollinella succinogenes in Biopsy Specimens from Children with Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS)

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Introduction. Enterohepatic Helicobacter species can initiate IBD in immunodeficient mice. Attempts to detect such organisms in human IBD have resulted in divergent results.

Aim. To determine the presence and spatial distribution of Helicobacteraceae (Helicobacter and Wollinella), in colonic biopsies from children undergoing colonoscopy.

Methods. Three biopsies were collected from 18 children, diagnosed with IBD [n = 12], IBS [n = 4] and 2 controls (without symptoms or inflammation). DNA from one biopsy was used for Helicobacteraceae-specific polymerase chain reaction (PCR) and subsequent sequencing; the second to examine the spatial distribution of Helicobacteraceae using specific rRNA fluorescent in situ hybridization (FISH) and for PAS staining to assess the depth of the mucus layer and the third for histology.

Results. Helicobacteraceae were detected in 12/12 children with IBD (11 CD, 1 UC), 4/4 with IBS and 0/2 controls. Sequencing of PCR products showed three to be closely related to H. hepaticus, six to H. trogontum, five to W. succinogenes, and two to an uncultured Helicobacter species previously detected in UC patients. PAS staining showed children with IBD to have a significantly thinner (p < .001) mucus layer (0.84 ± 0.08 µm) than children with IBS (3.30 ± 0.22 µm). Using FISH, a positive Helicobacteraceae signal was detected in the mucus layer of 3/16 PCR-positive children.

Conclusions. This is the first report of the detection of Wollinella species in the human gastrointestinal tract (GIT) and of the
Abstract no.: 15.04
Helicobacter hepaticus Infection in the TCR alpha− and TCR beta− Mouse Models of IBD is Associated with Increased Colonization of Altered Schaedler Flora in the Inflamed Colon

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Altered Schaedler flora (ASF), consisting of eight anaerobes recently speciated by our laboratory using 16S ribosomal RNA analysis, have historically been used to colonize germ-free mice to establish normal gut physiology. Little is known about potential interaction between ASF colonization, bacterial pathogens such as Helicobacter hepaticus, and intestinal disease in susceptible mice. H. hepaticus-infected TCRα− and TCRβ− mice develop typhlocolitis that emulates the dysregulated inflammatory response of human inflammatory bowel disease (IBD) patients to their intestinal flora. We used qPCR (quantitative polymerase chain reaction) based on 16S rRNA sequences to measure the levels of H. hepaticus, ASF361, ASF492, and ASF500 were also higher in β−/− mice compared to controls (p < .008). These results suggest that H. hepaticus colonization and/or the associated typhlocolitis favors increased colonization of select species of ASF in TCR knockout mice and warrants further study given the interest in probiotics for human IBD therapy.

Abstract no.: 15.05*
Detection of Helicobacter Species in Archival Human UC Colorectal Tissue by Fluorescent In Situ Hybridization (FISH)

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Background. Data are conflicting regarding the role of Helicobacter species in human IBD. This in part is due to the techniques used and to lack of definition of disease phenotypes. No studies have investigated tissue obtained at de novo presentation before therapy has been implemented.

Aim. To investigate archival paraffin-embedded tissue from patients with confirmed ulcerative colitis (UC) for the presence of Helicobacter species.

Materials and Methods. Fifty-seven UC patients (38 relapsing, 19 de novo) and 12 healthy controls had archival biopsies investigated. UC patient biopsies were taken from throughout the colon. Healthy controls were asymptomatic subjects undergoing colonoscopy and found to have macroscopically and histologically normal tissue. FISH assays were designed to differentiate H. pylori from non-pylori Helicobacter species. Sections were analyzed in triplicate.

Results. Eleven of 57 UC patients (19%) were exclusively positive for non-pylori Helicobacter, whereas only one was H. pylori-positive. None of the 12 controls had any Helicobacter (p < .001 versus UC). Helicobacter were predominantly detected in the left colon, especially in left-sided colitis cases. One of 19 de novo patients had evidence of non-pylori Helicobacter. Positive relapsing patients were almost always negative at first presentation, subsequently becoming positive upon relapse. Helicobacter were detected on the mucosa and in colonic crypts, in both inflamed and non-inflamed tissues.

Conclusions. Non-pylori Helicobacter species are commonly detected in UC patients, particularly in those with left-sided colitis. These species do not appear to play a role at disease onset but may be implicated in subsequent relapses.

Abstract no.: 15.06
Detection of Helicobacter Species in Liver Tissue of Polish Patients with Chronic Liver Diseases by PCR–DGGE and Sequence Analysis

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Background. DNA of number of Helicobacter species that have been isolated from the stomach, intestinal tract, and liver of a variety of animals, have been detected in human bile and liver samples. The aim of this study was to determine the possible presence of Helicobacter species in the liver tissue samples of patients with chronic liver diseases of different etiology.

Materials and Methods. Ninety-seven Polish patients (46 women, 51 men), aged 18–66 years (mean 41 ± 1) were admitted to the Hospital for Infectious Diseases in Gdansk, Poland because of a chronic liver disease. Liver biopsy specimens were examined for the presence of Helicobacter species by a genus-specific polymerase chain reaction (PCR) assay. PCR products of positive samples were subsequently characterized by denaturing gradient gel electrophoresis (DGGE) and DNA-sequencing.

Results. Using Helicobacter genus-specific PCR assay, Helicobacter DNA was detected in 69/97 (68%) of liver tissue samples. Among them, 45/70 (55%) positive samples were detected in patients chronically infected with hepatotropic viruses (HBV or HCV), 9/14 (64%) in patients with toxic liver damages and 9/13 (85%) in patients with autoimmune liver diseases. No correlation was found
between the frequency of Helicobacter PCR-positive results and etiology of liver diseases and signs of chronic liver inflammation. **Conclusions.** The presence of Helicobacter species DNA in liver tissue may suggest a possible role of Helicobacter infection in human chronic liver diseases.

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**Abstract no.: 15.07**

**Prevalence of Novel Helicobacter in Feces of Free-living Canada Geese in the Greater Boston Area**

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Canada geese have increased substantially in the past decade and have become a nuisance in some urban areas. Because of their close contact with humans in parks and areas adjacent to surface waterways, contact with their feces poses a zoonotic risk. A total of 97 geese from 10 separate geographical locales in the greater Boston area had their feces sampled for detection of Helicobacter spp. Positive identification of Helicobacter spp. based on 16S rRNA genus-specific helicobacter primers were noted in 39 of 97 (32%) DNA fecal extracts. Twenty-seven of these geese had helicobacters isolated (27.8%) from their feces. A urease-positive novel Helicobacter sp. C/B52 based on phenotypic and 16S rRNA analysis previously isolated from terns on the Massachusetts Atlantic shoreline was isolated from 21 geese from seven different gaggles. A second novel urease-negative Helicobacter sp. was identified in six geese. Four geese had both novel Helicobacter spp. using the same phenotypic and molecular characterization cultured from their feces. Whether these novel helicobacters pose a zoonotic risk, similar to other enteric helicobacters (e.g., *H. canadensis* previously isolated from diarrheic humans and from geese in Europe) will require further studies.

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**Abstract no.: 15.08**

**Identification of “Candidatus H. heilmannii” in Cats, Dogs, and Humans**

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“Candidatus Helicobacter helimannii” (CHh) is an uncultured spiral organism that has recently been identified in the stomach of wild carnivores and one human being [1]. In a previous study, a multiplex polymerase chain reaction (PCR) [2] was used to detect and differentiate *H. felis*, *H. bizzozeronii*, and *H. salomonis* in canine, feline, and human gastric samples, and a new genotype, designated HLO135, was identified [3]. It was the aim of the present study to determine the prevalence of CHh in cats, dogs, and humans and to study its phylogenetic relatedness with HLO135. Gastric canine (110) and feline (43) samples were therefore subjected to a CHh-specific PCR [1]. Cloning and sequencing analysis of the 16S rRNA and part of the urease gene were performed on a HLO135-positive feline sample. Thirty-four dogs and 29 cats tested positive for CHh. These animals were previously found to harbor HLO135 [2]. Sequencing the 16S rRNA gene and part of the ureB gene of HLO135 revealed 99% and 96% similarity with CHh, respectively, indicating that type HLO135 belongs to the same species.

Of 123 human gastric biopsies harboring spiral organisms as seen microscopically, nine were positive for HLO135 in the multiplex PCR, suggesting infection with CHh. The present study demonstrates the presence of CHh in cats, dogs, and humans. Additionally, these results prove that the previously developed multiplex PCR also enables us to identify CHh.

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**Abstract no.: 15.09**

**Spiral Organisms in the Stomach of Domestic Animals: True Zoonosis?**

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Non-Helicobacter pylori spiral organisms (NHPSO) have occasionally been associated with gastric disease in humans. There are indications that animals may act as a source of infection for at least some of these bacteria. In an attempt to obtain better insights in the epidemiology of these infections, spiral organisms in the gastric mucosa of cats (43), dogs (110), pet rabbits (23), and humans (123) were identified up to the species level. All samples were subjected to a multiplex polymerase chain reaction (PCR), enabling the identification of *H. felis*, *H. salomonis*, *H. bizzozeronii*, “Candidatus *H. suis*”, and “Candidatus *H. heilmannii*.”

*H. felis* and “Candidatus *H. heilmannii*” were identified as the most prevalent *Helicobacter* species in cats (62.8% and 67.4%, respectively), whereas *H. bizzozeronii* presented the main spiral organism in the canine stomach (70%). Three pet rabbits were found to harbor *H. felis* in their stomach. *H. salomonis* was detected in the stomach of 1 rabbit, 1 cat, and 10 dogs. In human gastric samples, *H. felis*, *H. salomonis*, *H. bizzozeronii*, “Candidatus *H. heilmannii*” and “Candidatus *H. suis*” were identified in 14.6%, 21.1%, 4.1%, 8.1%, and 36.6% of the samples, respectively.

These results indicate that pigs and cats most probably constitute a more important source of NHPSO infections for humans than dogs. More research is required to determine the source of *H. salomonis* infections for humans and to estimate the role of pet rabbits in the epidemiology of NHPSO infections.

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Abstract no.: 15.10

Serum Antibodies to Helicobacter hepaticus, Helicobacter bilis, and Helicobacter pullorum in Patients with Chronic Liver Diseases and in a Population with High Prevalence of Helicobacter pylori Infection


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Objectives. Enteric Helicobacter species might be a risk factor for chronic liver and biliary tract diseases.

Aim. To analyze serum antibody levels to three enteric Helicobacter species in patients with various chronic liver diseases (CLD); to compare them with corresponding parameters for population with high prevalence of Helicobacter pylori infection including blood donors and pediatric patients; to explore possible association of increased serum antibody levels to enteric Helicobacter species with chronic liver diseases.

Methods. Sera of 90 patients with various CLDs, 121 Estonian adult persons, 68 blood donors, and 50 consecutive pediatric patients were analyzed. Sera tested previously for IgG to Helicobacter species in patients with various chronic liver diseases were analyzed for IgG to Helicobacter species in patients with various chronic liver diseases (CLD); to compare them with corresponding parameters for population with high prevalence of Helicobacter pylori infection including blood donors and pediatric patients; to explore possible association of increased serum antibody levels to enteric Helicobacter species with chronic liver diseases.

Results. Liver patients showed a significantly higher prevalence of IgG to H. hepaticus, H. bilis, and H. pullorum. ELISA-positive sera were analyzed by immunoblot. To remove cross-reactive antibodies to H. pylori, sera were preabsorbed with a lysate of H. pylori cells.

Conclusion. Use of immunoblot for defining antibody response to the specific proteins of each Helicobacter spp. seems to be the most promising way to establish serodiagnosis of infections with these pathogens.

Abstract no.: 15.11

Detection of Gastric Helicobacter-Like Organisms (GHLOs) in Domestic and Stray Cats in Tehran Province via PCR

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The presence of spiral-shaped bacteria in the feline stomach has been recognized for over a century, but the identities and degrees of prevalence of such organisms in cats are still poorly documented. On the other hand, reports of domestic animal-to-human transmission and isolation of Helicobacter pylori from domestic cats have led to speculation that cats and dogs may serve as a reservoir for human infection. The zoonotic potential of Helicobacter heilmannii and feli has been the subject of considerable interest as well.

In order to investigate the presence and prevalence of gastric Helicobacter spp., feline gastric samples underwent rapid urease test (RUT), histopathology examinations, and genus strain-specific polymerase chain reaction (PCR). According to 16s rRNA-specific PCR, prevalence of rate of GHLO infection in domestic and stray cats was estimated as 100% and 56.7%, respectively. The most commonly found GHLOs in cats are H. feli and H. Heilmannii.

According to our results H. Heilmannii is the most prevalent infecting strain. No signs of H. pylori infection were detected in the studied cats. Prevalence of GHLO infection was significantly different between domestic and stray cats (81% versus 43.3%, respectively; p < .005). The comparison between different diagnostic tests revealed that Giemsa staining is the best method for initial screening of GHLO infection. Furthermore, gastric body is the best site for feline biopsy sampling. Unlike in humans, there was no correlation found between presence and degree of Helicobacter colonization and the development and severity of chronic gastritis in cats (p > .05).

Abstract no.: 15.12

PCR–DGGE Detection of Helicobacter Species in Human Colon Biopsies of Patients with Inflammatory Bowel Disease

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Background. Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract that exists in two forms: Crohn’s disease (CD) and ulcerative colitis (UC). As a microbial factor, some of Helicobacter and other bacterial species may play a role in IBD. The aim of this study was to determine the presence of Helicobacter species in colon biopsies of patients with IBD.

Methods. Forty-eight colonoscopic biopsies were collected from 24 patients undergoing colonoscopy as follows: 6 from UC, 3 from CD, 12 from other colon diseases, and 3 from patient’s colon of normal endoscopic picture. Samples were examined by using Helicobacter genus-specific PCR–DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) and DNA sequencing.

Results. Helicobacter species were detected in 11% of the patients with IBD, in 50% of the patients with other colon diseases, and in 33% of the patients with normal colon endoscopy. PCR–DGGE analysis of Lactobacillus DNA concentration was decreased in patients’ colon with IBD compared to other groups. Desulfovibrio DNA was detected in 50% of patients with UC and in none of the patients with CD.

Conclusions. The high PCR–DGGE detection of Helicobacter DNA in other colon diseases than in IBD suggests that Helicobacter species may not have a role at least in the late stage of this disease. A possible link of lactic acid bacteria and Desulfovibrio was found to IBD.

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Abstract no.: 15.13

Antibodies to Bile-Tolerant Non-pylori Helicobacter in Patients with Chronic Liver Diseases

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Aim. To study the seroprevalence of enterohepatic Helicobacter species in patients with chronic liver diseases.

Methods. Sera of 32 patients with primary biliary cirrhosis (12 cases), chronic active hepatitis (4), primary sclerosing cholangitis (1), alcoholic cirrhosis (2), cryptogenic cirrhosis (1), fatty liver (3), focal nodular hyperplasia (1), cholestatic hepatitis (4), hepatitis of unknown etiology (3), and normal liver status (1) (earlier elevated enzyme levels were slightly or moderately elevated (averages, ALT 19 patients, AST 50, alkaline phosphatase 366).

Seropositivity of the 32 patients to Helicobacter pylori, enteric Helicobacters spp., and non-Helicobacter species were uncommon (22% of EIA results were positive/borderline EIA results were further analyzed by immunoblot (IB). Sera were preabsorbed with H. pylori before EIA and IB analyses.

Results. Seropositivity of the 32 patients to Helicobacter spp. by EIA and IB.

Table 1 Helicobacter antibodies demonstrated by IB in IBD patients

<table>
<thead>
<tr>
<th>Patients with</th>
<th>H. pylori</th>
<th>H. bilis</th>
<th>H. hepaticus</th>
<th>H. pullorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn’s disease (n = 61)</td>
<td>13 (21%)/12</td>
<td>1 (1.6%)</td>
<td>1 (1.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Ulcerative colitis (n = 73)</td>
<td>17 (23%)/15</td>
<td>2 (2.7%)</td>
<td>2 (2.7%)</td>
<td>0</td>
</tr>
</tbody>
</table>

The seroprevalence of H. pylori was 22%. Of the H. pylori-positive, 90% were also CagA+. Two (3%) patients with Crohn’s disease and four (5%) with ulcerative colitis had antibodies to enterohelial Helicobacter species. Both of these Crohn’s disease patients had acute inflammatory findings in the ileum, whereas all the four seropositive ulcerative colitis patients had inactive bowel disease. Two of the six patients (one with H. bilis and the other with H. hepaticus antibodies) had no evidence of H. pylori infection.

In conclusion, infection with enteric non-pylori Helicobacter species is rarely detected in Finnish IBD patients. Our findings, however, do not exclude colonization/infection with these species at an earlier stage of IBD.

Abstract no.: 15.14

Detection of Helicobacter Antibodies in Patients with Inflammatory Bowel Disease

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The seroprevalence of enterohepatic Helicobacter species was studied in 61 patients with Crohn’s disease (median age 37.0 years, median duration of disease 6.3 years) and 73 patients with ulcerative colitis (median age 44.2 years, duration 11.4 years).

Abstract no.: 15.15

Isolation of Helicobacter spp. from Seawater, Plankton, and Oysters from Areas of the Caribbean Sea Subject to Fecal Contamination

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This study aimed at culturing Helicobacter species from a marine environment subject to fecal contamination. We collected oysters from a marine environment subject to fecal contamination. We collected oysters (Isognomus alatus and Crassostrea mangle), marine water filtrates, and marine plankton from a touristic coastal area of Venezuela. Detection was made using culture, Gel stained, transmission electron microscopy, and genus-specific polymerase chain reaction (PCR). Total and fecal coliforms and enterococci were measured using classical culture methods for the assessment of the microbiological quality of coastal recreational waters. We obtained cultures of Gram-negative curved and spiral rods, with a polar flagellum from marine water filtrates, plankton, and oysters (Isognomus alatus). We confirmed the identity of the cultures by PCR.

Our results show that viable Helicobacter species are present in marine areas subject to fecal contamination. To our knowledge, this is the first successful culture of Helicobacter species from the marine environment.
Abstract no.: 15.16
**Helicobacter cinaedi Bacteremia in a Previously Healthy Person with Cellulitis**

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**Helicobacter cinaedi** is an infrequent, but well-recognized course of gastroenteritis in immunosuppressed patients. Here we report a case of an extra-intestinal infection in a previously healthy 61-year-old heterosexual male.

The bacterium was cultured from blood twice within 1 week. Focus for the infection was most likely cellulitis on the lower right leg. The condition was at first considered to be erysipelas and treated with intravenous penicillin without any clinical response.

Five days after admission blood cultures were positive. The bacterium grew under aerobic conditions and was isolated on horse blood agar enriched with 5% yeast. Light microscopy and Gram staining identified a spiral organism. Electron microscopy of the isolate visualized bipolar flagella suggesting *Helicobacter cinaedi*. Partial DNA sequencing of the 16S rRNA gene established the species diagnosis. Fecal specimens were not available. Antibodies reacting against *H. pylori* were detectable in serum.

After the isolation of *H. cinaedi* and the antibiotic resistance tests performed treatment was changed to rifampicin for 2 weeks. At the end of treatment no *Helicobacter* were found in blood samples and the cellulitis had disappeared.

Source of infection is unknown; no contact to animals was reported. The only exposition to be account for was mice excrements, whereas cleaning the attic of his house.

Molecular bacterial identification methods have been proven beneficial in many settings. The fact that the patient did not respond to standard treatment regiments should always make one consider alternative etiology.

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Abstract no.: 15.17
**Bacterial Interaction Between Staphylococcus Strains and Helicobacter pylori Clinical Isolates**

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Objectives. 1, To study the inhibitory effect of 6 *Staphylococcus* strains against 10 *Helicobacter pylori* clinical isolates; 2, to determine whether this effect is caused by the microorganism itself or by something relapsed by it.

Methods. *H. pylori* strains were obtained from 10 gastric biopsies and processed by standard microbiological methodology. Six *Staphylococcus* strains were obtained from clinical specimens and identified by Gram stain and MicroScan (Dade Behring). To determine the effect of *Staphylococcus* on *H. pylori* we used the “drop” method. A blood agar plate was completely inoculated with *H. pylori* and 2 drops were deposited containing 10 µl of *Staphylococcus* after 0.5 MacFarland concentration and after 72 hours in BH1. One *Staphylococcus* isolate was selected for further studies. It was inoculated in 5 ml of BH1 overnight and the supernatant was collected by centrifugation (10 minutes at 13,000 g) and then filter-sterilized (0.45 µm pore size filter). It was also sonicated and filter-sterilized again. Plates were incubated under microaerobic atmosphere for 3–5 days.

Results. A *Staphylococcus epidermidis* strain showed an inhibitory effect against all the *H. pylori* tested. The inhibition zone was 27–36 mm depending on the concentration of the *Staphylococcus*. This effect was seen after concentration and sonication but not after filtration.

Conclusions. In our study *Staphylococcus epidermidis* is able to inhibit the growth of *Helicobacter pylori* in vitro. According to these data, the effect seems to be caused by the microorganism itself. Further studies are needed in order to confirm these results.

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Abstract no.: 15.18
**Detection of Enterohepatic Helicobacter Species and Wolinella succinogenes in Biopsy Specimens from Children with Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS)**

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Introduction. Enterohepatic *Helicobacter* species can initiate IBD in immunodeficient mice. Attempts to detect such organisms in human IBD have resulted in divergent results.

Aim. To determine the presence and spatial distribution of Helicobacteraceae (*Helicobacter* and *Wolinella*), in colonic biopsies from children undergoing colonoscopy.

Methods. Three biopsies were collected from 18 children, diagnosed with IBD (*n* = 12), IBS (*n* = 4), and two controls (without symptoms or inflammation). DNA from one biopsy was used for Helicobacteraceae-specific polymerase chain reaction (PCR) and subsequent sequencing; the second to examine the spatial distribution of Helicobacteraceae using specific rRNA fluorescent in situ hybridization (FISH) and for PAS staining to assess the depth of the mucus layer and the third for histology.

Results. Helicobacteraceae were detected in 12/12 children with IBD (11 CD, 1 UC), 4/4 with IBS and 0/2 controls. Sequencing of PCR products showed 3 children to be closely related to *H. hepaticus*, 6 to *H. trogontum*, 5 to *W. succinogenes* and 2 to an uncultured *Helicobacter* species previously detected in UC patients. PAS staining showed children with IBD to have a significantly thinner (*p < .001*) mucus layer (0.84 ± 0.08 µm) than children with IBS (3.30 ± 0.22 µm). Using FISH, a positive Helicobacteraceae signal was detected in the mucus layer of 3/16 PCR positive children.

Conclusions. This is the first report of the detection of *Wolinella* species in the human gastrointestinal tract (GIT) and of the detection of *Helicobacter* species or *W. succinogenes* colonizing the colonic mucus layer of children with IBD or IBS. Further work is required to clarify the roles of mucus-associated bacteria in IBD.
**Abstract no.: 15.19**

**Isolation of Helicobacter spp. from Seawater, Plankton, and Oysters from Areas of the Caribbean Sea Subject to Fecal Contamination**

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This study aimed at culturing Helicobacter species from a marine environment to fecal contamination. We collected oysters tissue (Isognomus alatus and Crassostrea mangle), marine water filtrates, and marine plankton from a touristic coastal area of Venezuela.

**Hepatobiliary Diseases**

**Abstract no.: 16.01**

**Biliary Lipid Compositions in Cholesterol Gallstone-Prone C57L Mice Infected with Certain Cholelithogenic Enterohepatic Helicobacter spp. Suggest Pro-Nucleating Organisms**

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We demonstrated previously (Gastroenterology 2005; 128) that infection with specific enterohepatic Helicobacter spp. contributes to cholesterol cholelithogenesis in C57L mice. In particular, coinfection with H. hepaticus and H. rodentium or mono-infection with H. bilis induced an approximately 80% prevalence of cholesterol gallstones compared to uninfected controls (~10%). To elucidate the mechanism(s) we analyzed lipids from hepatic bile of H. hepaticus and H. rodentium coinfected and uninfected mice. Helicobacter spp.-free male 4- to 5-week-old C57L mice were either infected or sham dosed (n = 10/group). Four weeks after infection, mice were fed a lithogenic diet for 6 weeks. At laparotomy, hepatic bile was collected for 20 minutes. Bile of infected and uninfected animals did not differ in total lipid content (3.14 ± 0.25 and 3.16 ± 0.31 g/dl, respectively). Similarly, CSIs (1.25 ± 0.10 and 1.25 ± 0.13) and cholesterol concentrations (3.29 ± 0.29 mmol/l) from infected and uninfected animals were identical, and bile salt (42.4 ± 4.3 and 41.8 ± 4.4 mmol/l) and phospholipid (9.5 ± 0.4 and 10.1 ± 0.9 mmol/l) concentrations were similar. The bile salt pool of infected animals demonstrated a significant loss of muriatic acids compared to uninfected animals (11.5 ± 0.6% and 13.7 ± 0.7%, p < .05) and consequently contained significantly more hydrophobic bile salts (p < .05). In vitro, bile salt hydrophobicity changes the phase diagram of bile and alters kinetics of cholesterol crystallization. These data, together with our earlier observations, imply that these bacteria act by making cholesterol monohydrate crystal formation more favorable by promoting heterogeneous nucleation and altering the hydrophobicity of bile.

**Abstract no.: 16.02**

**Unlike Some Enterohepatic Helicobacter Species, Helicobacter pylori Does Not Promote Cholesterol Gallstone Formation in Gallstone-Prone C57L Mice**

K. J. Maurer, A. J. Wiese, Z. Ge, A. B. Rogers, M. J. Carey and J. G. Fox

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We demonstrated recently that infection with specific enterohepatic Helicobacter spp. induces cholesterol gallstones (approximately 80%) in gallstone-prone C57L mice fed a lithogenic diet (Gastroenterology 2005; 128). In contrast, uninfected mice do not develop gallstones with appreciable frequency (approximately 10%). We wished to determine whether Helicobacter pylori would exhibit similar properties. Helicobacter-free, 4-week-old male C57L mice were infected with H. pylori SS1 (n = 10) or sham dosed (n = 10). Mice were fed a lithogenic diet containing 1% cholesterol and 0.5% cholic acid for 8 weeks. At necropsy, gallbladder bile was examined microscopically, and tissues examined histopathologically, molecularly, and by culture. Neither control nor infected animals exhibited a high prevalence of cholesterol gallstones (10% and 20%, respectively). Likewise neither group progressed along the choledocholithogenic pathway (10% cholesterol monohydrate crystals and 0% sandy stones for controls, and 20% cholesterol monohydrate crystals and 0% sandy stones for infected). Additionally, infected mice did not differ significantly from controls with respect to normalized gallbladder weight (0.75 ± 0.23 mg/g, 0.66 ± 0.12 mg/g) nor mucin score (0.95 ± 0.5 and 1.2 ± 0.2) and uninfected animals demonstrated slightly more mucin gel accumulation. Nested polymerase chain reaction (PCR) performed on the hepatobiliary tree revealed no H. pylori. Infected animals displayed moderate to severe gastritis and H. pylori was present in the stomach. These data demonstrate that H. pylori is not cholelithogenic and is not present in the hepatobiliary tree of gallstone susceptible mice. We hypothesize that organisms identified previously as H. pylori in patients with cholesterol gallstones were actually a cholelithogenic–enterohepatic Helicobacter species with high sequence homology to H. pylori.
Abstract no.: 16.03
Helicobacter pylori Gastric Infection and Histologic Hepatic Lesions in Patients with Hepatic Diseases

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There are studies demonstrating association between gastric Helicobacter pylori infection and cirrhosis in patients with hepatitis B or C. It has been postulated that increased blood pro-inflammatory cytokines associated with H. pylori infection would increase hepatic lesions predisposing to cirrhosis. Since there are no studies evaluating the effect of H. pylori gastric infection on the histologic hepatic lesions, we studied, prospectively, the liver histology of 110 consecutive patients (60 men; mean age, 52.4 ± 15.9 years; range 18–93) with hepatic diseases. Liver biopsy specimens were stained with hematoxylin and eosin (H&E), Gomori’s trichromic and reticulin to investigate the presence of necrosis, inflammatory cells, and fibrosis. H. pylori status was determined by ELISA and 13C–UBT. Association between H. pylori infection and fibrosis, alcholic hepatic diseases (AHD), HCV, HBV, autoimmune hepatitis (AIH) and drug-induced liver disease (DILD) was seen in univariate analysis, but H. pylori infection (p = .03, OR = 3.6, 95%CI = 1.1–11.0), AHF (p = .029, OR = 6.0 95%CI = 1.6–23.0), AIH (p = .03, OR = 14.3, 95%CI = 1.3–158.2), DILD (p = .02, OR = 15.3, 95%CI = 1.6–149.3), and HCV (p = .04, OR = 11.6, 95%CI = 1.1–120.8) remained independently associated with necrosis in multivariate analysis. In the univariate analysis, the presence of intra-acinar neutrophils was associated with H. pylori infection, liver metastasis, AHF, HBV, and DILD. In the multivariate analysis, only H. pylori infection (p = .03, OR = 3.2, 95%CI = 1.2–9.2) and DILD (p = .03, OR = 5.2, 95%CI = 1.2–24.2) remained independently associated with lobular neutrophils. H. pylori infection was neither associated with mononuclear cells, even when the analysis was topographic, nor with fibrosis (p = .5). We demonstrated independent associations between H. pylori gastric infection and hepatic histologic lesions, reinforcing the hypothesis that the infection may contribute to the course of liver diseases.

Abstract no.: 16.05
Detection of Helicobacter and Other Bacterial DNA in Biliary Tree of Kosovan Patients with Chronic Cholecystitis by PCR-DGGE

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Background. Helicobacter DNA has been detected in bile, biliary stones, and gallbladder mucosa and in association with stomach, liver, and pancreatic cancer. However, the correlation of Helicobacter species to some chronic biliary tree and liver diseases is still poorly understood. The aim of this study was to investigate the presence of Helicobacter species in bile and gallbladder biopsy specimens of Kosovan patients with chronic cholecystitis.

Materials and Methods. DNA was extracted from gallbladder and bile specimens, using a QIAamp DNA Kit (QIAGEN, Hilden, Germany). All samples were examined for the presence of Helicobacter species and other bacteria by genus-, group-, and prokaryote-specific polymerase chain reaction (PCR) assays. PCR products of positive samples were further characterized by DGGE and DNA-sequencing.

Results. Helicobacter DNA was detected in 19/32 and 26/84 of bile and gallbladder samples, respectively. DGGE analysis and DNA sequencing identified DNA of H. pylori (9), H. pullorum (1), H. cholecystus (4), H. muridarum (2), H. pametensis (1), and H. hepaticus (2) in bile samples. In gallbladders, the Helicobacter species DNA identified were H. pullorum (16), H. pylori (4), H. cholecystus (2), and H. bilis (1) and, H. pametensis (1). On the other hand, Lactobacillus species and Escherichia coli DNA were detected in only four and two of the bile samples, respectively.

Conclusions. The high prevalence of Helicobacter DNA in gallbladder and bile of Kosovan patients with chronic cholecystitis in comparison to a low prevalence of some gut microbe DNA highlights a possible role of Helicobacter species in chronic cholecystitis and associated cholangiocarcinoma.

Abstract no.: 16.04
Molecular Detection of Helicobacter and Other Bacterial Species in Liver Tissue of Patients with Malignant Liver Disease

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Background. Primary liver tumors are commonly divided into benign tumors including cavernous hemangioma, hepatocellular nodules, focal nodular hyperplasia, and adenoma. Malignant tumors are classified as hepatocellular carcinoma, cholangiocarcinoma, hepatoblastoma, and angiosarcoma. The aim of this study was to determine the possible presence of Helicobacter species in the liver tissue samples of patients with hepatocellular carcinoma (HCC) (n = 13), cholangiocarcinoma (CC), normal tissue surrounding the tumor (n = 34), and as controls, focal nodular hyperplasia (n = 52).

Materials and Methods. Paraffin-embedded liver specimens (112) were examined for the presence of Helicobacter species by a genus-specific polymerase chain reaction (PCR) assay, and other bacteria. PCR products of positive samples were further characterized by denaturing gradient gel electrophoresis (DGGE) and DNA-sequencing.

Results. Using Helicobacter genus-specific PCR assay, helicobacter DNA was detected in 7/13 normal tissue surrounding HCC, 8/13 normal tissue surrounding CCC, 1/34 inside the tumor, and 4/52 from the control group. DGGE analysis and DNA sequencing showed that 90% of the detected PCR products were H. pylori-like. Other bacterial DNA was detected in only 3% of total samples.

Conclusions. The presence of DNA of various Helicobacter species in liver tissue samples especially of HCC and CCC may indicate a possible role of Helicobacter species in chronic inflammation associated with human malignant liver diseases.
Abstract no.: 16.06
Serologic Analysis of Helicobacter hepaticus Infection in Patients with Chronic Viral Hepatitis Using New Monoclonal Antibodies

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Background and Aims. Infection of Helicobacter hepaticus has been associated with the development of hepatocellular carcinoma in mice. In humans, however, prevalence of H. hepaticus infection has not been studied. The aim of this study was to examine the prevalence of H. hepaticus infection in patients with chronic hepatitis serologically.

Methods. Serum samples obtained from 182 patients with chronic hepatitis C (106 men and 76 women, mean age 47.4) and 51 patients with chronic hepatitis B (33 men and 18 women, mean age 35.8) were studied. Sera were also obtained from 142 control subjects who were not infected with hepatitis virus (69 men and 73 women, mean age 60.0). Seropositivity of H. pylori and H. hepaticus was tested by E-plate and antigen-capture ELISA using monoclonal antibody to H. hepaticus antigen.

Results. H. hepaticus seropositivity was significantly higher in patients with chronic hepatitis C (29.4%) compared to control subjects (13.7%, p < .05), whereas H. pylori seropositivity was not different (67.0% and 77.4%, respectively). H. hepaticus seropositivity in patients with chronic hepatitis B was 14.0% and it was not different from control subjects who were below 50 years (18.8%). Higher H. hepaticus seropositivity was observed in H. pylori-seropositive patients (32.1%) than in H. pylori-seronegative patients (15.3%, p < .01).

Conclusions. Present results suggest the possibility that prevalence of H. hepaticus is high in patients infected with hepatitis C virus. Association of seropositivity may suggest the presence of similar transmission routes of H. hepaticus and H. pylori.

Abstract no.: 16.07
The Role of Helicobacter pylori Infection in Cirrhotic Patients: Facts or Fictions

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Aim. The purpose of this study was to evaluate the evidence of the pathogenic role of Helicobacter pylori infection in liver cirrhosis (LV).

Patients and Methods. Eighty patients (68 men, 12 women) mean age 48.5 year with LV were included in the study. All patients had upper gastroduodenal endoscopy and gastroduodenal pathology was identified. H. pylori infection was confirmed by gastric histology. The patients were divided into two groups. Group A, 40 cirrhotic patients with H. pylori; and group B, 40 cirrhotic patients without H. pylori infection. The age and sex of two studied groups were compared. The effect variables were: stay in the ICU, peptic ulcer disease, levels of serum ammonia, hepatic encephalopathy, mortality, and surgery treatment.

Results. The incidence of peptic ulcer disease was significantly higher in group A (74%) compared to group B (51%), p < .001. The mean levels of serum ammonia in group A was significantly higher (68%) compared to group B (39%), p < .001. The episodes of hepatic encephalopathy were significantly lower in group B (3, 6) compared to group A (8, 2), p < .001. The duration of stay in the ICU in group A (6.8 days) was significantly higher compared with group B (4.2 days), p < .001. No significant difference was found for surgical intervention and mortality into two groups.

Student’s t-test, Friedman, and Wilcoxon Signed-Ranks tests were used.

Conclusion. According to our results, we found that H. pylori infection is the major risk factors for peptic ulcers in LV. The mean levels of serum ammonia in patients with H. pylori infection were higher than in patients without H. pylori infection.