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Microbiology

Abstract no.: 01.01
Molecular Responses of *Helicobacter pylori* to Changes in Oxygen Tension

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Introduction. Bacteria of the genus *Helicobacter* are microaerophiles with habitats in the gastrointestinal tract of higher vertebrates; some species are pathogenic to humans and animals. *Helicobacter pylori* is the causative agent of serious stomach diseases. The bacterium grows optimally at low oxygen tensions, and host colonization is associated with the oxygen content of the growth atmosphere. The aim of this work was to understand the molecular responses of *H. pylori* to changes in oxygen tension.
Methods and Results. The effects of different oxygen concentrations on the transcriptome of *H. pylori* were investigated using microarrays. The data indicated that at higher oxygen tensions, 58 genes were up-regulated and 40 were down-regulated. Bioinformatic analyses for predicted operons and regulons showed that several were differentially transcribed. For example, at higher oxygen tensions, the thioredoxin system was up-regulated and genes encoding heat shock proteins were down-regulated. Functional classification analyses demonstrated that protein biosynthesis, oxidative phosphorylation, and redox processes were among the functions up-regulated at higher oxygen tensions, whereas primary metabolism and several DNA-related proteins were down-regulated under these conditions.
Conclusion. Transcriptomics combined with functional classification analyses allowed for a better understanding of the responses of *H. pylori* to oxygen stress. These data will serve for comparative studies of the responses of other microaerophiles to the same type of stress.

Abstract no.: 01.02
Regulation and Role in Iron Acquisition of the Two *Helicobacter mustelae* TonB Orthologs

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Background. *Helicobacter* species require iron for growth. Iron acquisition is an important bacterial virulence factor, as the mammalian host restricts iron availability as a nonspecific defense mechanism. Iron transport over the outer membrane in gram-negative bacteria is energized via the TonB-ExbB-ExbD complex. The *Helicobacter mustelae* genome sequence contains two genes encoding TonB orthologs, and here we describe the differential regulation and function of these two TonB orthologs.
Methods. Isogenic mutants in the tonB genes were created in *H. mustelae* by insertional mutagenesis. Strains were incubated on plates supplemented with paper discs with hemin, hemoglobin, or ferric-citrate as sole iron source, and the growth promotion zone was measured after 48 hours. Transcriptional regulation was assessed by Northern hybridization.
Results. Homology searches of the *H. mustelae* genome sequence allowed the identification of two TonB orthologs, tentatively named tonB1 and tonB2. Wild-type *H. mustelae* was able to utilize $FeCl_3$, ferric citrate, hemoglobin, and hemin as the sole iron source. Inactivation of tonB1 resulted in the inability to grow with hemin as sole iron source, but did not affect growth on the other tested iron sources. In contrast, inactivation of the tonB2 gene resulted in reduced growth with ferric citrate and hemoglobin. Transcription of tonB2 was iron repressed, whereas transcription of tonB1 was not affected by iron.
Conclusions. Hemin and hemoglobin uptake seems to require different TonB orthologs in *H. mustelae*. Expression of the *H. mustelae* TonB orthologs is differentially regulated, allowing fine-tuning of iron uptake, and may thus contribute to adaptation to the conditions encountered in the gastric mucosa.

Abstract no.: 01.03
Epinephrine and Norepinephrine, but not Gastrin, Induce Growth and Virulence Factor Expression in *Helicobacter pylori*

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Helicobacter pylori infection induces hypergastrinemia, and previous studies have described human gastin 17 (HG17) as a growth factor for *H. pylori*. Epinephrine (E) and norepinephrine (NE) are growth factors for *Escherichia coli*. Pre-*H. pylori* literature links stress with peptic ulceration. Also, smoking induces ulcers in *H. pylori*-infected people and increases gastric NE levels.

We aimed to assess whether HG17, E, or NE induced growth and virulence factor expression in *H. pylori*, and to explore possible mechanisms. HG17 did not stimulate growth of any *H. pylori* strains tested at any concentration in complex or defined media (CDM). Both E and NE enhanced *H. pylori* growth dose dependently (e.g., for CDM – NE OD = 0.15 ± 0.03 at 24 hours, CDM + NE OD = 0.42 ± 0.06, $p = .02$). NE also increased detectable intracellular adenosine triphosphate (by around 20%, 4 hours after NE exposure). Preliminary experiments showed that NE induced expression of VacA by a mean of 13% by 1.5 hours after exposure. Interestingly, the growth effect was only observed when growth of the untreated control culture was submaximal. Specific growth limitation by iron chelation was overcome by adding NE (which is a weak iron chelator) but not by the closely related compound, normetanephrine, which cannot chelate iron.

Abstract no.: 01.04
Lactic Acid from Gastric Mucosal Cell Induces the Proliferation of *Helicobacter pylori*

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Helicobacter pylori mainly inhabits the mucus layer in the gastric mucosa. However, mechanisms involving *H. pylori* colonization and proliferation in the gastric mucosa are not well established. When *H. pylori* were cocultured with the murine gastric surface mucosal cell line GSM06, a marked increase in the number of colony-forming unit of *H. pylori* was observed. Culture media conditioned by GSM06 cell growth stimulated *H. pylori* growth by approximately one-thousandfold. By bioassay-guided purification, a soluble factor that enhanced *H. pylori* growth was isolated from the conditioned medium of GSM06 cells and identified as L-lactic acid. When *H. pylori* and GSM06 cells were cocultivated, the enhanced growth of *H. pylori* correlated strongly with L-lactic acid concentrations in the conditioned media. The results of this study suggest that L-lactic acid secreted by gastric mucosal cells enhances the growth of *H. pylori*, and this L-lactic acid-dependent growth of *H. pylori* may be important to the long-term colonization of *H. pylori* in the stomach.

Abstract no.: 01.05
Action of a Novel Copper Biocide on the Viability of *Helicobacter pylori*

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Introduction. Owing to the increasing prevalence of antibiotic resistance in *Helicobacter pylori* and the falling eradication rate, novel antimicrobial agents are being actively sought. One novel biocide is a series of highly reductive copper complexes that have broad antimicrobial activity. These compounds have no effect on eukaryotic cell viability until an excess of 100 p.p.m. We have investigated the action of two of these compounds on the viability of *H. pylori*.
Methods. Fresh clinical isolates, clarithromycin-resistant, and metronidazole-resistant isolates and two reference strains [NCTC11637 (cagA positive), NCTC10567 (cagA negative) and J99] were used. A final inoculum of 10^6 colony-forming units per milliliter of *H. pylori* in sterile water was exposed to differing concentrations (0.5, 1.0, 5.0, 12, and 15 p.p.m.) of two copper compounds CuAL42 and CuPC33 for 15, 30, 60 and 120 minutes. At each time point, samples were withdrawn, decimal diluted into 0.25 strength ringers lactate, plated, and incubated.
Results. The product CuAL42 was more active than CuPC33. At 5 p.p.m., CuAL42 reduces the viable count by five to six logs over 120 minutes. At 12 p.p.m., CuAL42 reduces the viable count by five to six logs in 30 minutes and resulted in no growth in 60–120 minutes. Neither the cagA status nor the resistance to metronidazole or clarithromycin appeared to have any effect on the efficacy of either of the agents.
Conclusions. The high biocidal activity of these compounds against *H. pylori* warrants further investigation as a potential therapeutic.

Abstract no.: 01.06
Reducing Duration of Incubation from 72 to 31 Hours Improves Accuracy of Metronidazole Resistance Determination of *Helicobacter pylori* with the E-test

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Metronidazole sensitivity testing of *Helicobacter pylori* is difficult, partly because clinical effect also depends on variables that are not displayed in vitro, and partly because of problems related to procedure standardization. Optimal standardization thus remains a key issue, also when E-test is used.

We studied the usefulness of reducing the duration of incubation from 72 to 31 hours using 150 stored pretreated *H. pylori* isolates.The reproducibility achieved by dual testing with agar dilution and E-test was 85 and 41% for ±1 log2 dilutions after 31 and 72 hours incubation, respectively ($p < .0001$). For ±2 log2 dilutions, reproducibility was 92 and 70%, respectively, after 31 and 72 hours incubation ($p < .0005$). The maximum predictive value for prediction of triple drug treatment failure (metronidazole, tetracycline, and bismuth for 10 days) was 50%. Minimal inhibitory concentration values in the range 2–8 mg/L signified intermediate resistance (predictive value for treatment failure = 22%).

Thirty-one hours incubation, i.e., the time period between the first working hours in 1 day and the next day's last working hours is sufficient for ample growth of *H. pylori* with optimal growth conditions, and such a short period of incubation helps to improve the accuracy of metronidazole sensitivity testing of *H. pylori*. Further studies are needed to assess the clinical significance of intermediate metronidazole resistance.

Abstract no.: 01.07
Antimicrobial Activity of Plant Extracts against *Helicobacter pylori*

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Background. The development of resistance to antibiotics used to treat *Helicobacter pylori* infection and the poor availability of these drugs in developing countries prompted us to test the antimicrobial activity of natural substances to *H. pylori* strains with various expression of CagA.

Materials and methods. Samples were dissolved, sterilized by filtration, and double diluted in *Brucella* broth-bovine serum. Suspensions of four *H. pylori* strains were added to each dilution at the final suspension of approximately 10^6 colony-forming units per mL. After incubation overnight in microaerobic conditions at 37 °C, 3 µL of each dilution were deposited onto Columbia blood agar plates, which were incubated for 3–5 days. The lowest concentration in the broth, whose subculture on agar showed complete absence of growth, was considered the minimal bactericidal concentration (MBC) and was expressed in milligrams per milliliter.

Results. MBCs were the following: lipophilic (carotenoid fraction) and hydrophilic (poliphenolic fraction) tomato extracts (*Lycopersicon esculentum*), 0.075 and 0.312, respectively; lyophilized grape juice (*Vitis vinifera*), 6.25; soy extract (*Glycine max*), 1.04; thorny bush leaf extract (*Rubus ulmifolius*), 5.0; resveratrol, 16.0 µg/mL. The four *H. pylori* strains showed the same susceptibility, independently of the CagA status.

Conclusions. These substances constitute a rich source of bioactive chemicals; their good antioxidant and antibacterial activities make them proper candidates in the prevention of gastric mutagenic events and may help in the treatment of *H. pylori* infection.

Abstract no.: 01.08
Novel Inhibitors of Thymydilate Synthase ThyX Discovered Using an Automated Screening

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Until recently, thymidylate synthase ThyA (EC.1.1.145) was thought to be the only enzyme catalyzing the de novo formation of the essential DNA precursor thymidylate (dTMP) from deoxyuridine monophosphate (dUMP). Strikingly, genome-wide comparisons of a large number of microbial genome sequences revealed the absence of ThyA in up to 35% of microbial genomes. This observation led to the discovery of a novel family of thymidylate synthases, ThyX (EC.1.1.148), using in silico and experimental methods.

ThyX proteins are present in a large number of pathogenic bacteria (e.g., *Helicobater pylori, Campylobacter jejuni, Mycobacterium tuberculosis, Treponema pallidum, Chlamydia trachomatis,* and *Paramecium bursaria Chlorella* virus), but are absent in humans. Moreover, the two families of thymidylate synthases are structurally different; ThyA is a homodimer, whereas ThyX is a tetramer. They also have different reductive mechanisms; ThyX is a flavoprotein and produces tetrahydrofolate, whereas ThyA produces dihydrofolate, and is the reason why ThyX-containing species are "resistant" to trimethoprim.

Consequently, ThyX proteins provide an interesting target for specifically inhibiting microbial growth. We present an activity-based screening. This automated test is based on nicotinamide adenine dinucleotide phosphate oxidation by ThyX; we measured the decrease of absorbance at 340 nm or fluorescence during time. A screening of three libraries representing 2,370 molecules led to the identification of 12-nanomolar inhibitors (druglike compounds).

Abstract no.: 01.09
Antibacterial Activity of *Trachyspermum Copticum* against *Helicobacter pylori*

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Helicobacter pylori is a pathogen that infects half of the world population and causes antral gastritis, duodenal ulcers, and enhances the risk of gastric malignancies. The increasing rate of these infections and the emergence of antibiotic resistance in *H. pylori* strains lead us to seek new sources of treatment.

The aim of this research was to study the antimicrobial activity of *Trachyspermum copticum* against 80 clinical isolates of *H. pylori* from children and adults.

The effect of methanol, diethyl ether, petroleum benzene, MEP (a mixture of the three), and water extracts of *T. copticum* on *H. pylori* were examined using the disk sensitivity tests. Water, methanol, and MEP extracts of *T. copticum* showed significant antibacterial activity and had a minimum inhibitory concentration of 31.25–125 µg/mL and minimum bacteriocidal concentration of 62.50–125 µg/mL. The methanol extract was active after autoclaving for 30 minutes at 121 °C.

The results show that *T. copticum* extracts contain a heat stable agent(s) with possible therapeutic potential.

Abstract no.: 01.10
Anti-*Helicobacter pylori* Activities of 20 Iranian Plants

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Background. *Helicobacter pylori* is the major worldwide cause of bacterial gastrointestinal infections in adults and children. Antibiotic therapy and a combination of two to three drugs have been widely used to eradicate these infections. However, development of drug resistance in bacteria calls for new sources of drugs, and plants seem to be a logical source of new antibacterial compounds.

Methods. Anti-*H. pylori* activity of 20 Iranian native plants and seven antibiotics were determined against clinical isolates using the disk susceptibility assay. Minimum inhibitory concentrations were also measured for the biologically active extracts. One extract with the best anti-*H. pylori* activity was fractionated by silica gel and thin layer chromatography, and the active compounds were identified by hydrogen nuclear magnetic resonance spectroscopy.

Results. Ten plant extracts showed anti-*H. pylori* activity by the disk sensitivity method but the most active ones were from *Carum bulbocastanum*, *Carum carvi*, *Xanthium brasilicum*, *Mentha ligifolia*, *Saliva limba*, and *Thymus vulgaris*. In fact, the anti-*H. pylori* activity in the six extracts was superior to the disk antibiotic susceptibility profile. Minimum inhibitory concentrations were within the range of 31.25–500 μg/mL. Fractionation and chemical identification of the extract from *X. brasilicum* showed presence of a xanthanolide. **Conclusion.** As a result of the rise in antibiotic resistance, new sources of anti-*H. pylori* drugs are needed. The use of medicinal plants and/or their chemical components may be of potential use in eradicating such problems.

Molecular Genetics and Genomics

Abstract no.: 02.01
Sequence of the First *Helicobacter pylori* Strains Involved in Low-Grade Gastric Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma

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Gastric lymphoma development is directly linked to the *Helicobacter pylori* infection. Comparative genomics, based on the analysis of the genome of 120 clinical isolates including 43 strains associated with gastric lymphoma using DNA arrays, revealed that 50% of the mucosa-associated lymphoid tissue (MALT) isolates could be grouped together in a distinct class distinguishing them from strains associated with either gastritis, peptic ulcer, or metaplasia.

Sequencing of the genome of one strain (B38) representative of this cluster of MALT isolates was conducted. B38 strain is plasmid as well as cag pathogenicity island free, transformable, and capable of colonizing the mouse gastric mucosa. It was achieved with 340 cosmids and shotgun clones, providing a 13X coverage depth. Following automatic assembly, the MaGe System was used for annotation.

The genome contained 1.57 Mbases, 1596 coding sequences (CDSs) and a G+C% of 39. B38 lacks 119 of the 242 nonubiquitous genes of 26695 as well as IS605; 30 CDSs present both in 26695 and J99 were found as pseudogenes in the B38 genome (frameshift or stop codon). Fourty-three new CDSs that both 26695 and J99 lack were identified: 35 encode hypothetical proteins and eight encode phage-related proteins. Ten pseudogenes in 26695 and J99 were found as coding sequence in B38. At this stage of annotation and finition, the B38 genome is characterized by the presence of major rearrangements despite region of high syntheny as compared to the strains 26695 and J99 correlating with a genetic content almost restricted to the "core" of the *H. pylori* strains.

Abstract no.: 02.02
The Role of HP1230 Protein in the Regulation of *Helicobacter pylori* Chromosome Replication

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Chromosome replication is a crucial step in a cell cycle and is highly regulated to restrict chromosome duplication to once per generation. The bacterial chromosome replication is initiated by DnaA protein binding to *oriC* region, which leads to DNA unwinding. Key elements of the *H. pylori* chromosome replication have been characterized, but none of the replication regulatory proteins has been discovered so far. *H. pylori* lives in a protected ecological niche and possesses a relatively small genome, encoding very few regulatory proteins. Comparative genomics suggests that its mode of initiating and regulating replication differs from that of *Escherichia coli*. The yeast two-hybrid genome-wide screening revealed that dnaA interacts with HP1230 – a putative protein with no known function. We hypothesized that this protein might play a crucial role in the control of *H. pylori* chromosome replication. We showed that lack of HP1230 is lethal for *H. pylori and* that increased or decreased level of HP1230 did not disturb bacterial growth, whereas a drastic reduction in the protein amount stopped chromosome replication and cell proliferation. In vitro studies showed that HP1230 not only increased dnaA affinity to the *oriC* region but also reorganized the structure of the initiation complex. Electron microscopy experiments showed that HP1230 was able to join two dnaA-*oriC* complexes. We postulate that HP1230 plays a dual role – first it assists the formation of the orisome and influences DNA unwinding,and second, it might be responsible for *oriC* cohesion and prevention of premature re-replication.

Abstract no.: 02.03
Identification of a Novel Hot Spot for Genetic Exchange within the *Helicobacter pylori* Genome

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In a previous study, we reported the use of subtractive hybridization as a tool for comparative genomics between *Helicobacter pylori* strains. We isolated one original sequence with no protein sequence homology in databases.

An in-house genome-walking method was used to localize this sequence, then the identified genomic region was amplified by polymerase chain reaction and sequenced in 23 strains.

The sequence was reconstructed and localized, in comparison to *H. pylori* reference strains J99 and 26695, between JHP1069 (HP1141), which encodes for a putative methionyl-tRNA formyltransferase and JHP1071 (HP1143), which encodes for a conserved hypothetical protein. This new sequence formed a region composed of one putative gene with 2, 467 bp that replaced JHP1070 (HP1142), a conserved hypothetical protein. This new gene was present in 7 of 23 strains (30.4%) whereas in 4 strains (17.4%), we identified another putative gene with 2586 bp without nucleotidic sequence homology with JHP1070 (HP1142) or the putative gene initially identified. Finally, 12 strains (52.2%) harbored a JHP1070 (HP1142) homolog. Regardless of the gene considered, we found a low G+C% (29 to 31%). No correlation between the presence of these new genes and the disease outcome could be made.

In conclusion, a probable novel hotspot for genetic exchange in the *H. pylori* genome was identified, whereas this region was conserved in the two *H. pylori* reference genomes. It could be used as strain-specific marker. The putative role of these new genes, in particular while taking into account the neighboring genomic areas, remains to be determined.

Abstract no.: 02.04
E266K CARD4/NOD1 and Toll-like Receptor 4 Gene Polymorphism in *Helicobacter pylori*-Infected Patients with Duodenal Ulcer or Gastritis

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Background. *Helicobacter pylori* can be recognized in epithelial cells by the intracellular pathogen receptor NOD1 or extracellular LPS detecting toll-like receptor 4 (TLR4). The aim was to evaluate the frequency of NOD1 and TLR4 gene polymorphism in *H. pylori*-infected patients with duodenal ulcer (DU) and gastritis.
Patients. One hundred thirty-one *H. pylori*-positive patients with dyspeptic symptoms were examined by gastroduodenoscopy. *H. pylori* positivity was detected by ^{13}C-UBT and histopathology. DU was found in 58 and gastritis in 73 patients.
Methods. E266K CARD4/NOD1 (G to A) was determined by restriction fragment-length polymorphism, and the TLR4 (ASP/299/Gly and Thr/399/Ile) gene polymorphism by melting point analysis with a real-time polymerase chain reaction method.
Results. AA homozygote mutant variants of NOD1 were detected in 12 of 58 *H. pylori*-positive patients with DU (20%) versus 5 of 73 *H. pylori*-positive patients with gastritis (6.8%), the difference being significant (p = .034, OR: 3.42, 95% CI = 1.184–2.519). Conversely, the G alelle was significantly more frequent in patients with gastritis by 76% than in DU patients (62%) (p = .014, OR: 2.992, 95% CI = 1.574–5.789). However, no significant correlation in the frequency of the TLR4 gene polymorphism could be revealed between these two groups. The genotype frequency of AG heterozygotes concerning gene polymorphism ASP/299/Gly was 13.8% in the patients with gastritis versus 8.6% in DU patients (p = .490). Similarly, there was a 13.2% frequency of CT heterozygotes concerning the Thr/399/Ile gene polymorphism in the patients with gastritis versus 8.66% in DU patients (p = .568).
Conclusion. E266K CARD4/NOD1, but not the TLR4 gene polymorphism, increases the risk of peptic ulceration in *H. pylori*-positive patients.

Abstract no.: 02.05
Genetic Diversity of *Helicobacter pylori* Strains and their Hosts

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Humans of Asian ancestry arrived in the Americas some time over 10,000 years ago. Evidence of analysis from mtDNA supports that these human populations suffered a moderate bottleneck before their expansion in the continent. These groups have been subject to geographic isolation and show lower genetic diversity than people of European or South American mestizo ancestry. The aim of this work was to examine whether genetic variability of *Helicobacter pylori* is reduced when genetic variability of the host is reduced. We analyzed multilocus sequence typing (MLST) of seven housekeeping genes (*atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI*, and *yphC*) in 130 *H. pylori* isolates from the MLST database (pubMLST.org). The individual strains were from 19 Africans (Burkina Faso and Senegal), 15 East Asians (Korea, China), 42 Amerindians (Athabaskans, Inuit, Huitoto, and Piaroa), 36 Spaniards, and 18 South American mestizos from Venezuela and Colombia. We estimated pairwise genetic distances between isolates that were grouped by hosts or by assignment to bacterial populations, in MEGA3. The lowest genetic diversity was observed for hpEastAsian and hspAmerind strains, whereas hpEurope population had the highest diversity. When analyzed by host, the highest genetic diversity was observed in isolates from Spaniards and mestizos. Changes in hosts and *H. pylori* as a result of mixing are currently happening at very high speed, and we hypothesize that these phenomena are likely to affect disease outcome related to *H. pylori* colonization.

Abstract no.: 02.06
Polymorphisms of Innate Immune Response Genes and IL2 and Risk of *Helicobacter pylori*-Associated Diseases

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Several studies have assessed the relationship between proinflammatory polymorphisms and gastroduodenal diseases, mainly *Helicobacter pylori*-associated diseases. We aimed to add to the current knowledge by analyzing, in multivariate models, functional polymorphisms in genes associated with the innate immunity (*TLR2*+2029C/T, *TLR2*+2251G/A, *TLR4*+896A/G and *TLR5*+1174C/T) as well as *IL2*-330T/G together with the bacterial virulence factor *cag*A, in patients with duodenal ulcer (n = 241, 29 with perforated duodenal ulcer) and with gastric carcinoma (n = 168). We also included 540 blood donors as a control group. Polymorphism of *TLR2* at position 2029 was not found in the studied population. The alleles did not deviate significantly from the Hardy–Weinberg equilibrium in any of the studied loci that segregated independently in the control group. Neither the polymorphisms of *TLR* genes nor of the *IL2*-330 was associated with duodenal ulcer (p = .74 for *TLR4*+896A/G, p = .34 for *TLR2*+2251G/A, p = .74 for *TLR5*+1174C/T, and p = .79 for *IL2*-330T/G) or with gastric carcinoma (p = .10 for *TLR4*+896A/G, p = .72 for *TLR2*+2251G/A, p = .71 for *TLR5*+1174C/T, and p = .39 for *IL2*-330T/G) in the multivariate model. Otherwise, *IL2*-330 heterozygosity was negatively associated with *H. pylori*-negative status when duodenal ulcer patients plus gastric carcinoma patients were compared with *H. pylori*-negative blood donors, even after adjustment for age and gender (p = .04, OR = 0.69, 95% CI = 0.48–0.96). This result suggests that *IL2*-330T/G polymorphism is protective against *H. pylori* infection.
Grants. FAPEMIG and CNPq/Brazil.

Abstract no.: 02.07
Prevalence of a Second Urease Gene Cluster in *Helicobacter* Species Colonizing the Carnivore Stomach

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Background. The enzyme urease is an important virulence factor of *Helicobacter* species colonizing the gastric environment. Active urease consists of UreA and UreB subunits, and requires coexpression of the UreEFGH accessory proteins. Recently, a gene cluster encoding a putative second urease (*UreA2B2*) was identified in *Helicobacter felis*. In this study, we have investigated the prevalence of this second urease gene cluster in other *Helicobacter* species.

Methods. The prevalence of *ureB* genes in *Helicobacter* species was determined using Southern hybridization. The *Helicobacter mustelae* genome sequence was obtained from http://www.sanger.ac.uk/Projects/H_mustelae

Results. Genomic DNA of *H. mustelae*, *H. felis*, and *Helicobacter acinonychis* contained multiple fragments hybridizing to a *ureB* probe, suggesting the presence of additional *ureB* genes. Expression of two UreB-like proteins was detected in *H. mustelae* and *H. acinonychis* using immunoblotting. Analysis of the *H. mustelae* and *H. acinonychis* genome sequences revealed that in addition to a *ureABIEFGH* cluster, there was also a *ureA2B2* gene cluster lacking accessory genes. The *H. mustelae* and *H. acinonychis* UreB2 proteins are approximately 70% identical to the corresponding UreB proteins. Phylogenetically, *H. mustelae* and *H. acinonychis* UreB2 cluster together with *H. felis* UreB2, separately but close to *Helicobacter* UreB.

Discussion. *H. mustelae*, *H. acinonychis*, and *H. felis* contain a gene cluster potentially encoding a second urease. Such a second urease gene cluster seems to be absent in *Helicobacter pylori* and enterohepatic *Helicobacter* species and may only be present in *Helicobacter* species colonizing the carnivore stomach. This suggests a link between diet and genetic properties of *Helicobacter* species.

Abstract no.: 02.08
Lethal Phenotypes in *Helicobacter pylori* Studied by Generation of Merodiploid Strains Using *rdxA*

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Introduction and Aims. The identification of essential genes in *Helicobacter pylori* is of great interest for therapeutic purposes. Classification of genes as essential often relies on the absence of growth following gene disruption. For example, *gpt* is considered essential according to gene disruption studies. It encodes a xanthine-guanine phosphoribosyltransferase (XGPRTase) and the role of this enzyme in purine nucleotide salvage can theoretically be substituted for by the de novo purine biosynthesis pathway. In this work, the essential role of *gpt* was investigated by generating strains in which the gene was complemented elsewhere in the chromosome. The complementation utilized the *rdxA* gene, whose disruption is associated with metronidazole resistance in *H. pylori*.

Methods and Results. Plasmids were constructed using overlap polymerase chain reaction and employed to generate merodiploid *gpt* strains, and to knockout the wild-type *gpt* gene. The complementation vector contains *rdxA* and its flanking regions insertionally disrupted by *gpt*. Transformation of *H. pylori* strains, X47-2AL and 26695, with this construct produced a metronidazole-resistant phenotype. The knockout of wild-type *gpt* was performed using the kanamycin resistance cassette, *aphA3*. Viable transformants showed integration of the disrupted *gpt* only at the locus of the complemented gene. Sequencing confirmed homologous recombination in all transformants. Measurement of XGPRTase activity by spectrophotometry was performed to confirm the status of the enzyme activity in the various mutants.

Conclusion. The generation of merodiploid *H. pylori* strains served to provide evidence that *gpt* is essential to the survival of *H. pylori* without relying exclusively on negative results.

Abstract no.: 02.09
A Cluster Method Design for *Helicobacter pylori* Strain Typing Based on the Genomic Methylation Status

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There are over 370 complete sequenced bacterial genomes. A small group of species under 2.5%, has more than 15 putative methylatransferase genes (MTases), such as *Campylobacter upsaliensis* with 30 and *Helicobacter pylori* with 26. Expressed MTases may be determined by digesting the genomic DNA with restriction endonucleases (REases). We studied 51 strains of *H. pylori* isolated from Portuguese patients and digested their genomic DNA with 33 REases. The results were grouped as a binary matrix, where "0" indicates unmethylated DNA and "1" methylated DNA. Dendrograms were constructed following the UPGMA method and the Jaccard, Dice, and Simple matching coefficients. However, these coefficients do not reflect that for type II restriction-modification (RM) systems, there is a selective pressure imposed by the REase on the MTase. That pressure makes the loss of an RM system more difficult than its acquisition. In order to reflect that selective pressure, an algorithm for dendrogram construction after genomic methylation (GEME) analysis was developed using MATLAB® R12 software (The Mathworks, Inc.). Minimum common restriction modification system algorithm for GEME (MCRM-GEME) is a cluster method that starts by grouping strains sharing a common minimum set of RM systems (species specific or from a characteristic genetic pool) and progressively associates strains that share common MTases in an increasing number of matches. MCRM-GEME can be used as a tool for strain typing, reflecting the high diversity of RM systems found in *H. pylori* strains.

Abstract no.: 02.10
Polymorphisms of IL-1B, IL-1RN, and IL-2 Genes in Patients with Gastric Cancer and Peptic Ulcer Diseases in Korea

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Background and Aim. Interleukin-1 (IL-1) gene polymorphism has been reported to be associated with increment of gastric cancer (GC), and decrement of duodenal ulcer. In addition, IL-2 is well known to induce *Helicobacter pylori*-associated gastric atrophy, but it is not known whether IL-2 gene polymorphism increases the risk of GC or peptic ulcer disease (PUD). Therefore, we compared the genotypes of IL-1B, IL-1RN, and IL-2 gene polymorphisms with risk of GC and PUD in Korean population.

Methods. One hundred and twenty-two GC patients and 110 PUD patients were included and compared with 100 healthy controls. Polymorphisms of the *IL-1B*-511/-31 gene, the penta-allelic variable number of tandem repeats of the *IL-1RN* and *IL-2*-330 genes were analyzed by polymerase chain reaction with restriction fragment length polymorphism or confronting two-pair primers methods.

Results. The age-sex adjusted odds ratios (ORs) for *IL-1B*-511 T genotype relative to C/C genotype [OR = 0.82, 95% CI = 0.39–1.69], *IL-1RN* *2 genotype relative to L/L genotype [OR = 0.81, 95% CI = 0.38–1.73] and *IL-2-330* T genotype relative to G/G genotype [OR = 1.943, 95% CI = 0.761–4.959] were not increased in GC. There was also no significant difference in genotypes of these cytokine polymorphisms between study group (gastric ulcer, or duodenal ulcer) and control group. In addition, genotypic frequency was not associated with *H. pylori* positivity and histologic type of gastric cancer.

Conclusions. *IL-1B*-511, *IL-1RN*, and *IL-2* genetic polymorphisms were not the important contributors to pathogenesis of GC and PUD in Korea.

Abstract no.: 02.11
TLR4 Polymorphism D299G in Association with Gastric Cancer and Duodenal Ulcer in *Helicobacter pylori*-Infected Patients

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Background. Recent work supports a role for toll-like receptors (TLRs) in the initial response to *Helicobacter pylori*. Two polymorphisms of human *tlr4* has been described, an A-G substitution at 896 base pairs (bp) (D299G) and a C-T substitution at 1196 bp (T399I), both located in the extracellular domain. The aim of this study was to analyze whether the D299G polymorphism is associated with gastric cancer or duodenal ulcer.

Methods. We studied 261 unrelated Mexican patients infected with *H. pylori*: 140 with chronic gastritis (control group), 60 with precancerous lesions, 26 with cancer, and 35 with duodenal ulcer. We extracted DNA from peripheral blood mononuclear cells and D299G variants were screened for by allelic discrimination (SNP detection) using *Taq*Man probes, then randomly chosen, we sequenced the genotype of three DNAs of each variant. Frequencies were compared by χ^2 test, and odds ratios (OR) and 95% CIs were calculated.

Results. Of the 261 patients, 93.4% presented the D/D genotype and 6.6% the D/G genotype. There was a higher presence of the polymorphic variation in cases than in controls: 11.1% in cancer, 17.4% in duodenal ulcer, 5.8% in chronic gastritis, and 3.3% in precancerous lesions. OR was 2 (95% CI = 0.36–11; $p = .34$) for gastric cancer and 3.4 (95% CI = 0.84–14; $p = .09$) for duodenal ulcer.

Conclusions. In our population, the D299G polymorphism of the *tlr4* gene is a risk factor for gastric cancer and duodenal ulcer.

Abstract no.: 02.12
Search for Protein Complexes of the *Helicobacter pylori* Strain B38 Inducing Gastric MALT Lymphoma

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Helicobacter pylori induces different gastric pathologies. The strains J99 and 26695, whose genomes have been annotated, were isolated from patients with ulcer and gastritis, respectively, and B38 from a patient with gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The B38 genome has been sequenced, and is currently being annotated. MALT lymphoma-associated strains appear to have a particular genetic content, but no specific virulence factors have yet been identified.

The aim of this study was to compare the protein complexes of the three *H. pylori* strains cited in the previous discussion, in order to find the complexes specific to the B38 strain. We used the two-dimensional Blue Native polyacrylamide gel electrophoresis/ sodium dodecylsulfate polyacrylamide gel electrophoresis method to analyze cytosolic and membrane fractions of these three strains.

At the cytosolic level, numerous differences between the three strains were observed. Multi-homooligomeric and multi-heterooligomeric protein complexes common with these three strains were also identified. Concerning the membrane protein complexes, the B38 profile appears to be very different from that of the other strains. Twenty membrane protein complexes were visualized in the B38 strain, including 13 multi-heterooligomeric protein complexes.

All of the protein spots are being identified by mass spectrometry (LC-MS/MS). To obtain a better definition, new gel migrations are being performed on crude samples with different acrylamide concentrations. Fractions obtained after sample purification (gel filtration, isoelectrofocalization) will also be analyzed.

Abstract no.: 02.13
Antigen Production of Novel Variants CagA Protein from Malaysian *Helicobacter pylori* Strains

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Helicobacter pylori CagA protein is highly immunogenic. Patients infected with CagA protein expressed by certain strains developed serum Immunoglobulin-G (IgG) anti-CagA. The *cagA* gene sequence has been reported to be of great diversity and varies with geographical regions. The aim of this study was to characterize the novel *cagA* variants of Malaysian *Helicobacter pylori* isolates and to produce recombinant CagA protein that can be used as antigen in the development of a diagnostic kit. *H. pylori cagA* was amplified using polymerase chain reaction technique, and the products were cloned into pCR2.1 vector for sequencing. Expression of fusion CagA protein was performed using pQE30 expression vector and *Escherichia coli* strain M15[pREP4]. Ni-NTA agarose is used to purify the recombinant protein. Sequence analysis of the *cagA* gene showed the presence of four subtypes of *cagA* among Malaysian *H. pylori* isolates named as A1, A2, B, and C. Strains with *cagA* subtype A1 was predominantly isolated from Chinese patients who have higher risk to a more severe disease, and also patients with peptic ulcer. Variants recombinant CagA protein is expressed at 37 °C with 1 mmol/L of IPTG. Purified CagA protein will be coated onto microtiter plates, and the presence of IgG anti-CagA in the patients' sera will be determined using enzyme-linked immunosorbent assay technique. In this study, an attempt has been made to develop diagnostic kits using antigen isolated from local strains for the detection of antibodies to virulence factors of *H. pylori* associated with more severe disease. Antibody detection using this kit will provide healthcare providers with a noninvasive screening device, enabling them to select patients with high risk of peptic ulcer and gastric carcinoma for better management of the disease.

Abstract no.: 02.14
The Genetic Analysis of Chromosomal DNA and Genotyping *Helicobacter pylori* Isolation from Gastroduodenal Illnessess in Tajikistan

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The purpose of the present work was the genetic analysis of chromosomal DNA and genotyping of *Helicobacter pylori* strains isolated from biopsy materials at a pathology of organs of a gastrointestinal tract in Tajikistan.

The analysis of chromosomal DNA was made using the polymerase chain reaction (PCR) method. Strains have been analyzed if present in a chromosome of genes – cagA, cagH, ureC, ureB, vac s1, and vac s2.

The PCR analysis biopsy materials have allowed the distribution of *H. pylori* on 16 genotypes; from these, 15 genotypes are a combination of the genes *ureB*, *ureC*, *cagA*, *cagH*, *vacS1*, and *vacS2*, and one genotype has one gene (*ureC*). *H. pylori* was characterized as a genotype with presence at a chromosome of all six analyzed genes in three samples. Three strains of *H. pylori* were characterized as genotypes from a combination of five genes in a chromosome. The genotype from a combination of four genes – *ureB, ureC, cagA*, and *cagH* was most the frequently revealed (12 strains).

The genes coding major factors of virulence are widespread among strains of *H. pylori* that were isolated of from biopsy materials in various forms of gastroduodenal pathologies. *H. pylori* strains from various origins differ among themselves on the frequency of occurrence of various genes in a chromosome. Distribution of genotypes of *H. pylori* in regions of the Republic of Tajikistan has a significant geographic specificity. Combined *ureB, cagA*, and *cagH* genotypes of *H. pylori* are found more often in the central part of Tajikistan; predominant in the northern part are mainly genotypes *vacS1* and *vacA2*; in the southern regions is the *cagH* genotype of *H. pylori*.

Abstract no.: 02.15
Study of the Transcription Machinery and Gene Regulation in *Helicobacter pylori*

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We are interested in the transcription machinery (RNA polymerase and transcription factors) and the mechanism of transcription in *Helicobacter pylori*. Much progress has been made in the past years in elucidating the in vivo regulation of gene expression in *H. pylori*. However, there is no in vitro transcription system available to study the molecular details in control of transcription in the bacterium. As reported previously, RNA polymerase purified from *H. pylori* was inactive using in vitro transcription assays, indicating unique features and/or requirements in the transcription machinery of *H. pylori*. Expending our expertise from *Escherichia coli* studies, we are attempting to dissect the transcription machinery and to develop an in vitro transcription system for *H. pylori* using different approaches. Our progress in these attempts will be reported and/or discussed.

Virulence Factors and Pathogenesis

Abstract no.: 03.01
Matrix Metalloproteases-7 and -9 Production by *Helicobacter pylori*-Infected Gastric Epithelial Cells Show Differential Gene Participation from *cag* Pathogenicity Island

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Helicobacter pylori infection induces the expression of matrix metalloproteases (MMPs). CagA protein could play a pivotal role in the release of MMPs in gastric epithelial cells. The aim of this study was to identify the mechanism of expression of MMP-9 and -7 in reference to *cag* pathogencity island (*cag*PAI) genes. MMP-9 and -7 productions were measured by reverse transcriptase polymerase chain reaction and gelatin zymography or Western blot. Inducible CagA expression cell lines (a kind gift from Prof. Hatakeyama M. of Hokkaido University) was used. Also, a pair of cagA isogenic mutants with different tyrosine phosphorylation activities was used to check MMP production. *cagE* and *vacA* knockout mutants as well as *cag* PAI absent strain 8822 were used. *CagA*-positive strains are able to induce greater MMP-9 production. It was well correlated with the tyrosine phosphorylation status of the CagA protein. The expression of CagA in AGS cells by inducible system or transient transfection, gastric epithelial cells confirmed that the increased production of MMP-9 is phosphorylation dependent. On the other hand, the expression of MMP-7 is *cag*PAI dependent but CagA phosphorylation independent. Rather than phosphorylation of CagA, either presence of *cagA* and *cagE* or PAI may be important in increased production of MMP-7. The tyrosine phosphorylation of CagA influences the production of MMP-9 by gastric epithelial cells. The presence of *cagE* rather than the phosphorylated CagA seems to be an important factor for the expression of MMP-7. This explains the differential role of *cag*PAI genes' participation in MMPs production by *H. pylori*-infected gastric epithelial cells.

Abstract no.: 03.02
Dendritic Cell Maturation and Release of Cytokines, Chemokines, and Growth Factors in Response to *Helicobacter pylori*: Role of the Urease

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The nature of *Helicobacter pylori* infection is characterized by chronicity of carriage. Bacterial factors, which may play a role in immune escape mechanisms, should be present in every *H. pylori* strain. In this study, the influence of *H. pylori* on maturation and cytokine, chemokine, and growth factor release of dendritic cells (DCs) was investigated with a particular focus on the role of the urease.

Monocyte-derived immature DCs were stimulated for up to 48 hours with *H. pylori* isogenic strains (producing or lacking urease) both intact bacteria and lysates, and recombinant (r) UreA and UreB. Maturation of DCs was determined by flow cytometry. Cell supernatants were tested by the 17-Plex assay of BioRad, which allows for the simultaneous quantitation of 17 cytokines, chemokines, and growth factors. Bioactive TGF-β1 was quantified by conventional enzyme-linked immunosorbent assay.

All stimuli caused maturation of DCs. With the exception of the recombinant proteins, all stimuli induced considerably higher amounts of G-CSF, IL-8, and IL-10 than lipopolysaccharide (LPS) (positive control). In contrast, values of IL-12, IFNγ, and TNFα obtained with LPS were far above those with other stimuli and in particular with recombinant proteins. In contrast to all other stimuli, intact bacteria were shown to induce significant amounts of TGF-β1, which may play a role in gastrointestinal mucosal healing among others down-regulating the Th1 immune response. Furthermore, *H. pylori* urease was shown to up-regulate ($p < .05$) TGF-β1 and MCP-1, which promotes Th2 effector cells.

Thus, the interaction of *H. pylori* with DCs may favor the Th2 immune response.

Abstract no.: 03.03
Activation of Beta-Catenin Signal by *Helicobacter pylori* CagA

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Helicobacter pylori cagA gene encodes a 130–145-kDa CagA protein that is delivered into gastric epithelial cells via the bacterial type IV injection system. Translocated CagA localizes to the inner surface of the plasma membrane where it undergoes tyrosine phosphorylation by Src family kinases. Infection with CagA-producing *H. pylori* is closely associated with the development of gastric adenocarcinoma. Intestinal metaplasia, which is characterized by transdifferentiation of the gastric mucosa to an intestinal phenotype, has been considered to be a precancerous lesion from which gastric adenocarcinoma arises. Recent studies have shown that deregulated beta-catenin signal plays an important role in the pathological transdifferentiation of various cell lineages, including intestinal metaplasia. Also, germline mutation of E-cadherin, which forms a physical complex with beta-catenin, is responsible for the development of hereditary gastric carcinoma. Accordingly, we investigated the possible link between CagA and the E-cadherin/beta-catenin complex. By using the sequential immunoprecipitation-immunoblotting technique, we found that CagA interacts with the cytoplasmic domain of E-cadherin independently of CagA tyrosine phosphorylation. The CagA–E-cadherin interaction inhibits E-cadherin/beta-catenin complex formation, resulting in cytoplasmic/nuclear translocation of beta-catenin. Nuclear accumulated beta-catenin then transactivates beta-catenin–dependent genes that include intestinal-specific transcription factor cdx1. These results raise the possibility that deregulated activation of beta-catenin signal by CagA aberrantly induces *Cdx1*-dependent genes, which mediate the development of intestinal metaplasia, a premalignant gastric mucosal lesion associated with *H. pylori* infection.

Abstract no.: 03.04
Prevalence of Virulent *Helicobacter pylori* Strains in Patients with Ischemic Cerebrovascular Disease: A Multicenter Study

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Background and Aims. Previous studies suggested an association between ischemic cerebrovascular disease and *Helicobacter pylori* infection, in particular CagA-positive strains. Available data are contrasting and come from monocentric studies from referral tertiary centers. The aim of the present study was to assess the prevalence of CagA-positive *H. pylori* strains in patients with ischemic cerebrovascular infection with respect to controls without evidence of atherosclerotic-related diseases.

Methods. A total of 106 consecutive patients (age 76.4 ± 8 years; men 50%) with well-documented history of ischemic cerebrovascular disease and 97 sex-age (age 76.4 ± 8 years; men 45%) and social background-matched controls without relevant vascular diseases. Subjects come from five different regions of Italy. Risk factors for ischemic cerebrovascular disease (familiar history, arterial hypertension, smoking, diabetes mellitus, dyslipidemia, and obesity) were assessed in all subjects. *H. pylori* infection was assessed by ^{13}C-urea breath test (Altana, Milano, Italy). A serological assay for specific IgG against CagA was also performed (Radim, Pomezia, Italy).

Results. Prevalence of active *H. pylori* infection higher in cases (63%) with respect to controls (54%); however, without reaching statistical significance ($p = .2$, OR = 1.21, 95% CI = 0.85–2.61). A significant association was found between patients and controls as concerning Cag-A positivity (41.5% versus 17.5%; $p < .001$, OR = 1.65, 95%CI = 1.74–6.40).

Conclusions. This is the first study assessing the prevalence of active *H. pylori* infection and CagA-positive strains in the setting of the general population. Our findings suggest that CagA-positive, more cytotoxic strains of the bacterium are significantly associated to ischemic stroke.

Abstract no.: 03.05
In Vitro Study of the Role of *Helicobacter pylori* Strains Involved in Low-Grade Gastric Mucosa-Associated Tissue (MALT) Lymphoma in the Proliferation of Lymphocytes

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Helicobacter pylori is involved in the pathogenesis of gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Hussell et al. showed that *H. pylori* can induce in vitro a proliferation of cells obtained from gastric lymphoma biopsies. However, Gerhard et al. provided evidence for an inhibition of lymphocyte proliferation by a secreted *H. pylori* protein. The aim of our study was to test the in vitro proliferation of lymphocytes from different origins in the presence of different *H. pylori* gastric MALT lymphoma strains.

Lymphocytes from peripheral blood mononuclear cells (PBMC) or tonsil cells were isolated by density-gradient centrifugation and cultured in complete RPMI 1640 with 10% fetal calf serum. *H. pylori* sonicates or viable bacteria were added to the lymphocytes with or without PHA/IL-2 (cell proliferation assay versus T-lymphocyte proliferation inhibition assay). Lymphocyte proliferation was determined by BrdU incorporation measured by flow cytometry.

We did not obtain a significant lymphocyte proliferation in the presence of viable *H. pylori* bacteria or sonicates alone. However, all *H. pylori* MALT strains were able to significantly inhibit T-lymphocyte proliferation. This antiproliferative effect was not obtained with culture supernatants nor with other bacteria (*Escherichia coli* and *Campylobacter jejuni*) used as controls. It was also abolished after tryspin treatment of the sonicates.

In conclusion, lymphocytes from PBMC of nongastric MALT lymphoma patients are not able to respond to *H. pylori* MALT strain antigenic stimuli in contrast to lymphocytes obtained from

gastric lymphoma biopsies (Hussel et al.). As described by Gerhard et al., *H. pylori* gastric MALT lymphoma probably harbors an antiproliferative protein.

Abstract no.: 03.06
Helicobacter pylori Induces Gastric Epithelial Cell Invasion in A c-Met and *cag* PAI-Dependent Manner

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Numerous studies have shown that *Helicobacter pylori* is able to interact with gastric epithelial cells, activating signaling pathways, modifying host cellular functions, and inducing cell phenotypes important for carcinogenesis. One of the less explored cell phenotypes induced by *H. pylori* is cellular invasion, and little is known about the mechanisms involved in this process.

Our aim was to investigate the role of *H. pylori* on gastric epithelial cancer cell invasion, and the mechanisms underlying this process. We also examined whether there were differences between strains in their ability to stimulate cell invasion, especially to assess the role of *cag* pathogenicity island (*cag* PAI), CagA, and VacA in cell invasion.

We found that *H. pylori* induced in vitro AGS cell invasion in two well-established invasion assays, collagen type I gels, and Matrigel filters. *H. pylori*-mediated cell invasion was blocked by the c-Met receptor inhibitor NK4, and by silencing c-Met expression with small interference RNA. Supernatants of cells cultured with *H. pylori* showed increased matrix metalloproteinase (MMP)-2 and MMP-9 activity, which was also suppressed by silencing the c-Met receptor. Studies with different *H. pylori* strains revealed that cell invasion, c-Met tyrosine phosphorylation, and increased MMP-2 and MMP-9 activity, were all dependent on the presence of an intact *cag* PAI.

Our findings suggest that *H. pylori* strains with an intact *cag* PAI activate c-Met and induce MMP-2 and MMP-9 activity, possibly increasing extracellular matrix degradation and leading to subsequent invasion of cancer cells.

Abstract no.: 03.07
Diversity in the Genomic Plasticity Zone in *Helicobacter pylori* Strains from Patients with Chronic Gastritis, Duodenal Ulcer, and Gastric Cancer

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Background. Comparison of the two genome sequences from *Helicobacter pylori* has shown that approximately 6–7% of the genes present in one strain is absent from the other and vice versa. About half of the strain-specific genes is found in the plasticity zone (PZ).

Objective. To determine the diversity in the PZ in *H. pylori* strains isolated from patient with gastritis (G), duodenal ulcer (DU), and gastric cancer (GC) and determine if there is any relation between the presence or absence of some genes and disease.

Experimental. Microarrays consist of 1,660 genes covering the J99 and 26695 genomes and were performed to 42 *H. pylori* isolates from 10 patients with G, 10 with DU, and 9 with GC. DNA from each strain was labeled with CY3 and hybridized with a mixture of J99 and 26695 labeled with CY5. Log_2 ratio = Cy5/Cy3 $\geq$ 0.5 value indicates presence of the gene. The association between presence or absence of genes and diseases was analyzed using χ^2.

Results. The gene core was found to be 1319, and 341 (20.5%) were strain-specific genes. Among the variable genes, 30 were statistically associated with one of the diseases: gastritis, ulcer, or cancer ($p < .10$). Many of these genes were on the PZ. These genes included unknown function, restriction-modification systems, outer membrane proteins, and *cag* PAI.

Conclusions. There is variability in the gene content within the PZ in *H. pylori* from individuals with different diseases and some of them are significantly associated with G, DU, or GC.

Abstract no.: 03.08
The Level of FHIT Gene Expression versus Cytotoxicity of *Helicobacter pylori* Strains

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Helicobacter pylori infection is the main cause of inflammation of the gastric mucosa and the development of gastric cancer. *H. pylori* displays considerable genetic diversity, including the presence of strains that produce cytotoxins VacA and CagA. One of the markers to evaluate the risk of gastric cancer is assessment of the fragile histidine triad (FHIT) proapoptotic protein. Inactivation of

the *FHIT* gene is observed in most human neoplasms and many precancerous conditions.

The aim of this study was to evaluate FHIT protein gene expression in the gastric mucosa of patients with symptoms of nonulcer dyspepsia (below 60 years of age), in correlation to the genotype of *H. pylori* strains *cagA* and *vacA*, as compared to the control group without coexisting infection.

The presence of *H. pylori cagA* and *vacA* genes was determined in bacterial DNA samples isolated from 25 patients (50 bioptates), with polymerase chain reaction method, using primers specific for both genes and strain types (s1, s2, m1, m2). The level of FHIT mRNA was established as a ratio to glyceraldehyde-3-phosphate dehydrogenase mRNA with real time reverse transcriptase-PCR method in 100 bioptates, exactly distributed from the antrum and the corpus.

The level of FHIT mRNA was found to be lower in all *H. pylori*-infected patients. A tendency of greater decrease of FHIT mRNA level for gastric mucosa of patients infected with *vacA*+ genotype (s1/m1) and *cagA*-negative *H. pylori* strains than those with *cagA*-positive strains was observed. The obtained results may be valuable in diagnosis and predicting the risk of gastric cancer development.

Abstract no.: 03.09
Adhesin-Receptor Interactions Involved in Adherence of *Helicobacter pylori* to Different Topographical Regions of the Human Stomach

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Background. Infection of the human stomach by *Helicobacter pylori* usually occurs in two main topograpical regions: the antrum and the fundus. Although the distribution of *H. pylori* is known to be influenced by acid, little work has been carried out to determine whether regional variation in the expression of cell-surface receptors also has a role to play; which was the aim of this study.
Methods. Both immunohistochemistry and binding assays (using *H. pylori* mutants lacking the adhesins BabA, SabA, AlpA, AlpB, OipA, and HopZ) were used to determine the presence of *H. pylori* receptors in biopsies from both the antrum and fundus of 10 patients with inflamed stomachs.
Results. Binding to BabA was reduced in all patients and SabA in two patients. Binding to AlpA and OipA was unaffected. Binding to AlpB was reduced in all patients and to HopZ in three patients. No differences were seen between the fundus or the antrum.
Discussion. The results suggest that the same receptors necessary for *H. pylori* adhesion are present in both the antral and fundal regions of inflamed human stomachs. Both BabA and AlpB made significant contribution to binding in all patients. SabA and HopZ was important in two and three patients, respectively. AlpA and Oip A made no contribution to binding.

Abstract no.: 03.10
Helicobacter pylori BabA Expression, Gastric Mucosal Injury, and Clinical Outcome

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Background. BabA, the blood group antigen-binding adhesion, has been proposed to play a role in disease pathogenesis. This hypothesis is based on the functional BabA status as determined by polymerase chain reaction (PCR) analysis to distinguish functional *babA2* genes from nonfunctional *babA1* genes.
Methods. We compared the ability of published PCR-based methods to assess BabA status with BabA immunoblotting and Lewis b (Le^b)-binding activity assays. We also used immunoblotting to examine the relationship between clinical presentation and levels of BabA expression.
Results. Immunoblotting and Le^b binding assays for 80 strains revealed three levels of BabA expression: BabA-high producers (BabA-H) with Le^b-binding activity, BabA-low producers (BabA-L) without Le^b-binding activity, and BabA-negative. BabA-negative strains lacked the *babA* gene. PCR methods to determine BabA status yielded poor results. *babA1* Sequences were never detected. BabA expression was examined in 250 strains from Western countries and 270 from East Asia. The results failed to confirm any relationship between triple-positive status (*cagA*-positive/*vacA* s1/BabA-H) and clinical outcome. BabA-negative strains were typically *cagA*-negative/*vacA* s2 and were associated with gastritis. BabA-L strains exhibited a higher level of mucosal injury and were more frequently associated with duodenal ulcer and gastric cancer than the other groups.
Conclusions. Information gained from currently used PCR-based methods must be interpreted with caution. Le^b-binding activity does not accurately reflect the severity of mucosal damage or the clinical outcome. Quantitation of BabA expression revealed that Le^b-nonbinding BabA-L strains are associated CagA and with higher levels of mucosal injury and clinical outcome.

Abstract no.: 03.11
Relationship of HP-Urease Enzyme with Oxidative Burst and T-cell Migration

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Introduction. In *Helicobacter pylori* gastritis, neutrophil activation plays a central role in the pathogenesis of the disease, and the immune response in the control of infection outcome. Urease is a major component of *H. pylori* surface proteins, and its participation in oxidative stress and inmune response intensity is unknown.
Objectives. To investigate the ability of *H. pylori* urease enzyme on oxidative burst and T-cell migration toward the gastric mucosa.

Materials and Methods. AGS were cultured with two *H. pylori* strains (10^7–10^8 CFU/mL/24 hours), one of them without the *ureC* gene ($HP_{not\ ureC}$).

Lymphocytes and polymorphonuclear leukocyte (PMNL) erythrocytes were separated on Ficoll-Hypaque gradient and PMNLs recuperated after erythrocyte lysis.

PMNLs were incubated with supernatants of AGS/*H. pylori* cultures 90′/37 °C /5% CO_2.

Oxidative stress was assessed with H_2-DCF-DA and MitoSOX. Analyses were performed by flow cytometry and confocal microscopy. For chemotaxis assays, lymphocytes and supernatants were placed in the upper and lower wells, respectively, separated by cellulose nitrate filters (5 µm pore). After 120′ incubation 37 °C/5%CO_2, chemotactic index was calculated as cells migrating toward supernatants or toward medium.

Results. Reactive oxygen species (ROS) synthesis in PMNLs was dose and urease dependent (32% *H. pylori* and 13.5% $H. pylori_{not\ ureC}$ with respect to control) but in AGS, a 1.3-fold increase was observed with all of the strains.

Conclusions. Urease is involved in T-cell recruitment and in oxidative stress observed in mucosa, increasing the ROS synthesis in PMNLs but not in epithelial cells.

	Control	$H. pylori_{not\ ureC}$ 10^7	$H. pylori_{not\ ureC}$ 10^8	*H. pylori* 10^7	*H. pylori* 10^8	FMLP
CI	1	0.64	1.75	1.25	2.61	3.53

FMLP: positive control.

Abstract no.: 03.12
Lack of Association between *dupA*-positive *Helicobacter pylori* Strains and Duodenal Ulcer/ Gastric Carcinoma in Brazilian Patients

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Recently, Lu et al. (2005) described a new putative *Helicobacter pylori* virulence marker (*dupA*) associated with an increased risk for duodenal ulcer (DU) and reduced risk for gastric cancer (GC). We investigated the presence of *cagA* and *dupA* (*jhp0917–0918*) in 482 *H. pylori* strains from Brazilian children (34 DU, 97 gastritis) and adults (126 DU, 144 gastritis, 81 GC). We constructed test on two sets of primers to test again *jhp0917–0918*–negative strains. PCR products from 89 strains were sequenced in order to detect the insertion of C or T (after position 1385) in the *jhp0917* 3′ region that characterizes *dupA*. *jhp0917–jhp0918* Were present in 445 (92.3%) and absent in 29 (6.0%) strains. Because an insertion of T or C was observed in 96.6% of the *jhp0917–jhp0918* positive strains, the presence of the two genes was considered to be *dupA* positive. Strains with only one gene were not included in the analysis. All samples from children with and without DU were *dupA* positive. No association was observed among the strains from adults with gastritis (92.3%), DU (87.3%), and GC (87.6%). Conversely, *cagA*-positive status was independently associated with DU (in adults and children) and with GC in logistic analysis. When children and adults were compared, the presence of *dupA* was significant higher in children, even when only the group of gastritis was analyzed (p = .01). In conclusion, *dupA* is highly frequent and it is not associated with GC or DU in both Brazilian adults and children, which points to regional differences in the distribution of *dupA*.

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Abstract no.: 03.13
Stability and Variability of *cagA* and its Correlation with Disease Outcome

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CagA of *Helicobacter pylori* has been associated with peptic ulcer disease and gastric cancer. CagA can be phosphorylated on tyrosine residues within EPIYA motifs, leading to cell spreading and elongation, a phenomenon termed hummingbird phenotype (HBF). In *H. pylori* strains, the number of phosphorylation sites (PHS) in CagA varies and has been associated with an increase in the extent of HBF. We investigated whether the encoding region of the EPIYA motifs is identical in isolates from different parts of an individual's stomach using isolates from fundus, corpus, and antrum of four patients. Pairs of *H. pylori* strains obtained between 7 and 10 years apart from seven other patients were used to determine the stability of the region through time. We also investigated whether the number of PHS in CagA correlated with disease outcome in 49 Mexican patients with differing gastrointestinal conditions. The *cagA* 3′ region was amplified by polymerase chain reaction, and results were analyzed using agar gel electrophoresis. Sequencing of 10 representative strains from the Mexican patients corroborated a correlation between higher molecular weight bands in gel electrophoresis and the presence of additional EPIYA motifs. The *cagA* 3′ region was identical in *H. pylori* strains isolated from different gastric regions studied, and over time. There was no correlation between the number of PHS in the translated CagA protein and severity of patient's gastrointestinal condition. We conclude that the number of PHS is stable in the predominant isolates over the time period and in the locations tested, but were not associated with clinical outcome.

Abstract no.: 03.14
Characterization of the Number and Type of Repeating EPIYA Phosphorylation Motifs in the Carboxyl Terminus of CagA protein in *Helicobacter pylori* Clinical Isolates

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We aimed to map the EPIYA tyrosine phosphorylation motifs (TPMs) *A*:EPIYAKVNK, *B*:EPIYAQVAKK, and *C*:EPIYATIDDLG in CagA protein, which have been proposed to enhance *cagA*-dependent pathogenicity. Sixty-five *Helicobacter pylori* clinical strains isolated from adults with nonulcer dyspepsia (n = 13), esophagitis (n = 12), gastric ulcer (n = 11), and duodenal ulcer (n = 29) were analyzed. In the 48 cagA-positive strains, the 3′ variable region of *cagA* gene was amplified and sequenced and the EPIYA motifs were mapped in the deduced protein sequences. *H. pylori* colonization and the associated gastritis were evaluated by the modified Sydney system and statistical analysis performed by χ^2 test and Fisher's exact test.

The majority of strains harbored the *ABC* (54.5%) and the *ABCC* combination of TPMs (13.6%). Only four strains were found to harbor additional TPM in the *ABCCC* (n = 3) or *ABABC* (n = 1) combinations. Eighty-five percent of strains isolated from gastroduodenal ulcers harbored *ABC* or *ABCC* combinations of TPMs. EPIYA presence in the CagA protein was correlated significantly with the development of gastroduodenal ulcer ($\chi^2 = 11.617$, $p = .0007$) and in particular with the presence of duodenal ulcer ($p = .0016$). There was significant positive association with the severity of chronic inflammatory infiltration ($p = .039$) and the activity of chronic gastritis ($p = .013$) in the antrum, but not with higher levels of *H. pylori* colonization ($p = .136$). In conclusion, the severity of chronic inflammatory infiltration and the activity of chronic gastritis developed in the antrum of *H. pylori*-positive patients may be associated with the presence of EPIYA TPMs in the CagA protein, irrespective of the levels of *H. pylori* colonization in the gastric mucosa.

Abstract no.: 03.15
Status of *dupA* Gene in Iranian *Helicobacter pylori* Isolates Recovered from Patients with Gastric Cancer

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Introduction. *jhp0917* and *jhp0918* genes are recently identified in the plasticity region, which form one continuous gene designated as *dupA* (duodenal ulcer promoting gene A). *dupA* Is identified as a novel marker of *Helicobacter pylori* associated with an increased risk for duodenal ulcer (DU) and reduced risk for gastric atrophy and cancer. We aimed to determine the prevalence of *dupA* gene in Iranian strains, with concentration on its relationship with histopathologic indices.

Methods. One hundred cases including 35 gastric cancer (GC), 48 nonulcer dyspepsia (NUD), 8 DU, and 9 gastric ulcer (GU) cases were studied. The amplification of *jhp0917* and *jhp0918* genes was performed and the *dupA* genotype was determined. In addition, histologic indices, including *H. pylori* load, atrophy, intestinal metaplasia, and lymphoid follicles were studied.

Results. The *dupA* gene was detected in 44% of Iranian *H. pylori* isolates (4% of isolates were positive for *jhp0917* gene but negative for *jhp0918*). More than half of the isolates from cardia GC patients (55%) and 33% of noncardia GC cases possessed the *dupA* gene, whereas 50% of DU, 41.7% of NUD, and 22.2% of GU cases were *dupA* positive. Among cancer patients, 58% of cases with atrophy and 52.6% of cases with intestinal metaplasia were *dupA* negative. Although the presence of the *dupA* gene was associated with reduced intestinal metaplasia and atrophy, this association was not statistically significant. There was no association with other pathologic indices.

Conclusion. Our preliminary findings indicate that the *dupA* gene can be informative of DU development and reduced risk of atrophy and intestinal metaplasia. However, further studies are required.

Abstract no.: 03.16
Study of the Serum Antibody Response against the Vacuolating Cytotoxin (VacA) from *Helicobacter pylori* in a Mexican Population

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VacA activity differs among isolates as a result of polymorphism in its gene. Its p58 subunit may be type m1 or m2. Type m1 binds more extensively to cells and has been associated more closely with gastric adenocarcinoma than type m2. We aimed to study the antibody response against p58 in a Mexican population.
Methods. Serum and gastric biopsies were obtained from 147 infected individuals. These included 92 cases with chronic gastritis only, 34 with precancerous lesions (intestinal metaplasia, atrophy, or dysplasia), 19 cases with duodenal ulcer, and 2 with gastric cancer. Twenty-seven noninfected individuals were used as controls. Strains were *vacA* genotyped by polymerase chain reaction and recombinant m1 and m2 p58 subunits were used in enzyme-linked immunosorbent assay (ELISA) for serology.
Results. Of 147 infected individuals, 104 were seropositive for VacA; sera from 65 of these recognized both m1 and m2 antigens and sera from 39 recognized one only. From the 39, 37 (95%) had ELISA results that correctly identified the genotype found in isolates (p = .008 Fischer Exact Test). Intensity of the IgG response was higher for m1 than m2 ($p < .05$). Levels of p58 antibodies in precancerous lesions were significantly higher than for duodenal ulcer ($p < .01$).
Conclusion. Mixed serological response to both m1 and m2 antigens is frequent. The m1 toxin induces higher levels of antibodies than m2. High antibody response is significantly associated with precancerous lesions. VacA serotyping is accurate when there is a serological response to only one toxin type.

Abstract no.: 03.17
Increased Amidated Gastrin-17 (PGL) Systemic Levels and Gastric Epithelial Cell Proliferation (GECP) in Dyspeptic Patients with CagA-Positive (CagA+) *Helicobacter pylori* Infection

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Background and Aim. High gastrin systemic levels and augmented gastric epithelial cell proliferation (GECP) are involved in gastric carcinogenesis. Infection with CagA-positive *Helicobacter pylori* is associated with an increased risk of gastric cancer (GC) too. We aimed to verify the possible influence of the CagA status on both these parameters.
Methods. Blood samples and antral gastric biopsies were taken from 116 dyspeptic patients. We determined the *H. pylori* status by histology, serology [enzyme-linked immunosorbent assay (ELISA)] and urease rapid test on biopsies, anti-CagA antibodies by Western blotting, basal amidated gastrin-17 (PGL) by ELISA and epithelial proliferation by anti-proliferating cell nuclear antigen immunostaining as percentage of labeled cells in gastric pits.
Results. Sixty-nine patients (59.4%) were infected and anti-CagA antibodies were detected in 51 of them (73.9%). PGL in patients infected with CagA-positive strains (8.5 ± 3.1 pmol/L) were significantly higher than in patients infected with CagA-negative strains (4.6 ± 1.5 pmol/L) (p = .05) and in uninfected patients (4.8 ± 2.1 pmol/L) (p = .05). Proliferation scores in patients infected by CagA-positive *H. pylori* were also higher in than in CagA-infected and in uninfected patients ($p < .001$, χ^2 test for linear trend).
Conclusions. In *H. pylori*-infected patients, both hypergastrinemia and increased gastric cell proliferation seem to be related to the CagA status rather than to the infection itself. Our results suggest that the increased GC risk run by CagA-positive *H. pylori*-infected individuals could be partially attributed to an up-regulation of growth factors such as gastrin.
Acknowledgements. This study was partly funded by the Siena University grant PAR 2004, "*Helicobacter pylori* infection, host's aplotypes of inflammatory cytokines and risk of ischemic heart disease".

Abstract no.: 03.18
Detection and Quantification of *Helicobacter pylori cagA* Gene in Gastric Biopsies of Chilean Patients by Real-Time PCR

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Background. The *cagA* gene is one of most studied in *Helicobacter pylori* because of its association with atrophic gastritis, intestinal metaplasia, and gastric cancer. The latter, being the second most common cause of death in the world.

In this work, we have used real-time polymerase chain reaction (PCR) technology because of its higher sensitivity and specificity for the analysis of gastric biopsy samples.

The aim of this work was to detect and quantify *cagA* on gastric biopsies from Chilean patients with diverse histopathologies (nonatrophic chronic gastritis, atrophic gastritis, intestinal metaplasia, neoplasia, and gastric cancer).
Methods. We analyzed 88 gastric biopsy samples (one from the antrum and one from the corpus, from 44 patients) positive for the 16Sr RNA gene of *H. pylori*, then the *cagA* gene was detected and quantified by real-time PCR.
Results. *cagA* Was detected in greater percentage, 83% (29/35), in the most aggressive lesion (gastric atrophy, metaplasia, neoplasia, and gastric cancer) when compared with nonatrophic chronic gastritis, 56% (5/9). On the other hand, *cagA* was detected equally in the antrum and corpus. These results also suggest an assosiation between the severity of the gastric injury and the *cagA* gene quantity. That is to say, *cagA*-positive bacteria are more abundant in severe lesions than they are in less severe lesions.

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Abstract no.: 03.19
Distribution and Allelic Diversity of a Novel Peptic Ulcer Marker Candidate in Different Geographical Regions

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Background. *jhp*0870 Was correlated with peptic ulcer disease (PUD) in Portuguese children, and was strongly associated with the more virulent genotypes, whereas its 90% homologue, *jhp*0649, was strongly associated with gastritis and the less virulent genotypes. Both code putative outer membrane proteins. Allelic variation was observed in the midregion of these genes.
Aims. Study prevalence and allelic diversity of both genes in *H. pylori* strains from different countries.
Materials and Methods. Two hundred ninety-one *H. pylori* strains from PUD, nonulcer dyspepsia (NUD) or gastric adenocarcinoma (GC) (Portugal n = 115, Brazil n = 57, France n = 50, USA n = 10, Colombia n = 10, Japan n = 10, Korea n = 10; England n = 7, Sweden n = 7, Norway n = 4, Burkina-Faso n = 11) were genotyped by polymerase chain reaction and sequencing.
Results. Both genes presented heterogeneous distribution, being the major differences in Asian strains (90% for *jhp*0870, 15% for *jhp*0649). *jhp*0870 Correlated with PUD compared to NUD in Brazil, Colombia, and Korea. It showed high prevalence among Norwegian and English PUD strains but not in Swedish ones. It has no difference between PUD and NUD for Portuguese, French, and USA strains and is present in all Japanese strains. It is significantly lower in Portuguese and Brazilian GC strains than overall prevalence in these countries. *jhp*0649 Associated with GC in Portugal and presented low prevalence in Colombian, Japanese, and Korean PUD strains.

Each gene presented predominant but distinct allelic variant.
Conclusions. Both genes displayed a varied worldwide distribution. None constitutes a universal disease-specific marker. *jhp*0870 Tends to be more associated with PUD and *jhp*0649 to GC. Allelic conservation observed in a polymorphic genetic region suggests its importance for the function of proteins coded by these genes.

Abstract no.: 03.20
Prevalence of Virulence-Associated Genes among *Helicobacter pylori* Isolated from a Chilean Population and Its Relationship with Gastric Mucosa Lesions

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Background. *Helicobacter pylori* induces an inflammatory response in the stomach that persists for decades. The biological costs for patients include an increased risk for peptic ulceration and gastric carcinoma. The identification of specific strains by genetic tools that stimulate particular diseases is the main goal for many laboratories. Moreover, the identification of a relationship between virulence markers and geographical areas is also crucial. The aim of this work was to evaluate the prevalence of selected virulence genes *cagA*, *vacA* (alleles s1a, s1b, s2, m1, and m2), *iceA* (alleles 1 y 2), *babA2*, and its relationship to a specific gastric lesion intensity.
Methods. Two hundred sixty biopsy samples (50% from the antrum and 50% from the corpus) were studied. Samples were analyzed by conventional polymerase chain reaction using specific primers that included both a 16S-23S rDNA hypervariable region and ureC markers for species identification.
Results. Forty-eight percent and 57.7% of the samples contain *H. pylori* as detected by 16S-23S rDNA and ureC primers, respectively. Virulence genes identified were as follows: *cagA* 32.7%, *vacAs1a* 40.7%, *vacAs1b* 20.7%, *vacAs2* 32.7%, *vacAm1* 42.0%, *vacAm2* 44.7%, *iceA1* 26.0%, *iceA2* 67.7%, and *babA2* 5.3%, respectively. There was no significant difference in the positive rates of gene detection and the intensity of the lesion in patients. In addition, no differences were also observed among genetic markers between the antrum and the corpus isolates in the same patient.
Conclusion. In Chilean patients, no correlationship between virulence markers of the clinical isolates of *H. pylori* and gastric lesions intensity was observed.

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Abstract no.: 03.21
Relationship between *Helicobacter pylori* CagA and VacA Serological Status and Bacterial Density on Gastric Mucosa

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Aim. To evaluate the effects of CagA and VacA seropositivity on *Helicobacter pylori* bacterial density in bioptic samples of gastric mucosa.
Methods. Study was carried out on 207 patients undergoing upper endoscopy. All patients were *H. pylori* positive based on results of histology and serology. To all patients, serum antibodies to p 120 (CagA) and p 95 (VacA) proteins of bacteria *H. pylori* were assessed by using Western blot method. Histological data were analyzed according to updated Sydney classification with special attention to the grade of *H. pylori* colonization. Statistical analysis was performed using χ^2 test.
Results. the study population consisted of 91.3% (189/207) CagA antibody positive and 75.8% (157/207) VacA antibody-positive patients. The distributions of CagA- and VacA-positive and -negative patients according to *H. pylori* bacterial load grade are presented in Table 1.
Conclusions. CagA and VacA seropositivity have no influence on bacterial load grade in the gastric mucosa.

Table 1 CagA and VacA serological status and bacterial density

	Corpus			Antrum		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Garde 3
CagA+	108	50	31	66	69	54
CagA–	13	2	3	9	4	5
VacA+	92	40	25	55	62	40
VacA–	30	12	8	20	11	19

No statistically significant difference in grades of bacterial load was found between CagA-positive and CagA-negative patients in the corpus and antrum of the gastric mucosa ($\chi^2 = 2.180$, d.f. 2, $p < 1.0$; $\chi^2 = 1.986$, d.f. 2, $p < 1.0$). The same results were obtained for VacA-postive and VacA-negative patients ($\chi^2 = 0.0515$, d.f. 2, $p < 1.0$; $\chi^2 = 5.634$, d.f. 2, $p < 1.0$).

Abstract no.: 03.22
Vacuolating Activity of Different *Helicobacter pylori* Colonies Isolated from the Same Dyspeptic Patients with Peptic Ulcer (PU) or with Chronic Gastritis Only

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Background. Infection by vacuolating cytotoxic *Helicobacter pylori* strains (VacA+) increases the risk of peptic ulcer (PU). As infected patients may simultaneously harbor various *H. pylori* genotypes, we tested four to eight different *H. pylori* colonies per patient to better understand the pathogenic role of VacA.
Material and Methods. We examined 78 *H. pylori* strains, 38 from 11 patients with PU and 40 from 11 patients without ulceration (WU). Forty-eight–hour agitated broth cultures were centrifuged and sterilized by filtration. Filtrates were added to Vero, HeLa, and Chinese hamster ovary cells in vitro at dilutions ranging from 1 : 2 to 1 : 32, in triplicate. Strains were considered VacA positive if ≥ 30% of cells of whatever line were vacuolated after 24 and 48 hours of incubation. The "s" and the "m" subtypes of the *vacA* gene were determined by polymerase chain reaction.
Results. In only 15 of the 22 patients that all strains from the same patients were either cytotoxic or noncytotoxic; 25 of 38 strains from PU patients were VacA positive (65.7%), versus 15 of 40 strains from WU patients (37.5%) ($p = .013$; OR = 3.21, 95% CI = 1.15 to 9.06). Almost all VacA positive strains were s1/m1 *vacA* subtype.
Conclusions. A fair proportion of patients harbors either VacA-positive and VacA-negative strains at the same time. Oscillation in the predominance of VacA-positive and VacA-negative bacterial population may account for spontaneous healing and recrudescence of PU disease.
Acknowledgements. This study was partly funded by the Siena University grant PAR 2004, "*Helicobacter pylori* infection, host's aplotypes of inflammatory cytokines and risk of ischemic heart disease".

Abstract no.: 03.23
Cyclooxygenase-2 Expression in *Helicobacter pylori*-Infected Early Gastric Carcinoma

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Introduction. Previous studies demonstrate that increased cyclooxygenase-2 (cox-2) expression is observed in both *Helicobacter pylori*-related gastritis and gastric carcinoma. Little is known about the cox-2 expression in *H. pylori*-infected gastric carcinoma.
Aim. We studied the expression of cox-2 in *H. pylori*-infected early gastric cancer tissue after being successfully treated with endoscopic mucosal resection and their paired adjacent mucosa, as well as their follow-up endoscopically biopsied mucosa after *H. pylori* eradication.
Method. The expression of cox-2 in 20 patients with *H. pylori*-infected early gastric carcinoma (15 men and 5 women; mean age, 63.8 years) and their follow-up biopsy (mean follow-up period, 27.4 months) was assessed by immunohistochemical stain. Immunoreactive score was calculated by multiplication of the grade determined by the percentage of positive cells and the staining intensity (scale, 0–9).
Results. Slightly increased expression of cox-2 was found in cancer tissue compared to their respective paired *H. pylori*-infected normal mucosa (4.5 ± 3.7, 3.2 ± 1.9), but statistically not significant ($p = .07$). After *H. pylori* eradication, the cox-2 expression was markedly decreased (1.5 ± 1.3, $p < .05$).
Conclusion. Cox-2 overexpression may contribute to an early event of *H. pylori* infected gastric carcinoma.

Abstract no.: 03.24
Distribution of Virulence Marker of *Helicobacter pylori* Patients with Functional Dyspepsia in Pakistan

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Aim. To determine the distribution of virulence markers of *Helicobacter pylori cagA, vacA,* and its allele types in patients with dyspepsia.
Methodology. Endoscopic-negative functional dyspepsia patients were enrolled from January to December 2005. Gastric antral biopsies were obtained for rapid urease test, histopathology, culture, and polymerase chain reaction (PCR) for *H. pylori* virulence markers *cagA, vacA,* and its allele types. PCR for *cag A* and *vacA* alleles were performed using primers previously described.
Results. Of 94 patients, 64 (68%) were men, age range 18–70 years and mean age 40 ± 11.3 years. Epigastric pain syndrome was present in 81 (86%) and postprandial stress syndrome in 13 (14%). Esophagogastroduodenoscopy showed hyperemia in all 94 (100%). Rapid urease test was positive in 89 (95%). Histopathology was only performed in 53 (56%) and it showed *H. pylori*-associated moderate gastritis in 36 (68%) and mild gastritis in 12 (23%) whereas nonspecific gastritis in 5 (9%). The PCR for *cagA* was positive in 39 (41%) and negative in 55 (59%). The *vacA* allelic types were *s1a m1* 41 (44%), *s1a m2* 22 (23%), *s1b m1* 8 (9%), and *s1b m2* 19 (20%); however, it could not be obtained in four

isolates. *CagA s1a m1* was positive in 16 (17%), *cagA s1a m2* 11 (12%), *cagA S1b m1* in 3 (3%), and *cagA S1b m2* in 8 (9%).
Conclusion. In functional dyspeptic *H. pylori,* infection was frequently with *cagA*-negative strains. However, the dominant virulence pattern in these isolates was cagA-positive vacA S1a m1 allelic type.

Abstract no.: 03.25
Virulence Genotypes of *Helicobacter pylori* in Palermo, Italy

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Aims. To determine the distribution and/or the functional status of *s1-vacA, cagA, oipA, babA2, sabA,* and *hopQ* genotypes of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Palermo, Italy, and to explore the association between the different genes and their usefulness for predicting the clinical outcome of infections.
Methods. Forty-three strains of *H. pylori* were isolated from gastric biopsies of 24 patients with gastritis (16) and/or duodenal ulcer (8); only 28 of these strains were considered for evaluation; 15 were excluded as considered duplicates after genotyping. The virulence genotypes were determined by polymerase chain reaction.
Results. The *s1-vacA, cagA*$^+$ (all *s1-vacA*), *s1/m1-vacA,* and *s1/m1-vacA/cagA* positive genotypes were detected in 75% (21/28), 64.3% (18/28), 42.9% (12/28), and 32.1% (9/28), respectively, of the *H. pylori* strains. The *oipA* "on" status, exhibited by 31.8 (7/22) of the strains, showed only a weak association with the *cagA* gene (71.4% of the *oipA* "on" strains were *cagA* positive, 35.7% of the *cagA*-positive strains were *oipA* "on"); the *babA* positive and the *sabA* "on" genotypes, present in less than 25% of the strains, were strongly associated with each other and with the *cagA*-positive genotype; the type I *hopQ* allele was predominant, and predominantly (but not always) associated with the *s1-vacA/cagA*-positive genotype: in 29.2% (7/24) of the strains. After amplification, both types I and II *hopQ* products were observed.
Conclusions. Many results are concordant with data in literature. Differences, to be correctly evaluated, might be confirmed by additional isolations.

Abstract no.: 03.26
Functionally Active *Helicobacter pylori* Vacuolating Cytotoxin in *Escherichia coli*

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Background and Aims. Although the expression of VacA toxin in *Escherichia coli* has been attempted, the production of a functionally active recombinant version has been rare. In this study, we produced the active recombinant VacA to gain further insights into the pathological activities of VacA in gastric epithelial cells.
Methods. A soluble form of about 90-kDa VacA s1/m1 genotype fused with 8X histidine tagged at the C terminus was expressed in *E. coli* at low temperature. The antisera was raised in a rabbit by intradermal injections of this recombinant protein.
Results. The recombinant VacA was able to induce vacuolation and apoptosis in AGS cells and HeLa cells. Anti-rVacA antibody reacted with supernatants from *Helicobacter pylori* carrying the s1/m1 *vacA* gene in an enzyme-linked immunosorbent assay and an immunoblot with a 88-kDa protein. Furthermore, the immunoglobulins neutralized the vacuolating activity completely and inhibited cell death induced by both rVacA and supernatant of *H. pylori* in AGS cells.
Conclusion. These data indicate that this recombinant VacA has function and structure similar to those of native VacA, which could be useful in exploring the pathogenic role of VacA.

Epidemiology and Transmission

Abstract no.: 04.01
Environmental and Host Factors Associated with Risk of *Helicobacter pylori* Infection in Brazil

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There are few studies evaluating host genetics as risk factors for *Helicobacter pylori* infection. We investigated associations between functional polymorphisms in genes linked to the innate and adaptative immune response and susceptibility to HP infection in logistic models, adjusting for demographic and environmental factors. We included 541 blood donors; 370 (68.4%) were *H. pylori* positive by enzyme-linked immunosorbent assay. *IL1B*-511C/T, *TNFA*-307G/A, *TLR4*+896A/G and *TLR2*+2251G/A were genotyped by polymerase chain reaction (PCR)-restriction fragment-length polymorphism, *IL1RN* by PCR, *IL1B*-31T/C, and *IL2*-330T/G by PCR-confronting two-pair primer (CTPP) and *TLR5*+1174C/T by allele-specific PCR. All results were confirmed by sequencing. *IL1B*-511 and *IL1B*-31 were in almost complete linkage disequilibrium, whereas the other loci segregated independently. The models were well fitted and in the univariate analysis, age ($p = .001$), gender ($p = .02$), crowding index ($p = .20$), socioeconomic

level (p = .00), *TNFA* (p = .11), and *IL2* (p = .02) polymorphisms were selected. In the multivariate analysis, age (p = .02, OR=1.03, 95% CI = 1.01–1.05), socioeconomic level (p = .00, OR = 0.58, 95% CI = 0.42–0.78) and polymorphic allele of *IL2*-330 (p = .04, OR = 0.67, 95% CI = 0.45–0.95) remained independently associated with the infection. The *IL2*-330 heterozygote genotype was overrepresented in the group of *H. pylori*-negative subjects. These results were confirmed when groups of *H. pylori*-positive patients with gastritis (n = 307), duodenal ulcer (n = 240), and gastric cancer (n = 164) were compared with the *H. pylori*-negative blood donors (p = .04), and *H. pylori*-positive ones (p = .50). It is worth emphasizing that according to Matezans et al., *IL2*-330 heterozygote genotype is the highest IL-2 producer. Considering the importance of IL-2 in the immune response including lymphocyte activation and proliferation, cell cycle advancement, and apoptosis, it is possible that subjects harboring this polymorphism mount a more effective immune response that protects them against *H. pylori* infection.

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Abstract no.: 04.02
Shift Work Increases the Ulcerogenic Potential of *Helicobacter pylori* Infection

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Background. Previous studies showing an association between shift work and peptic ulcer disease (PUD) did not take into account the role of *Helicobacter pylori*. The aim of the present study is to verify whether shift work may increase the ulcerogenic potential of *H. pylori* infection.

Patients and Methods. Four hundred fifteen consecutive active workers (129 shift workers and 286 daytime workers) with persistent dyspeptic symptoms, and tested positive for *H. pylori* infection at urea ^{13}C breath test, were considered for upper gastrointestinal endoscopy to evaluate the prevalence of PUD. Twenty-eight shift workers and 39 daytime workers did not meet the inclusion criteria for endoscopy (no treatment with gastric antisecretory drugs or antibiotics during the previous month and no regular nonsteroidal anti-inflammatory drugs use) and were therefore excluded. At endoscopy, three biopsies were taken from the gastric antrum and from the body. At each site, *H. pylori* infection was diagnosed if culture was positive and/or if the organism was concomitantly detected at histology (Giemsa staining) and urease testing.

Results. The prevalence of duodenal ulcer was higher in shift workers than in daytime workers (29 out of 101, 28.7% versus 23 out of 247, 9.3%; $p < .0001$), and persisted after multivariate analysis, taking into account age, sex, smoking, familial history of peptic ulcer disease, length of work, and social status as possible confounders. Odds ratio 3.97, 95% CI = 2.08–7.23).

Conclusions. Shift working increases the ulcerogenic potential of *H. pylori*. Application of a test and treat strategy in these workers may have strong health and economic implications.

Abstract no.: 04.03
Work-related *Helicobacter pylori* Infection among Sewage Workers of Municipal Wastewater Treatment Plants (WWTPs) in Belgium

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Introduction. Studies among sewage workers of wastewater treatment plants (WWTPs) have found higher prevalence of gastrointestinal symptoms. *Helicobacter pylori* has been detected in pre- and post-treated wastewater (sewage). Health and hygiene questionnaire result found higher prevalence of peptic ulcers among Belgian WWTP operators and maintenance workers.

Objectives. To assess prevalence of *H. pylori* infection in Belgian (Flemish) sewage workers of municipal WWTPs to determine whether occupational exposure to sewage is an important risk factor for the acquisition of *H. pylori* and possible association with gastrointestinal symptoms.

Methods. Seroprevalence study of *H. pylori* antibodies was conducted among 317 WWTP employees. Information about demographic variables, possible *H. pylori* risk factors (educational level of worker and parents, childhood characteristics, exotic journeys), working history data, and history of gastrointestinal symptoms during the last 3 months was obtained by questionnaire. Presence of *H. pylori* immunoglobulin G (IgG) was investigated with enzyme-linked immunosorbent assay. Results were compared with those of 250 employees of a Flemish pharmaceutical company.

Results. Prevalence of *H. pylori* IgG antibodies among sewage workers of WWTPs was 16.7% compared to 13.6% among the pharmaceutical control group. In logistic regression model controlling for both age and educational level, odds ratio study/control group was 1.02 (95% CI = 0.58–1.80 with p = .93). No significant associations were found between *H. pylori* seropositive status and the gastrointestinal symptoms, occupational exposures in different tasks, or with hygienic practices.

Conclusion. Our data do not support the concept that *H. pylori* infection is a probable cause of gastrointestinal symptoms among municipal WWTP workers.

Abstract no.: 04.04
Evolution of Yearly Prevalence of *Helicobacter pylori* Infection: A 15-Year Prospective Study in Consecutive Outpatients (Brussels 1990–2005)

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Background. Prevalence of *Helicobacter pylori* infection is declining in developing countries since the last 4–5 decennia as a result of

higher standards of living, higher levels of education, better sanitation, and probably also as the consequence of the frequent use of antibiotics for different medical problems. This observational study allows an indirect estimation of the decreasing prevalence of *H. pylori* infection in our population.

Method. All consecutive adult patients, without history of recent intake of antimicrobials, PPI, or Bi salts, attending the outpatient endoscopy clinic since 1988 are prospectively tested for the presence of *H. pylori* infection. *H. pylori* status is assessed in antral and body biopsies by urease test, histology, and culture.

Results. Nine thousand nine hundred fifty-one consecutive patients were included from January 1, 1990 to December 31, 2005. The prevalence of *H. pylori* infection in this population attending the endoscopy clinic decreased gradually from 41% in 1990 to 21.9% in 2005, as reported in Table 1.

Conclusion. During this 15 consecutive year observational study, the prevalence of *H. pylori* infection in naïve patients attending the endoscopy clinic decreased significantly.

This observation confirms the declining prevalence rate of *H. pylori* infection in developed countries, which probably contributed to declines in gastro-duodenal ulcer disease and gastric cancer in these countries.

Table 1

	Year								
Population	1990	1991	1992	1993	1994	1995	1996	1997	1998
Nb patients screened	863	787	859	807	725	639	606	543	549
Nb patients *H. pylori*+	354	307	310	278	261	224	204	175	179
H. pylori+ (%)	41.0	39.0	36.1	34.4	36.0	38.0	33.7	32.2	32.6

	Year							
Population	1999	2000	2001	2002	2003	2004	2005	Total
Nb patients screened	526	564	519	556	491	497	420	9951
Nb patients *H. pylori*+	172	162	145	138	127	139	92	3267
H. pylori+ (%)	32.7	28.7	27.9	24.8	25.9	25.9	21.9	32.8

The declining prevalence of *H. pylori* was statistically significant (< .05; χ^2 test, Spearman rank correlation test).

Abstract no.: 04.05
The Polymorphisms of Pro-Inflammatory Genes Associated with Gastric Cancer: A Meta-analysis

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Background. Many studies have investigated the association between gastric cancer risk and pro-inflammatory cytokine gene polymorphisms. We have conducted a series of meta-analyses to estimate the association between IL-1B, IL-1RN, IL-8, IL-10, TNF-α single nucleotide polymorphisms (SNPs), and gastric cancer, to establish more precise estimates of risk.

Methods. We have identified all published literature and meeting abstracts from: MEDLINE, PubMed, EMBASE, Web of Science, and BIOSIS databases between 1990 and 28 April 2006. No language limitations were set and where data are missing, authors are being contacted. Sixteen thousand two hundred sixteen references have been included in the REFERENCE MANAGER version 11 (ThomsonResearchSoft2005) with predefined inclusion criteria.

Results. Eight thousand three hundred forty-seven duplicate reports were found with the remainder divided into groups of exclusion categories. A second investigator was included to provide independent review of 1,500 potentially eligible references. To date, we have analyzed IL-10-1082 and IL-10-592 in a subset of reports where no contact with authors was needed. In analyses of SNP-1082, nine studies were used for the calculation of the odds ratio (OR) in a random effects model. ORs were 1.06 (95% CI = 0.87–1.31), 1.60 (95% CI = 0.70–3.64), and 1.24 (95% CI = 0.89–1.73) for AG, GG and G*, respectively, with substantial heterogeneity. Seven studies were included in analyses of SNP-592, OR were 0.85 (95% CI = 0.62–1.16), 0.84 (95% CI = 0.58–1.20) and 0.93 (95% CI = 0.76–1.14) for AC, AA and A*, respectively, with low or no heterogeneity.

Conclusion. To date, our results have shown no statistically significant relationship between SNP IL-10-1082, and -592 and gastric cancer.

Abstract no.: 04.06
The Prevalence of *Helicobacter pylori* Infection in Children in Poland – Epidemiological Studies

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Introduction. *Helicobacter pylori* is one of the most widespread bacterial infection all over the world.

Objective. The estimation of *H. pylori* occurrence depending on age, sex, and place of living.

Material and Methods. Nine thousand children aged 0 to 18 years, were selected randomly in six centers, 1500 in each. Children were chosen from various regions: voivodeship city, district town, and from the rural area, 500 from each, respectively. Children were selected in five age groups, taking into consideration proportion in a given population and sex. For examination, 3432 children were submitted. Anti-*H. pylori* antibodies (IgG) above 24 U/mL was

assumed as the base for infection. In 209 children, genetic examination of *H. pylori* strains was performed (polymerase chain reaction).
Results. The prevalence of *H. pylori* infection in Poland in children aged 0 to 18 years was 30.4%. A more frequent occurrence of infection in the first three years of life, and its increase along with the age since 4 years of age was demonstrated. No influence of sex on the infection frequency was found. Statistically significantly more frequent infections were observed in children living in the rural area and small towns than in children living in large cities (socioeconomic factors). Among 209 cases studied for *H. pylori* strains, gene *cagA*(+)*s1m1* was observed in 34%, *cagA*(+)*s1m2* in 32%, *cagA*(−)*s2m2* in 14%, *cagA*(−)*s1m1* in 3.3%, *cagA*(−)*s1m2* in 5%, mixed genotype, 9.7%.
Conclusions. The rate of *H. pylori* infection in children aged 1 to 18 years was 30.4%. Infections in the studied group in most cases were caused by *H. pylori* strains of greater harmfulness (*cagA*(+)*s1m1*, *s1m2*).

Abstract no.: 04.07
Does *Helicobacter pylori* Infection Affect the Occurrence of Dyspeptic Symptoms in the General Population? A Multicenter Study

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Background. The role of *Helicobacter pylori* infection in functional dyspepsia remains controversial.
Aim. To asses the incidence of dyspeptic symptoms in *H. pylori*-infected representative group of Polish population (a multicenter study).
Methods. The *H. pylori* infection incidence was assessed in 3307 adult subjects selected randomly from the population of big cities, small towns, and villages in proportion to age, basing on the anti-*H. pylori* IgG antibody titers determined by enzyme-linked immunosorbent assay. Every subject was interviewed using questionnaire regarding dyspeptic complaints – bloating, rumbling, fullness, discomfort centered in the upper abdomen (discomfort c.u.a.), vomiting, and early satiety.
Results. The incidence of the infection in observed group was 2784/3307 (84.2%). The frequency of all analyzed symptoms was similar in both *H. pylori*-positive and *H. pylori*-negative subjects (Table 1).
Conclusion. *H. pylori* infection seems to have no impact on the occurrence of some dyspeptic symptoms in the general population.

Symptoms	n	*H. pylori* positive (%)	*p*
bloating (+)	1059	902 (85.2%)	NS
bloating (–)	2247	1881 (83.7%)	
rumbling (+)	698	590 (84.5%)	NS
rumbling (–)	2607	2193 (84.1%)	
fullness (+)	456	390 (85.5%)	NS
fullness (–)	2847	2392 (84.0%)	
discomfort c.u.a. (+)	581	484 (83.3%)	NS
discomfort c.u.a. (–)	2720	2293 (84.3%)	
vomiting (+)	165	141 (85.4%)	NS
vomiting (–)	3140	2641 (84.1%)	
early satiety (+)	701	602 (85.9%)	NS
early satiety (–)	2602	2178 (83.7%)	

Abstract no.: 04.08
Amplification of *babA* and Urease genes of *Helicobacter pylori* from Oral, Gastric, and Vaginal Yeasts

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Background. Yeasts, mainly *Candida* species, are carried in the oral cavity, stomach, anus, groin, and vagina of healthy individuals. Occurrence of *Helicobacter pylori* the oral yeast has been demonstrated. In this study oral, gastric, and vaginal yeasts were examined for the presence of *H. pylori* genes, using the polymerase chain reaction (PCR) method.
Methods. Seven oral and seven gastric yeasts were isolated from dyspeptic referrals to the endoscopy unit. Five vaginal yeasts were isolated from patients in gynecology ward. DNAs were extracted from yeasts and PCR was performed for detection of *babA* (in three oral and seven gastric yeasts) and ure G+H (in four oral and five vaginal yeasts). PCR products were analyzed by electrophoresis.
Results. Amplification products of *babA* gene from yeasts was 1056 bp and homologous to that of *H. pylori*. This product was obtained from one of three of oral and five of seven of gastric yeasts. PCR product of urease gene with 600-bp size was amplified from four of four oral and three of five vaginal yeasts. The size of product was homologous to that of control *H. pylori*.
Discussion. Ubiquitous yeasts are well known for their sophisticated lifestyle and persistency against environmental stresses, such as antibiotics. Yeasts develop stable or changing populations within individuals, indicating their easy transmission through close contact or food consumption. Furthermore, yeasts have been demonstrated as natural reservoirs of *H. pylori*. Results of this study showed the existence of *H. pylori* in the oral, gastric, and vaginal yeasts. Accordingly, yeasts could be considered as potent vehicles for the transmission of *H. pylori*.

Abstract no.: 04.09
Helicobacter pylori IgG Antibody Positivity at Dokuz Eylül University Hospital, Turkey

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Helicobacter pylori infection rate is high in developing countries compared to the developed ones. The aim of this study is to determine the seroprevalance of serum IgG levels of *Helicobacter pylori* in our hospital. Between April 2002 and December 2005, the 3168 sera of the patients (mean age, 43.3 ± 20.1 years) admitted to different outpatient clinics at Dokuz Eylul University Hospital were included in this study. Among these patients, 1957

of them (61.8%) were women and 1211 (38.2%) were men. *H. pylori* IgG EIA (Radim®) was used as a screening test. The seroprevalance of *H. pylori* IgG was 43.8%, the negative population was 47%, and the equivalent population was 9.2%. Anti-*H. pylori* IgG seropositivity among women were 45.6%, and 40.9% in men, but no significant statistical difference on *H. pylori* seroprevalance was found between the two sexes ($p = .83$). *H. pylori* seroprevalance during puberty (0–18 years) period was 11.7%, between 19–40 years was 45.5%, and among the other age groups (41 years and over), the ratio was 51.2%. These results were significant. As a result, no significant difference on the seroprevalance of *H. pylori* was found between men and women. Besides this, the seroprevalance of *H. pylori* increased with increasing age. It was very interesting to find out the low rate of *H. pylori* IgG seropositivity in our region. This could be the result of the high socioeconomic status of the people around this area, antimicrobial treatment for *H. pylori* eradication, a different treatment regimen, and recently or previously antibiotics used.

Abstract no.: 04.10
Specific Geographic Genotypes among *Helicobacter pylori* Strains

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Helicobacter pylori, a genetically diverse bacterium, has been shown to have geographic specific genotypes. This may be relevant as there also are differences in clinical outcome among *H. pylori*-positive persons from different geographical origins.

The aim of this study was to identify a marker for African origin based on the sequence of a housekeeping gene (*hspA*) and if so, to establish possible differences in virulence attributes between African and non-African strains.

Using polymerase chain reaction (PCR) amplification, *hspA* was analyzed with direct sequencing of the PCR products in 168 strains from patients of diverse ethnic origins, including East Asian (29), Amerindian (28), Caucasian (17), African American (27), and Hispanic (67). To assess for virulence, the *cag* pathogenicity island (*cag* PAI) was analyzed by three PCR amplifications focused on differing parts of the island.

hspA Was very informative for differentiating strains of diverse ethnic origin; in particular, genotypes defining African and East Asian origin were. A second marker for East Asian origin was found upstream of *hspA*. Based on the presence of the *cag* PAI, African American strains and East Asian strains were likely be most virulent. East Asian strains amplified smaller PCR products than African American strains at the *cag* PAI.

Abstract no.: 04.11
Detection of *Helicobacter pylori ureAB* Gene in the Oral Yeasts from Behçet's Patients

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Background. Polymerase chain reaction (PCR) has been recruited to confirm occurrence of *Helicobacter pylori* in oral cavity. *H. pylori* colonization in oral cavity might lead to oral diseases or reinoculation of gastric mucosa after antimicrobial therapy. Furthermore, oral yeasts have been demonstrated as natural reservoirs of *H. pylori*. In this study, occurrence of *H. pylori* in the oral yeasts of Behçet's patients was assessed by PCR. The aim was to examine the possible correlation between yeast and/or *H. pylori* and aphthous ulcer.

Methods. Five yeasts were isolated from oral cavity of five Behçet's patients. Yeasts were identified on CHROMagar as *Candida albicans*. Nested PCR was performed for detection of *H. pylori ureAB* gene in DNAs extracted from yeasts. *H. pylori* and *Escherichia coli* were used as positive and negative controls. Amplified products were analyzed by electrophoresis.

Results. PCR products that were amplified from four out of five oral yeasts from Behçet's patients were 406 bp in size and homologous to the one amplified from control *H. pylori*. This product was not obtained from control *E. coli*.

Discussion. *H. pylori* has been found in dental plaque, saliva, and the lingual site. Accordingly, oral cavity could be an adequate reservoir for this bacterium. Yeasts thrive in the oral cavity of a considerable number of individuals. Results of this study showed that four of five yeast isolates from the oral cavity of Behçet's patients contained *H. pylori*. Further studies are needed to elucidate the importance of yeast and *H. pylori* in Behçet's disease, such as aphthous ulcers.

Abstract no.: 04.12
Coccoid Morphology as a Possible Manifestation of *Helicobacter pylori* Adaptation to Adverse Environments

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The pleomorphic nature of *Helicobacter pylori* has been the subject of intensive debate over the last 10 years. Because conversion from spiral to the coccoid shape is induced with exposure to detrimental environmental circumstances, the wider view up until a few years ago was that the latter represented a degrading, nonviable form of the bacterium.

When studying the adhesion of water-exposed *H. pylori* to abiotic substrata, we have found that the bacterium would

assume different shapes depending on the material it was adhered to. On copper, for instance, cells would retain their spiral morphology for at least 2 months, whereas on PVC and other polymeric materials, transformation to the coccoid form was nearly complete after 192 hours. Tests with viability stains and standard cultivation methods on six different *H. pylori* strains have shown, however, that coccoid cells maintained membrane integrity for longer and that the cultivability of the bacterium increased when it was attached to PVC rather than on copper.

The spiral shape maintenance of *H. pylori* on copper can therefore be interpreted as a fast, biocidal effect of the metal upon the pathogen, killing the cell before it has time to undergo shape modification. On the other hand, for PVC coupons, the transformation into the coccoid morphology appears to be in fact a manifestation of cell adaptation to the environment. These results will allow a more effective search for the viable bacterium in drinking water distribution systems.

Abstract no.: 04.13
Detection of *Helicobacter pylori ureAB* Gene in Gastric Yeasts

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Background. *Helicobacter pylori,* inspite of being a fastidious microorganism and difficult to culture in laboratory conditions, is quite prevalent among human populations. This indicates that *H. pylori* might have a protective niche in the environment that facilitates its transmission to the human stomach. Previous studies indicated that intracellular symbiosis of *H. pylori* with oral yeast might play an important role in the survival and transmission of *H. pylori*. In this study, polymerase chain reaction (PCR) was recruited to examine yeast isolates from gastric biopsies for the existence of intracellular *H. pylori.*

Methods. Seven yeasts were isolated from *Brucella* blood agar plates when gastric biopsies from seven patients were cultured for detection of *H. pylori.* DNAs were extracted from yeasts using phenol-chloroform method. Nested PCR was performed for the amplification of the *ureAB* gene of *H. pylori* from total yeast DNAs. PCR products were analyzed by electrophoresis.

Results. From seven biopsy cultures that were positive for yeast, two were also positive for *H. pylori*. Gel electrophoresis showed that the size of amplified products of all seven gastric yeasts and control *H. pylori* was 406 bp, which corresponded to the *H. pylori* urease gene. This product was not amplified from control *Escherichia coli*.

Discussion. The question of how *H. pylori* survives outside the human stomach has not been answered yet. Results from this study showed that gastric yeasts harbor intracellular *H. pylori*. The symbiotic relationship of *H. pylori* with yeast might lead to the protection of bacterium against environmental stress and its transmission to the human stomach.

Abstract no.: 04.14
A Nested Case-Control Study on *Helicobacter pylori* Infection, Serum C-reactive Protein Level, and Risk of Gallbladder Cancer Death

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Aim. To evaluate relationships between *Helicobacter pylori* infection, serum C-reactive protein (CRP) levels, and risk of gallbladder cancer death in a nested case-control study.

Subjects and Methods. This cohort study was conducted throughout 45 areas of Japan with 127,477 participants who were 40–89 years of age at baseline and followed up for 11 years. The cases were 25 subjects who subsequently died from gallbladder cancer and controls were 69 subjects who were randomly selected from cohort participants. Serum samples were collected at baseline. We measured *H. pylori* IgG antibody and serum CRP levels by enzyme-linked immunosorbent assay. The subjects were divided into four groups by the median of serum CRP level and *H. pylori* serology, and odds ratios (ORs) of gallbladder cancer death were calculated using a conditional logistic regression model. Then, CRP levels were compared using Mann–Whitney test.

Results. *H. pylori* seroprevalence among the cases and controls were 84% and 76.1%, respectively. Compared with the *H. pylori*-negative and CRP-low subjects, ORs were 0.80 (95% CI = 0.09–6.98) in *H. pylori*-negative and CRP-high subjects, 2.02 (0.43–9.39) in *H. pylori*-positive and CRP-low subjects, and 0.68 (0.12–3.82) in *H. pylori*-positive and CRP-high subjects. Among *H. pylori*-positive subjects, CRP levels were higher in cases than in controls ($p = .03$).

Conclusions. No significant association was observed between *H. pylori* infection and risk for gallbladder cancer death, whereas low CRP levels were associated with a decreased risk of gallbladder cancer death among *H. pylori*-positive subjects.

Abstract no.: 04.15
Differences in the 3' *cagA* Region between *Helicobacter pylori* Strains of East Asian and African Origin

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Among the virulence factors in *Helicobacter pylori, cag* pathogenicity island (PAI) is one of the most important. In particular the last gene of *cag*PAI, *cagA* that encodes a protein that is translocated inside the epithelial cells using the type IV secretory machinery encoded by the *cag*PAI. CagA undergoes tyrosine phosphorylation and its functionality depends of the number and type of EPIYA motifs located in the carboxyl terminal.

The aim of the study was to determine differences in the *cag*A region in *H. pylori* strains of East Asian and African origin.

Polymerase chain reaction (PCR) amplification of the *cagA* 3′ region was performed in 12 *H. pylori* strains of East Asian origin and 11 strains of African origin. The characteristics of either East Asian or African type were confirmed by *hspA* genotyping, presence or not of the 180bp insert, and vacA s and m typing. Specific primers were required for the amplification of the *cagA* 3′ region of the East Asian strains. PCR products from both East Asian and African strains had similar size, and sequencing analysis confirmed that strains from both groups had nearly identical number of EPIYA motifs. However, amino acid sequences following the EPIYA motifs were different between both groups. Co-infection of AGS cells with *H. pylori* strains induced IL-8 production, but levels were similar between strains of African and East Asian origin. We were unable to confirm if the East Asian *H. pylori* strains are more virulent than the African strains.

Abstract no.: 04.16
Ethnic Variation in cagA EPIYA Motifs

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Background. While more than half the world is infected with *Helicobacter pylori*, only a small percentage progress to gastric cancer (GC). Currently, why this progression occurs remains unclear. Recent studies have postulated that specific *cagA* EPIYA motifs may be associated with GC.

Aim. To determine the prevalence of cagA and specific cagA EPIYA motifs in subjects from three ethnic backgrounds (Chinese, Indian, and Malay) diagnosed with functional dyspepsia (FD) and GC who are residents in Malaysia and Singapore.

Methods. In total, 139 *H. pylori* isolates cultured from 119 FD and 20 GC subjects were included in the study. DNA was extracted and the presence of cagA and the types of cagA EPIYA motifs determined using polymerase chain reaction.

Results. cagA Was detected in 96 and 100% FD and GC isolates, respectively. No significant difference in prevalence existed between ethnic groups. The type of EPIYA motifs showed considerable variation. EPIYA-A and -B were present in > 80% of isolates. EPIYA-C (Western) was observed in 83, 14, and 75% of Indian, Chinese, and Malay FD isolates, respectively. EPIYA-D (East Asian) was observed in 10, 84, and 25% of Indian, Chinese, and Malay FD isolates, respectively. Furthermore, 31% of Indian FD isolates possessed repeats of EPIYA motifs, as compared with 7% Chinese FD isolates. No significant difference was found between FD and GC isolates.

Conclusion. Substantial genomic variation exists in EPIYA motifs in the three ethnics groups. Based on our small number of GCs, the type of EPIYA motif does not relate to GC development; however, larger numbers of subjects are clearly required.

Abstract no.: 04.17
Work-related *Helicobacter pylori* Infection among Workers of Nursing Homes for the Elderly in Belgium

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Introduction. Elderly persons have higher prevalence of *Helicobacter pylori* infection. Close personal contact inherent to patient care of elderly and activities in nursing homes may expose nursing home workers to increased occupational infection risk.

Objectives. To assess the prevalence of *H. pylori* infection in Belgian (Flemish) workers of nursing homes to determine whether work is a possible risk factor for acquisition of *H. pylori* and possible associations with specific job tasks or occupational exposures.

Methods. Seroprevalence study was conducted among 198 employees of four nursing homes. Information about sex, age, country of origin, history of gastrointestinal symptoms in the last 3 months, and job was obtained by questionnaire. Presence of *H. pylori* immunoglobulin G (IgG) was investigated with enzyme-linked immunosorbent assay. Results were compared with those of 250 employees of Flemish pharmaceutical company.

Results. Prevalence of *H. pylori* IgG antibodies among nursing home workers was 14.6% versus 13.6% among the control group. Age standardized prevalences among study and control group were not statistically significant different, 14.2% versus 14.9%, respectively. Univariate analyses of seropositivity showed no significant associations with self-reported frequency of contact with feces or vomit, washing or feeding, handling nasogastric tubes, animation activities, or washing linen. In multivariate logistic regression analysis, earlier employment in health care for ≥ 10 years remained a significant infection risk factor.

Conclusions. Our study found no significant higher *H. pylori* seroprevalence among nursing home workers than among pharmaceutical workers. No significant associations between *H. pylori* seropositive status and job tasks, or occupational exposures were demonstrated.

Abstract no.: 04.18
Immune Response to Specific Antigens of *Helicobacter pylori* in Children of Algiers Region

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Introduction. *Helicobacter pylori* possesses a number of antigens that induce specific immune response. Among those, some are considered as virulence factors such as CagA and VacA.

In the Algeriers Region, studies have shown that *H. pylori* infection is acquired early in childhood and increases with age but prevalence of anti CagA and anti-VacA antibodies in infected children is unknown.

Aim. To investigate the seroprevalence of antibodies to CagA, VacA as well as to other specific *H. pylori* antigens in children from the Algiers Region.

Materiel and Methods. Sera of 76 children aged from 6 months to 16 years (mean age 7.5 years) *H. positive* positivity by enzyme-linked immunosorbent assay (Biorad) are examined by immunoblotting test using Helicoblot 2.1 of Genelabs, which detects antibody-specific response to 19.5 kD, 30 kD (Urease), 37 kD, 89 kD (VacA), and 116 kD (CagA) antigens.

Results. Percentage of children with antibody response to every antigen according to age are shown on Table 1.

Conclusion. The immune response to every antigen increases with age.

Table 1 The highest responses are obtained with urease and Cag A

Age of children	19.5 kD	30 kD (erease A)	35 kD	37 kD	VacA	CagA
6m*–23m n = 9	11	33,5	0	11	22	44,5
2–6 y** n = 20	41	66,5	29,5	26	33,5	63
7–11 y n = 20	55	75	35	35	40	50
12–16 y n = 20	5	60	35	35	50	65
Total n = 76	43,5	63	29	29	38	58

*m, month **y, years.

Abstract no.: 04.19
Incidence of *Helicobacter pylori* Recurrent Infection and Influencing Factors in Thailand

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Aims. To determine the rate of *Helicobacter pylori* recurrence after successful eradication in a 4-year follow-up study and to evaluate the influencing factors for reinfection.

Patients and Methods. A total of 37 patients (age range 20–74 years; average 49.06 ± 14.03 years). There are 35.1% men and 64.9% women. All patients had been proved successfully treated for *H. pylori* infection. Annually, urea breath test was assessed to determine *H. pylori* status after eradication. Age, sex, eating habit, water drinking, number of child, and treatment regimens against *H. pylori* were recorded. A breath test was also performed on the patient's spouse.

Results. The *H. pylori* recurrence occurred in 5/37 (13.51%) of patients observed. There were 2 patients in the first year, 1 patient in the second year, 1 patient in the third year, and 1 patient in the fourth year, respectively. The cumulative reinfection rate was 5.41% at 1 year, 8.11% at 2 years, 10.81% at 3 years, and 13.51% at 4 years, respectively. *H. pylori* infection of spouses was also frequent (80%). Even if the spouse was infected, 88.89% of patients will remain uninfected after 4-year *H. pylori* eradication. No influencing factors for infection recurrence were detected.

Conclusions. The risk of reinfection after *H. pylori* eradication is low in Thai patients after a 4-year follow up. Annual reinfection rate was 3.38%. No dependent factors were associated with a recurrence.

Inflammation and Host Response

Abstract no.: 05.01
TLR4-dependent NF-κB Activation and Mitogen and Stress-Activated Protein Kinase 1-Triggered Phosphorylation Events are Central to *Helicobacter pylori* Peptidyl Prolyl *cis-*, *trans-* Isomerase (HP0175)-Mediated Induction of IL-6 Release from Macrophages

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Helicobacter pylori infection is associated with the local production of chemokines and cytokines of which interleukin 6 (IL-6) is overexpressed at the margin of gastric ulcer in *H. pylori*-positive gastritis and is a cytokine that is central to the inflammatory arm of the innate immune response. Our study shows for the first time that a secreted peptidyl prolyl *cis-*, *trans-* isomerase (PPIase), HP0175 elicits *IL-6* gene expression and IL-6 release from macrophages. An isogenic strain inactivated in the *HP0175* gene was attenuated in its IL-6-inducing ability. IL-6-inducing ability of the bacterium was restored upon complementation of the *HP0175*-negative mutant with ectopically expressed HP0175. HP0175-induced *IL-6* gene expression was critically dependent on NF-κB activation, which occurred in a TLR4/MyD88/TRAF6/TAK1/NIK/IKK-dependent manner. The MAPK, ERK1/2 was activated by PI3K/Ras/Raf/MEK signaling. p38 Mitogen-activated protein kinase activation depended simultaneously on PI3K/Rac/PAK and TAK1/MKK3/6 signaling. Extracellular signal-regulated kinase and p38 MAPK signaling converged upon activation of mitogen and stress-activated protein kinase 1 (MSK1). The central role of MSK1 was borne out by the fact that silencing of MSK1 expression abrogated HP0175-mediated NF-κB-dependent *IL-6* gene transcription. MSK1 phosphorylated p65 on serine 276 and histone H3 on serine 10. These modifications in turn regulated p300-mediated acetylation of p65 (at lysines 221 and 310) and H3 (on lysine 14), and their subsequent association with the *IL-6* promoter. HP0175 therefore regulated *IL-6* gene transcription through chromatin modification at the *IL-6* promoter.

Abstract no.: 05.02
Specificity of CD4+ T-Cell Response in Murine *Helicobacter pylori* Infections

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Helicobacter infection is characterized by the development of chronic gastritis dominated by lymphocytic infiltration into the gastric mucosa. Although a Th1-biased cellular response is widely reported in *Helicobacter pylori*-induced gastritis, the antigen specificity of this response is poorly understood, particularly because *H. pylori* is usually noninvasive and is hence spatially removed from infiltrating immune cells. In our study, we generated T-cell hybridomas with CD4+ T cells from the paragastric lymph nodes of *H. pylori* SS1-infected mice. *H. pylori*-reactive CD3+CD4+ hybridoma lines were cloned by limiting dilution and six clones were chosen for further characterization. Analysis of cytokine production by these clones after *H. pylori* SS1 stimulation revealed mixed Th1/Th2 responses. TCR Vb expression screening by flow cytometry showed that three of the six clones used Vb2. Antigen presentation assays proved the specificity of T-cell hybridomas for *H. pylori*, and confirmed that they were not activated by gastric bacterial flora or *Helicobacter bilis*, a resident *Helicobacter* species. The clones showed varying strain-specific responses when stimulated with other *H. pylori* strains. We noted that coccoid (i.e., nonviable) *H. pylori*-stimulated clones to a similar extent as spiral *H. pylori*. To refine the antigen specificity, we fractionated *H. pylori* into separate protein compartments by ultracentrifugation and found that IL-2 production was highest when clones were stimulated with the membrane protein preparations, compared to cytosolic or secreted proteins. These proteins will be further separated by isoelectric focusing in order to elucidate the antigen specificity of the T-cell hybridoma clones.

Abstract no.: 05.03
Secretory Leukocyte Protease Inhibitor (SLPI), A Target Gene of *Helicobacter pylori* Infection, Regulates NF-κB Signaling in Gastric Tumor Cell Lines and Leukocyte-derived Elastase Activity in the Gastric Mucosa of *H. pylori*-infected Subjects

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Background. Recently, secretory leukocyte protease inhibitor (SLPI) was identified as target gene in *Helicobacter pylori* infection.
Aim. To investigate functional implications and mechanisms regulating gastric SLPI expression in *H. pylori* infection.
Methods. SLPI expression and NF-κB signaling of *H. pylori*-infected gastric cell lines were studied by enzyme-linked immunosorbent assay and reverse transcriptase-polymerase chain reaction. Antral SLPI levels of patients with gastrointestinal diseases were investigated with respect to the CagA status determined by immunoblot, and the neutrophil-derived elastase activity, a target protease of SLPI.
Results. The infection of cell lines with *H. pylori* resulted in a significant decrease (–80%, $p < .001$) of the SLPI protein levels in AGS and MKN-28 and a similar trend for MKN-45 and NCI-N87, whereas corresponding transcript levels were induced up to fivefold ($p < .01$). The *H. pylori*-mediated induction of NF-κB was dose-dependently reduced by SLPI in two cell lines. Antral biopsies of *H. pylori*-infected subjects contained about 30-fold higher neutrophil-derived elastase activity than those from *H. pylori*-negative or -eradicated subjects ($p < .01$). The presence of anti-CagA antibodies in *H. pylori*-infected patients was strongly associated with decreased antral SLPI levels (1944 ± 287 pg/50 µg protein, $p < .0001$) compared to *H. pylori*-negative subjects (4255 ± 370 pg/50 µg), whereas *H. pylori*-infected without anti-CagA antibodies revealed only slightly reduced SLPI levels (3210 ± 589 pg/50 µg, n.s.).
Conclusions. The inverse correlation between antral elastase activity and SLPI levels, as well as the decrease of *H. pylori*-induced NF-κB signaling by SLPI illustrate the functional relevance of SLPI for the gastric mucosa. The association between anti-CagA antibodies and reduced SLPI levels suggest a pathogenicity island-dependent mechanism for the *H. pylori*-mediated down-regulation of SLPI.

Abstract no.: 05.04
The Toll-Like Receptor Signaling Pathway in Host Cells is Affected by *Helicobacter pylori pldA* Phase Variation

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Background. *Helicobacter pylori* phospholipase A (OMPLA) degrades bacterial phospholipids to lysophospholipids. The phospholipase A gene (*pldA*) displays phase variation resulting in bacterial variants with high ($pldA_{ON}$) or low ($pldA_{OFF}$) OMPLA activity. Isolates with high OMPLA activity release the virulence associated factors urease and VacA, adhere to and invade cells in vitro, and are significantly associated with ulcer disease in vivo. In order to further investigate the host cell response to virulent phase variant ($pldA_{ON}$), the gene expression profile in AGS cells infected with both the *pldA* phase variants was studied.
Methods. AGS cells were cultured in appropriate maintenance media. At 80% confluency, the cells were infected for 12 hours with *H. pylori* at a 300 : 1 bacterium/cell ratio in antibiotic-free cell culture medium. Total RNA was isolated using Trizol RNAeasy method and amplified with low input RNA fluorescent linear amplification kit. Hybridization was carried out using Agilent 60-mer 44k whole human genome array and RNA from uninfected AGS cells as a reference. Data analysis was performed by Bayesian statistical algorithm. Immune profile LDA card from Applied Biosystems confirmed the oligo array results.
Results. High- and low-density arrays showed up-regulation of several genes in the toll-like receptor signaling pathway in the $pldA_{ON}$ variant, including IL-8 as one of the most significantly differentially up-regulated gene.

Conclusion. Results indicate that the *H. pylori* $pldA_{ON}$ variant induces stronger virulent-associated response in host cells. Up-regulation of several genes in the $pldA_{ON}$ variant comparing with the $pldA_{OFF}$ variant may suggest a faster and more aggressive infection course.

Abstract no.: 05.05
V75Q576 IL-4Ra Variant and IL-4 -588T Allele Favor cagA-positive *Helicobacter pylori* Infections

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Interleukin 4 (IL-4), which acts by binding to its receptor, IL-4Ra, affects the immune system and can modify gastric acid secretion. Our aim was to elucidate the relationships between IL-4 and IL-4Ra genetic polymorphisms, considered singly or as haplotypes, and *Helicobacter pylori* infection or *H. pylori*-associated diseases. *IL-4*-588CT, *IL-4R* 148AG and *IL-4R* 1652AG were assayed in 170 patients with gastritis (107 *H. pylori positive*), 75 duodenal ulcer (66 *H. pylori positive*), 144 noncardia gastric cancer (91 *H. pylori positive*). *H. pylori ureA* and *cagA* were polymerase chain reaction amplified from mucosal DNA; gastritis grade was assessed (hematoxylin and eosin). Haplotypes were estimated using ARLEQUIN software. Allele frequencies were −588C = 0.86, −588T = 0.14, 148A = 0.55, 148G = 0.45, 1652A = 0.84, and 1652G = 0.16. All SNPs were in Hardy–Weinberg equilibrium. *IL-4* or *IL-4R* single genotypes were neither correlated with disease diagnosis nor with *H. pylori* infection or gastritis grade, considering gastritis patients *IL-4*-588T allele was *cagA* associated ($\chi^2 = 9.12$, $p < .01$). *IL-4R* haplotype frequencies, considering the 148AG and 1652AG loci, were AA = 48.6%, AG = 6.0%, GA = 35.2%, GG = 10.2%. The GA haplotype (V75Q576 amino acids combined in the same protein) was correlated with *cagA* ($\chi^2 = 4.42$, $p < .05$). After patients with or without at least a GA haplotype were subdivided into different groups, the association between *cagA* and *IL-4*-588T was confirmed only in those without GA haplotype ($\chi^2 = 4.04$, $p < .05$).

Conclusions. *IL-4R* GA haplotype and *IL-4*-588T allele favor *cagA* infections. The V75Q576 combination resulting from this haplotype may affect receptor functioning, whereas *IL-4*-588T allele may affect the amount of the secreted cytokine. These genetic variants may modulate IL-4 effect on gastric acid secretion and/or Th2 response.

Abstract no.: 05.06
Pattern of Transcription Factor Activation in *Helicobacter pylori*-Infected Mongolian Gerbils

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Background. Transcription factors regulate cellular signaling pathways. The pattern and timing of transcription factor activation in *Helicobacter pylori*-infected gastric mucosa remain unclear.

Methods. Eight-week-old male Mongolian gerbils were orally inoculated with TN2GF4 *H. pylori* or its isogenic *cagE* mutants. One, 3, 9 and 18 months after inoculation, gerbils were sacrificed and nuclear and cytoplasmic proteins were extracted from the gastric mucosa. We examined 54 transcription factors by DNA/protein array and electrophoretic mobility shift assay. The phosphorylation of mitogen-activated protein kinases and IκB was measured by immunoblot.

Results. Ten transcription factors were up-regulated by *H. pylori* infection; six including AP-1 and cAMP response element binding protein (CREB) reached maximal levels at 3 months, and four including NF-κB and ISRE reached maximal levels at 18 months. Only AP-1 and CREB levels were strongly correlated with cellular inflammation and with the presence of ulceration. In contrast, NF-κB and ISRE levels correlated with the presence of severe atrophy. Levels of transcription factors induced by *cagE* mutants were generally similar to those in uninfected control; only four factors including NF-κB and ISRE were up-regulated by *cagE* mutants but at levels significantly lower than with wild-type *H. pylori*. Phosphorylation of extracellular signal-regulated kinase was correlated with activation of AP-1 and CREB; phosphorylation of Jun N-terminal kinase and p38 correlated with activation of NF-κB and ISRE.

Conclusion. The pattern of induction of gastric mucosal transcription factors induced by *H. pylori* infection differs throughout the infection and in different outcomes (e.g., AP-1 and CREB levels in inflammation and ulceration versus NF-κB and interferon-stimulated response element in atrophy).

Abstract no.: 05.07
Phenotypic Changes Provoked by *Helicobacter pylori* at Cellular and Mitochondrial Levels

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Introduction. Mitochondria dynamics are controlled by the equilibrium between fusion/fission processes, which can be altered as a result of apoptotic stimulus.

A morphogenic phenotype called stress fiber associated (SFA) can be observed in cells infected by bacteria as a consequence of the interaction with the actin filaments.

Aims. In vitro demonstration that *Helicobacter pylori* can trigger the phenotype changes occurring at mitochondria and cellular levels. Establishing its relationship with oxidative stress.

Materials and Methods. Human gastric epithelial cells AGS (ATCC CRL-1739) were cultured with an *H. pylori* strain (ATCC-51932)

to a concentration of 10^7CFU/mL for 24 hours with or without vitamin E (10^{-4}M).

Oxidative stress was observed by H_2-dichlorofluorescin diacetate (DCF)-DA (5 µM, flow cytometry) and by the MitoSOX Red reagent [5 µM, confocal microscopy (CM)] whose fluorescences are proportional to the reactive oxygen species present in the cells.

Mitochondrial fission was assessed with nonyl acridine orange (NAO, 100 nmol/L) by CM.

The SFA phenotype was analyzed by phase contrast microscopy.
Results. At 24 hours, *H. pylori* caused 1.3-fold increase in DCF fluorescence (206 versus 167 AUF in control). VitE pre-incubation reduced it (178 AUF). CM images corroborated it.

H. pylori changed mitochondrial phenotype [nonyl acridine orange (NAO) staining] breaking the mitochondrial network in round and isolated mitochondria (punctiform phenotype). The antioxidant avoided this alteration.

The SFA-like cellular phenotype was characterized by spreading and loss of adhesion, what it was not modified by VitE pretreatment.
Conclusions. *H. pylori* provokes morphological changes at the cellular level (similar to SFA phenotype) being independent of oxidative stress and at the mitochondrial level (punctiform phenotype) caused by a ROS synthesis increased as antioxidants treatment avoided it.

Abstract no.: 05.08
Helicobacter pylori Inhibits Proliferation but not Activation of T cells

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Background. *Helicobacter pylori* infection always leads to chronic inflammation of the gastric mucosa. The mechanisms by which *H. pylori* is able to overcome and survive such a response are still not fully understood. Experimental animal infection models indicate that a specific T-cell response is crucial for clearance of the infection. The aim of this study was to investigate whether *H. pylori* affects T-cell responsiveness, proliferation, and cell division.
Methods. Purified T cells obtained from blood of healthy volunteers were stimulated with either photohemagglutinin or CD3/CD28 antibodies in the presence or absence of *H. pylori*. The effect of *H. pylori* on expression levels of CD25 and CD69 activation markers was determined by fluorescence-activated cell sorting analysis, and the effect of *H. pylori* on proliferation and cell division of T cells were measured by [^{3}H]-thymidine incorporation and carboxyfluorescein succinimidyl ester (CFSE) dilution.
Results. *H. pylori* did not affect induction of CD25- and CD69-expression on T cells on day 1 ($p = .11$; n = 3). In contrast, *H. pylori* inhibited the proliferation of stimulated T cells in a dose-dependent way, with 75% inhibition at multiplicity of infection (MOI) 5 (n = 5; $p = .04$). The CFSE dilution assay showed that *H. pylori* suppressed cell division of stimulated T cells. Upon stimulation with CD3 and CD28 antibodies, the number of generations of CD4+ helper T cells after 3 days was reduced from seven to six and in CD8+ cytotoxic T cells from four to three.
Conclusion. Although *H. pylori* does not inhibit initial activation, it does affect proliferation of T cells. This effect may contribute to the persistence of *H. pylori* in the immune-competent host.

Abstract no.: 05.09
Comparison of the Host Gene Expression Profile in *Helicobacter pylori*-infected and Noninfected Mice

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We have previously reported that *Helicobacter pylori* infection induced a gastric mutagenic effect in mice after 6 months. In an attempt to understand the molecular mechanisms involved in the pathology and the genotoxicity associated to a chronic infection, the host genes expression profile was compared in *H. pylori* SS1-infected and noninfected mice after 6 and 12 months, using the Affymetrix GeneChip® mouse expression system.

All infected stomachs exhibited a chronic gastritis. The expression of 120 and 175 genes was found modulated by the *H. pylori* infection after 6 and 12 months, respectively, according to the required criteria (an expression fold change, ≥ 2 and an adjusted $P \leq 0.001$). Among them, the pancreatic phospholipase A2 group IB and the ATPase H+/K+ alpha and beta subunits are among the most down-regulated by the infection by 19- and 10-fold, respectively. Thirty percent of the genes commonly regulated at 6 and 12 months are involved in the immune response and characterized by an interferon gamma-dependent expression including genes coding for MHC II components. Other genes with an interferon gamma-dependent expression, as genes coding for the indoleamine pyrrole 2,3 dioxygenase (Ido), the tryptophanyl-tRNA synthetase, and the carcinoembryonic antigen-related cell adhesion molecule 1, were also up-regulated by the infection. All these data have been validated concomitantly by reverse transcriptase-polymerase chain reaction analysis.

Experiments are now in progress to investigate the relevance of these genes in the host response to the infection. This study will allow the identification of new targets for further functional studies on *H. pylori*-associated pathogenesis.

Abstract no.: 05.10
Inhibitory Effect of Enterohepatic *Helicobacter hepaticus* on Innate Immune Responses of Mouse Intestinal Epithelial Cells

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Enterohepatic *Helicobacter* species infect the intestinal tract and biliary tree of various mammals, including mouse and human, and are associated with various chronic inflammatory diseases of the intestine, gallstone formation, and malignant transformation. The recent analysis of the whole genome sequence of the mouse enterohepatic species, *Helicobacter hepaticus*, allows for the functional analysis of bacterial factors that may play a role in these diseases. We tested the hypothesis that *H. hepaticus* may suppress or evade innate immune responses of mouse intestinal epithelial

cells, thus allowing this pathogen to induce or contribute to chronic inflammatory disease. We demonstrate in the present study that innate immune responses of intestinal epithelial cells to lipopolysaccharide (LPS) via toll-like receptor (TLR)-4 are reduced by *H. hepaticus* infection through soluble bacterial factors. The presence of the genomic island HHGI1 or the cytolethal distending toxin do not play a role in this effect. In particular, *H. hepaticus* lysate and the soluble subcomponent LPS were able to suppress/antagonize TLR4-mediated immune responses of intestinal epithelial cells. Suppression of innate immune responses by *H. hepaticus* LPS may thus affect intestinal responses to resident microbial flora, epithelial homeostasis, and intestinal inflammatory conditions.

Abstract no.: 05.11
Interleukin-18 Expression and Macrophage Infiltration of Gastric Mucosa in Children with *Helicobacter pylori* Infection

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Objectives. The aim of our study was to analyze the expression of interleukin 18 (IL-18) and its association with the macrophage infiltration in the gastric mucosa in *Helicobacter pylori*-infected children.

Material and methods. Children and adolescents referred for endoscopy with clinical symptoms indicating a possible pathology in the upper gastrointestinal tract were included in the study. From each child, six antral biopsies were obtained for histology, culture, and semiquantitative analysis of IL-18 mRNA and CD14 mRNA expression by reverse transcriptase-polymerase chain reaction.

Results. A total of 43 patients (age range 5–18 years) were included in the study, 25 (58%) of whom were *H. pylori* positive. Children with *H. pylori* infection had significantly higher number of macrophages in the antral mucosa (as determined by CD68 staining and CD14 mRNA expression), which correlated with colonization density and severity of gastric lesions. Additionally, *H. pylori*-infected patients showed higher expression of IL-18 mRNA compared to uninfected children, and IL-18 mRNA levels correlated with all histologic parameters of *H. pylori* gastritis and with CD14 mRNA expression.

Conclusions. These results suggest the presence of possibly lipopolysaccharide-induced, macrophage-dependent, and rather procytotoxic mechanisms of innate immunity in the gastric mucosa of *H. pylori*-infected children.

Abstract no.: 05.12
In vivo and in vitro Expression of the TLR-4 and TLR-5 Receptors during *Helicobacter pylori* Infection

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Our aim was to determine the expression in vivo and in vitro of *TLR4* and *TLR5* receptors during *Helicobacter pylori* infection.

Methods. In vivo study. We enrolled 40 dyspeptic patients, and *H. pylori* status was determined by rapid urease test, histology, and culture.

In vitro study. AGS cells were co-infected with 24 *H. pylori* strains form our collection. Strains were isolated form patients with gastric cancer (GC), antral ulcer (AU), duodenal ulcer (DU), intestinal metaplasia (IM), atrophic gastritis (AG), and superficial gastritis (G); (four forms each group).

In both studies, strains were genotyped for *cagA,* and *vacA*. TLR-4 and *TLR-5* expression was examined by reverse transcriptase-polymerase chain reaction in biopsies and AGS cells for the in vivo and in vitro studies.

Results. In the in vivo assay, 74.1% and 82.6% of *H. pylori* positive and 76.5% and 94.1% of *H. pylori* patients expressed *TLR-4* and *TLR-5,* respectively.

For the in vitro assay, all the strains induced the expression of *TLR4,* but only three failed to stimulated *TLR5* expression. These strains were isolated for patients with G (n = 2) or AG (n = 1). No correlation between strain genotype and expression of *TLR4* or *TLR5* either in vivo or in vitro was observed.

Conclusion. There were no differences in the expression in vivo of *TLR4* and *TLR5* related to the *H. pylori* status and the strain genotype. Induction of *TLR5* in vitro with strains from patients with benign pathologies was significantly lower than expression of *TLR5* by strains from patients with severe pathologies ($p = .03$).

Abstract no.: 05.13
Th1 Cytokines and Reduced Expression of EP3 Receptors May Facilitate *Helicobacter pylori*-Induced Corpus Gastritis and Atrophy

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Acid secretion plays a pivotal role in *Helicobacter pylori* colonization patterns and localization of pathological changes in the stomach. *H. pylori*-induced inhibition of acid secretion or lack of the regulatory feedback that maintains sufficient acid output may be responsible for the spread of pathological changes to the corpus of the stomach. Using a mouse model of acid secretion, we have previously shown that only Th1 cytokines, namely interleukin-2 and interferon-γ possess antisecretory properties and because C57BL/6 mice respond to *H. pylori* infection with a predominantly Th1 response, the extent of acid inhibition and its consequences is also more marked in this strain. Furthermore, fluctuations of the gastric surface pH provide a feedback mechanism for the regulation of acid secretion and this physiological process is

mediated by prostaglandins. Alkalinization of the luminal surface stimulates gastrin and subsequent increase in acid output, which in turn prevents microbial colonization of the oxyntic mucosa. However, we have shown that C57BL/6 mice do not respond to PGE2 because they have a markedly reduced number of gastric EP3 receptors (AJP-Gastro&Liver Physiol 2005, 288,G1110-G1117), suggesting that antral alkalinization in this strain may not trigger a prostaglandin-dependent physiological increase in acid output, enabling spread of the infection. This is consistent with the observed *H. pylori* colonization of oxyntic mucosa and gastritis pattern in the C57BL/6 mouse model. We propose that a prostaglandin-mediated physiological feedback mechanism for regulating acid secretion may explain the pattern of *H. pylori* colonization, which leads to gastric atrophy and its consequences.

Abstract no.: 05.14
Humoral Markers of Host Response to *Helicobacter pylori* Lipopolysaccharide (LPS) in Patients with Coronary Artery Disease (CAD)

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The role of *Helicobacter pylori* in atherosclerosis may be related to humoral response to bacterial proteins and Lewis (Le) determinants of lipopolysaccharide (LPS) of such bacteria. LPS may contribute to atherogenesis via soluble CD14 (sCD14) and LPS binding protein (LBP), mediating the interactions of LPS with TLR4 host receptor. We estimated the immunoglobulin (IgG and IgA to glycine acid extract-GE of Hp antigens, IgG to Hp LPSs with or without LeX or LeXY determinants, immune complexes-ICs LeX-anti-LeX IgG, and LeY-anti-LeY IgG), and nonimmunoglobulin markers (sCD14, LBP) of host response to LPS. An active metalloproteinase 9 (MP9) was also measured. The investigated groups consisted of 140 patients with coronary artery disease (CAD) and 60 healthy subjects. The enzyme-linked immunosorbent assay tests were used for detection of serum proteins and polymerase chain reaction for the detection of –159 C/T CD14 polymorphism. Anti-*H. pylori* humoral response in CAD group was characterized by higher levels of IgG to *H. pylori* LPS of LeXY type and LeY-anti-LeY IgG ICs as compared to controls. The elevated concentration of LBP and sCD14 in CAD group was linked to *H. pylori* infection. There was no difference in sCD14 level between CAD and control groups regarding –159C/T CD14 polymorphism. In the CAD patients, the MP9 levels were higher than in controls. *H. pylori* LPS could be involved in atherogenesis by inducing strong humoral response to LeXY determinants and by increased levels of sCD14 and LBP. Additionally, MP9 may contribute to tissue injury in the acute myocardial incidences.

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Abstract no.: 05.15
Association of *Helicobacter pylori* Infection with Cardiovascular Risk Factors in Korean Adults who were Diagnosed with Coronary Heart Disease

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Background. Chronic inflammation is known to be an important factor in the pathophysiology of atherosclesrosis. Among the many factors associated with chronic inflammation, *Helicobacter pylori* is thought to be the major factors for gastric disease in Korean people. The purpose of this study is to evaluate the association of *Helicobacter* infection with cardiovascular risk factors in Korean adults who were diagnosed with coronary heart disease.

Method. One hundred eighteen subjects with coronary heart disease were divided into two groups; one is CLO test (+), the other is CLO test (–). Then the association of *H. pylori* infection with cardiovascular risk factors was evaluated in the subjects. *H. pylori* infection status was determined using CLO test. Serum lipid profiles, serum WBC count, serum glucose, coagulation time, serum uric acid and hsCRP were tested.

Result. Forty of 118 (38%) of participants were found to be infected with *H. pylori* in the CLO test. Serum low-density lipoprotein (LDL) level showed significant difference between CLO (–) groups and CLO (+) groups, 90.77 mg/dL, and 107.29 mg/dL, respectively ($p < .05$). The mean values of other risk factors such as triglyceride (125.88 mg/dL and 129.52 mg/dL), high-density lipoprotein (55.51 mg/dL and 46.00 mg/dL), total cholesterol (174.23 mg/dL and 184.95 mg/dL), glucose (125.83 mg/dL and 133.14 mg/dL), and serum uric acid (5.78 mg/dL and 5.38 mg/dL) showed no significant differences between two groups ($p > .05$).

Conclusion. The results of this study show that *H. pylori* infection is independently correlated with cardiovascular risk factor such as LDL in atherogenic way in subjects with cardiovascular disease. This conclusion may support the hypothesis that *H. pylori* infection might be one of the important risk factors of atherosclerosis in Korea.

Abstract no.: 05.16
Prevalence of *Helicobacter pylori* and CagA Antibodies in Patients with Pre cancerous Gastric Lesions, Gastric Cancer, and Duodenal Ulcer in Mexico

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Background. Gastric cancer is the fourth most common cancer and the second cause of cancer death worldwide. Risk for gastric

cancer and duodenal ulcer is enhanced by infection with *Helicobacter pylori* cagA-positive strains.
Aim. The aim of this work was to study *H. pylori* infection and CagA seropositivity as possible risk factors for precancerous lesions, gastric cancer or duodenal ulcer.
Methods. The work is a case-control study. A total of 618 serum samples from patients were studied; 25 with chronic atrophic gastritis, 103 with intestinal metaplasia, 18 with gastric dysplasia, 63 with gastric cancer, 59 with duodenal ulcer, and 350 controls with chronic gastritis. *H. pylori* infection and CagA seroprevalence were determined by enzyme-linked immunosorbent assay.
Results. OR for CagA seropositivity showed an increasing gradient: gastritis (1.0), atrophic gastritis (1.1), intestinal metaplasia (2.4); which then decreased: intestinal metaplasia (2.4), dysplasia (1.7), gastric cancer (1.2). *H. pylori* showed a similar pattern. The magnitude of the IgG response to both *H. pylori* and CagA was significantly higher in patients with intestinal metaplasia than in those with other lesions. In patients with duodenal ulcer, CagA (OR 1.8) and *H. pylori* infection (OR 3.0) were found as risk factors.
Conclusion. This study shows that *H. pylori* infection and CagA response show a gradient response, increasing in early precancerous lesions and decreasing in advanced precancerous lesions and cancer. A high IgG response to both *H. pylori* and CaGA is suggestive of intestinal metaplasia.

Abstract no.: 05.17
Antiphagocytic Activity of *Helicobacter pylori* Lipopolysaccharide

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In this study we asked whether *Helicobacter pylori* lipopolysaccharide (LPS) with or without Lewis (Le) determinants, released from bacterial cells during their lysis, may influence the course of phagocytosis of these bacteria by human granulocytes. Phagocytosis was estimated fluorimetrically using fluorescein isothiocyanate-labeled *H. pylori* cells and human blood polymorphonuclear leukocytes (PMNs) as phagocytes. The ingestion of bacterial cells by PMNs was expressed in relative fluorescence units (RFU) (using 485/530 nm filters), after quenching the fluorescence of extracellularly bound bacteria with crystalline violet. Usually, PMNs treated with LPS ingested a lower number of *H. pylori* bacteria than untreated phagocytes. The effect was independent on Le determinants. Phagocytes showed a strong oxidative burst activity appearing shortly after introduction of *H. pylori* LPS to the culture. However, longer (1 hour) pretreatment of phagocytes with *H. pylori* LPS resulted in diminished ability of PMNs to reduce tetrazolium salt (MTT), indicating that *H. pylori* LPS down-regulated the metabolic activity of granulocytes although did not induce apoptotic symptoms in the cells. It has been shown that recombinant laminin binding protein (rLBP) significantly reduced antiphagocytic effect of *H. pylori* LPS. This could have been caused by the neutralization by rLBP of LPS toxicity or preventing of over-activation of PMNs. The LPS binding with rLBP may be important for the activation of PMNs on CD14-dependent pathway and for effective ingestion of *H. pylori*. In conclusion, LPS released from *H. pylori* cells may be involved in the maintenance of *H. pylori* infection by reducing the effectiveness of phagocytosis.

Abstract no.: 05.18
Tissue Ferritin and Cytokines in *Helicobacter pylori* – Positive Peptic Ulcer Disease

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Background. Although tissue ferritin and cytokines have been suggested to play an important role in the pathogenesis of *Helicobacter pylori*-associated peptic ulcer disease, few studies have investigated the contents of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and ferritin in the gastric mucosa.
Aim. To investigate IL-6, TNF-α, and ferritin in gastric mucosa before and after 1 month *H. pylori* triple eradication therapy (omeprazole 40 mg + clarithromycin 500 mg + tinidazole 500 mg during 1 week) in *H. pylori*-positive peptic ulcer patients.
Methods. The antral biopsies were obtained from an area of endoscopically intact mucosa in 28 *H. pylosi*-positive peptic ulcer patients (men/women = 15/13; mean age 37.1 ± 13.2 years) and six healthy volunteers (m/f = 4/2; mean age 23.9 ± 5.0 years) for histology and CLO-test. Levels of IL-6, TNF-α, and ferritin in supernatants of mucosal biopsies were determined by enzyme-linked immunosorbent assay.
Results. The findings are shown in the table. (Table 1)
Conclusions. Significant differences in TNF-α content for controls versus *H. pylori*-positive and *H. pylori*-negative groups patients. The contents of ferritin as "protein of an acute phase," is reduced after eradication therapy.

	Groups of patients		
	Control	Before therapy (Hp+)	After therapy (Hp–)
IL-6 (pg/g)	49.6 ± 33.3	88.9 ± 73.9	46.7 ± 32.7
TNF-α (pg/g)	42.2 ± 7.2	86.3 ± 33.8†	55.1 ± 25.0*
Ferritin (ng/mL)	314.9 ± 163.0	1675.0 ± 346.0†	685.0 ± 197.7*†

*significant differences before and after therapy ($p < .05$), †significant differences with control group ($p < .01$).

Abstract no.: 05.19
Evaluation of Selected Cytokines in Cell Culture Supernatants from Children with *Helicobacter pylori*-Associated Gastritis

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Introduction. The contribution of cytokines in the pathogenesis of *Helicobacter pylori*-associated chronic gastritis and duodenitis is not entirely clarified in children.
Objectives. To estimate the selected cytokines in *H. pylori*-associated chronic gastritis in children and to correlate cytokines concentration in the supernatants of peripheral blood mononuclear cells and histopathology gastritis score of as well as *H. pylori* density.
Material and methods. A cytometric bead array test was used for the evaluation of L-1β, TNF-α, and IL-10 in supernatants of peripheral blood lymphocyte culture in 41 children aged 5–18 years (27 with and 14 without with *H. pylori* infection). All of them underwent upper gastrointestinal endoscopy for chronic dyspepsia. Histopathology inflammation score, *Helicobacter* density have been assessed as well and correlated with those cytokines.
Results. In the infected children, the mean level of TNF-α and IL-β was significantly lower than in the control group ($p < .05$). The levels of TNF-α and IL-1β correlated with each other in both groups. In the group of children without *H. pylori* infection, a negative correlation of IL-1β with the degree of gastric mucosa inflammation was shown, which was not observed in the *H. pylori*-positive group. The results may confirm the ability of *H. pylori* strains to reduce the systemic cellular response in children, what may play a role in the failed infection elimination in childhood.

Abstract no.: 05.20
Chronic Gastritis and Persisting *Helicobacter pylori* Antibodies after Successful Eradication Therapy

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Aim. To study the presence of gastritis and positive *Helicobacter pylori* serology after eradication therapy.
Patients and Methods. One hundred ten patients (85 women; median age, 65 years) with successful *H. pylori* eradication therapy 0.1–15.4 (median 6.5) years earlier were gastroscopied and biopsies taken from the antrum and the corpus. Serum samples from 79 patients were tested for *H. pylori* IgG antibodies (in-house EIA).
Results. None of the patients had acute inflammation, but 37 (33%) had chronic gastritis. Less than 5 years after eradication therapy, 22 (58%) of 38 patients (group A) had chronic gastritis and still ≥ 5 years after eradication, 15 (21%) of 72 patients (group B) had chronic gastritis (5 in antrum, 1 in corpus, and 9 both in the antrum and corpus gastritis). One in group A and none in group B of the patients had corpus atrophy. Intestinal metaplasia in the antrum was found among 18 (47%) in group A (13 with chronic inflammation) and among 14 (19%) in group B (4 with chronic inflammation). In group A, 14 of 25 with sera available (56%) and in group B, 17 of 54 (31%) were still seropositive. Of the seropositive patients, 13/14 in group A but only 3/17 in group B had chronic inflammation. Antral intestinal metaplasia was detected in 64% in group A and 12% in group B of the seropositive patients.
Conclusion. Chronic gastritis, intestinal metaplasia in the antrum, and persisting *H. pylori* antibodies are still often found ≥ 5 years after successful eradication therapy. However, these phenomena do not seem to be associated with each other.

Abstract no.: 05.21
Serum Pepsinogens in *Helicobacter pylori* Chronic Gastritis: Relationship with Density of the Bacterium, Inflammation, and Atrophy

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Background. *Helicobacter pylori* chronic gastritis has a key role to develop severe gastro-duodenal diseases. The evaluation of this condition by means of serum pepsinogens is important for the first management of dyspeptic patients but it imposes an optimal comprehension of the factors influencing pepsinogen excretion.
Aim. To study the relationship between serum pepsinogens and different histopathological features of *H. pylori*-related chronic gastritis.
Patients and Methods. One hundred forty-nine consecutive outpatients referred for dyspeptic symptoms were studied. All patients underwent endoscopy with biopsies. Serum pepsinogens I and II were measured by immunoassay method.
Results. Serum pepsinogen levels were significantly correlated with *H. pylori* density, both of the corpus (sPGI: $r = .32$, $p < .001$; sPGII: $r = .56$, $p < .001$) and the antrum (sPGI: $r = .41$, $p < .001$; sPGII: $r = .43$, $p < .001$), as well as with chronic inflammation (sPGI: $r = .26$, $p < .001$; sPGII: $r = .49$, $p < .001$) and activity (sPGI: $r = .38$, $p < .001$; sPGII: $r = .50$, $p < .001$) in the antrum. Only sPGII was correlated with chronic inflammation ($r = .44$, $p < .001$) and activity ($r = .40$, $p < .001$) in the corpus. SPGI was inversely correlated with atrophy ($-r = .33$, $p < .001$) and intestinal metaplasia ($-r = .37$, $p < .001$) in the corpus.
Conclusions. Serum pepsinogens I and II are linked to the gastric histopathologic features in different ways. Serum pepsinogen II levels could be considered as markers of gastric inflammation all over in the stomach. Serum pepsinogen I levels are inversely related to atrophic body gastritis.

Abstract no.: 05.22
Relation between the Pattern of Serum Pepsinogen Changes and the Magnitude of the Immune Response to *Helicobacter pylori* Infection in a Cohort of Young Mexican Children

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Background and Aim. Serology is the main tool for the noninvasive diagnosis of *Helicobacter pylori* infection in young children. Its correlation with other serologic biomarkers can help to better define the magnitude and physiologic consequences of such an infection. The aim of this study was to determine if the magnitude of the immune response is related with the degree in change of serum pepsinogen levels in a cohort of Mexican children.
Methods. Forty-four children between 3 to 5 years of age were recruited in Mexico City. A serum sample was obtained. IgA, IgM, and IgG against *H. pylori* were determined by Western blot (WB). Pepsinogen (PI) and PII levels were determined with an enzyme-linked immunosorbent assay commercial kit. PI : PII ratio was also determined. Kruskall–Wallis test was used to compare groups.
Results. All children had acute and/or secondary immune responses versus *H. pylori*. Children were divided in three groups: (a) high PI and/or PII levels; (b) low PI and/or PII levels; and (c) normal PI and PII levels. A higher rate of high molecular weight (HMW) bands was seen in the groups with high and low pepsinogen levels when compared with the normal level group ($p = .04$). A strong positive relation was seen between IgA and IgG antibodies versus virulence protein bands with PII levels and a strong inverse relation with PI : PII ratio.
Conclusions. All study children had serum immune responses against *H. pylori*. A strong relation was seen between an intense immune response and the presence of *H. pylori* virulence markers with PII and PI : PII ratio. Together, these biomarkers can help to better define noninvasively the magnitude and physiologic consequences of *H. pylori*-induced gastric damage in children.

Abstract no.: 05.23
Proinflammatory Cytokines and *Helicobacter pylori* Infection in Patients Suffering from Rheumatoid Arthritis

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Introduction. Cytokines are hormone-like substances acting mostly in the region of secretion. They play significant role in local and general inflammatory response, also in gastrointestinal diseases. Proinflammatory cytokines like interleukin 1β (IL1β), tumor necrosis factor α (TNFα) and interleukin 8 are reported to be causes and markers of gastritis like in *Helicobacter pylori* infection.
Aim of the Study. The evaluation of the level and expression of proinflammatory cytokines in *H. pylori*-related gastritis, suffering from rheumatoid arthritis.
Material and Methods. One hundred twenty-seven patients with rheumatoid arthritis were enrolled into the study.
The enzyme-linked immunosorbent assay method was used to measure the level of proinflammatory cytokines in serum and tissue homogenates. Cytokine expression in gastric mucous was evaluated using immunohistochemical reaction. Statistical analysis was performed with χ^2, Fischer, and Mann–Whitney U tests.
Results. Elevated level of IL-8 was found in patients with *H. pylori* infection compared with noninfected patients (not statistically significant). *H. pylori* infection had no influence on the level and expression of cytokines in studied patients with rheumatoid arthritis.
Conclusion. In studied group of patients with rheumatoid arthritis, *H. pylori* infection was not connected with increased level or expression of inflammatory cytokines.

Abstract no.: 05.24
CD14 in Bacteria-Driven Chronic Pathological Inflammation

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Helicobacter pylori and *Mycobacterium tuberculosis* are responsible for most common infections in humans. The majority of these infections are "silent" and produce no symptoms. The chronic diseases develop in about 15% of *H. pylori*- and 5% of *M. tuberculosis*-infected subjects. Why these pathogens do not cause diseases in every infected person is not known. *H. pylori*-associated peptic ulcers and *M. tuberculosis*-induced leaking granuloma contain activated macrophages and neutrophils with a dominance of Th1 lymphocytes. A sensing of pathogen-associated molecular patterns (PAMPs) by CD14 pattern recognition receptor precedes an inflammatory response. The aim of this study was to elucidate a possible role of CD14 in the cellular responses to and outcome of the infections from *H. pylori* and mycobacteria. CD14 exists as a 55-kDa polypeptide attached to the membrane of macrophages and neutrophils (mCD14) and as a soluble serum protein (sCD14). A signaling cascade following interaction between CD14 and PAMP plays a critical role in inflammatory response. A polymorphism of CD14-159 gene, the concentration of serum sCD14, and the expression of mCD14 on macrophages were evaluated for *H. pylori*-infected volunteers with various gastric symptoms, the tuberculosis patients and healthy BCG-vaccinated volunteers with positive or negative responses to tuberculin. Chronic gastritis and tuberculosis were associated with a significant decrease and increase in sCD14, respectively. A relation between the polymorphism of CD14-159 gene and development of delayed-type hypersensitivity to tuberculin and *H. pylori*-induced active gastritis was also demonstrated.

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Abstract no.: 05.25
Levels of Serum Pepsinogen Correlate with the Immune Response to *Helicobacter pylori* in a Cohort of Mexican Children

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Background. Serology is the main noninvasive diagnostic test for *Helicobacter pylori* infection in young children. Its correlation with other serologic biomarkers would help define the magnitude and physiologic consequences of this infection. We looked for an association between the magnitude of the immune response and changes in serum pepsinogen levels in a cohort of Mexican children.
Methods. Serum was obtained from 44 children belonging to a 5-year follow-up cohort in Mexico City. Hp IgA, IgM, and IgG were determined by Western blot (WB). Pepsinogen (P) I and II levels were determined using a commercial enzyme-linked immunosorbent assay; PI : PII ratio was calculated. Kruskall–Wallis test was used for comparison between groups.
Results. All children had acute and/or secondary immune responses to *H. pylori*. Three groups were observed: (a) high PI and/or PII levels; (b) low PI and/or PII levels; and (c) normal PI and PII levels. Higher rates of high molecular weight bands were seen in groups with high and low pepsinogen levels when compared with the normal level group ($p = .04$). IgG and IgA antibodies to virulence proteins (CagA, VacA, BabA) were positively associated with PII levels, and inversely associated with PI : PII ratio.
Conclusions. All children had serum immune responses against *H. pylori*. A strong association was seen between an intense immune response, the presence of *H. pylori* virulence markers, PII levels, and PI : PII ratio. Together, these biomarkers can help to elucidate the magnitude and physiologic consequences of *H. pylori*-induced gastric damage in children.

Pathology and Pathophysiology

Abstract no.: 06.01
Anti-*Helicobacter pylori* Therapy Improves Iron Status and Gastric Acid Output in Young Bangladeshi Women with *H. pylori*-Associated Hypochlorhydria and Iron Deficiency Anemia

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Background. Hypochlorhydria is a risk factor for iron deficiency anemia (IDA), which affects infants and young women. *Helicobacter pylori*-associated gastritis may lead to hypochlorhydria.
Aim. To determine if hypochlorhydria contributes to IDA and to evaluate if IDA and hypochlorhydria can be managed by *H. pylori* eradication.
Method. We studied 96 women (20–40 years), infected with *H. pylori* (positive urea breath test) and measured hemoglobin (Hb), serum ferritin (SF), and soluble transferrin receptor (sTfR) to assess iron status (IS). Gastric acid output (GAO) (in mmol/hour) was measured during a 1-hour basal period (BAO) and for an additional hour stimulated period (SAO) after pentagastrin. GAO and IS were re-evaluated in those who exhibited both IDA (Hb < 110 g/L, plus SF < 12 µg/L, and/or sTfR > 8.3 mg/L) and hypochlorhydria (SAO < 14.0 mmol/hour), 60 days after a 2-week course of anti-*H. pylori* therapy.
Results. Of the 96 infected women, 57 (59%) had hypochlorhydria and 39 (41%) had normochlorhydria (SAO > 14.0 mmol/hour). Compared to normochlorhydric women, the prevalence of IDA was significantly higher in hypochlorhydric women (23% versus 49%, $p < .001$). In women who exhibited both hypochlorhydria and IDA (n = 28), anti-*H. pylori* therapy resulted in an improvement of BAO and SAO accompanied with improvement of geometric mean of SF [(µg/L) 10.6 versus 16.3, $p = .02$] and sTfR [(mg/L) 10.2 versus 6.5, $p < .0001$]. In women with hypochlorhydria, 15 (54%) became normochlorhydric and 68% became nonanemic following anti-*H. pylori* therapy.
Conclusion. Our findings indicate that hypochlorhydria and IDA is *H. pylori* associated; both problems can be managed by appropriate eradication therapy.

Abstract no.: 06.02
Is *Helicobacter pylori* Gastritis a Risk Factor for Chronic Pancreatitis in Mongolian Gerbils?

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We investigated the effects of *Helicobacter pylori* cag-PAI on the development of chronic atrophic gastritis, peptic ulcer, and chronic pancreatitis in Mongolian gerbils. They were infected with *H. pylori* type I-strain or an isogenic cagY mutant for several months. Special stains were applied to investigate the metaplastic changes of the mucosa and fibrous changes in the pancreas tissue. Frozen biopsies were used for real time reverse transcriptase-polymerase chain reaction analyzing the expression of H+/K+-ATPase and the cytokines IL-1β, IFN-γ, and KC.

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 wild type-infected gerbils developed peptic ulcer and metaplastic changes. Transmural inflammation and pancreatitis were developed in 56% and 33% of wild-type-infected and mutant-infected gerbils, respectively. Typical histologic parameters of a chronic pancreatitis were present.

The cag-PAI of *H. pylori* is responsible for a severe inflammation that results in corpus dominant atrophic gastritis, intestinal metaplasia, as well as 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 already fibrous changes as shown in collagen and vimentin positivity of paraffin-embedded 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 an *H. pylori*-associated pancreatitis might reveal clinical relevance.

Abstract no.: 06.03
Effects of *Helicobacter pylori* Infection on Gut Appetite Peptide Expression (Leptin, Ghrelin) in Elderly In-patients

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Objective. Recent evidence indicates that chronic gastritis may affect the expression of gastric satiety inducible peptides such as leptin and ghrelin, which may play a role in the regulation of food intake. The aim of our study was to investigate the relationship between chronic gastritis associated with *Helicobacter pylori* infection, leptin, and ghrelin levels, and nutritional parameters in elderly patients.

Methods. Patients over 75 years old undergoing an endoscopy were included. Data on nutritional status, and on *H. pylori* infection diagnosis [serology, ^{13}C-urea breath test, culture, histology, and polymerase chain reaction (PCR) on gastric biopsies] were gathered. Gastric mRNA expression of leptin and ghrelin were quantified by real-time PCR.

Results. A total of 62 patients were included (age: 84.7 ± 5.2 years). *H. pylori* infection was associated with a decreased gastric expression of leptin ($p = .021$), ghrelin ($p = .002$), and plasma ghrelin levels ($p = .018$). Atrophy was associated with decreased levels of gastric leptin ($p = .007$) and ghrelin ($p = .02$). Gastric ghrelin production was significantly lower in CagA-positive patients ($p = .0001$). *H. pylori* infection correlated negatively with the patient's energy intake ($r = -.36$; $p = .001$) and BMI ($r = -.34$; $p = .018$).

Conclusions. The negative association between ghrelin production and *H. pylori* infection may be related to a higher prevalence of atrophy, and raises the possibility that *H. pylori* may contribute to under-nutrition in some older people.

Abstract no.: 06.04
High-Resolution Magnification Endoscopy Can Identify the Normal Gastric Mucosa, *Helicobacter pylori*-Infected Stomach, and Gastric Atrophy

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Background. Endoscopic visualization of *Helicobacter pylori* infection and gastric atrophy is not always feasible with conventional endoscopy. Our aim was to describe the magnified endoscopic findings in human stomach, to correlate them with *H. pylori* gastritis and gastric atrophy, and to evaluate the inter-/intra-observer agreement in the assessment of the magnified endoscopic patterns seen.

Methods. Ninety-five consecutive dyspeptic patients underwent upper gastrointestinal endoscopy with a magnifying endoscope. The endoscopists classified the magnified endoscopic patterns seen and correlated them with histology. Two hundred images were shown to five endoscopists to examine the inter- and intra-observer variability.

Results. Magnified endoscopic findings in gastric body were categorized into four types: (a) honeycomb-type subepithelial capillary network (HTSECN) with regular arrangement of collecting venules (CVs) and regular round pits; (b) HTSECN/regular round pits/loss of CVs; (c) loss of normal HTSECN/CVs with white enlarged pits surrounded by erythema; and (d) loss of normal HTSECN/round pits with irregular arrangement of CVs. The sensitivity, specificity, positive and negative predictive value of (a) type 1 for predicting: the normal gastric mucosa was 92.7, 100, 100, and 83.8%; (b) types 2–3 for predicting the *H. pylori*-infected stomach was 100, 92.7, 83.8, and 100%; and (c) type 4 for predicting gastric atrophy was 90, 96, 85.7, and 97.3%, respectively. The kappa values for inter-/intra-observer agreement in predicting normal gastric mucosa, *H. pylori* gastritis, and gastric atrophy were 0.864, and 0.913 respectively.

Conclusion. High-resolution magnification endoscopy can identify the normal gastric mucosa, *H. pylori*-associated gastritis, and gastric atrophy in a Western population.

Abstract no.: 06.05
Pathophysiological Role of *Helicobacter pylori* γ-Glutamyltranspeptidase

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Helicobacter pylori produces γ-glutamyltranspeptidase (GGT), which induces apoptosis on gastric epithelial cells. To explore molecular mechanism of GGT-mediated pathogenesis and physiological function of GGT in metabolism in *H. pylori*, we examined the enzymatic property of purified GGT protein. *H.*

pylori 26695 GGT was expressed in *Escherichia coli* BL21(DE3) and purified with a Ni chelating affinity column, a cation exchange column, and a gel filtration column. The purified protein exhibited hydrolysis activity for glutamine and glutathione. Glutamate was released from both glutamine and glutathione by the hydrolysis reaction. *K*m and *V*max of glutamine hydrolysis were 182 ± 21 nmol/L and 492 ± 14 nmol/minute/mg protein, respectively, at pH 7.0. *K*m of glutathione hydrolysis was below 100 nmol/L and *V*max was 574 ± 8 nmol/minute/mg protein at pH 7.0. Live *H. pylori* 26695 exhibited hydrolysis activity for external glutamine and glutathione, and GGT knockout strain completely lost this activity, indicating that *H. pylori* GGT exerts its enzymatic activity for external substrates. Glutamate produced by the hydrolysis reaction was incorporated by *H. pylori* cells in a Na-dependent manner. Glutamate was then mainly incorporated into the tricarboxylic acid (TCA) cycle and partially utilized for glutamine synthesis in cytosol. These results suggest that *H. pylori* GGT functions to produce glutamate to be used by *H. pylori* cells. On the other hand, *H. pylori* GGT would cause exhaustive consumption of glutamine and glutathione at gastric niche, as *K*m values of the hydrolysis reactions were very low. Deprivation of glutamine and glutathione would be detrimental to the host cells.

Abstract no.: 06.06
Impairment of Inhibition of HSP70 by *Helicobacter pylori* in Human Epithelial MKN7 Line

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Heat shock proteins (HSPs) are considered as a key component of signaling pathways involved in gastric mucosal defense but their interaction with *Helicobacter pylori* remains unknown. We determined the alterations in mRNA and protein expression for HSP70 induced by live strains of *H. pylori* (cagA+ vacA+) and *H. pylori* (cagA– vacA–) in the epithelial MKN7 cells incubated for a period of 3 hours up to 72 hours without or with addition of exogenous recombinant protein CagA. The incubation of the MKN7 cells with CagA protein alone failed to affect significantly the expression of HSP70, whereas strain *H. pylori* (cagA+ vacA+) inhibited the expression of mRNA for HSP70 in a time-dependent manner. When the MKN7 cells were coincubated with *H. pylori* (cagA+ vacA+) and CagA, the disappearance of the signal for HSP70 after 48 hours was observed. The incubation of MKN7 with *H. pylori* (cagA–, vacA–) also significantly attenuated the expression of HSP70 with the most pronounced inhibitory effect observed at 72 hours of incubation with this *H. pylori* strain. Addition of the recombinant CagA to *H. pylori* (cagA–, vacA–) completely suppressed the expression of HSP70 at 48 hours and 72 hours after the end of incubation periods. We conclude that (a) both *H. pylori* (cagA+, vacA+) and *H. pylori* (cagA–, vacA–) inhibit expression of HSP70 in MKN7 human gastric epithelial cells independently of the presence or absence of cagA gene, and that (b) recombinant CagA protein may exert biological activity in vitro via acceleration of inhibitory effect of *H. pylori* (cagA– vacA–) on HSP70 expression in epithelial cells infected with this bacteria.

Abstract no.: 06.07
Gastrin-17: The Key in Order to Understand Relationship between *Helicobacter pylori* and Gastroesophageal Reflux Disease

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Background. The relationship between gastroesophageal reflux disease (GERD) and *Helicobacter pylori* infection is not still clear. Some studies showed worsening of the reflux symptoms after *H. pylori* eradication. High basal acid output can determine very low gastrin17 (G-17) levels as a result of the feedback relating antrum and corpus mucosa. Serum pepsinogen I and II (sPGI and sPGII) are markers of acid output and gastric inflammation.
Aim. To evaluate G-17, sPG I and II levels in patients with GERD, both *H. pylori* positive and *H. pylori* negative, compared with other acid-related disorders.
Patients and Methods. One hundred twenty-four consecutive outpatients (60 men, mean age: 39 ± 9.4 years, range 21–59) with upper gastrointenstinal (GI) symptoms, and not under proton pump inhibitor (PPI) treatment, were enrolled and divided, on the basis of upper-GI endoscopy, symptoms and medical history, into: 84 with GERD, 42 (17 *H. pylori* positive) with esophagitis (ERD); 42 (19 *H. pylori* positive) with nonerosive disease (NERD), 42 with other acid-related disorders 6 (5 *H. pylori* positive) with duodenal ulcer (DU), 28 (14 *H. pylori* positive) with gastritis (GAS), and 6 (3 *H. pylori* positive) with gastric ulcer (GU). In all patients, *H. pylori* status was assessed, and a blood sample was taken to evaluate serum G-17 and sPGs-levels (EIA, Biohit, Helsinki, Finland).

Mann–Whitney U-test was used.

Results. All *H. pylori*-positive patients showed significantly increased sPG II levels (*H. pylori* positive 15 ± 4; *H. pylori* negative 9 ± 3 pmol/L, $p < .001$). The mean of G-17 levels showed a marked decrease in group GERD versus other acid-related disorders (GERD: 2 ± 2, other: 5 ± 3 pmol/L, $p < .001$). Notably, patients with GERD confirmed significant lower G-17 levels also in this *H. pylori* positive subcohort (GERD 2.3 ± 2; other 5 ± 2 pmol/L, $p < .001$). No difference was found between ERD and NERD according to G-17 and sPGs. Decreasing levels of sPGI were observed from DU (124 ± 33 pmol/L), to GAS (109 ± 36 pmol/L), to GERD patients (93 ± 35 pmol/L), to GU (82 ± 35 pmol/L).
Conclusion. *H. pylori* is not related with risk of GERD: G-17 could be useful to the management and diagnosis of GERD, highlighting a new risk factor of acid exposure. Moreover, pepsinogen II is good serological biomarker to assess mucosal inflammation and gastric hypersecretion.

Abstract no.: 06.08
Gastric Corpus Atrophy with and Without Evidence of *Helicobacter pylori* Infection

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Aim. To study histology and serum findings of atrophic corpus gastritis patients.
Patients and Methods. Among 345 repeatedly gastroscopied patients, 38 showed atrophic corpus gastritis. Serum samples available for 23 patients were tested for *Helicobacter pylori* IgG antibodies (in-house enzyme immunoassay), parietal cell antibodies (Varelisa, Pharmacia), as well as pepsinogen-1 and gastrin-17 levels (Gastropanel, Biohit).
Results. Corpus atrophy was classified to be *H. pylori*-associated in 16 patients (median age 70 years); 10 had active infection as shown by histology or culture and/or active gastritis and 6 patients had evidence of an earlier infection. Twenty-two patients (median age 64 years) had no evidence of *H. pylori* in history, in former laboratory tests, or in earlier gastroscopies. Of the 16 patients with *H. pylori*-associated atrophic corpus gastritis, grade of corpus atrophy was mostly mild (grade 3 in only 31%), and chronic gastritis (in 75%) and intestinal metaplasia (25%) were often found in antrum. Of patients with sera available, 7/9 still had elevated *H. pylori* antibodies, and low pepsinogen and high gastrin levels were detected in 22%. However, in patients with atrophic corpus gastritis not associated with *H. pylori*, grade 3 corpus atrophy was often present (77%), whereas antrum was only occasionally affected (chronic gastritis in 27% and intestinal metaplasia in 5%). Of these patients, none showed elevated *H. pylori* antibodies but the majority had low pepsinogen 1 and high gastrin 17 levels (79%) as well as parietal cell antibodies (79%).
Conclusion. In corpus atrophy not associated with *H. pylori*, gastritis is rare in the antrum but parietal cell antibodies are frequent.

Abstract no.: 06.09
Gastric Epithelial Proliferation in *Helicobacter pylori* Gastritis: The Role of *cagA* Status and Apoptosis

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Background. *Helicobacter pylori* role in gastric carcinogenesis may be related to its capacity to interfere with gastric mucosa cell turnover.
Aims. To analyze gastric epithelial proliferation in *H. pylori*-associated gastritis in relationship to *cag*A status and apoptosis.
Patients and Methods. Fifty patients (22 men, age 40 ± 14 years) presenting *H. pylori* gastritis (30 *cag*A+) and eight noninfected patients were studied prospectively. *H. pylori* and *cag*A status were determined by polymerase chain reaction and immunoblot analysis. Proliferation and apoptosis were studied by immunohistochemistry in biopsies from the antrum, incisura, and corpus. The percentage of Ki67+ nuclei [(proliferative index (PI)] was determined; anti-apoptotic (Bcl2 and Bclx) and pro-apoptotic (Bax and Bak) proteins expression was scored (0 to 4).
Results. PI was higher in *H. pylori*-positive (32.8%) than in *H. pylori*-negative (17.7%) cases and in *cag*A-positive (35.6%) compared to *cag*A-negative patients (27.4%) ($p \leq .0001$). In all the groups, proliferation was significantly more intense in the antrum than in the corpus ($p \leq .002$), and in the antral lesser curvature than in the other sites, irrespectively of *cag*A status ($p \leq .03$). Patients with atrophy (n = 24) had higher PI (35.3%) than those without atrophy (n = 26; 29.3%) ($p < .01$). Proliferation correlated significantly with pro-apoptotic protein expression in infected patients ($r = .395$; $p = .005$), in those with atrophy ($r = .492$; $p = .01$), and in the antral lesser curvature and incisura ($p = .04$).
Conclusions. In *H. pylori*, gastritis epithelial proliferation is higher in the antral lesser curvature, and is related to *cag*A status, atrophy, and over-expression of pro-apoptotic proteins in the lesser curvature.
Financial support: CNPq, Fapemig

Abstract no.: 06.10
Evaluation of Subclinical Vascular Alterations in *Helicobacter pylory* (*H. pylori*) Positive Patients

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Background. Inflammation has been demonstrated to play a major role in the pathogenesis of atherosclerosis trough several mechanisms, including endothelial dysfunction. The association between *Helicobacter pylori* infection and atherosclerosis has not yet been established.
Aim of Study. Abnormal function of the endothelium and the associated structural changes in the wall of the arteries result in a loss of elasticity. We aimed to evaluate the presence of early preclinical arterial elasticity alterations in *H. pylori*-positive patients without clinical alterations or risk factors for cardiovascular diseases.
Materials and Methods. We enrolled 24 *H. pylori*-positive patients (12 men and 12 women, mean age 31.3 ± 8.5 years). The patients were studied with endoscopy and the *H. pylori* status determined. Indices of large-artery elasticity, small-artery elasticity, systemic vascular resistance, and total vascular impedance were obtained through a tonometry applanation with the HDI/PulseWave™ CR-2000 at baseline and after eradicant therapy.
Results. In table, below.

Parameters	Before therapy	*H. pylori* positive after therapy	*H. pylori* negative after therapy
C1 (mL/mmHg × 10)	16.56 ± 4.12	17.94 ± 2.15	19.85 ± 3.13
C2 (mL/mmHg × 100)	5.22 ± 2.50	6.39 ± 2.37	7.25 ± 2.41
RVS (dina × sec × cm^{-5})	1464.57 ± 186.15	1301.83 ± 252.91	1025.33 ± 115.42
IVT (dina × sec × cm^{-5})	112.35 ± 25.84	110.17 ± 27.58	96.49 ± 24.12

Conclusion. Our preliminary data indicate a reduction of arterial elasticity and increased systemic vascular resistance during *H. pylori* infection. The presence of subclinical vascular alterations in

persistent *H. pylori* positivity status seems to include *H. pylori* in the process of the atherosclerosis.

Abstract no.: 06.11
Proton Pump Inhibitor-Based Triple Therapy in *Helicobacter pylori*-positive Patients with Gastric Ulcers: Histologic Analysis

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Introduction. Further information is required concerning the cellular benefits associated with eradication of *Helicobacter pylori* infection among patients with gastric ulcer (GU).
Aims and Methods. Histologic analysis was completed as part of a randomized, double-blind, multicenter study that aimed to investigate the effects of proton pump inhibitor (PPI)-based triple eradication therapy on healing and long-term relapse in *H. pylori*-positive patients with GU (HELIX; study code: D9612C09991). Patients were treated with twice-daily esomeprazole 20 mg + amoxicillin 1000 mg + clarithromycin 500 mg for 1 week, or esomeprazole therapy alone. Biopsy specimens (corpus and antrum) were taken for histologic analysis at baseline, after eradication therapy, and at 6 and 12 months follow-up (for healed patients). All biopsies were assessed by a central reader (who was blinded to treatment) in accordance with the upgraded Sydney system.
Results. Biopsy specimens were evaluable for 401 patients (intention-to-treat population). At baseline, evidence of corpus gastritis was observed in the majority of patients (79 to 83%), as was antrum gastritis (88 to 93%). Eradication of *H. pylori* and marked reductions in the level of inflammatory activity, chronic inflammation, and overall severity of gastritis in both the corpus and antrum were observed with triple eradication therapy. These effects were sustained during 12 months follow-up. In contrast, esomeprazole monotherapy did not eradicate *H. pylori* and had relatively small, temporary, effects on histological parameters.
Conclusion. Histological evidence of antrum and corpus gastritis is reduced after successful eradication of *H. pylori* with PPI-based triple therapy in patients with GU.

Abstract no.: 06.12
Helicobacter pylori, Intestinal Metaplasia, and Gastric Atrophy: Role of Age and Gender

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Background/Aim. Criteria for characterization of population that would most benefit from *Helicobacter pylori* eradication, in preventing gastric tumor, are still uncertain. We estimate the relation between *H. pylori* infection, intestinal metaplasia (IM), and gastric atrophy (GA), considering age and gender.
Materials/Methods. From June 1998 to June 2000, 1310 patients (654 men and 656 women, median age 59.8 ± 17 years), never treated for *H. pylori,* have been studied by esophagogastroduodenoscopy (EGDS) with four biopsies from the gastric antrum and corpus. Odd ratios of association (OR) were calculated with logistic regression. The age has been stratified on two levels: < 45 and > 45 years and in four bands: < 50, 50–62, 63–72, 72 years.
Results. Association between *H. pylori* and presence of IM is greater in women (OR adjusted for age = 1.7; $p = .01$) than in men (OR = 1.3; $p = .22$). Association between *H. pylori* and GA is also more obvious in women (OR = 1.6; $p = .03$), than men (OR = 0.8; $p = .26$). Controlling for the gender, IM, and GA are meaningfully associated with *H. pylori* in the first class of age (< 49 years): IM OR = 2.9 ($p = .003$), GA OR = 2.5 ($p = .001$). In the other classes, such associations are more weak; age 50–62: IM OR = 1.08 ($p = .94$) and GA OR = 0.9 ($p = .97$); age 63–72 years: IM OR = 1.2 ($p = .45$) and GA OR = 1.0 ($p = .89$); age > 72: IM OR = 1.3 ($p = .31$), and GA OR = 0.8 ($p = .85$).
Conclusions. The association between *H. pylori* infection and potentially precancerous gastric lesions is meaningful only in youngest subjects (< 50 years). These results suggest that *H. pylori* eradication could be useful only in this class of population and may be used to select patients who need treatment.

Abstract no.: 06.13
Genoypes of Colombian *Helicobacter pylori* Strains and Relationship to Chronic Active Gastritis

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The main objective of this study was to investigate the Helicobacter *pylori vacA* and *cagA* alleles in 75 Colombian patients with chronic active gastritis. Two antrum gastric biopsy samples were obtained for rapid urease test and bacterial culture. *vacA* and *cagA* genotypes were determined by polymerase chain reaction. The average age was 51 years, the frequency of *H. pylori* 81.3% (61/75). Of the *H. pylori* strains isolated from the antrum specimens, 51.4% were *cagA* gene positive. The proportions of *vacA* gene subtypes were s1/m1 (25.7%), s1/m2 (34.3%), s2/m2 (31.4%), and s2/m1 (8.5%). The polymorphism of the gene s1 was s1a 62% and s1b 38%. The most frequent genotype was cagA negative s1/m2 (22.8%). The proportion of *cagA* positive s1/m1 genotype was 20%. Infected with two or three *H. pylori* strains with different genotypes were 73.3%. However, no statistically differences among *cagA* occurrence and the different *vacA* subtypes s1 and m1 could be found ($p = .08$), s2 and m2 ($p = .19$). There was no statistically significant association among the expression of *vacA* s1/m1 versus s2/m2 and the presence or absence of the *cagA* gene ($p = .12$). No statistically significant differences among the proportion of positive and negative strains for *cagA* gene be found ($p = .81$).

These findings show that there was not a predominant genotype in the patients with chronic active gastritis; co-infections with different *H. pylori* strains are found. Different *H. pylori* strains are not closely associated with severity of chronic active gastritis.

Abstract no.: 06.14
Relationship between Virulence Factors CagA and VacA and Grade Atrophy of *Helicobacter* Chronic Gastritis

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Background. The glandular atrophy that starts at first in the antrum results in the majority of the cases from chronic gastritis in *Helicobacter pylori.*
Aim. To correlate the virulence factors CagA and VacA to grade atrophy.
Methods. Study was prospective with co-observation by five driven pathologists using two antral and two corpus biopsies from adults with epigastric pain. Patients were submitted to other tests: URT, serology, culture, HpSA, CagA, and VacA.
Results. Two hundred one (sex ratio = 0.7). The mean age was 35 years (18–74). Histology showed *H. pylori* and chronic gastritis in 201 (100%).

In the antrum, chronic gastritis was diagnosed with 73 (36%) mild atrophy, 121 (60%) moderate, and 7 (3%) severe. Intestinal antral metaplasia and severe dysplasia were observed in all severe atrophy. Fifty-six (28%) patients were CagA positive VacA positive, 83 (41%) CagA positive VacA negative, 12 (6%) CagA negative VacA positive, and 50 (25%) CagA negative VacA negative. CagA positive was found in 47/73 (64%) mild atrophy, 83/121 (69%) moderate, and 7/7 severe (100%). VagA positive was found in 24/73 (33%) mild atrophy, 41/121 (34%) moderate, and 2/7 severe associated to CagA positive.
Conclusion. In this study, atrophy was very frequent, predominant in antrum, almost moderate, and rarely severe. CagA was frequently found in moderate atrophy and always associated to severe atrophy.

Abstract no.: 06.15
Observer Agreement on Grading Atrophy in *Helicobacter pylori* Chronic Gastritis

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Background. The glandular atrophy presents problems of reproducibility. Currently, atrophy is defined better but its appreciation is variable with: gastric sites, multifocality of lesions, quality of the withdrawals, histologic techniques and observer.
Aim. To evaluate the atrophy according to updated Sydney system and to determine the concordance of atrophy using kappa statistics.
Patients and Methods. Forty-eight (sex ratio = 0.6) with epigastric pain from prospective study (2000–2006) of 565 patients. The mean age was 36 years (18–74). Two antral and two corpus biopsies were first co-observed by five driven pathologists. In second time, two driven pathologists independently examined again these biopsies to evaluate atrophy.
Results. Histology showed *Helicobacter pylori* in 45/48 (94% and chronic gastritis in 48 (100%).

In the antrum, chronic gastritis was diagnosed in all patients, without atrophy in 4 (9%). Atrophy was detected in 41 (91%) with 19 (46%) mild, 20 (49%) moderate, 2 (5%) severe. Intestinal antral metaplasia and dysplasia were observed in all severe atrophy.

In corpus, mucosa was not modified in 7(16 %) and presenting gastritis without atrophy in 11 (24%). Atrophy was observed in 27 (60%) with 10 (37%) mild, 17(63%) moderate and 0(0%) severe. Intestinal metaplasia and severe dysplasia were not noted in corpus. Coefficient kappa of atrophy variations was 0.77 in antrum and 0.6 in corpus.
Conclusion. The concordance of the atrophy *H. pylori* chronic gastritis is remarkably good because all biopsies were reviewed jointly by five driven pathologists for 6 years.

Abstract no.: 06.16
Pathological Role of Vacuolating Cytotoxin of *Helicobacter pylori* in the Onset of Gastric Cancer Correlates with Intestinal Metaplasia

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Background and Aims. The roles of VacA in gastric diseases caused by *Helicobacter pylori* infection are still controversial. To gain further insight into the pathological activities of VacA in the development of gastric abnormalities, we investigated the histopathological features of gastric biopsy specimens.
Methods. The expression of *H. pylori* antigen and VacA in 219 gastric biopsy specimens from routine gastroendoscopy and 51 gastric mucosa from patients with gastric cancer were examined using immunostaining.
Results. Histological features by immunohistochemistry with anti-*H. pylori* and anti-VacA were closely similar to each other in gastric glands, intestinal metaplasia, and infiltrated plasma cells. However, the positive reactions by anti-*H. pylori* and anti-VacA did not appear in normal mucus-secreting cells. Some peculiar positive spots by anti-rVacA were observed in the parietal cells of gastric glands in contrast to diffuse staining by anti-*H. pylori* in those were observed. Positive cases by anti-*H. pylori* were significantly frequent in most biopsy specimens among gastric caner patients ($p < .0001$), whereas those by anti-VacA antibody were specifically higher only in intestinal metaplasia ($p < .009$). Statistically, the OR of the presence of VacA in chronic inflammation and intestinal metaplasia in the biopsy specimens from gastric cancer were significantly higher than the normal counterpart ($p < .05$ and $p < .01$, respectively).
Conclusion. It shows VacA is an immunodominant marker protein of *H. pylori* and is specifically observed in precancerous lesion such as intestinal metaplasia, which implies that VacA may be involved in the transformation of normal cells into precancerous conditions.

Preneoplastic and Neoplastic Diseases

Abstract no.: 07.01
DNA Methylation in the Gastric Mucosa of *Helicobacter pylori*-Infected Patients and the Effect of Eradication

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Background. Gene promoter methylation is an epigenetic event particularly relevant in human carcinogenesis.
Aim. To evaluate the methylation of some tumor-related genes in *Helicobacter pylori*-infected patients before and after eradication.
Patients and Methods. Fifty-seven dyspeptic outpatients (51 ± 12 years) underwent endoscopy with three biopsies (antrum, angulus, and corpus) to evaluate *H. pylori*, intestinal metaplasia (IM), and DNA methylation (MSP) of 5 tumor-related genes (CDH1, p16, hMLH1, APC, and COX2). All infected patients were given a 7-day standard triple therapy and were asked to repeat endoscopy after 1 year.
Results. All patients had an endoscopic diagnosis of nonulcer dyspepsia. *H. pylori* infection was found in 45 patients and IM in 17 out of 45 (38%). After 1 year, 23 out of 45 patients repeated endoscopy and 17 out of 23 (74%) were found *H. pylori* negative. The mean number of methylated genes and the proportion of patients with CDH1, p16, hMLH1, APC, and COX2 methylation in the different subgroups at baseline and before and after eradicating treatment are shown in Table 1.
Conclusion. *H. pylori* infection is associated with promoter methylation and silencing of genes, which are relevant in the initiation and progression of gastric carcinogenesis. Although CDH1 methylation seems to be an early event in *H. pylori* gastritis, MLH1 methylation occurs late along with IM. *H. pylori* eradication is able to reduce gene methylation, thus delaying or reversing *H. pylori*-induced gastric carcinogenesis.

Table 1

Patients (n)	*H. pylori*-/IM– (12)	*H. pylori*+/IM– (28)	*H. pylori*+/IM+ (17)
No. meth genes	0	1.1 ± 0.9	1.6 ± 0.9
CDH1	0%	68%	71%
p16	0%	25%	29%
APC	0%	7%	35%
MLH1	0%	0%	12%
COX2	0%	14%	12%

Patients (n)	*H. pylori*+/IM– (9) Eradicated	*H. pylori*+/IM+ (8) Eradicated	*H. pylori*+/IM – or + (6) Noneradicated
No. meth genes	2.2 ± 0.7 → 1.2 ± 0.8	1.6 ± 0.5 → 1.2 ± 0.9	0.1 ± 0.3 → 0.4 ± 0.5
CDH1	89% ± 89%	75% ± 63%	50% ± 100%
p16	67% ± 22%	25% ± 13%	17% ± 83%
APC	22% ± 11%	37% ± 37%	0% ± 33%
MLH1	0% ± 0%	0% ± 13%	0% ± 0%
COX2	44% ± 0%	25% ± 0%	0% ± 0%

Abstract no.: 07.02
Regression of Antral Intestinal Metaplasia after *Helicobacter pylori* Eradication in 9 Years of Follow-up

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Background. Intestinal metaplasia (IM) is an important stage in the pathogenesis of gastric cancer. The purpose of the study was to evaluate how *H. pylori* eradication modifies the severity of gastritis and IM in antrum in 9 years of observation.
Material and Methods. A group of patients with ulcer disease successfully eradicated were followed up prospectively. In 23 cases, antral IM was diagnosed before the treatment. Endoscopy with antral biopsies was carried out 3 and 9 years after eradication. Severity of gastritis and IM was graded according to the Sydney classification.
Results. No reinfection of *H. pylori* was observed. Total regression of IM was found in 61% of patients 3 years after *H. pylori* eradication and in the same patients after 9 years. Mean intensity of IM was 1.87 ± 0.69 at the start, 0.65 ± 0.93 after 3 years, and 0.56 ± 0.56 after 9 years. The mean grade of intensity of gastritis declined from 2.61 ± 0.66 to 1.48 ± 0.66 after 3 years and 1.35 ± 0.50 after 9 years. The mean grade of activity of gastritis decreased from 2.52 ± 0.73 to 1.39 ± 0.66, and 1.30 ± 0.56, respectively. The mean grade of atrophy decreased from 2.17 ± 0.72 to 1.65 ± 0.78, and 1.43 ± 0.73 after 3 years and 9 years. All the differences in comparison to the results before eradication were statistically significant.
Conclusions. During the 9 years follow-up after successful *H. pylori* eradication in 61% of patients, total regression of antral IM was seen and in the rest of the patients, no progression of IM was observed. Stable reduction of antral gastritis was achieved.

Abstract no.: 07.03
Helicobacter pylori and Gastric Premalignant Conditions: Results of the Japanese Intervention Trial (JITHP)

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Aim. To determine the regression/progression of mucosal atrophy and intestinal metaplasia in eradicated and noneradicated groups both endoscopically and histologically. The Japanese Intervention Trial was originally designed to evaluate premalignant conditions and cancer as end points; however, because of the final number of recruitments, the endpoints were reduced to one.
Material and Methods. *Helicobacter pylori*-infected patients between 20–59 years of age recruited in 119 institutions around Japan were randomized to receive a 7-day therapy. Atrophy was evaluated endoscopically according to the Kimura–Takemoto classification. For histological evaluation, the "mean stomach score" was calculated from the score of the three biopsies of the

antrum, angulus, and gastric body. The scores for regression, no change, and progression were determined as +1, 0, and −1, respectively.
Results. A total of 379 and 372 subjects enrolled in the eradication and noneradication groups, respectively. Subjects followed up for more than 4 years were evaluated. Endoscopically, there were no differences in regression/progression of gastric premalignant conditions among the groups. On the other hand, histopathologic analysis revealed significant regression of atrophic gastritis and metaplasia in eradicated subjects. Individual analysis revealed that significant regression of atrophic gastritis in eradicated subjects was observed regardless gender or age ($p < .001$). In relation to intestinal metaplasia, although significant regression in the eradicated group was not observed in the overall analysis, significant regression in eradicated subjects more than 40 years was observed.
Conclusion. Our results suggest that *H. pylori* eradication reverses premalignant conditions and is a way to prevent gastric cancer.

Abstract no.: 07.04
The Chain 1 Interferon Gamma Receptor (IFNGR1)-56C/T Gene Polymorphism is Associated with Increased Risk of Early Gastric Carcinoma

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It was demonstrated that polymorphisms within inflammation-related genes are associated with risk of gastric carcinoma (GC) in *Helicobacter pylori*-infected individuals. Recently, polymorphisms in the gene encoding the chain 1 of the interferon gamma receptor (*IFNGR1*) were found to be associated with increased susceptibility to *H. pylori* infection. We aimed to determine the association between the -56C/T polymorphism in the *IFNGR1* gene and risk of GC. In a case-control study including 733 controls and 393 GC patients, the *IFNGR1*-56C/T polymorphism was genotyped. The effect of the -56C/T promoter polymorphism in the level of expression of the *IFNGR1* gene was evaluated by an *IFNGR1*-56C/T allele-specific luciferase reporter assay. In individuals with early-onset GC (defined as having less than 40 years of age at the time of diagnosis), we found a significant over-representation of the *IFNGR1*-56*T/*T homozygous genotype with an odds ratio of 4.1 [95% confidence interval (CI) 1.6–10.6]. In the luciferase reporter assay, we observed a 10-fold increase ($p < .001$) in luciferase expression associated with the *IFNGR1*-56*T allele. Our results indicate that the *IFNGR1*-56C/T polymorphism is a relevant host susceptibility factor for GC development. Our data also indicate that this genetic polymorphism is functionally relevant and may be related with early development of GC. The -56*T allele is associated with significantly increased expression of the *IFNGR1* gene and may therefore play an important role in modulating the host inflammatory response to *H. pylori* infection.

Abstract no.: 07.05
Constant Frequency of Epigenetic Alterations in Patients with *Helicobacter pylori*-Associated High Risk Gastritis Before and After *H. pylori* Eradication Therapy

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Background. Epigenetic alterations are observable in a portion of patients with *Helicobacter pylori*-associated high-risk gastritis. We observed recently that hypomethylation of genome-wide CpG-sites can be detected in gastric cancer and in patients with high-risk gastritis (corpus-predominant/pangastritis with or without intestinal metaplasia). The aim of this study was to evaluate the methylation status of CpG-sites in patients with high-risk gastritis before and after *H. pylori* eradication therapy.
Methods. Gastric DNA was analyzed from patients with CpG (n = 15, *H. pylori* positive, 8 with additional IM, 2 with atrophy), patients with previous CpG after *H. pylori* eradication therapy (n = 24, at least 4 years after *H. pylori* eradication therapy) and controls (n = 17) without *H. pylori* infection. In all patients a M.SssI-enzyme assay for methylation of genome-wide CpG-sites with s-adenosyl-L-[methyl-^{3}H]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 ratio of methylated CpG sites was not significantly different between high-risk gastritis (86.8%), previous high-risk gastritis after *H. pylori* eradication therapy (86.7%), and controls (88.7%). Severe hypomethylation was found in 36% of patients with high-risk gastritis and in 33% of patients with previous high-risk gastritis *H. pylori* after eradication therapy.
Conclusions. A substantial part of patients with *H. pylori* infection and advanced changes in gastric mucosa have decreased levels of global methylation of CpG sites, indicating that in those patients, frequent epigenetic alterations have been already accumulated. The portion of patients with those severe changes in methylation level remains constant after *H. pylori* eradication therapy; therefore the epigenetic alterations might be not reversible.

Abstract no.: 07.06
Efficacy of *Helicobacter pylori* Erradication Treatment in Gastric MALT Lymphoma: A Systematic Review

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Objective. To perform a systematic review of studies evaluating the effect of *Helicobacter pylori* eradication treatment on the histological regression of gastric mucosa-associated lymphoid tissue (MALT) lymphoma.
Methods. Bibliographic searches were conducted in MedLine, and studies evaluating the effect of *H. pylori* eradication treatment on the histological regression of gastric MALT lymphoma were included.

Results. Forty-one studies were identified, including a total of 1446 patients. After *H. pylori* eradication, complete remission was achieved in 74% of the cases, partial remission in 10%, and no response in 16%, during a mean follow-up of 23 months (range 12 to 35 months). After complete remission, tumoral relapse was detected in 6% of the cases; of these, 25% were associated with *H. pylori* re-infection, and in 15% a high-grade MALT lymphoma component was identified. When only patients with MALT lymphoma stage EI (confined to stomach) and with purely low-grade histologic type were included, complete remission was achieved in 80% (95% CI, 77–82%) of the cases. In stage EI1 (confined to mucosa or submucosa), the response to eradication treatment was higher than in stage EI2 (beyond submucosa): 84% versus 31%; $p < .0001$.
Conclusion. *H. pylori* eradication in patients with gastric low-grade MALT lymphoma and stage EI achieves complete remission in 80% of the cases. This figure increases up to 84% in EI1 lymphomas, but is only 31% in EI2 cases.

Abstract no.: 07.07
Early Results from the European Cancer Prevention Organization Intestinal Metaplasia Intervention Study

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Aims and Methods. The aims were to test two primary hypotheses; that *Helicobacter pylori* eradication and/or ascorbic acid supplementation for 3 years causes a statistically significant change in the pattern of intestinal metaplasia (IM) (type, extent, etc.). Six hundred twelve patients with biopsy-proven IM, recruited from 26 centers in 9 European countries, were divided into two groups based on *H. pylori* status. Using a factorial design, *H. Pylori*-positive patients were randomized on a 2 : 1 basis to active or placebo *H. pylori* eradication treatment followed by a 3-year supplementation phase (2 g vitamin C or placebo daily). *H. Pylori*-negative patients were randomized to either active or placebo vitamin C. Endoscopy with antral and incisural biopsies was carried out at baseline, 1, and 3 years. IM status was determined locally and by a central laboratory using the Sydney classification. *H. pylori* status was assessed using a ^{13}C-urea breath test 4–6 weeks after completion of eradication therapy.
Results. Results are presented for 188 patients *H. pylori* positive at entry, where full central histological evaluation was available. There was no difference at 3 years in progression of IM extent or change in IM type between patients who had *H. pylori* eradicated and those who remained *H. pylori* positive.
Conclusions. *H. pylori* eradication did not result in a significant IM improvement 3 years after study entry. A combination of *H. pylori* eradication and vitamin C supplementation did not alter the extent of IM over a 3-year period. These results await confirmation by analysis of all eligible patients.

Abstract no.: 07.08
The Effect of Eradication Therapy for *Helicobacter pylori* Infection on the Incidence of Gastric and Other Cancers

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Background. *Helicobacter pylori* infection is strongly associated with gastric cancer; associations with pancreas, biliary tract, colorectal and lung cancers have also been reported.
Aim. To study whether eradication therapy for *H. pylori* infection would influence the subsequent incidence of gastric and other cancers.
Methods. The standardized incidence ratio (SIR) of cancers was determined in two subgroups: 3650 patients with successful eradication therapy, verified by a significant fall of antibody titers within 4–12 months (SUC-Th) and 11,216 infected patients with no information about therapy (NOI-Th). All microbiological *Helicobacter* data we determined in clinical samples in 1973–1998 were linked with the Finnish Cancer Registry. Follow-up ended at death or at the end of 2004; by then, 120,534 observation years had accumulated.
Results. The incidence of gastric cancer was lower in the SUC-Th cohort, but higher in the NOI-Th cohort than the national incidence. The $SIR_{SUC\text{-}Th}/SIR_{NOI\text{-}Th}$ ratio decreased as years passed and was 0.16 (95% CI = 0.004–0.99) for the follow-up from the sixth year on. The $SIR_{SUC\text{-}Th}/SIR_{NOI\text{-}Th}$ ratios of all other cancers studied (cardia, esophagus, pancreas, liver, biliary tract, colon, and all cancers together) were close to 1 except for lung cancer, which was significantly lower (0.63, 95% CI 0.41–0.94).
Conclusions. In patients whose *H. pylori* infection was eradicated, besides the expected decrease in the subsequent incidence of gastric cancer – most obvious a few years later – also a significant decline in the incidence of lung cancer was seen.

Abstract no.: 07.09
Helicobacter pylori-negative Gastric MALT Lymphoma: Results of Long-Term Follow-up

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Background/Aims. *Helicobacter pylori*-negative gastric MALT lymphoma is a rare disease and still a therapeutic challenge. We

analyzed long-term outcome of *H. pylori*-negative gastric mucosa-associated lymphoid tissue (MALT) lymphoma in comparison with that of *H. pylori*-positive cases.
Methods. We retrospectively studied 156 patients of gastric MALT lymphoma (25 *H. pylori* negative and 131 *H. pylori* positive) with various modalities at the Seoul National University Hospital between January 1996 and April 2006.
Results. *H. pylori*-negative rates were 15% (20/133) among the low-grade patients and 21.7% (5/23) among the high-grade patients ($p = .007$). In *H. pylori*-negative cases, percentage of early stage (IE) was significantly lower than that of *H. pylori*-positive cases (40% versus 89.3%, $p = .002$). In *H. pylori*-positive cases, complete remission (CR) was obtained in 88.6% of early-stage (101/114, three follow-up loss) and 71.4% of late-stage patients (10/14). Three patients showed relapse during follow-up (3–106 months). Although 30.8% of late-stage *H. pylori*-negative patients (4/13, two follow-up loss) failed to achieve CR, all of the early-stage *H. pylori*-negative patients (10/10) showed CR. One patient (stage IV) suffered a relapse after 21 months during the follow-up (3–75 months). Compared to *H. pylori*-positive cases, CR rate of *H. pylori*-negative patients showed no significant difference ($p = .851$).
Conclusions. Regardless of treatment modality, *H. pylori*-negative patients show favorable long-term prognosis (5 years 92.0%). To deduce strategic guidelines, further data through large-scale prospective studies is necessary.

Abstract no.: 07.10
Helicobacter pylori Infection and Gastric Carcinoma (GC): Importance of the Bacterial Genotypes and the Genetic Polymorphism (GP) of the Host's Inflammatory Cytokine (IC), Interleukin 1 Beta (IL-1b)

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Background. *Helicobacter pylori* infection is a main determinant for gastric carcinoma (GC) development, and infected individuals with particular interleukin 1 beta (IL-1b) genetic polymorphism (GP) may run an increased GC risk. We determined both bacterial genotype and host's haplotypes of IL-1b promotor gene in this area with high GC incidence.
Patients and methods. We examined 190 GC patients (GCP) who underwent gastrectomy and 180 age- and gender-matched controls (Co). *H. pylori* and CagA status were determined serologically by enzyme-linked immunosorbent assay and Western blotting. In 45 patients and 60 controls, we determined the *cagA* structural types of isolates by polymerase chain reaction (PCR). IL-1b haplotypes were determined by PCR with primers specific for the promoter region at positions −31 and −511. Amplicons were digested with restriction enzymes and analyzed by electrophoresis.
Results. One hundred seventy-one GCP (90%) and 130 Co (72.2%) were infected ($p < .001$, OR = 3.4); 146 infected GCP (85.3%) and 88 infected Co (67.6%) were CagA seropositive ($p < .001$; OR = 2.7); *cagA* was present in 45 GCP (100%) and in 42 Co (70.0%, $p < .001$, OR indefinite). The prevalence of *cagA* structural types were similar in the two groups; *cagA* with a small deletion was present in 6.6% of GCP versus 23.3% of Co ($p = .042$, OR = 4.2). Pro-inflammatory IL-1b haplotypes were similarly prevalent in GCP and Co; e.g., 21 GCP (11.0%) and 20 Co (11.1%) had the hyper-inflammatory haplotype −511 C/C (NS).
Conclusions. The increased GC development risk seems to be linked to the bacterial determinants of virulence rather than the polymorphism of the host's IL-1b gene.
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Abstract no.: 07.11
Methylation of *CDH1*, *p16/INK4A*, *APC*, *MLH1*, and *COX2/PTGS2* Genes in Sera and Neoplastic and Non-neoplastic Gastric Mucosa of Patients with Stomach Cancer

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Background. Silencing of tumor suppressor and tumor-related genes by methylation of gene promoters is one of the major epigenetic events in human carcinogenesis.
Aims and methods. To evaluate the methylation status of multiple genes in peripheral blood lymphocytes (PBL) and surgical samples of cancerous tissue and corresponding non-neoplastic mucosa from 40 consecutive patients (32 men; mean age: 65 ± 12 years) with advanced (stage II or III) noncardia gastric carcinoma (GC). DNA methylation of five tumor-related genes (*CDH1*, *p16*, *MLH1*, *APC*, and *COX2*) was evaluated by methylation-specific polymerase chain reaction (MSP). Non-neoplastic gastric mucosa was evaluated for intestinal metaplasia (IM) and *Helicobacter pylori* infection.
Results. Among GC samples, 30 (75.0%) were classified as intestinal-type and 10 (25.0%) as diffuse-type carcinoma. IM was more frequent in intestinal-type than in diffuse-type GC (26/30; 86.6% versus 8/10; 80%, p = ns). Most GC patients (18/34; 52.9%) had type III IM. All but 9 patients (77.5%) were *H. pylori* infected. There was no difference between the frequency of *H. pylori* infection in intestinal-type GC (23/30; 76.6%) and diffuse-type GC (8/10; 80.0%). No DNA methylation was detected in PBL. The number (and proportion) of patients with methylation of the five tumor-related genes in neoplastic and non-neoplastic tissues sorted by the histologic type of tumor are shown in the Table 1. No significant difference in gene methylation rate was observed when data were analyzed according to patients' age, *H. pylori* infection, and tumor stage.
Conclusions. Aberrant DNA methylation of tumor suppressor and tumor-related genes initially occurs in non-neoplastic gastric epithelia and may predispose tissues to GC initiation and progression. Epigenetic silencing of CDH1, p16, and APC, which occurs early in non-neoplastic gastric mucosa could be a predictor of malignancy in the stomach.

Table 1.

	Intestinal-type GC (n = 30)		Diffuse-type GC (n = 10)	
Genes	Non-neoplastic	Neoplastic	Non-neoplastic	Neoplastic
CDH1	16 (53%)*	4 (13%)*††	6 (60%)	4 (40%) ††
p16	16 (53%)†	7 (23%)†¶	10 (100%)§	6 (60%)§¶
APC	16 (53%)	18 (60%)	6 (60%)	4 (40%)
MLH1	0	4 (13%)	0	0
COX2	8 (27%)‡	16 (53%)‡	4 (40%)‡‡	8 (80%) ‡‡

*$p < .001$; †$p < .025$; ‡, ¶ $p < .03$; § $p < .05$; †† ‡‡ $p = .06$

Abstract no.: 07.12
Prevalence of *Helicobacter pylori* Infection in Gastric MALT Lymphoma: A Systematic Review

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Objective. To perform a systematic review of the studies evaluating *H. pylori* prevalence in patients with gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and to analyze the factors influencing it.
Methods. Bibliographic searches were conducted in MedLine, selecting the articles that studied *Helicobacter pylori* prevalence in patients with MALT lymphoma.
Results. Thirty-eight studies were identified, including 1844 patients. The mean prevalence of *H. pylori* infection was 79%. In patients diagnosed with *H. pylori* infection using two or more methods, the prevalence was 85%, whereas it was 77% when only one diagnostic method was performed ($p < .0001$). The *H. pylori* prevalence in patients diagnosed with histology was 75%, but this figure increased up to 85% when serology was used ($p < .0001$). The *H. pylori* prevalence in high-grade lymphomas was 60%, and 79% in low-grade lymphomas ($p < .0001$). *H. pylori* infection was detected in 74% of MALT lymphomas confined to the mucosa or submucosa (EI1), but in only 44% of those beyond submucosa ($p < .0001$).
Conclusions. The *H. pylori* prevalence in patients with MALT lymphoma is variable, which seems to depend, at least partly, on the number and the type of diagnostic methods used to detect the infection, on the histologic grade and on the stage of tumoral invasion. If the adequate diagnostic methods are performed, and if only low-grade MALT lymphomas are considered, the *H. pylori* prevalence is remarkably high, nearly 90%, which reinforces the role of this microorganism in the pathogenesis of gastric MALT lymphoma.

Abstract no.: 07.13
Meta-analyses on the Relationships between *Helicobacter pylori* Infection and Extra-gastric GI Cancers

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Background. *Helicobacter pylori* is an important causative factor in gastric carcinogenesis. However, its role in extra-gastric gastrointestinal (GI) malignancies, such as esophageal and colonic cancer, is controversial. The main aim of this study was to explore the relationships between *H. pylori* infection and these malignancies by meta-analyzing all relevant cohort and case-control studies. Secondary aims were to investigate the possible sources of heterogeneity between studies and to look for the existence of publication bias.
Methods. Extensive Medline English language medical literature searches for human studies were performed through May 2006, using suitable keywords. Pooled estimates were obtained using fixed or random-effects models as appropriate. Heterogeneity between studies was evaluated with the Cochran Q test, whereas the likelihood of publication bias was assessed by constructing funnel plots. Their symmetry was estimated by the Begg and Mazumdar adjusted rank correlation test and by the Egger's regression test.
Results. For colon cancer the pooled odds ratio (OR) with 95% confidence intervals (CI) were 1.44 (1.02–2.02), test for overall effect $Z = 2$, $p = 0.038$. The heterogeneity Q value was 10.5, $I^2 = 52.3$, $p = .062$. There was no publication bias (Begg and Mazumdar adjusted rank correlation test p two-tailed value 0.7, Egger's regression test p value .09). For esophageal cancer the pooled OR with 95% CI were 0.54 (0.41–0.72), test for overall effect $Z = -4.17$, $p < .0001$. The heterogeneity Q value was 10.93, $I^2 = 36$, $p = .14$. There was no publication bias (Begg and Mazumdar adjusted rank correlation test p two tailed value 0.27, Egger's regression test $p = .19$).
Conclusion. The results of our study showed a small statistically significant relationship between *H. pylori* infection and colon cancer. On the contrary, there was an inverse statistically significant relationship between *H. pylori* infection and esophageal cancer, suggesting that *H. pylori* infection has no etiological role in this malignancy.

Abstract no.: 07.14
Oxidative DNA Damage and the Efficiency of DNA Repair in Patients Infected by *Helicobacter pylori*

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The aim of this study was to the evaluate the relationship among oxidative DNA damage, efficiency of DNA repair, amount of *Helicobacter pylori*, and the relevance of *cagA, vacA,* and *iceA* genotype of *H. pylori* gastric epithelial cells and lymphocytes from 24 noninfected patients, 41 *H. pylori*-infected patients with gastritis, and 61 with gastric cancer. Oxidative DNA damage and the efficiency of DNA repair were analyzed by the Comet assay and real-time polymerase chain reaction (PCR). Amount of *H. pylori* was measured by quantitative real-time PCR. Oxidative DNA damage was significantly higher in *H. pylori*-infected patients with gastritis and cancer than in noninfected patients. Infected patients by *H. pylori cagA*$^+$, *vacAs1m1*, and *iceA1* genotype showed

higher levels of oxidative DNA damage than infected patients with *H. pylori cagA*$^-$, *vacAs2m2*, and *iceA2* genotypes and noninfected patients. The efficiency of DNA repair of gastric epithelial cells from infected patients with moderate or severe gastritis was lower than non-infected patients. Our results indicate that *H. pylori* infection, *H. pylori cagA*$^+$, *vacAs1m1*, and *iceA1* genotype is associated with oxidative DNA damage and inhibition of DNA repair. Together, such factors can favor gastric carcinogenesis.

Abstract no.: 07.15
Gastritis is Important for Gastric Carcinogenesis Induced by *Helicobacter pylori* Infection

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Aim. *Helicobacter pylori* is recognized as a definite carcinogen of gastric cancer. Host factor and/or bacterial factor modify gastric carcinogenesis. We investigate the role of bacterial factor for gastric carcinogenesis using the Mongolian gerbil model. In addition, the importance of gastritis for carcinogenesis is evaluated in this model.

Materials and methods. Six-week-old male specific-pathogen-free (SPF) Mongolian gerbils (n = 78) were used. *H. pylori* TN2GF4 and SS1 were selected as a pathogenic bacterium and an attenuated bacterium in Mongolian gerbils, respectively. TN2GF4 has *cagPAI* and *vac*A s1/m1 phenotype. SS1 has *cagPAI* and *vac*A s2/m2 phenotype. The *cagPAI* of SS1 was not working. N-methyl-N-nitrosourea (MNU) 30 ppm was provided as the drinking water on alternate weeks for 10 weeks (total exposure: 5 weeks). Fifty-six gerbils were inoculated with *H. pylori* (TN2GF4 or SS1) 1 week after the completion of methylnitrosourea (MNU) treatment. Gerbils divided into three groups, MNU+TN2GF4 (n = 26), MNU+SS1 (n = 26), and MNU alone (n = 26). All animals were sacrificed at week 50. In addition, differences of gastritis induced by TN2GF4 and SS1 were examined.

Results. Microscopically, 47.8% (11/23), 26% (7/26) and 0% (0/26) animals have gastric adenocarcinoma in the MNU+TN2GF4 group, the MNU+SS1 group, and the MNU alone group, respectively. Severe severity of gastritis was observed in TN2GF4 infectious group as compared with SS1 group.

Conclusion. *H. pylori* promoted susceptibility of chemical gastric carcinogenesis in Mongolian gerbil. Gastritis is important for gastric carcinogenesis. The fact that the MNU+SS1 group had gastric adenocarcinoma suggested not only *CagPAI* and VacA but also other factors modified gastric carcinogenesis.

Abstract no.: 07.16
Interleukin 8-251T/A Polymorphism is Associated with Risk for Gastric Carcinoma Development in Populations of Asian Origin but not of Caucasian Origin

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It has been demonstrated that polymorphisms within inflammation-related genes are associated with risk of gastric carcinoma (GC) in *Helicobacter pylori*-infected individuals. Recently, several studies have reported conflicting results regarding the association between the interleukin (IL)8-251T/A polymorphism and risk of GC. In the present study, we performed a case-control analysis, including 693 controls, 187 chronic gastritis cases, and 333 GC cases in order to determine the association between the IL8-251 polymorphism and risk of chronic gastritis and GC. We found no significant association between the IL8-251 polymorphism and increased risk of chronic gastritis or GC. The estimated effect of the polymorphism under analysis was not significantly different in subgroups of GC cases defined by histologic type and anatomic site of the tumors and by sex of the subjects. The retrospective analysis of published data shows that the association between the IL8-251 polymorphisms and risk of GC is reproducible in populations of Asian origin but cannot be demonstrated in populations of Caucasian origin. In conclusion our results indicate that although the IL8-251 polymorphism might be a relevant host susceptibility factor for GC development, this association is likely to be ethnic specific.

Abstract no.: 07.17
CagA and VacA *Helicobacter pylori* Antibodies in Gastric Cancer

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Background. Infection with different genotypes of virulent *Helicobacter pylori* strain, CagA and/or VacA positive, can play a role in the development of atrophic gastritis, duodenal ulcer (DU), and gastric carcinomas (GC). This study was undertaken to investigate whether patients with GC with *H. pylori*-negative histologic Giemsa staining had a past infection by virulent strains of *H. pylori* CagA and/or VacA positive.

Methods. Twenty-three GC, (mean age ± SD) 68.14 ± 9.8 years old *H. pylori* negative to histology took part in the study. Two control groups were included: 19 *H. pylori*-infected patients with DU eradicated 10 years before, 58 ± 18.2 years. *H. pylori*-negative

status was determined every year with histology and follow-up after therapy at 120 ± 32 months; range 96–144 months. Twenty asymptomatic children, 7 ± 4.47 years, with *H. pylori*-negative fecal test. The immunoblot assay was used to detect serum antibodies against CagA and VacA.

Results. Prevalence of CagA and VacA seropositivity was 82.6% and 73.91% in GC, 84% and 84% in DU *H. pylori* negative, 25% and 5% in *H. pylori*-negative children. CagA and VacA antibody positivity was not significantly different between GC and patients with DU eradicated 10 years before. A true significant positivity was found against children (χ^2 test; $p < .0001$). Statistical difference was found in age between groups $p < .03$.

Conclusion. Patients with GC, although *H. pylori* negative at present, could be infected by *H. pylori* before the appearance of the disease as confirmed by CagA and VacA seropositivity. These data reinforce the idea that *H. pylori* may be a direct carcinogenetic agent of GC.

Abstract no.: 07.18
E-cadherin-dependent Activation of EGFR is Responsible for Enhanced Cell Motility and Invasion in Gastric Cancer

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Helicobacter pylori infection is the main risk factor for gastric carcinoma development. In the diffuse subtype, E-cadherin mutations represent the most common genetic alteration and are associated with invasion and metastasis during cancer progression. Nevertheless, the underlying molecular mechanism remains poorly understood. Because epidermal growth factor receptor (EGFR)-mediated signaling plays a major role in cell migration and invasion, and increasing evidence supports the existence of a bidirectional cross-talk between E-cadherin and EGFR, we challenged the hypothesis that E-cadherin deregulation interferes with EGFR activity, thus increasing cell ability to migrate and invade.

To test this hypothesis, we used cells stably expressing either wild-type E-cadherin or its hereditary diffuse gastric cancer-associated germline missense mutations *T340A*, *A634V*, *P799R*, and *V832M* as in vitro model. We previously showed that the four mutations impair in vitro the E-cadherin ability to mediate cell to cell adhesion and suppress cell invasion. We performed co-immunoprecipitation and kinase analysis, as well as wound healing and matrigel invasion assays.

Our results support the idea that EGFR interacts independently with both E-cadherin and β-catenin. In particular, whereas the EGFR/β-catenin complex seems to involve the membrane-unbound β-catenin fraction, for the EGFR/E-cadherin interaction an intact E-cadherin extracellular domain appears to be required. Upon interaction with EGFR, E-cadherin exerts an inhibitory function modulating the kinase activity of the receptor in an adhesion-independent manner. E-cadherin-dependent activation of EGFR is responsible for enhanced cell motility and invasion, in a mechanism involving decreased focal adhesion kinase phosphorylation and RhoA activation.

Abstract no.: 07.19
Random Mucosectomy Role to Maintain Patients Free from Gastric Noninvasive Neoplasia (Category 3 of Vienna Classification)

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Introduction. Low-grade dysplasia (LGD) is a preneoplastic lesion that may progress. There is no consensus on which is the best treatment.

Methods. From February 2001 to March 2006, 26 *Helicobacter pylori*-negative patients (16 men, 10 women, mean age 66 years ± SD) previously diagnosed as LGD at standard biopsies underwent antral endoscopic mucosal resection (EMR) by "cup and suction technique." All patients underwent endoscopic surveillance by standard biopsies every 6 to 12 months. In case of persistent or relapsing LGD, a second EMR was performed.

Results. Thirty-five EMRs were performed on 26 patients. Seventeen patients (65%) were LGD concordant at the first EMR. Fourteen patients followed up for 33 months had a mean of three (2–6) control endoscopies with standard biopsies. In these, seven patients were LGD negative (group A) and seven were LGD positive (group B) at the first EMR (Table 1). Nevertheless, at control endoscopies, a second mucosectomy was performed in 3/7 pts (group A) and in 2/7 (group B) for recurrent dysplasia in the biopsies. At the last follow-up, 100% of patients in group A and 57% in Group B were LGD negative. At 33 months of follow-up, 11/14 (79%) patients were free of dysplasia.

Conclusion. A close follow-up and one or more ERMs seems effective to maintain patients free of dysplasia in *H. pylori*-negative patients.

Table 1 Displasia-negative patients after mucosectomy

	No. patients	II° Mucosectomy	Dysplasia-negative at 33 months
Group A: LGD negative at first mucosectomy	7	3	7
Group B: LGD positive at first mucosectomy	7	2	4
Total	14	5	11

Abstract no.: 07.20
A Model to Infer the Pathogenic Significance of CDH1 Germline Missense Variants

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The majority of gastric carcinomas appear in a sporadic setting and are associated to *Helicobacter pylori* infection. Nevertheless, in about 10% of cases, a clear familial clustering is observed. In this regard, germline mutations of the E-cadherin gene (*CDH1*) are the

genetic cause of hereditary diffuse gastric cancer (HDGC). Total prophylactic gastrectomy is the only option offered to mutation carriers in genetic counseling. In this regard, *CDH1* germline mutations of the missense type represent a clinical burden, as their pathogenic relevance is not straightforward. In the present study, we have outlined a multivariate approach to infer the significance of such variants.

We reviewed all HDGC-associated E-cadherin germline missense mutations reported to date. Information collected included: cosegregation of the mutation within pedigrees, frequency in healthy population control, recurrence in independent families, and functional in vitro and in silico data. We used the neighbor-joining method to group mutations according to the collected information and assessed the robustness of mutation clusters with a bootstrap test.

Nineteen *CDH1* germline missense variants were classified according to the parameters defined in the multivariate analysis. This analysis allowed the distribution of the variants into two distinct groups: neutral variants versus mutations. The model here described provides an important tool that can ultimately improve the genetic counseling offered to the carriers of the germline CDH1 missense variants.

Abstract no.: 07.21
Gastric Cancer Mortality in Hungary – Cluster analysis in Periods 1986–1993 and 1994–2004

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Introduction. Causative environmental factors are more likely to occur in the developing countries where people continue to live in a relatively static environment and pursue their traditional lifestyle and habits.

Patients and methods. Spatial differences in mortality from gastric cancer (ICD-10:C16) in both sexes in the age groups of 35–64 years and over 65 years was studied by cluster analysis using the spatial scan statistic (SCAN) method based on the generalised additive models (GAM)-K method. The association of mortality and 28 socioeconomic factors from the 2001 census on settlement level as well as the type of soil (upper layer) was studied by spatial logistic regression method.

Primary care physicians in six counties in Hungary used rapid serological test (Signify *Helicobacter pylori* Test-Biotech Inc.) during the diagnostic procedure of 898 patients (mean age: 50.22 y, 18–80 years) with recurrent dyspeptic symptoms.

Results. Clusters of gastric cancer mortality accumulated in West Transdanubia, northern Hungary and the northeastern part of the country. Agricultural workers significantly correlated with higher mortality. Gastric cancer mortality was lower in settlements with piped drinking water and sanitation. On the contrary, mortality was higher in settlements with soil of good or high water retention compared to sandy soil.

H. pylori positivity rate was 56.57% in the examined population. Lowest prevalence was found in Bács-Kiskun county (44%) with sandy soil and the highest in Hajdú-Bihar (67%) with clay and loamy type soil. The difference was significant ($p = .000$), as was the difference between the prevalence of Bács-Kiskun and Somogy, resp. Heves county, both characterised by clayey soil.

Conclusions. Statistical analysis showed that gastric cancer mortality is associated with social status, agricultural type of work, with sanitation, and type of soil.

Abstract no.: 07.22
The Association between Interleukin-1ß Polymorphisms and Metastatic Spread in *Helicobacter pylori*-Positive Gastric Cancer Patients

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Background. The association between interleukin 1 (IL-1) polymorphisms, *Helicobacter pylori* infection and gastric cancer progression remains to be defined.

Aim. To compare the prevalence of IL-1 polymorphisms in gastric cancer individuals with different tumor stage.

Material and methods. One hundred twenty-one gastric cancer patients, all *H. pylori* infected were included in the study. Genomic DNA was isolated from blood samples and typed for polymorphisms at position −511, −31 in the interleukin-1β gene (IL-1β) using primer extension and mass spectrometry. Analysis of the variable number of tandem repeats in intron 2, in its receptor antagonist gene (IL-1RN) was performed by polymerase chain reaction and agarose gel electrophoresis. For the main statistical analysis, χ^2 test was used. Additionally, Hardy–Weinberg equilibrium of alleles at individual loci was assessed by Fisher's exact testing using the program GENEPOP. Linkage disequilibrium coefficients D′ = D/Dmax and χ^2 were calculated for all genotypes.

Results. All patients were successfully genotyped for three gene loci. IL-1β-511 was found to be in reverse linkage disequilibrium with IL-1β-31. The differences between gastric cancer patients with divergent tumor stage concerned only heterozygous variant of IL-1β and were related to metastatic status. Thus, CT genotype was significantly more prevalent in gastric cancer patients with no metastases than in those with lymph node involvement or/and distal metastases (61.9% versus 34.3%, $p = .008$). No differences were noted between patients with early and advanced tumors.

Conclusion. Our data support the view that IL-1β may be involved in gastric cancer progression. The findings may suggest potential prognostic significance of IL-1β assessment in gastric cancer patients.

Esophageal and Extradigestive Diseases

Abstract no.: 08.01
Helicobacter pylori Eradication and Serum Level of Homocysteine and Folic Acid – 1 Year Intervention Study

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Background. The relationship between *Helicobacter pylori* infection and heart diseases is a field of interest of many studies but the responsible mechanism is still not clear.
Aim. To evaluate if *H. pylori* eradication changes homocysteine serum level over 12 months observation time. In addition, serum vitamin B12, folic acid, and CRP levels were measured. The role of the grade of gastritis was also considered.
Material and methods. Seventy-three patients with dyspeptic symptoms, normal upper endoscopy results, and successful result of *H. pylori* eradication treatment were included. Serum homocysteine, folic acid, vitamin B12, and CRP levels were measured at the start, 6, and 12 m after eradication.
Results. The changes of homocysteine and folic acid levels after *H. pylori* eradication were dependent to the results of histological examination of the gastric mucosa. In patients with the highest improvement of inflammation and inflammatory activity, the highest lowering of homocysteine and highest increasing of folic acid level were found. In patients with atrophy, the differences were not statistically significant (Table 1).
Conclusions. In the group of patients studied, decreasing serum level of homocysteine and increasing level of folic acid were found after *H. pylori* eradication.

Table 1

	0 m	6 m	12 m
Homocysteine (mmol/L)	11.66 ± 4.97	10.38 ± 4.51	10.41 ± 4.16*
Folic acid (ng/mL)	10.56 ± 3.84	12.40 ± 5.40*	13.20 ± 4.99*
Vitamin B12 (pg/mL)	466.7 ± 194.2	485.2 ± 186.3	477.9 ± 170.7
CRP (mg/L)	1.87 ± 1.59	1.86 ± 1.68	1.63 ± 1.54

*$p < .05$ (in comparison to the entry).

Abstract no.: 08.02
Helicobacter pylori Infection and Density of Gastric Ghrelin-producing Cells in Obese and Nonobese Patients

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Ghrelin is a peptide that affects appetite and weight control. It is produced mainly in the gastric mucosa. *Helicobacter pylori* eradication is reported to increase the number of gastric ghrelin-producing cells (GhrCell) in subjects with normal body mass index (BMI) subjects but this effect still have not been studied in obese patients. The present study was designed to investigate the influence of *H. pylori* on the number of GhrCell in the oxyntic and antral mucosa of obese (Ob) and dyspeptic, nonobese patients. Ob patients, 48 subjects, BMI > 40, 79.2% female, mean age, 35.4 years; and nonobese patients, 49 subjects, BMI < 30, 65.3% female, mean age 39.8 years. Tissue specimens from the gastric mucosa were sectioned for histology (H&E and Giemsa), and for immunoperoxidase staining using polyclonal antibodies against human ghrelin (Phoenix, USA). GhrCell density in oxyntic mucosa of Ob and nonobese patients was similar, respectively 102.6 versus 91.4 cells/mm^2 ($p = .11$), whereas in the antral mucosa of Ob patients, there was a higher density of GhrCell in comparison with nonobese patients, 47.0 versus 14.2 cells/mm^2 ($p = .001$).The density of GhrCell in the oxyntic mucosa of *H. Pylori*-negative patients was higher than in *H. Pylori*-positive patients in both Ob (108.5 versus 87.1 cells/mm^2; $p < .026$), and nonobese patients (117.0 versus 75.3 cells/mm^2; $p < .0007$). Antral GhrCell was not affected by *H. pylori* infection in Ob patients. The results of the present study indicate that *H. pylori* infection may be considered a factor that affects the number of ghrelin-producing cells of the stomach in either obese and nonobese patients.

Abstract no.: 08.03
Role of CagA-Positive Strains of *Helicobacter pylori* in Patients with Stable and Unstable Angina Pectoris

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Introduction. Previous studies have reported that CagA-positive strains of *Helicobacter pylori* are prevalent in patients with ischemic heart disease. The aim of this study was to assess the prevalence of CagA-positive strains and the antibody titer in patients with stable (SA) and unstable (UA) angina pectoris and to verify whether anti-CagA antibodies cross-react with antigens of coronary atherosclerotic plaques.
Methods. Thirty-eight (30 men, 64 ± 11 years) patients with UA, 25 patients with SA (21 men, 62 ± 10 years), and 50 healthy volunteers (38 men, 62 ± 10 years) were enrolled. Prevalence of *H. pylori* infection and CagA-positive strains and the antibody titers were evaluated through enzyme-linked immunosorbent assay. Fresh fragments of coronary atherosclerotic plaques were obtained from all patients through directional coronary atherectomy, and prepared for immunohistochemistry using monoclonal antibodies anti-CagA.
Results. Prevalence of either *H. pylori* infection or CagA-positive strains was significantly higher in patients with SA and UA compared to controls (60% in SA, 61% in UA versus 38% of controls, $p < .04$ and 44% in SA, 50% in UA and 18% in controls,

$p < .001$, respectively). Moreover, the titer of anti-CagA antibodies was significantly higher in UA patients than SA (161 ± 120 RU/mL versus 78.7 ± 63.1 RU/mL; $p < .03$). Interestingly, anti-CagA antibodies recognized antigens localized in the cytoplasm of fibroblasts and lymphocytes inside atherosclerotic plaques.
Conclusions. Anti-CagA antibody titer is significantly higher in UA patients than SA. Interestingly, anti-CagA antibodies specifically recognize antigens localized inside coronary atherosclerotic plaques of the same patients. The binding of anti-CagA antibodies to those antigens could influence the destabilization of coronary atherosclerotic plaques.

Abstract no.: 08.04
Immobilization of *Helicobacter pylori* Cells on the Carbonic Sorbent Surface for Increase of Effectiveness of Urea Elimination from Blood Plasma

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The purpose of the work is the immobilization of *Helicobacter pylori* cells on the carbonic sorbent surface for increase of effectiveness of urea elimination from blood plasma.

For immobilization, the suspension of microbial cells of *H. pylori* clinical cultures in saline solution was mixed with carbonic hemosorbent (SKN), and was shaken for 6 hours at 25 °C. The unabsorbed cells were removed by multiple repeated washes with distilled water and 0.06 mol/L solution of the phosphate buffer at pH 6.8. The modified sorbent effectiveness was detected by addition of 10 mL of the urea solution (20 mmol/L) to 1 g of the obtained preparation and then this blend was shaken at 25 °C for 2 hours. For a control experiment we used the blend in the same ratio of the initial sorbent with the urea solution.

The main process of the urea elimination from the solution occurred during the first 30 minutes. Using *H. pylori* in 1 minute, the urea concentration decreased up to 88.7% of the initial and up to 73.8% in 30 minutes ($p < .001$). In the control experiment, urea concentration in 1 minute decreased to 95.9% of the initial one, in 30 minutes – 96.8%. The similar results were also obtained in model experiments with blood samples.

Thus, the modification of the carbonic sorbents by the immobilization of *H. pylori* cells essentially increases their effectiveness on the urea elimination that in the future could be applied at treatment of the patients with uremia.

Abstract no.: 08.05
Association of *Helicobacter pylori* and Chronic Idiopathic Urticaria

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Background. *Helicobacter pylori* chronic colonization is associated with many extragastric diseases.
Goal. To demonstrate that *H. pylori* is associated with the dermatological disease chronic idiopathic urticaria (CIU).
Methods. We studied 60 patients with CIU diagnosis at baseline and 6 weeks after therapeutic intervention to eradicate *H. pylori* infection established by UBT-C14 and we performed enzyme-linked immunosorbent assays to determinate the antibodies' response to CagA and *H. pylori* whole-cell (WC) antigens.
Results. Forty-four patients were UBT positive and 16 were UBT negative; no statistically significant differences were founded in the mean age, gender, duration of disease, and symptoms between both groups. No correlation between *H. pylori* density [UBT disintegrations per minute (DPM) mean value] and symptoms severity was found. Serology correlated in 88.6% of UBT-positive patients and five (11.4%) of them were false-negative for WC enzyme-linked immunosorbent assay (ELISA) but four (80%) of those patients were positive for CagA ELISA. In contrast, only five (31.3%) of UBT-negative patients shows concordance in the serology (WC & CagA negative); with four (25%) false-positive for WC and CagA serology and nine (43.7%) were false-positive for CagA. A decrease in WC optical density (OD) net values was find in the UBT-positive patients with clinical improvement after *H. pylori* eradication (100%) compared with UBT-negative patients ($p < .05$), but not for CagA OD net values. Severity of symptoms showed a correlation with increasing OD values of WC and CagA in the UBT-negative group ($p > .05$).
Conclusion. Eradication of *H. pylori* correlated with clinical improvement, but not with the severity of CIU.

Abstract no.: 08.06
Helicobacter pylori Eradication: Relation with Blood Lipids and Fibrinogen in Patients with Functional Dyspepsia

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Background. In patients with atherosclerosis-related cardiovascular diseases, higher incidence of *Helicobacter pylori* infection was confirmed. The mechanisms of relationship between *H. pylori* and higher risk of ischemic heart disease are discussed.
Aim. The aim was to evaluate the effect of *H. pylori* eradication on serum level of triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol and fibrinogen in a group of patients with functional dyspepsia.

Material and methods. Forty-nine patients with dyspeptic symptoms, normal result of upper endoscopy, and presence of *H. pylori* infection were included. Eradication therapy was implemented. In 4–6 weeks after treatment ^{13}C-urea breath test was performed. Serum level of triglycerides, total cholesterol, LDL and HDL cholesterol, and fibrinogen were measured three times.
Results. Eradication was successful in 42 patients. No statistically significant changes in serum level of evaluated parameters before and after treatment were found. Mean triglycerides level before treatment is 1.39 ± 0.76 mmol/L, 1.52 ± 1.26 mmol/L 1 month after treatment, 1.51 ± 0.98 mmol/L 3 months after treatment; total cholesterol level before treatment is 5.34 ± 1.08 mmol/L, 5.42 ± 1.07 mmol/L 1 month after treatment, 5.59 ± 0.87 mmol/L 3 months after treatment; HDL cholesterol before treatment is 1.26 ± 0.31 mmol/L, 1.29 ± 0.34 1 month after treatment, 1.32 ± 0.31 mmol/L 3 months after treatment; LDL cholesterol before treatment is 3.44 ± 1.10 mmol/L, 3.29 ± 1.22 mmol/L 1 month after treatment, 3.67 ± 0.71 mmol/L 3 months after treatment; fibrinogen before treatment is 2.86 ± 0.88 g/L, 2.95 ± 0.74 mmol/L 1 month after treatment, 3.17 ± 0.72 mmol/L 3 months after treatment.
Conclusion. *H. pylori* eradication did not change serum level of triglycerides, total cholesterol, LDL and HDL cholesterol, and fibrinogen during 3 months observation in the group of patients with functional dyspepsia.

Abstract no.: 08.07
Clinical Spectrum and Risk Factors of Erosive and Nonerosive GERD in Health Check-up Subjects

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Background. This study was prospectively performed to evaluate the clinical spectrum and the risk factors of gastroesophageal reflux disease (GERD) in health check-up subjects.
Methods. A prospective survey was performed for 752 subjects, aged 18–79 years, who visited health promotion centers. The subjects were asked to complete questionnaire, and the risk of GERD was calculated by logistic regression regarding several variables including smoking, alcohol, exercise, body mass index, fasting glucose, cholesterol, triglyceride, and anti-*Helicobacter pylori* immunoglobulin G (IgG). Nonerosive reflux disease (NERD) was defined as the presence of heartburn and/or acid regurgitation at least once per week.
Results. Seven hundred fifty-two subjects were classified into three groups: 65 erosive reflux disease (ERD) subjects (8.6%), NERD 66 (8.8%), and control group 621 (82.6%). In the 65 ERD subjects, typical reflux symptoms were found in 19 (29.2%), less frequent reflux or atypical symptoms in 38 (58.5%), and no symptoms in 8 (12.3%). They showed Los Angeles Grade A Reflux Oesophagitis in 48 (73.8%), B in 11 (17.0%), C in 6 (9.2%). The positive rate of *H. pylori* IgG in the ERD was 36.4%, significantly lower than those in the NERD (60%) and in the control group (65.3%), resulting in the odds ratio of ERD in the absence of *H. pylori* infection, 5.079 (95% CI 1.907–13.530).
Conclusions. The prevalence rate of GERD was 17.4%. There was no correlation between the grade of ERD and severity of reflux symptoms. The relative risk of GERD in Korea was significantly low in *H. pylori* IgG-positive subjects.

Abstract no.: 08.08
Influence on *Helicobacter pylori* Infection on Reflex Esophagitis in Japan

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Background. *H. pylori* infection has also been negatively associated with reflex esophagitis (RE). We examined the *Helicobacter pylori* infection rate and correlation of *H. pylori* infection with RE.
Method. The subjects were 418 patients who received upper gastrointestinal endoscopy (UGIE) and had their serum IgG *H. pylori* antibody examined.
Result. Total *H. pylori* infection rate was 33.5% (140/418). The percentage of RE among those *H. pylori*-negative subjects was 23.4% (19.0% in age 20–29, 24.0% in age 30–39, 24.3% in age 40–49, and 29.4% in age 50–59), which was significantly higher than the percentage (12.1%) of RE among *H. pylori*-positive subjects (0% in age 20–29, 16.7% in age 30–39, 12.2% in age 40–49, and 10.5% in age 50–59). The percentages of LA-A, B, C, D grades among *H. pylori*-negative subjects were 74, 22, 4, and 0%, respectively (91, 9, 0, and 0% in age 20–29; 50, 42, 8, and 0% in age 30–39; 92, 4, 4, and 0% in age 40–49; and 80, 20, 0, and 0% in age 50–59). On the other hand, the percentages of LA-A, B, C, D grades among *H. pylori*-positive subjects were 59, 29, 12, and 0%, respectively (0, 0, 0, and 0% in age 20–29; 71, 29, 0, and 0% in age 30–39; 50, 33, 17, and 0% in age 40–49; and 50, 25, 25, and 0% in age 50–59).
Conclusio. In this study, increase in RE was recognized with *H. pylori*-negative patients. On the other hand, it is possible that *H. pylori* infection influences advance of severity of RE.

Abstract no.: 08.09
Helicobacter pylori Infection as A Cause of Acute Pancreatitis: Fact or Fiction?

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Aim. To evaluate the evidence of the pathogenic role of *Helicobacter pylori* in patients with acute pancreatitis (AP).
Methods. Sixty patients (58 men/12 women) mean age 48.5 years with AP admitted to an intensive care unit (ICU). The patients were divided into two groups. Group A: 30 patients with *H. pylori* infection and group B: 30 patients without *H. pylori* infection. Both groups of patients were comparable in age, sex, body mass index, Ransons criteria, elevated serum C-reactive protein, etiology, and levels of amilasemia and amylasuria. All patients received upper gastroduodenal endoscopy and gastroduodenal pathology was identified. *H. pylori* infection was confirmed by

gastric histology. All patients had also computed tomography (CT) scan within 72 hours of onset. For each group, the incidence of pancreatic sepsis, duration of stay in the ICU, need for surgical intervention, and mortality rate were recorded.

Results. The incidence of pancreatic sepsis was much less in patients of group B, 5 of 30 (16%) versus 11 of 30 patients (36%) in group A ($p < .001$).The duration of stay in ICU in group B was 7.2 days versus 13.4 in group A ($p < .001$). No significant difference was found in surgical intervention between two studied groups [in group A, 3 patients of 30, in group B, 2 patients of 30 ($p < .08$)]. A difference in mortality rates between two groups has not yet been demonstrated.

Conclusion. Our results demonstrate evidence of an injurious effect of *H. pylori* infection of the stomach in the course of AP. Patients with *H. pylori* infection showed higher risk of developing a pancreatic sepsis, and prolonged stay in the ICU. With further study, our understanding of the relationship between the pancreas and *H. pylori* will improve, undoubtedly clarifying the real role of *H. pylori* infection in pancreatic diseases.

Abstract no.: 08.10
Helicobacter pylori Infection and Iron Deficiency Anemia (IDA) in Nigeria

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Background. *Helicobacter pylori* has recently been implicated in iron deficiency anemia (IDA) when hookworm infestation and other causes of anemia are ruled out. In Nigeria, this study has not been undertaken before.

Aim. The study is therefore aimed at screening for the presence of *Helicobacter pylori* infection in patients with IDA.

Methods. Informed consent had been secured from patients before obtaining their blood screened for serum ferritin, hemoglobin, and occult blood tests, whereas their stools were screened for stool antigen tests for *H. pylori* and ova of parasites.

Results. Of 31 patients with gastritis, ten were men, whereas 21 were women. From our study, IDA was determined as hemoglobin concentration of < 14g/dL for men and < 12 g/dL for women. The ferritin concentration used according to manufacturer's instructions (Diagnostic Automation, Inc, CA, USA). Out of the ten men, two (20%) had anemia and had no evidence of parasite seen or occult blood. They had one high ferritin value and the other low ferritin. Out of 21 women, 7 (33%) had anemia, whereas out of the seven with anaemia, three (43%) had low serum ferritin levels. There was no occult blood or parasite seen. All were positive for *H. pylori*. However, 87% (27/31) of the patients screened in this study had *H. pylori* using HpSA kit.

Conclusion. Although not all the patients with *H. pylori* had IDA, the presence of *H. pylori* infection in patients with IDA should be looked at critically in Nigeria most especially as other causes of anemia were excluded in this pilot study.

Abstract no.: 08.11
Helicobacter in Idiopathic Parkinsonism: A Template for Intervention in the Role of Inflammation in Neuropsychiatric Disease

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Background. Following *Helicobacter* eradication, "malignant" idiopathic parkinsonism (IP) appears converted to "benign." Although a minority of probands are positive for urea breath test, the serum immunoblot *Helicobacter pylori* antibody profile is predictive of risk, presence, severity, and deterioration: low-density infection may be important. Lymphopenia in probands and spouses, with spouses being down the way to parkinsonism on measurement of multifarious facets, supports an infective etiology [1–3]. Biochemical calling cards of gastritis/atrophy consolidate the picture.

Methods and Results. We report follow-up to a mean of 440 days after deblinding (scheduled 1 year post-treatment) of the randomized, double-blind, placebo-controlled efficacy study of *H. pylori* eradication on time course of measures of IP in 30 recruits [taking no or stable, long-t($^1/_2$), anti-parkinsonian medication]. Those still infected following placebo, or failed blinded active treatment, received open active. Marked deterioration had followed failed blinded active (in two) or open active (further two). Despite this, intention-to-treat analysis showed a difference in time trend in the primary outcome criterion, mean stride length (7.5 (95% CI = 3.9, 11.2) cm/year, $p < .001$) in favor of blinded active over placebo. Those initially drawing placebo responded to open active ($p = .001$), and the improvement gained after blinded active did not fall off. Disappointing recovery from lymphopenia (4 (0.8) % per year) post-*Helicobacter* eradication favors another etiologic (perhaps virus driven) layer or autonomous autoimmunity. However, dependence on persistent cross-reactive antigen or inflammatory products, is favored by reversal of the deficit (23/124 cm) in stride length 12 weeks after open eradication therapy in a severely disabled, urea breath test/culture negative, but molecular-biology positive, untreated proband.

Conclusions. Pharmaceutical interventions in the pathogenesis of IP have hitherto been limited by adherence to a simple remote insult model.

References

1 Dobbs, R. J. et al. Role of Chronic Infection and Inflammation in the Gastrointestinal Tract in the Etiology and Pathogenesis of Idiopathic Parkinsonism. Part 1: Eradication of *Helicobacter* in the Cachexia of Idiopathic Parkinsonism. *Helicobacter* 2005, 10, 267–275.
2 Bjarnason, I. T. et al. Role of Chronic Infection and Inflammation in the Gastrointestinal Tract in the Etiology and Pathogenesis of Idiopathic Parkinsonism. Part 2: Response of Facets of Clinical Idiopathic Parkinsonism to *Helicobacter pylori* Eradication. A Randomized, Double-Blind, Placebo-Controlled Efficacy Study. *Helicobacter* 2005, 10, 276–287.
3 Weller, C. et al. Role of Chronic Infection and Inflammation in the Gastrointestinal Tract in the Etiology and Pathogenesis of Idiopathic Parkinsonism. Part 3: Predicted Probability and Gradients of Severity of Idiopathic Parkinsonism Based on *H. pylori* Antibody Profile. *Helicobacter* 2005, 10, 288–297.

Paediatric Issues

Abstract no.: 09.01
IL2-330 T/G Polymorphisms and Risk of *Helicobacter pylori* Infection and Associated Diseases in Children

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Helicobacter pylori infection is mainly acquired in childhood. Among the cytokines involved in the host response to the infection, IL-2, produced by activated T cells, has a powerful immune regulatory effect on a variety of immune cells. Thus, we evaluated a functional polymorphism in *IL2* (-330T/G) in the risk of *H. pylori* infection and of duodenal ulcer in children. We studied 393 children: 227 *H. pylori* negative and 166 *H. pylori* positive (57 with duodenal ulcer). Single nucleotide polymorphism (SNP) of *IL2*-330T/G was evaluated by polymerase chain reaction-confronting two-pair primers (CTPP) and the results were confirmed by sequencing. The data were analyzed in logistic models. The alleles did not deviate significantly from Hardy–Weinberg equilibrium in the control group. The models were well fitted. In the univariate analysis, the age, gender, *IL2*-330 T/G genotype, and the allele 2 of *IL1RN* and the allele 2 of *TNFA*-307 were selected. In the multivariate analysis, the age, gender, and the allele 2 of *IL1RN* remained associated with duodenal ulcer, but not the *IL2*-330 T/G genotype (p = .11, OR = 1.56, 95% CI = 0.91–2.66). Otherwise, *H. pylori*-negative children significantly differed from *H. pylori*-positive children without duodenal ulcer after adjusting for age and gender (p = .01, OR = 0.61, 95% CI = 0.31–0.87). Heterozygosity T/G was more frequently seen in the group of *H. pylori*-negative children similarly to that we observed in symptomatic and asymptomatic adults from our country. Thus, the *IL2*-330 T/G genotype seems to be a protective factor against the acquisition of *H. pylori* and against the persistence of the infection.

Grants: FAPEMIG and CNPq/Brazil.

Abstract no.: 09.02
Evaluation of A Novel Rapid One-Step Monoclonal Enzyme Immunoassay for Detection of *Helicobacter pylori* Antigen in Stool in Children

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Background. Monoclonal stool antigen enzyme immunoassay (EIA) (FemtoLab *Helicobacter pylori* Cnx) showed an excellent accuracy (98.7%) in diagnosing *H. pylori* in children.

Aim. To evaluate a new monoclonal rapid office-based test (RAPID *H. pylori* StAR, DakoCytomation, UK) for detection of *H. pylori* antigen in stool of children against invasive diagnostic methods and to compare it with a monoclonal EIA (Amplified IDEIA *H. pylori* StAR, DakoCytomation, UK).

Methods. Pretreatment: Stool from 118 symptomatic children (0.28–18.75 years) was frozen at time of endoscopies; 54 children were *H. pylori* infected defined by positive culture and/or two other tests (UBT, histology, rapid-urease test), 64 children showed negative results. Post-treatment: Thirty-four children (4.8–17.8 years) were monitored with UBT (five remained positive) 6–8 weeks post-therapy. Stool tests were performed on coded samples according to manufacturer's instruction. The immunoassays were independently read by two investigators.

Results. The EIA showed excellent test characteristics (Table 1). The rapid immunoassays showed poor sensitivity, but good specificity. The observers agreed in 38 positive and 93 negative results, were discordant in 12, whereas 9 times the tests were invalid.

Conclusions. The new office-based immunoassay for diagnosing *H. pylori* should be modified to improve sensitivity, inter-observer variability, and some technical problems. In contrast, the monoclonal EIA is highly reliable pre- and post therapy, and equivalent to the UBT.

Table 1.

		Sensitivity* [95%CI]		Specificity* [95%CI]		Accuracy†		Invalid tests	
		Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
Pretherapy (n = 118)	RAPID *H. pylori* StAR	**71.1%** [55.7–83.6]	**63.8%** [48.5–77.3]	**91.1%** [80.4–97.0]	**96.2%** [87.0–99.5]	**76.8%** (n = 109)	**79.3%** (n = 109)	**7.6%** (n = 9)	**7.6%** (n = 9)
(n = 105)	Amplified IDEIA *H. pylori* StAR	**98.0%** [89.1–99.9]		**100.0%** [93.6–100.0]		**99.1%** (n = 105)			
		Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
Post-therapy (n = 34)	Rapid *H. pylori* StAR	**20.0%** [0.5–71.6]	**0.0%** [0.0–60.2]	**100.0%** [88.1–100.0]	**100.0%** [88.1–100.0]	**77.0%** (n = 34)	**–** (n = 34)	**0.0%** (n = 0)	**0.0%** (n = 0)
(n = 30)	Amplified IDEIA *H. pylori* StAR	**100%** [39.8–100.0]		**96.2%** [80.4–99.9]		**94%** (n = 30)			

equivocal results: *excluded for calculation, †considered as false results.

Abstract no.: 09.03
Active Forms of Nitrogen and Oxygen in Children with Atrophic Gastritis (CAG)

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Introduction. Knowledge about the role of oxidants and nitric oxide (oxygen-nitric stress) in gastrointestinal disorders is still insufficient, particularly, in CAG in children.
Aim of the study. To evaluate the concentrations of nitric oxide (NO), malonyldialdehyde (MDA), and hydrogen peroxide (H_2O_2) as the exponents of active forms of nitrogen and oxygen in children with CAG.
Material and methods. The study comprised 44 children aged 8–18 years with CAG in groups: GI, 24 with CAG and *Helicobacter pylori* infection; GII, 10 with CAG without *H. pylori* infection; GIII, 6 with CAG, *H. pylori* infection, and food hypersensitivity; GIV, 4 with CAG and food hypersensitivity; and GV, 30 controls. Concentration levels of NO (Oxis test), MDA (Okhawa's method), and H_2O_2 (enzymatic-calorimetric method) in serum were performed before treatment (day 0) and 6 weeks after its cessation (day 50).
Results. High NO concentrations were stated before treatment compared to controls ($p < .0001$), which correlated with the degree of atrophy and *H. pylori* infection. All examined children had, before treatment, statistically significant high MDA concentrations compared to controls ($x = 24.3 \pm 4.64$ nmol/L). Four times higher concentration of H_2O_2 compared to GV before treatment was observed in children with *H. pylori* infection and food hypersensitivity ($x = 28.3 \pm 8.0$ mol/L). It was three times higher in children with *H. pylori* infection. MDA versus NO and H_2O_2 versus NO relations are statistically significant ($p < 0.0001$).
Conclusions. CAG involves biochemical mediators. *H. pylori* infection may initiate and maintain the inflammation. The studies on NO and MDA in children with gastritis are of great importance in the evaluation of intensity and activity of the inflammation.

Abstract no.: 09.04
The Effect of *Helicobacter pylori* Eradication Therapy on Gastric Myoelectrical Activity in Children with Functional Dyspepsia

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Introduction. *Helicobacter pylori*-induced inflammation of the gastric mucosa may affect gastric motility. *H. pylori* eradication can improve gastrointestinal motility and effect on dyspeptic symptoms in children.
Objective. The aim of this study was to evaluate the value of electrogastrography (EGG) in children with functional dyspepsia (FD) before and after *H. pylori* eradication therapy.
Methods. Thirty-six children, aged 7 to 18 years, with FD, and 16 healthy children were studied. FD children were divided into two groups, depending on the presence of *H. pylori* infection: I-24 children without *H. pylori* infection (FD-*H. pylori* negative), II-12 children with *H. pylori* infection (FD-*H. pylori* Positive). Gastric myoelectrical activity was measured using surface EGG. Based on Fourier transformation, the following parameters were analyzed: dominant frequency/power and percent of normal frequency. In FD-*H. pylori*-positive children, EGG was performed before and 4 weeks after the eradication.
Results. Basing on EGG parameters, preprandial bradygastria and dysrythmia were more frequent in FD-*H. pylori*-positive and in FD-*H. pylori*-negative children than in healthy controls ($p < .05$). In the FD-*H. pylori*-positive group, postprandial bradygastria and dysrythmia were also observed. In the FD-*H. pylori*-positive children 4 weeks after the eradication, significantly lower percentage of bradygastria and increase of normogastria both pre- and postprandial were noticed ($p < .05$).
Conclusions. In FD-*H. pylori*-positive children, significantly lower percentage of bradygastria and increase of normogastria both pre- and postprandial were observed after *H. pylori* eradication therapy. The normalization of gastric myoelectrical activity may be one explanation for the significant symptom improvement of the dyspepsia population after *H. pylori* eradication. The results should be confirmed by further investigations.

Abstract no.: 09.05
The Prevalence of Iron Deficiency Anemia and Iron Deficiency in The Group of Children with *Helicobacter pylori* Infection Depending on Somatic Development

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Introduction. *Helicobacter pylori* infection plays a role in pathogenesis of iron deficiency anemia and iron deficiency.
Aim. Prevalence of iron deficiency anemia (IDA) and iron deficiency (ID) in children with *H. pylori* infection, depending on somatic development, was analyzed.
Methods. A study group consisted of 99 children aged 7 to 18 years with *H. pylori* infection, treated in the clinic in 2004. All children were hospitalized as a result of recurrent abdominal pain and/or short stature. The control group consisted of 23 children without *H. pylori* infection with the same symptoms. *H. pylori* infection was diagnosed based on positive gastric mucosa culture. The children were divided into two groups depending on the somatic development. Group I consists of 48 children with proper somatic development and group II is composed of 48 children with short stature (height < 5th percentile). Frequency of ID and IDA in both groups was analyzed.
Results. Collectively in the group with *H. pylori* infection, ID was present in 24.2% of children, IDA in 11.2%; the difference was statistically significant as compared with controls. The remaining results are presented in Table 1.
Conclusions. In 24.2% of children with *H. pylori* infection, ID was observed, IDA in 11.2% of children was noticed. In the group with *H. pylori* infection and short stature, statistically significant higher prevalence of ID and IDA was observed as compared with children with proper somatic development.

Table 1.

	H. pylori positive (%)	*H. pylori* negative (%)	
	Proper development	Short stature	Control group
ID	18.7*	29.4*	8.7
IDA	6.25	15.6*	4.3

*Statistically significant.

Abstract no.: 09.06
Apoptosis in Children Infected with *Helicobacter pylori*

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Background. Apoptosis has been related to atrophy in patients infected with *Helicobacter pylori*. Most studies on apoptosis have been performed in adults.
Aims. To analyze apoptosis in children infected with *H. pylori* with duodenal ulcer and gastritis.
Patients and methods. We studied 54 children (age 10 ± 3.7 years, 44 *H. pylori* positive), 18 with duodenal ulcer (DU), and 26 with *H. pylori* gastritis. Immuno-expression (sABC) for anti-apoptotic (Bcl2) and pro-apoptotic protein (Bax) was scored (0 to 3) in gastric biopsies from the antrum. *H. pylori* status was determined by polymerase chain reaction.
Results. Bcl2 expression was weak or negative in 9 patients with DU, 24 with gastritis, and 5 noninfected patients; Bax expression was higher than Bcl2 in most cases ($p = .02$). Among *H. Pylori*-positive group, Bax expression was significantly higher in patients with gastritis compared to those with DU ($p = .006$). No significant difference was observed in Bcl2 expression in patients with gastritis compared to those with DU ($p = .8$), and in noninfected patients compared to DU ($p = .9$) and gastritis patients ($p = .1$).
Conclusion. Our results suggest that in children, apoptosis is predominantly related to chronic gastritis, which may have a role on atrophy development on adulthood in some of these patients.
Financial support. CNPq, Fapemig

Abstract no.: 09.07
Gastropanel: A Useful Screening Test in Pediatric Populations

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Aim. Ascertain the role of GastroPanel (GP) in screening children for gastric inflammation.
Patients. One hundred ninety-five children (101 girls, 94 boys; mean age 7 years) underwent esophagogastroduodenoscopy (EGDS) for chronic abdominal pain. At endoscopy, 105 had no evident lesions, 43 antral erythema, 15 diffuse gastritis, 4 duodenitis, 11 esophagitis, 10 micronodular gastritis, and 7 mosaic duodenal mucosa. *Helicobacter pylori* was histologically diagnosed in 17.6% of cases. In fasting sera pepsinogen A (PGA), pepsinogen C (PGC), gastrin-17 (G17), anti-*H. pylori* Ab were assayed and analyzed by the GastroSoft for Excel (Biohit®, Helsinki, Finland).
Results. One hundred eight of 195 children had a histologically normal mucosa, 68 had gastric inflammation, and 19 had extragastric lesions. PGC and anti-*H. pylori* correlated significantly with *H. pylori* infection ($t = 4.22$, $p < .001$ and $t = 5.28$, $p < .001$). GastroSoft provided five categories: normal, antral gastritis, antral atrophy, antral+body atrophy, and body atrophy. GP sensitivity in identifying children with gastric inflammation was 29.4%, the specificity toward extragastric diseases was 68.4%, whereas that toward controls was 95.4% (predictive positive value 83.9%; predictive negative value 70.7%). None of the single parameters discriminated gastric inflammation (under ROC curves areas: 0.60 for PGA, 0.63 for PGC, 0.57 for G-17, 0.72 for anti-*H. pylori*). Six of seven children with mosaic duodenal mucosa were biochemically classified as having corpus atrophy; two had autoimmune gastritis and four celiac disease.
Conclusion. GP might be a useful noninvasive test for screening gastric inflammation in children. This test seems able to indicate also in children the presence of corpus atrophy that has probably to be histologically searched, especially in subjects with celiac disease.

Abstract no.: 09.08
Symptomatic *Helicobacter pylori* Infection in Children – Clinical and Endoscopic Manifestations

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Introduction. The causal relationship between chronic abdominal pain and *Helicobacter pylori* in children remains unclear.
Aim. The purpose of this study was to assess the clinical and endoscopic findings in *H. pylori*-infected children.
Methods. This prospective study included 465 consecutive children (298 girls; age range 3–18 years) with abdominal complaints requiring endoscopic evaluation. Clinical symptoms and socioeconomic conditions were analyzed. *H. pylori* infection was evaluated by urease test and histologic examination. The diagnosis of gastritis was based on the updated Sydney system.
Results. Two hundred seventy of 465 children (58.06%; mean age 13.2 years) had documented *H. pylori* infection. The rate of *H. pylori* colonization was inversely correlated with the socio-economic status ($p < .005$) and directly with age ($p < .002$). Endoscopic nodular gastritis was observed in 73.33% infected children compared with 5.92% uninfected ($p < .001$). Peptic ulcer disease was detected only in infected children (7.03%). Endoscopic nodular gastritis was significantly associated with higher grades of gastritis severity, observed during histologic examination ($p < .001$). Symptoms characteristics have not differentiated infected from uninfected children, except for epigastric pain associated with nocturnal ($p < .001$) and fasting abdominal pain ($p < .001$), more frequent in infected patients.

Conclusions. This endoscopic series reveals a high rate of *H. pylori* infection (58.06%) and suggests that infected children can present abdominal pain with distinctive features. Epigastric pain with nocturnal awakening and fasting pain relieved by food may represent a warning alarm to screen for *H. pylori*. Endoscopic nodular gastritis was strongly associated with *H. pylori* infection (73.33%) and with higher grades of gastritis severity ($p < .001$).

Abstract no.: 09.09
Assessment of the Eradication Rate of *Helicobacter pylori* Infection in Children

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Introduction. *Helicobacter pylori* is acquired mostly in childhood. Its eradication may prevent serious gastrointestinal diseases.
Aim. This prospective study aims at assessing the efficacy of three first-line triple therapies in children, consisting in omeprazole (O) plus two of the following antibiotics: clarithromycin (C), amoxicillin (A), and metronidazole (M).
Methods. Two hundred seventy consecutive children (163 girls, range 3–18 years) were selected from 465 children (58.06%) with uninvestigated dyspepsia referred for gastroscopy. They were randomized in three groups to receive three first-line triple therapies for 7–10 days with omeprazole plus two antibiotics (OCM, OAC, OAM). *H. pylori* status was assessed before and 4 weeks after eradication treatment, by urease test and histopathology. In patients failing to be cured by a first treatment, a second alternative triple therapy was applied.
Results. Patients' baseline characteristics were similar in the three groups. All infected children had chronic gastritis, most of them having antral nodularity (73.33%). *H. pylori* was eradicated in 229 patients (84.81%), with no significant statistical differences related to the type of the triple therapy used, varying between 81.60% (OCM) to 88.06% (OAC). A second alternative triple therapy was applied in 41 children (15.19%) with persistent infection. Seven patients (2.59%) with resistant strains thereafter were retreated in combination with rifabutin or quinolone.
Conclusion. *H. pylori* infection (58.06%) remains a major problem in developing countries. The eradication rate after the first treatment was 84.81%, with the best results for the OAC protocol (88.06%). The OAM protocol (84.77%) is a good alternative for clarithromycin resistant strains.

Abstract no.: 09.10
How is *Helicobacter pylori* Infection Influenced by Immunosuppressive Therapy?

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Introduction. *Helicobacter pylori* infection is an important factor in gastric metaplasia/cancer and gastrointestinal lymphoma. Adhesion molecules and proliferation factors play a significant role in these disorders; however, there is no data of those under immunosuppressive therapy.
Aim. To investigate the prevalence of *H. pylori* and the expression of ICAM-1 and Ki-67 proliferation marker in children with *H. pylori* infection under immunosuppressive therapy.
Patients and Methods. ^{13}C-urea breath test was performed to screen *H. pylori* infection. Twenty-two children after kidney transplantation (NTX), (mean age, 16.1 years, 12 men) were enrolled in our study. ICAM-1 and Ki-67 expressions were determined by means of immunohistochemistry. To compare data of NTX+*H. pylori*, *H. pylori*-positive and *H. pylori*-negative controls were involved.
Results. Urea breath test was positive in 5/22 children (23%), indicating higher incidence for *H. pylori* infection than in controls (1/22). Two out of five *H. pylori*-positive patients had no gastrointestinal complaints; however, all biopsies showed chronic antral mucosal inflammation with the presence of *H. pylori*. We found significantly increased expression of ICAM-1 in children with NTX+*H. pylori* and *H. pylori* infection when compared with *H. pylori*-negative controls (median, 7.2, 7.3, and 3.2%, respectively). The highest crypt proliferation rate was found in NTX+*H. pylori* in comparison to both *H. pylori*-positive and –negative controls (median, 22.2, 17.1, and 5.3%, respectively).
Conclusion. We found higher incidence in *H. pylori* infection under immunosuppressive drugs following NTX, even in the absence of gastrointestinal symptoms. Increased crypt proliferation rate observed in NTX may indicate a risk for gastric metaplasia. Based on these, our findings indicate to screen for *H. pylori* in children after kidney transplantation.

Abstract no.: 09.11
Helicobacter pylori Infection in First Infancy in Algerian Population: About 65 Cases

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Helicobacter pylori infection, early acquired in infancy, is widespread in developing countries. Studies are lacking in Algeria.
Aim. To evaluate *H. pylori* prevalence infection among symptomatic children < 4 years and to establish clinical, endoscopic, and histological infection profile.
Methods. From 2002 to 2004, 65 children (boys: 39, mean age 30 months; 6 months to 4 years) were enrolled for recurrent abdominal pain, vomiting, chronic diarrhea, delay of growth, and anemia. Every patient had an upper gastrointestinal endoscopy with gastric biopsies for histology, rapid urease test (RUT), culture, and antibiotic microbial sensitivity (E-test), serology (IgG antibodies, enzyme-linked immunosorbent assay), and stool antigen test (HpSA). Diagnosis of *H. pylori* infection was assessed if culture and/or histology and an other test were positive.
Results. Infection was present in 42 patients (65%). The infection had no specific clinical or endoscopic feature. Nodular endoscopic aspect was noted in five cases; all these patients were *H. pylori* positive.
Histologicaly. Chronic gastritis, activity, follicles, atrophy (mild or moderate) were respectively observed in 100, 92, 55, and 47% of cases.

Conclusion. *H. pylori* prevalence is very high in infancy in Algeria. Nodular aspect is rare. Gastric atrophy unusually reported is very frequent.

Abstract no.: 09.12
Apoptosis in Antral Epithelial Cells in Chronic Gastritis with *Helicobacter pylori* Infection in Children – A Pilot Study

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Introduction. Gastric cancer is generally believed to develop from a multistep progression from chronic gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and subsequently to cancer. Apoptosis and proliferation play a vital role in physiology and pathophysiology of human gastrointestinal tract.
Aim of Study. The aim of study was to evaluate apoptotic activity of antral gastric epithelial cells in chronic gastritis in *Helicobacter pylori*-positive children.
Material and Method. The total number of 40 patients (aged 8–17 years old) underwent the assessment. In all cases, endoscopic examination was performed basing on a history of upper gastrointestinal tract complaints. Endoscopic examination, urease test, and pathology report were the baseline to dived studied population into three groups: (I) chronic gastritis with *H. pylori* infection, 20 children; (II) chronic gastritis and absent *H. pylori* infection, 10 children; (III) no inflammation of gastric mucosa and no present *H. pylori* infection, 10 children (no morphologic changes present either in endoscopy nor pathology report). Paraffin-embedded biopsy specimens were stained with hematoxylin and eosin and in accordance with Mallory`s method. Apoptosis was evaluated with the use of the terminal desoxynucleotidyl-transferase mediated dUTP nick-end labeling histochemistry method.
Results. The apoptic index (AI) in the studied groups were as follows: group I, 95–120 (mean 100.5); group II, 40–50 (mean 45); group III, 20–30 (mean 25).
Conclusion. The number of apoptotic positive cells in chronic gastritis is significantly higher in patients with *H. pylori* colonization compared to cases with no *H. pylori* infection.

Abstract no.: 09.13
Presence of *Helicobacter pylori* Genetic Material in the Gastric Mucosa of Children with *H. pylori* Infection Diagnosed with Conventional Methods

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Helicobacter pylori is a well-recognized pathogenic factor of chronic gastritis and class I carcinogen. This bacterium presents large variety in the structure of its genome. Despite mutual structural and biochemical features, *H. pylori* strains demonstrate different virulence. The most pathogenic are those of *cagA+/vacA+* genotype.

The aim of the project was to estimate the detection of *H. pylori* genes in the gastric mucosa in children with polymerase chain reaction method (PCR).

The study comprised 148 children, aged 5–18 years (mean 13.6), in whom based on serum anti-*H. pylori* antibodies, urease test, and histologic examination, *H. pylori* infection was diagnosed (97 patients) or excluded (51 patients). *H. pylori* genes, *ureA*, *vacA*, and *cagA* were identified in gastric mucosa specimens using the PCR method.

H. pylori ureA gene was detected in the gastric mucosa in 84.5% of children from *H. pylori*-positive group and in 15.7% from *H. pylori*-negative group ($p < .001$). The presence of *H. pylori cagA* gene was found in 21.6% bioptates *H. pylori*-positive children and in 2% *H. pylori*-negative children ($p < .001$). The presence of *H. pylori vacA* gene was discovered in 23.7% of *H. pylori*-positive and in 11.8% *H. pylori*-negative patients (not significant). *H. pylori* strains type I (*cagA+/vacA+*) were identified in the gastric mucosa of 15.5% *H. pylori*-positive patients. The compatibility of standard evaluation of *H. pylori* and the diagnoses by PCR method was 84.5% in children from *H. pylori*-positive group and 84.3% in *H. pylori*-negative group.
Conclusions. The testing of *H. pylori ureA* gene with PCR technique in mucosal biopsies is a sensitive method and enables diagnosis of *H. pylori* infection even in patients with the infection not detected by standard methods.

Abstract no.: 09.14
Gastric and Duodenal Ulcers and Erosions in Children with and without *Helicobacter pylori* Infection in the Last Two Decades

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Background. Peptic disease (PD) is a complex entity encompassing ulcers and erosions of gastric and duodenal mucosa. In adults, prevalence of *Helicobacter pylori* infection in PD is decreasing, but little is known about similar changes in children.
Aim. The aim of this study is to report data on PD in children undergoing endoscopy in the last 19 years. We analyzed clinical and endoscopic features of 2259 symptomatic children, and evaluated whether changes occurred over the last two decades.
Results. Two hundred fifty-five (11.5%) children (boy/girl 138/117, median age 8.5 years) received a PD diagnosis: erosions were seen in 184 and ulcers in 71 children. Erosions were gastric in 98, duodenal in 73 and gastroduodenal in 13 children. Ulcers were gastric in 24, duodenal in 45, and gastroduodenal in 2 children. Prevalence of *H. pylori* was 28% in gastric and 59% in duodenal ulcers in the first decade and was null in gastric and 36% in duodenal ulcer in the second decade (not significant). More frequent presenting symptoms were recurrent abdominal pain in 38%, hematemesis in 20.3%, failure to thrive in 13.8%, melena in 5%, vomiting in 3.4%, and anemia in 3%. Vomiting, melena, and failure to thrive were significantly more frequent in children younger than 5 years, and anemia and recurrent abdominal pain (RAP) in older children.

Conclusions. Prevalence of *H. pylori* infection in PD in children in the last two decades was similar, being always lower in gastric than in duodenal ulcers (26% versus 53%, $p = .02$) and in gastric than in duodenal erosions (10% versus 33%, $p = .0002$). Prevalence of symptoms did not change over the years.

Abstract no.: 09.15
Helicobacter pylori Infection and Gastritis in Children with Abdominal Complaints in 1980–2006 in Estonia

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Background. The prevalence of *Helicobacter pylori* infection has declined significantly in successive birth cohorts in Western Europe in the 20th century. In Estonia, the birth cohort effect was not evident in adults thorough the last century and *H. pylori* infection is very common. However, among children born since 1991, after the re-establishment of Estonian independence, the prevalence of *H. pylori* infection has decreased significantly.
Aim. To evaluate the dynamics of the prevalence of *H. pylori* infection and changes in the histopathology of gastritis among gastroscopied children with abdominal complaints in 1980–2006 in Estonia.
Patients and Methods. Altogether, 658 children underwent gastroscopy with obtaining biopsy specimens from both gastric corpus and antrum mucosa in Tartu University Children's Hospital in 1980–2006 resulting from abdominal complaints. Histologic examinations of biopsy specimens were performed according to the updated Sydney system. Altogether, 577 children (mean age 12 ± 3 years) were included in the study, 81 were excluded because of incomplete data.
Results. Altogether, 342 patients (59%) were *H. pylori* positive. There was a very good concordance between presence or absence of the *H. pylori* infection and the chronic inflammatory infiltration both in the antrum and corpus mucosa ($p < .0001$). No significant changes occurred in the age-adjusted prevalence of *H. pylori* infection in 1980–2006; however, only 18 (3.1%) of studied children were born since 1991. Over time, the proportion of children with severe chronic gastritis increased.
Conclusion. Decrease of the prevalence of *H. pylori* infection is not evident among gastroscopied children with abdominal complaints in 1980–2006.

Abstract no.: 09.16
Follow-Up of Children with *Helicobacter pylori* Infection

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The diffusion of *Helicobacter pylori* infection in children, and its therapy, are the most important clinical outcomes of affected children.

The aim is the follow up of patients with *H. pylori* and different therapy.

Eighty-eight *H. pylori*-positive patients by invasive tests, with positive fecal antigen in 96.5% were treated with triple therapy (group A, 18 patients: amoxicillin, tinidazole, omeprazole for 2 weeks; group B, 15 patients: amoxicillin, clarithromycin, omeprazole for 2 weeks; group C, 26 patients: amoxicillin, clarithromycin, omeprazole for 1 week; group D, 29 patients: amoxicillin, tinidazole, omeprazole for 1 week). The eradication of *H. pylori* was evaluated by *H. pylori* fecal antigen and by ^{13}C-urea breath test; all the patients also received pediatric check. Follow-up is for 18 months; 23 patients were lost to follow-up. Eight showed positive fecal antigen. The other symptomatic: two received a second esophagogastroduodenoscopy (EGDS) for the presence of severe symptoms caused by a moderate *H. pylori*-positive gastritis in the first children and by esophagitis in the second one; the other three patients received a second cycle of therapy. The 57 patients negative to *H. pylori* stool antigen, one patient presented epigastric pain after 3 months, and recurrence of *H. pylori* infection; he repeated therapy for 1 week. Twenty patients had recurrent abdominal pain, half of them presented constipation, which we have treated, whereas three patients presented gastroesophageal reflux disease after 6 months.

There were no differences between the various groups; the evaluation of the follow-up suggest that a lot of patients had persistent symptoms after the eradication, not related to the presence of *H. pylori*.

Abstract no.: 09.17
The Incidence of *Helicobacter pylori*-associated Gastritis in Symptomatic Children: 13 Years Experience in One GI Center from West Virginia

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Background. The prevalence of *Helicobacter pylori* infection in children from developed countries has been steadily declining over the last decade. This finding may be skewed by the limited asymptomatic population surveyed and by noninvasive methods used.
Aim. To investigate the prevalence of *H. pylori* infection in symptomatic children, utilizing histology as the diagnostic method (gold standard).
Methods. A retrospective review of all diagnostic upper endoscopy procedures (1993–2005), was carried out. Biopsies from the stomach (antrum, body) were available in all. Positive *H. pylori* diagnosis was defined by histology (+), and (+) urease test. Gastritis was assessed according to modified Sydney criteria.
Results. A total of 1,743 procedures were included in the study. A steady increase in esophagogastroduodenoscopy per year was noted. The average patient age per year was 10.1 and the average male : female ratio/year was 1.1 : 1.0. There was no significant variation in the patient's mean age or gender distribution between the studied years. Duodenal ulcer was noted in 52 (3%) procedures. A significant decrease in the rate (%) of *H. pylori*-associated gastritis was noted between 1993–1999 versus 2000–2005 (18.3 versus 7.3, respectively; $p < .001$).

Years	No. EGD	H. pylori+G+(%)
1993–1996	78 ± 7	16 ± 4
1997–1999	125 ± 16	21 ± 3
2000–2002	170 ± 29	06 ± 2
2003–2005	179 ± 16	08 ± 2

Conclusion. A significant decrease in *H. pylori*-associated gastritis in children was noted. Whether our findings are representing a local or a national trend is yet unknown.

Abstract no.: 09.18
Strategic Treatment of *Helicobacter pylori* in Algerian Children

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Background. *Helicobacter pylori* prevalence and resistance to antibiotics is high in Algerian children.
Aim. To compare the antibiogram guided to a random treatment.
Methods. Through 2002 to 2004, 67 children (29 boys, 38 girls, mean age 10.3 years, range 14 months to 16 years) diagnosed with *H. pylori* gastritis according to results obtained by histology (100%+), rapid urease test (87%+), culture (61%+) serology (83%+), and HpSA (56%+) were treated. Global resistance to antibiotics was as high as 61%: metronidazole (M), 58%; clarithromycin (C), 16%; M+C, 13%. Different daily treatments were used combining omeprazole (O) 10 or 20 mg b.i.d., C 15 mg/kg, amoxicilan (A) 50 mg/kg, and a high dose of M 40 mg/kg. In group I, a random treatment OAM during 7 or 10 days was used in 36 children. In group II, 31 children were treated with OAM7 except in M-resistant strains, according to antibiogram, treated with OAM10. OAC7 was only used as rescue treatment in failures of eradication.
Results. Group I: eradication in 17/36 (94%) with OAM7 and in 10/19 (84%) with OAM10. Group II: eradication rate of 87%. There were no significant differences between the two groups and between long and short treatments. Total eradication rate after rescue treatment reached 97%.
Conclusion. In regions with high prevalence of M-resistant strains, successful eradication can be obtained with high doses of metronidazole in children with gastritis.

Abstract no.: 09.19
Mannan-Binding Lectin in Children with *Helicobacter pylori* Infection

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Helicobacter pylori is considered to be a major etiological agent causing chronic gastritis (CG). Mannan-binding lectin (MBL) is one of the key factors of the innate immunity, enhances phagocytosis by direct opsonization, and activates complement via the lectin pathway, which protects the host from infection. The serum concentration and activity of MBL markedly depends on *mbl-2* gene point mutations and polymorphisms.

In this report, we investigated the correlation between heterogeneity of the *mbl-2* gene and MBL serum concentration. In addition, *mbl-2* gene expression in gastric biopsies has been estimated.

The study group comprised 119 children with CG aged 6–17 years, among them 78 children had *H. pylori* infection, whereas in 41, infection with *H. pylori* was excluded. The control group consisted of 77 healthy children. *H. pylori* was detected with both: urease test and histopathology. Genomic DNA was extracted from blood samples according to the GTC method. *mbl*-2 Genes (exon-1 and promoter regions) were identified with polymerase chain reaction. Determination of serum MBL concentration was performed using enzyme-linked immunosorbent assay.

The frequency of *mbl-2* gene mutations and serum protein concentrations did not differ significantly among groups. We found an expression of *mbl-2* gene in stomach biopsies of patients with CG, which was stronger in children *H. pylori*-positive compared to *H. pylori*-negative.

Results presented in this report suggest that *mbl-2* gene mutations do not enhance the risk of gastritis associated with *H. pylori* infection in children. Higher *mbl-2* gene expression in *H. pylori* infection-associated gastritis children may suggest an involvement of MBL in the local immunological response against *H. pylori.*

Abstract no.: 09.20
Helicobacter pylori Infection in Algerian Children: About 275 Cases

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Background. *Helicobacter pylori* infection prevalence increase with age. Few data are available in Algeria.
Aim. To evaluate *H. pylori* prevalence infection among symptomatic children and to establish clinical, endoscopic, histologic, and bacteriologic infection profile.
Methods. From 2002 to 2004, 275 children (123 boys, range age 6 months to 16 years) were enrolled for several complaints. Every patient had an upper gastrointestinal endoscopy with gastric biopsies for histology, rapid urease test (RUT), culture, and antibiotic microbial sensitivity (E-test), serology (IgG antibodies, enzyme-linked immunosorbent assay), and stool antigen test (HpSA). Diagnosis of *H. pylori* infection was assessed if culture and/or histology and an other test were positive.
Results. Positivity of diagnostic tests for histology, serology, RUT, culture, HpSA was 99, 80, 73, 57, and 38%, respectively. Infection was present in 212 patients (82%). The infection had no specific clinical feature except for recurrent abdominal pain (RAP) found in 43%. Eighty-four patients (40%) exhibited endoscopic nodularity in the antral mucosa. Histologicaly, chronic gastritis, activity, follicles, and atrophy (mild or moderate) were observed in 100, 94, 58, and 45% of cases, respectively. Metronidazole had the highest frequency of resistance (48%) and the rate of clarithromycin resistance was 12.5%.
Conclusion. *H. pylori* prevalence is very high. There is a strong association of *H. pylori* infection and RAP. Nodular gastritis is meaningful of the *H. pylori* infection in child. Gastric atrophy is abnormally frequent in our study. Future studies are required for more clarification.

Abstract no.: 09.21
Helicobacter pylori and ABO Blood Groups in Russia: Looking for Any Association with Childhood Duodenal Ulcer

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Aim. To determine ABO blood groups of unselected dyspeptic pediatric patients with evidence of peptic ulceration/chronic *Helicobacter pylori*-associated gastritis and to compare the phenotypes of those without *H. pylori* infection.
Methods. Blood samples were obtained from 231 children of 4–12 years (mean age 9.6 ± 1.1 years; 115 girls) referred to endoscopy because of severe dyspeptic complaints. ABO status was determined by standard hemagglutination assay. Sera were taken for the assessment of IgG antibodies against *H. pylori* with enzyme-linked immunsorbent assay kits (Roche, Zurich, Switzerland). Statistical analysis was carried out with the χ^2 test.
Results. Duodenal ulcer was revealed in 32 of our patients, all of them were *H. pylori*-positive. A total of 180 (77.9%) subjects had antibodies against *H. pylori*, the remainder (51 or 22.1%) were seronegative. Of the total infected patients, 26.6% (48/180) were type O and 35.5% (64/180) were type A. Blood groups B and AB occurred in 44 (24.4%) and 24 (13.3%) subjects, respectively. Of the 51 uninfected patients, 33.3% (17/51) were type O and 37.2% (17/51) were type A. The remainder of uninfected patients (15) were types B (10 or 19.6%) and AB (5 or 9.8%). Among the ABO blood groups, higher prevalences were found for O (17/32 or 53.1%) and A (9/32 or 28.1%) types. Blood groups B and AB occurred in 4/32 (12.5%) and 2/32 (6.25%) patients, respectively.
Conclusion. There were no significant differences in blood group distribution among seropositive or seronegative dyspeptic pediatric patients. The spread of blood group antigens among children with ulcers was not significantly different from ABO proportions in ulcer-free seropositive and seronegative children.

Abstract no.: 09.22
Use of Different Therapies for *Helicobacter pylori* Eradication in Children

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Aim. To know the efficacy of four therapies in the eradication of *Helicobacter pylori* in paediatric population.
Methods. Eighty-eight patients (52 female) from 4–17 years were diagnosed by positive urea breath test (UBT) from 2004 to 2005. Histology and culture were also performed. Eighty-two patients were treated with therapies between 10 and 14 days. Two groups were created: group 1 (G1): 44 children (4–10 years), group 2 (G2): 44 children (11–17). Forty-one children (19 G1, 22 G2) were treated with OCA: omeprazole (1 mg/kg), amoxicillin (50 mg/kg), and clarithromycin (20 mg/kg). Twenty patients (14 G1, 6 G2) with BMA: bismuth subcitrate (8 mg/kg), amoxicillin (50 mg/kg), and metronidazole (20 mg/kg). Four children (1 G1, 3 G2) with OAM: omeprazole (1 mg/kg), amoxicillin (50 mg/kg), and metronidazole (20 mg/kg). Seventeen patients (9G1, 8 G2) with OACTA as follows: 5 days with OAC and 5 days with OAT. UBT was carried out 6 weeks after therapy.
Results. Eradication rates were as follows: 9 out of 41 (22%), 16 out of 20 (80%), 3 out of 4 (75%), and 6 out of 17 (35%) with OCA, BAM, OAM, and OACTA, respectively. We did not find statistically significant differences between both groups of age. Nineteen patients who failed *H. pylori* eradication with OCA were treated using BAM, obtaining an eradication rate of 84.2% (16/19).
Conclusions. Therapies that included metronidazole were effective in *H. pylori* eradication. The disappointing results with clarithromycin oblige us to reconsider the inclusion of this agent in the treatment of *H. pylori* in the pediatric population.

Abstract no.: 09.23
Pepsinogen (PG) Levels and PGI/II Ratios in Sera of Japanese Children without and with *Helicobacter pylori* Infection

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Background. Infection of *Helicobacter pylori* causes gastritis that leads to gastric atrophy, but the time course of the diseases is largely unknown. We determined serum pepsinogen (PG) in *H. pylori*-infected and noninfected children, as increase in serum PGI and PGII levels and decrease in PGI/II ratios in adults are proposed as reliable markers of gastritis and gastric atrophy, respectively.
Subjects and methods. One hundred and fifty-nine serum samples from 149 Japanese children (mean age, 7.9 years; age group, 1–5, 6–10, 11–15, 16–20 years) without gastric symptoms were used, including 70 *H. pylori*-positive and 79 *H. pylori*-negative children as judged by serum antibody (EIA: JHM-CAP Scimedx Corp., Denville, NJ). Serum PG levels were measured by radioimmunoassay (Riabead Kit, Dainabot Corp., Tokyo, Japan).
Results. The mean PGI levels of noninfected and infected children increased from 33.4 (1–5 years) to 49.7 ng/mL (16–20 years), and 48.1 (1–5 years) to 71.9 ng/mL (16–20 years), respectively. The mean PGII levels of noninfected children ranged from 5.6 ng/mL (16–20 years) to 7.5 ng/mL (1–5 years), whereas those of infected children ranged from 16.0 (6–10 years) to 17.8 ng/mL (1–5 years). The PGI/II ratios in noninfected children increased from 5.5 (1–5 years) to 8.9 (16–20 years), whereas those of infected children ranged from 3.0 (1–5 years) to 5.2 (11–15 years).
Conclusions. Children with *H. pylori* infection showed significantly higher levels of serum PGI and PGII and lower PGI/II ratios than those without infection in all age groups. These data suggest that gastritis, and possibly atrophic gastritis, may start early in childhood.

Diagnosis

Abstract no.: 10.01
Re-infection after *Helicobacter pylori* Eradication Among Algerian Population

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Background. Few data about re-infection after successful eradication of *Helicobacter pylori* are available among adults in developing countries.
Aim. To determine the re-infection rate of *H. pylori* after eradication among adults in Algeria, a country with a very high prevalence of infection.
Methods. We prospectively studied 62 patients (mean age = 32.8 years; range: 16–62 years; men = 16; duodenal ulcer (DU) = 14, and gastritis = 48) in whom *H. pylori* was eradicated. ^{13}C-urea breath test (UBT) and 10–12 biopsies for histology, rapid urease test, and culture were performed 8–12 weeks after end of therapy. Eradication was assessed by the negativity of all tests. Patients were controlled by UBT at 1 and 2 years after eradication. Re-infection was defined by a positive UBT.
Results. Follow-up was 1 year for all patients and 2 years for 44 patients. Re-infection occurred in five patients (8%) during the study (men, 2; mean age, 31.2 years (16–43); DU, 4; and gastritis, 1). Two of the 62 patients were re-infected at 1 year (3.2%) and 3 others of the 44 patients at 2 years (6.8%). Annual re-infection rate was 4% per patient per year.
Conclusions. The risk of re-infection after successful eradication of *H. pylori* is 4% annually in Algerian adult population. It is higher than in developed countries.

Abstract no.: 10.02
Accuracy of Monoclonal Stool Antigen Test for the Diagnosis of *Helicobacter pylori* Infection: A Systematic Review and Meta-analysis

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Objective. To perform a systematic review and a meta-analysis of accuracy of monoclonal stool antigen test (SAT) for the diagnosis of *Helicobacter pylori* infection.
Methods. Selection of studies: assessing the accuracy of monoclonal SAT for the diagnosis of *H. pylori* infection. Search strategy: electronic and manual bibliographical searches. Data extraction: independently carried out by two reviewers. Data synthesis: meta-analyses combining the sensitivities, specificities, and likelihood ratios (LRs) of the individual studies.
Results. Twenty-two studies, including 2,499 patients, evaluated the monoclonal SAT before eradication therapy. Pooled sensitivity, specificity, LR positive and LR negative were 0.94 (95% CI, 0.93–0.95), 0.97 (0.96–0.98), 24 (15–41), and 0.07 (0.04–0.12). The accuracy of both monoclonal and polyclonal SAT was evaluated together in 13 pretreatment studies, and higher pooled sensitivity was demonstrated with the monoclonal technique (0.95 versus 0.83). Twelve studies, including 957 patients, assessed the monoclonal SAT to confirm eradication after therapy. Pooled sensitivity, specificity, LR positivity and LR negativity were 0.93 (0.89–0.96), 0.96 (0.94–0.97), 17 (12–23), and 0.1 (0.07–0.15). Both tests were evaluated together in eight post-treatment studies and, again, the monoclonal technique showed higher sensitivity (0.91 versus 0.76). Heterogeneity among studies disappeared when a single outlier study was excluded. Subanalysis depending on the reference method, the study population, or the study quality showed similar results.
Conclusion. Monoclonal SAT is an accurate noninvasive method both for the initial diagnosis of *H. pylori* infection and for the confirmation of its eradication after treatment. The monoclonal technique has higher sensitivity than the polyclonal one, especially in the post-treatment setting.

Abstract no.: 10.03
Is There Any Correlation between ^{13}C-urea Breath Test Values and Response to First-Line and Rescue *Helicobacter pylori* Eradication Therapies?

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Aim. To study if there is a correlation between ^{13}C-urea breath test values prior to treatment and the response to first-line and rescue *Helicobacter pylori* eradication therapies.
Methods. Six-hundred patients with peptic ulcer or functional dyspepsia infected by *H. pylori* were prospectively studied. Pretreatment *H. pylori* infection was established by ^{13}C-urea breath test. Three-hundred and twelve patients were treated with first-line eradication regimen, and 288 received a rescue regimen. *H. pylori* eradication was defined as a negative ^{13}C-urea breath test 8 weeks after completion of treatment.
Results. *H. pylori* eradication was achieved in 444 patients. No statistically significant differences were demonstrated when mean δ^{13}C-urea breath test values were compared between patients with eradication success and failure (49.4 ± 33 versus 49.2 ± 31). Differences in mean pretreatment $\delta^{13}CO_2$ between patients with eradication success/failure were not demonstrated either when first-line or rescue regimens were prescribed. With the cut-off point of pretreatment $\delta^{13}CO_2$ set at 35 units, sensitivity and specificity for the prediction of *H. pylori* eradication success was 43% and 60%. The area under the ROC curve evaluating all the cut-off points of the pretreatment $\delta^{13}CO_2$ for the diagnosis of *H. pylori* eradication was 0.5. Finally, $\delta^{13}CO_2$ values did not influence the eradication in the logistic regression model.
Conclusion. No correlation was observed between ^{13}C-urea breath test values before treatment and the response to first-line and rescue *H. pylori* eradication therapies. Therefore, we conclude

that quantification of $\delta^{13}CO_2$ prior to treatment is not useful to predict the success or failure of eradicating therapy.

Abstract no.: 10.04
Long-term Follow-up of ^{13}C-urea Breath Test Result after *Helicobacter pylori* Eradication: Frequency and Significance of Borderline $\delta^{13}CO_2$ Values

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Aim. To quantify the ^{13}C-urea breath test (UBT) result for several years following *Helicobacter pylori* eradication, and to evaluate the frequency and the significance of borderline $\delta^{13}CO_2$ values.
Methods. Two-hundred *H. pylori* eradicated patients confirmed by ^{13}C-UBT (100 mg of urea, citric acid), and having had repeated this test yearly up to 5 years, were studied. $\delta^{13}CO_2$ values between 2‰ and 5‰ were considered as borderline results.
Results. Eight *H. pylori* recurrences were observed during 406 patient years of follow-up (1.97% yearly). In 2/8 re-infected patients, the re-infection was preceded by a negative $\delta^{13}CO_2$ value > 2‰. Borderline $\delta^{13}CO_2$ values were detected in 4% of the 606 UBTs performed, and in 25% when only patients in whom *H. pylori* recurrence was detected in subsequent UBTs were included ($p < .05$). The negative predictive value of a post-treatment $\delta^{13}CO_2$ > 2‰ for the diagnosis of *H. pylori* recurrence was 99%.
Conclusion. Positive and negative UBT results tend to cluster outside of the range between 2‰ and 5‰. Nevertheless, a borderline UBT δ value (e.g., very close to the selected cut-off point) should be cautiously interpreted, and the result should probably be confirmed either by repeating the UBT or by other diagnostic methods. On the contrary, a $\delta^{13}CO_2$ value < 2‰ very confidently confirms *H. pylori* eradication.

Abstract no.: 10.05
Accuracy of *Helicobacter pylori* Diagnostic Tests in Patients with Bleeding Peptic Ulcer: A Systematic Review and Meta-analysis

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Objective. To perform a systematic review and a meta-analysis of diagnostic accuracy of the different tests aimed to detect *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding (UGIB).
Methods. Selection of studies: assessing the accuracy of *H. pylori* diagnostic methods in patients with UGIB. Search strategy: electronic bibliographical searches. Data extraction: independently carried out by two reviewers. Data synthesis: meta-analyses of the different tests were performed combining the sensitivities, specificities, and likelihood ratios (LRs) of the individual studies.
Results. Studies showed a high degree of heterogeneity. Pooled sensitivity, specificity, LR+ and LR– (95% CI) for the different methods were: rapid urease test (16 studies/1,417 patients): 0.67 (0.64–0.70), 0.93 (0.90–0.96), 9.6 (5.1–18.1), and 0.31 (0.22–0.44). Histology (10 studies/827 patients): 0.70 (0.66–0.74), 0.90 (0.85–0.94), 6.7 (2.5–18.4), and 0.23 (0.12–0.46). Culture (3 studies/314 patients): 0.45 (0.39–0.51), 0.98 (0.92–1.00), 19.6 (4–96), and 0.31 (0.05–1.9). Urea breath test (8 studies/520 patients): 0.93 (0.90–0.95), 0.92 (0.87–0.96), 9.5 (3.9–23.3), and 0.11 (0.07–0.16). Stool antigen test (6 studies/377 patients): 0.87 (0.82–0.91), 0.70 (0.62–0.78), 2.3 (1.4–4), and 0.2 (0.13–0.3). Serology (9 studies/803 patients): 0.88 (0.85–0.90), 0.69 (0.62–0.75), 2.5 (1.6–4.1), and 0.25 (0.19–0.33).
Conclusion. Biopsy-based methods, such as rapid urease test, histology, and culture, have a low sensitivity, but a high specificity in patients with UGIB. The accuracy of ^{13}C-urea breath test remains very high in these patients. Stool antigen test is less accurate in UGIB. Although serology seems not to be influenced by UGIB, it cannot be recommended as the first diagnostic test for *H. pylori* infection in this setting.

Abstract no.: 10.06
Comparison of Diagnostic Accuracy of Commercial Kits for *Helicobacter pylori* Infection in a Japanese Population

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Background and Aims. Recently, many enzyme-linked immunsorbent assay kits for the detection of *Helicobacter pylori* antibody or antigen have become available and have provided highly reliable results in Western countries. However, the diagnostic accuracy of these commercial kits made in Western countries has been reported to be lower when used among Asian populations. To gain a better understanding of the performance variation of these kits in a non-Western population, we compared the diagnostic accuracy of commercial kits among Japanese subjects.
Methods. Serum and stool samples were obtained from 79 Japanese subjects without the effect of *H. pylori* inhibitors such as proton pump inhibitors and H2-blockers. We used Japanese *H. pylori* strains (J-HM-CAP) (HM-CAP with the antigen derived from Japanese clinical isolate) and HM-CAP (imported kit) in serological assays, and TestMate pylori antigen EIA (domestic kit) and Premier Platinum HpSA (imported kit) in stool antigen assays. Using the ^{13}C urea breath test results as a gold standard, we evaluated the diagnostic accuracy of these kits.
Results. In serological assays, the sensitivity and the specificity of J-HM-CAP were 97.1% and 86.4%, respectively, and those of HM-CAP were 94.3% and 93.2%, respectively. In stool antigen assays, the sensitivity and the specificity of TestMate pylori antigen enzyme immunoassay were 91.4% and 93.2%, respectively, and those of Premier Platinum HpSA were 65.7% and 97.7%, respectively.
Conclusions. The diagnostic accuracy for *H. pylori* infection may differ considerably depending on the origin of the antigen or antibody used in the commercial kits both in serological and stool antigen assays in Japan.

Abstract no.: 10.07
^{13}C-urea Breath Test During Hospitalization for the Diagnosis of *Helicobacter pylori* Infection in Peptic Ulcer Bleeding

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Objective. To evaluate the prevalence of *Helicobacter pylori* infection in patients with peptic ulcer bleeding with ^{13}C-urea breath test (UBT) performed during hospitalization, and to assess the effect of proton pump inhibitors (PPIs) on diagnostic accuracy.
Methods. Patients hospitalized with peptic ulcer bleeding and treated with intravenous high-dose omeprazole, were prospectively included. A first UBT was performed the day after the patient resumed oral feeding. Patients with a negative UBT at the hospitalization underwent a repeated UBT 15 days after stopping PPIs.
Results. The first UBT during hospitalization was positive in 86.3% of 131 patients. Time between admission and performance of the test was longer in patients with negative versus positive UBT (5.2 ± 0.7 versus 4.3 ± 0.5 days). Eight out of 18 *H. pylori*-negative patients had borderline UBT results (2.0–5.0‰). The repeated UBT became positive in 15/18 (83.3%) patients with a negative first UBT. Therefore, *H. pylori* could finally be detected in 98% of the patients. In the multivariate analysis, the only variable associated with a negative first UBT was the time elapsed between admission and performance of the test (odds ratio = 6.6; 95% CI = 2.9–15.1).
Conclusion. Most of *H. pylori*-positive ulcer bleeding patients have a positive UBT (performed just after the patient resume oral feeding) despite previous treatment with high-dose intravenous PPIs. Nevertheless, to preclude false-negative results as a result of PPI therapy, the UBT should be performed as early as possible. If the infection cannot be demonstrated with this first UBT, *H. pylori* still needs to be definitively excluded with a second UBT performed 2 weeks after stopping these drugs.

Abstract no.: 10.08
Helic Device for Children Noninvasive Breath Diagnostics of *Helicobacter pylori*

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Recently, increasing interest in noninvasive *Helicobacter pylori* diagnostics is revealed. Computerized indicator for noninvasive *H. pylori* express diagnostics by detecting ammonia level in breath, Helic-device is successfully applied in Russia.

During our study, 104 children were examined by endoscopy, rapid urease test, morphology, urease breath Helic tubes, and Helic device.

H. pylori presence was proved if two or more methods were positive and endoscopy confirmed them. For *H. pylori*-negative patients, two or more methods gave negative results.

For 96 *H. pylori*-positive children Helic device showed the average basal ammonia level 43.5 ± 22.22 relative units; average load level, 88.8 ± 29.56; average increase, 18.2 ± 17.41 ($p < .001$).

We noticed that high basal ammonia level (more than 60) together with a minor increase should be evaluated as *H. pylori* positive.

Helic device results coincided with Helic tubes in 94 cases, discrepancy of Helic device and morphology was detected in four cases. Thus, Helic device sensitivity proved to be 96%.

For eight *H. pylori*-negative children, Helic device presented average basal ammonia level 47 ± 16.86; average load level, 48.88 ± 16.27; average increase, 1.75 ± 2.1 ($p > .05$).

The results of all used methods agreed and were negative. Thus, Helic device exhibited specificity 100%.

Helic device is a highly sensitive and highly specific method of *H. pylori* diagnostics. Increase in ammonia level less than 4 relative units should be considered as *H. pylori* negative, if the increase more than 4, *H. pylori* positive. High basal ammonia level (more than 60) should be evaluated as a positive result even if increase of ammonia level is less than 4.

Abstract no.: 10.09
Usefulness of RAPID *Helicobacter pylori* StAR™ for Diagnosing *H. pylori* Infection in Patients Undergoing Dialysis for Chronic Renal Failure

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Background. *Helicobacter pylori* infection in chronic renal failure patients has been linked to peptic ulcer and gastric neoplasia after kidney transplantation. It may also contribute to the accelerated arteriosclerosis that is usual in this population. Few data are available on the usefulness of fecal antigen detection for noninvasive diagnosis of *H. pylori* infection in dialyzed patients. The objective of the study was to determine the efficacy of two new monoclonal fecal tests: an immunochromatographic test *RAPID H. pylori* StAR™ (DakoCytomation, Cambridge, UK) and an ELISA-Amplified IDEIA™ *H. pylori* StAR (DakoCytomation, Cambridge, UK) for diagnosing *H. pylori* infection in chronic renal failure patients.
Methods. Eighty-five patients were included in a cross-sectional study. Urea breath test, *RAPID H. pylori* StAR™, and Amplified IDEIA™ *H. pylori* StAR were performed. *H. pylori* status was determined by urea breath test. Sensitivity, specificity, and positive and negative predictive values were calculated for each test. Spearman test and Kappa statistics estimated correlations between *RAPID H. pylori* StAR readings and concordance between both kits, respectively.
Results. Sensitivity, specificity, positive, and negative predictive values were 72–73, 80–84, 73–77, and 80–81% for *RAPID H. pylori* StAR™; and 78, 96, 93, and 86%, for amplified IDEIA™ *H. pylori* StAR. Readings from both readers were highly correlated; Spearman correlation test was 0.966 ($p < .001$). Concordance between tests showed a good correlation. Kappa statistics were 0.8 for observer 1 and 0.78 for observer 2.

Conclusions. Both *RAPID H. pylori* StAR™ and amplified IDEIA™ *H. pylori* StAR have an acceptable performance in patients with renal failure.

Abstract no.: 10.10
Development and Application of a Novel Peptide Nucleic Acid (PNA) Probe for the Specific Detection of *Helicobacter pylori*

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The standard methods for accurate diagnosis of *Helicobacter pylori* infection consist in either culturing of the pathogen and/or concordant positive results obtained by histology and the rapid urease test or the ^{13}C-urea breath test (UBT), as none of these diagnostic tests except bacterial culturing are 100% specific, fluorescence in situ hybridization (FISH) might be a good complement.

In this work, the development of a new peptide nucleic acid (PNA) FISH probe for the detection of *H. pylori* is reported. The 15-mer PNA probe was connected to an Alexa Fluor 546 dye and target a specific 16S rRNA sequence of the bacterium.

PNA probes are a recent technology that has been shown to provide for additional specificity and sensitivity in FISH procedures when compared to the standard DNA counterparts.

The probe was tested against several *H. pylori* and non-*H. pylori* strains, and was shown to be specific for the microorganism of interest. This technique was optimized for different types of supports such as slides, membrane filters, and also coupons of various materials where *H. pylori* was present. Tests performed showed a better sensitivity of the probe than the standard plating procedures for *H. pylori* detection. We are currently optimizing the application of this new probe for paraffin-embedded gastric biopsy specimens.

When completely optimized, this technique could be a useful method, as it is rapid, sensitive, and specific, for an even more accurate diagnostic of *H. pylori* infection, and could also be used for distinguishing *H. pylori* from other *Helicobacter* species.

Abstract no.: 10.11
Evaluation of Rapid Antibody Tests for the Diagnosis of *Helicobacter pylori* Infection in Thailand, Japan, and United States

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Objective. The aim of this study was to compare the performance characteristics of rapid antibody tests for *H. pylori* infection in Thailand, Japan, and the United States.

Methods. Total of 247 patients (50 gastric cancer, 78 peptic ulcer, and 119 gastritis) were enrolled in this study. The patients were gathered from Thailand (78 patients), USA (93 patients), and Japan (76 patients). The gold standard for diagnosis of *H. pylori* infection were ^{13}C-urea breath test for patients from USA and Japan and HM-CAP for patients from Thailand. Capillary blood obtained by fingerstick from all patients was tested with ASSURE™ *H. pylori* rapid test.

Results. A total of 204 patients (82.6%) were infected with *H. pylori*. The prevalence of *H. pylori* infection by the gold standard tests in gastric cancer, peptic ulcer disease, and gastritis were 94, 97.4, and 68%, respectively. The ASSURE™ *H. pylori* rapid test in Thailand, USA, and Japan had sensitivity of 88.9, 90.5, and 91%; specificity of 80, 100, and 100%; and accuracy of 87.2, 92.5, and 92.1%, respectively. In addition, this rapid test had overall sensitivity of 90.2%, specificity 93%, and accuracy of 90.7% in all diseases and countries. On the other hand, this rapid test had limited specificity (33%) for detecting *H. pylori* infection in gastric cancer patients.

Conclusion. ASSURE™ *H. pylori* rapid test provides accurate diagnosis of *H. pylori* infection in Thailand, Japan, and USA. However, these tests have limited specificity for *H. pylori* detection in gastric cancer especially in Thai patients.

Abstract no.: 10.12
Helicobacter pylori Infection among Belgian Sewage Workers: A Validity Study with Three Noninvasive Diagnostic Tests

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Introduction. In a health and hygiene questionnaire among Belgian (Flemish) sewage workers of municipal wastewater treatment plants (WWTPs) gastrointestinal symptoms, peptic ulcers and esophagogastroduodenoscopies were more prevalent than among nonsewage-exposed workers employed at same employer.

Objective. To evaluate validity, performance, and acceptance of three noninvasive diagnostic *Helicobacter pylori* test methods in a nonclinical, occupational setting among Belgian WWTP workers.

Methods. *H. pylori* infection status was determined on sera by Enzygnost Anti-Helicobacter II/immunoglobulins A (IgA) and G (IgG) (Behring Diagnostics). Fecal stool antigen enzyme immunoassay Premier Platinum PP HpSA™ test (Meridian Diagnostics-Bioscience) on feces. ^{13}C-urea breath test (UBT) was performed at workplace using instructions by Powerpoint™ presentation.

Results. One hundred of 146 (70.5%) WWTP workers participated voluntarily. Ninety-two remained for analysis because 11 were excluded because of possible interference with PP HpSA or UBT (antibiotics, proton pump inhibitors, partial gastrectomia). Of these, 21.9% and 13.0% had seropositive (= 10 IU/mL) IgG and IgA status, respectively. PP HpSA was positive in 18.5% of samples. 23.3% of UBTs had positive result. Evaluation of three tests by comparison with gold standard "at least two of three tests positive result" is given in Table 1.

Conclusions. Acceptance of serology and PP HpSA was good. UBT was performed correctly at the workplace without guidance of laboratory worker. UBT had highest sensitivity, precision, accuracy, and positive and negative predictive value. PP HpSA had highest specificity.

Table 1 Results of three noninvasive diagnostic tests against gold standard

%	**Specifity**/ sensitivity	**Precision**/ accuracy	**Positive predictive value**/ negative predictive value
Serology	**95.8**/89.5	**77.3**/94.5	**85.0**/97.2
Faecal antigen EIA test	**96.0**/94.1	**80.0**/95.7	**84.2**/98.6
Premium Platinum PP HpSA™ ^{13}C-urea breath test	**92.9**/100	**85.7**/96.7	**85.7**/100

Abstract no.: 10.13
Immunostaining Confirms that Urease Based Tests are Superior to Giemsa and Warthin Starry Silver Stain for Confirming Eradication of *Helicobacter pylori* in Peptic Ulcer Disease

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Confirming *Helicobacter pylori* eradication with conventional histology and urease based tests often gives conflicting results resulting in retreatment. The study aimed at determining the specificity and negative predictive value (NPV) of rapid urease test (RUT), urea breath test (UBT), and histology (Giemsa stain, Warthin Starry silver stain) when compared to immunohistochemistry (IHC) for detecting *H. pylori* following eradication therapy in peptic ulcer disease (PUD). Adult patients with *H. pylori*-related PUD were given esomeprazole 40 mg b.i.d., amoxycillin 1 g b.i.d., and clarithromycin 500 mg b.i.d. for 2 weeks. Four weeks following therapy, antral and corpus biopsies were taken for RUT, Giemsa stain, silver stain, immunohistochemistry, and UBT were performed. The gold standard for defining eradication of *H. pylori* was immunohistochemistry. The study group included 52 patients (mean age 38.9 (12.8) years, 42 men) and mean symptom duration of 4.2 (0.1–25) years. Post-therapy, Giemsa stain was positive in 38 (73.1%), Warthin starry in 16 (30.8%), RUT in 1 (1.9%), UBT in 1 (1.9%), and immunohistochemistry in 12 (23.1%) cases. The specificity and NPV were: Giemsa stain (18.4%, 63.6%); Warthin starry stain (60.5%, 74.2%), RUT (92.1%, 71.4%), and UBT (97.3%, 72.5%). RUT and UBT demonstrated a high specificity of 92% and 97.3%, whereas Giemsa stain led to false-positive in 31 and false-negative results in 4 cases.

Conclusion. Conventional histology stains are not predictive of *H. pylori* eradication status. If facilities for immunohistochemistry are unavailable, urease-based tests can be used to monitor eradication status of *H. pylori*.

Abstract no.: 10.14
Detection of *Helicobacter pylori* Infection by Invasive Methods and Two Different Urine Antibody Tests in Turkish Dyspeptic Patients

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Helicobacter pylori infection is one of the most common infectious diseases in the world. Two different *H. pylori* urine antibody tests as noninvasive diagnostic methods were used and compared with invasive methods. Forty-six dyspeptic patients (12 men, 34 women; mean age, 47.80 ± 13.37 years) were studied between April and May 2006. Two antrum and corpus biopsies were taken from each patient. Rapid urease test (RUT) and histopathology were applied to all patients as gold standard methods for the diagnosis of *H. pylori* infection. URINELISA and RAPIRUN (Otsuka Pharmacautical, Tokyo, Japan) tests were used in urine specimens of these patients for detection of anti-*H. pylori* IgG antibody. Thirty-five of 46 patients (76.1%) were diagnosed as positive and 11 (23.9%) were negative for *H. pylori* infection by the gold standard methods. Twenty-six (65.0%) patients were positive and 20 (35.0%) were negative for anti-*H. pylori* IgG antibody by URINELISA test. The sensitivity, specificity, and positive and negative predictive values were 71.4, 90.9, 96.2, and 50.0%, respectively (k = 0.48). However, anti-*H. pylori* IgG antibodies were positive in 27 (58.7%) and negative in 19 (41.3%) by RAPIRUN test. The sensitivity, specificity, and positive and negative predictive values were 68.6, 72.7, 88.9, and 42.1%, respectively (k = 0.33). We conclude that URINELISA, which was more sensitive (71.4%) and specific (90.9%), could be used as a routine diagnostic tool in the microbiology laboratory for assessing clinical significance and diagnosis of *H. pylori*.

Abstract no.: 10.15
Helicobacter pylori Infection Diagnosis in Gastroduodenal Disease In La Habana, Cuba

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There is a great paucity of information about *Helicobacter pylori* infection in the countries of the Caribbean basin. Our aim was to determine the prevalence of the infection in a group of 46 consecutive patients submitted to upper digestive tract endoscopy clinics in the Institute of Gastroenterology in La Habana, Cuba. Information concerning the age, gender, and main symptoms were collected and a serum sample obtained. *H. pylori* serology

(IgG) and (IgM) were performed using Microwell ELISA from Diagnostic Automation INC (USA).

Patients had the following endoscopic diagnosis: duodenal ulcer: 10; gastric ulcer, 2; nonulcer dyspepsia, 34; including gastritis, 23; hiatal hernia, 2; biliary reflux, 2; nonabnormality, 7. The mean age was 51 years with 26 men and 20 women. Sera were maintained at −70 °C before being tested.

Among the 46 sera tested, 29 were positive (63.04%). The prevalence of *H. pylori* infection in the symptomatic population of La Habana is the same as reported for other developing countries. These results indicate the importance for further studies to identify factors influencing the prevalence in the Caribbean.

Clinical Trials and Novel Treatments

Abstract no.: 11.01
Third-line Rescue Therapy with Levofloxacin or Rifabutin after Two *Helicobacter pylori* Treatment Failures

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Aim. In patients with a first eradication failure, a second (rescue) therapy still fails in > 20% of the cases. Both rifabutin and levofloxacin have been suggested to be effective in these refractory cases. Our aim was to compare two rescue regimens based on these antibiotics in patients with two consecutive eradication failures.
Methods. Patients in whom a first treatment with omeprazole-clarithromycin-amoxicillin and a second trial with omeprazole-bismuth-tetracycline-metronidazole (or ranitidine bismuth citrate with these antibiotics) had failed, received 10 day treatment with either rifabutin (150 mg b.i.d.) or levofloxacin (500 mg b.i.d.), plus amoxicillin (1 g b.i.d.), and omeprazole (20 mg b.i.d.). Cure rates were evaluated by ^{13}C-urea breath test.
Results. Forty patients were included (mean age, 56 years, 36% men; 19% peptic ulcer, and 81% functional dyspepsia): 20 received rifabutin and 20 levofloxacin. All the patients returned for follow-up. Compliance in the rifabutin group was 100%. Four patients in the levofloxacin group did not correctly take the medication (in two cases as a result of adverse effects: myalgias and rash). Side effects in the rifabutin and levofloxacin groups were reported in 60% and 50% of the cases. Five patients (25%) treated with rifabutin presented leucopenia, and six patients (30%) treated with levofloxacin presented myalgias. Per-protocol cure rates were 45% (95%CI, 26–66%) in the rifabutin group, and 85% (64–95%) in the levofloxacin group ($p < .01$). Intention-to-treat cure rates were, respectively, 45% (26–66%) and 81% (57–93%) ($p < .05$).
Conclusions. After two previous *Helicobacter pylori* eradication failures, 10-day triple levofloxacin-based rescue regimen is more effective than the same regimen with rifabutin.

Abstract no.: 11.02
Levofloxacin/Azithromycin-Based Triple Therapies as First-Line Treatment for *Helicobacter pylori* Eradication

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Background. The failure of anti-*Helicobacter pylori* therapies is the result of antibiotic resistance. Levofloxacin and azithromycin are administrable in single daily dose and could increase patients compliance. The aim of the study is to compare the efficacy of a 3- and 7-day levofloxacin/azithromycin-based regimen against standard therapy.
Material and Methods. Ninenty *H. pylori*-positive patients were randomized to receive: group A (30 patients), levofloxacin, azithromycin, and esomeprazole for 3 days; group B (30 patients), levofloxacin, azithromycin, and esomeprazole for 7 days; group C (30 patients) clarythromicin, amoxicillin, and esomeprazole. *H. pylori* status was rechecked by ^{13}C-UBT 6 weeks after end of therapies.
Results. *H. pylori* eradication rate in group A was 86.7% (26/30 patients), 93.3% (28/30 patients) in group B, 70% (21/30) in group C. Eradication rate of 7-day levofloxacin/azithromycin-based triple therapy was significantly higher than that observed using standard triple therapy (93.3% versus 70%; $p < .05$). A trend, even if not statistically significant in higher eradication rate, was observed using 3-day levofloxacin/azithromycin-based triple therapy compared to standard therapy (86.7% versus 70% $p = .06$). Incidence of side effects was lower in groups A and B than in group C. Moreover, prevalence of side effects resulted higher in the group B than in group A.
Conclusions. According to the present data, a 7-day levofloxacin/azithromycin-based triple therapy may be considered a highly effective therapy for *H. pylori* eradication. Interestingly, a short course of treatment antibiotics (3-day levofloxacin/azithromycin) may be suggested to patients with high incidence of side effect instead of standard treatment.

Abstract no.: 11.03
Ranitidine Bismuth Citrate- Versus Levofloxacin-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, levofloxacin has remarkable activity in vitro against *H. pylori*. Our aim was to compare, by a randomized trial, two different 7-day triple rescue regimens based on RBC or levofloxacin.
Methods. Patients in whom a first eradication trial with omeprazole-clarithromycin-amoxicillin had failed were randomized, in this single-centre 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) levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.), and omeprazole (20 mg b.i.d.). Cure rates were evaluated by ^{13}C-urea breath test.
Results. One hundred patients were included (mean age, 47 years, 34% men; 18% peptic ulcer, and 82% functional dyspepsia); 50 received the RBC regimen, and 50 the levofloxacin one. Groups were comparable in terms of demographic variables. Two percent of the patients (one in each group) did not return for follow-up. Compliance was similar in both groups (90% took correctly all the medications). Side effects (only mild/moderate) in the two groups were also comparable (38% with the RBC regimen and 36% with the levofloxacin). Per-protocol cure rates were 69% (95% CI, 54–80%) in the RBC group and 71% (57–82%) in the levofloxacin group. Intention-to-treat cure rates were, respectively, 68% (59–79%) and 68% (59–79%) (nonstatistically significant differences).
Conclusions. Both 7-day RBC- and levofloxacin-based rescue regimens represent effective alternatives to quadruple therapy in patients with omeprazole-clarithromycin-amoxicillin failure.

Abstract no.: 11.04
An Updated Meta-Analysis of Different Duration of First-Line, Clarithromycin-Based Triple Therapy for *Helicobacter pylori* Infection

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Aim. To evaluate the efficacy of first line, clarithromycin-based triple therapy of different length for treating *Helicobacter pylori* infection by an updated meta-analysis of comparative trials.
Methods. Computer-assisted and manual bibliographical searches were performed through May 2006. Randomized controlled trials comparing different length of identical triple therapies were included. Study quality was assessed using the Jadad scale. Meta-analysis was performed combining the risk ratio (RR) of the individual studies.
Results. Twenty-four RCTs were included in the analysis: 10 trials (six high-quality trials) comparing 7 versus 10 days (1098 versus 1064 patients); 16 trials (five high-quality trials) comparing 7 versus 14 days (1416 versus 1386 patients). The majority part of the studies was performed in Europe (16), then in Asia (4), North America (3), and Africa. The meta-analysis showed superiority of prolongation the duration of therapy: 7 versus 10 days relative RR of 0.95 (95% CI = 0.91–0.99) with an NNT of 28; 7 versus 14 days relative RR of 0.91 (0.88–0.95) with a NNT of 14. This difference reached, in both cases (7 versus 10; 7 versus 14), statistical significance. A meta-analysis performed considering only high-quality studies showed similar efficacy between different length of therapy: 7 versus 10 days relative RR of 0.95 (95% CI: 0.90–1.00); 7 versus 14 days relative RR of 0.98 (0.92–1.04), lacking statistical significant difference.
Conclusion. Increasing the length of first-line, clarithromycin-based triple therapies beyond 7 days does not improve treatment efficacy when only high-quality trials are considered into meta-analyses. More high-quality studies, especially from developing countries, are needed.

Abstract no.: 11.05
Eradication of *Helicobacter pylori* Infection with Two Triple-Therapy Regimes of 7, 10, and 14 days; Four Years Experience

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Aim. The efficacy of the most frequently used triple-therapy regimes (pantoprazole 40 mg, amoxicillin 1 g, metronidazole 500 mg, or clarithromycin 500 mg b.i.d.) of 7, 10, and 14 days duration, was investigated in 596 Croatian patients with gastric (GU), duodenal ulcer (DU), and nonulcer dyspepsia (NUD), treated from January 2002 until December 2005.
Methods. One hundred seventy-two GU (M/F 100/72), 282 DU (M/F 176/106), and 138 NUD (M/F 59/79), *Helicobacter pylori*-positive patients, underwent endoscopy with histology and culture at the beginning and 4–8 weeks after the end of the treatment. They were randomly assigned to six treatments groups: PAM groups, A for 14 days (n = 58), B for 10 days (n = 118), C for 7 days (n = 122); PAC groups, D for 14 days (n = 58), E for 10 days (n = 118), F for 7 days (n = 122). Five hundred sixty patients (94%) completed the study.
Results. The results of *H. pylori* eradications are presented in Table 1.
Conclusion. In all groups (7, 10, 14 days), the triple-therapy containing clarithromycin had greater *H. pylori* eradication rate than that containing metronidazole. The eradication rate exceeding 80% in ITT and 90% in PP calculation was achieved only by 14 and 10 days of PAC and only by 14 days of PAM. No statistical differences were found among all six groups in ulcer-healing rate, and clinical improvement rate was slightly higher in patients with ulcers than in patients with nonulcer dyspepsia. (*$p < .05$ was found between A and C groups, and between D and F groups).

	Eradication rate (ITT)	Eradication rate (PP)
A	55/58 (95%)*	55/56 (98%)*
B	98/119 (83%)	98/109 (90%)
C	92/122 (75%)*	92/113 (81%)*
D	54/58 (93%)*	54/57 (95%)*
E	93/118 (79%)	93/112 (83%)
F	90/122 (74%)	90/113 (80%)*

Abstract no.: 11.06
Impact of *Helicobacter pylori* Eradication Regimen Tailored for Clarithromycin Susceptibility in Japan

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Background. Decrease in eradication rate of proton pump inhibitor (PPI)/AC therapy for *Helicobacter pylori* was recognized. It is mainly induced by increase in clarithromycin (CAM)-resistant *H. pylori*. It is reported that PPI+AMPC+MNZ (PPI/AM) therapy have high eradication rate for CAM-resistant *H. pylori*. We investigated the usefulness of the regimens tailored for CAM-susceptibility using human feces.
Method. Fifty-four *H. pylori*-positive patients were recruited. We divided two groups. In one group, patients received PPI/AC (LPZ 60 mg + AMPC 1500 mg + CAM 800 mg 1 week) regimen without investigation into CAM susceptibility before treatment (control group). In another group, patients received PPI/AC regimen for CAM susceptibility (S), or PPI/AM regimen (RPZ 20 mg + AMPC 1500 mg + MNZ 500 mg 1 week) for CAM resistance (R) with investigation into CAM susceptibility before treatment (tailored group). CAM susceptibility test was conducted using patient feces by restriction fragment-length polymorphism–nested polymerase chain reaction (Rimbara E, et al. *Curr Microbiol* 2005; 51: 1–5).
Result. Eradication rates (ITT, intention to treat) were 92.6% and 66.7% in the tailored group and control group, respectively, with this difference being significant. Moreover, eradication rates were 90.0% and 94.1% for the PPI/AC regimen for CAM-S and PPI/AM regimen for CAM-R in tailored group, respectively, with no significant difference
Conclusion. Tailored *H. pylori*-eradication therapy for CAM-susceptibility is very useful regimen in recently Japan with CAM resistance rate more than 20%.

Abstract no.: 11.07
Third-line Rescue Therapy with Levofloxacin after Two *Helicobacter pylori* Treatment Failures

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Aim. Eradication therapy with proton pump inhibitor, clarithromycin, and amoxicillin 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 third-line levofloxacin-based regimen in patients with two consecutive *Helicobacter pylori* eradication failures.
Methods. Design: Prospective multicenter study. Patients: In whom a first treatment with omeprazole-clarithromycin-amoxicillin and a second with omeprazole-bismuth-tetracycline-metronidazole (or ranitidine bismuth citrate with these antibiotics) 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. Outcome: Eradication was confirmed with ^{13}C-urea breath test 4–8 weeks after therapy.
Results. One hundred patients were initially included, and nine were lost for follow-up. All patients but five took all the medications correctly. Per-protocol and intention-to-treat eradication rates were 66% (95% CI = 56–75%) and 60% (50–70%). Adverse effects were reported in 25% of the patients, mainly including metallic taste (8%), nausea (8%), myalgia/arthralgia (5%), and diarrhea (4%); none of them were severe.
Conclusion. Levofloxacin-based rescue therapy constitutes an encouraging empirical third-line strategy after multiple previous *H. pylori* eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole, and tetracycline.

Abstract no.: 11.08
Reinfection of *Helicobacter pylori* in Type 1 Young Diabetic Patients: A Long-Term Follow-Up Study

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Introduction. Several studies have demonstrated that *Helicobacter pylori* eradication does not affect metabolic control in type 1 diabetic patients. The prevalence of *H. pylori* infection in diabetic patients seems to be higher than in general population and adult patients with diabetes present higher re-infection rates than dyspeptic patients. Aims of our study were to evaluate a long-term *H. pylori* re-infection rate after having performed the eradicating protocol in a group of young diabetic patients.
Methods. We enrolled 75 patients affected by type 1 diabetes and 99 healthy controls in which we had evaluated *H. pylori* infection prevalence and performed an eradicating therapy in *H. pylori*-positive patients. In all recruited patients we have re-investigated *H. pylori* presence by means of ^{13}C-urea breath test and metabolic control through the evaluation of glycosylated hemoglobin A levels and daily insulin requirement.
Results. The re-infection rate was higher in patients with diabetes than in healthy controls of similar age, gender, and socioeconomic status (33.3% versus 4.5%; $p < .05$). *H. pylori* infection appeared to be related to socioeconomic factors evaluated by means of annual income. Metabolic control was not affected by *H. pylori* status.
Conclusion. No association has been found between *H. pylori* gastric infection and type 1 diabetes mellitus; young diabetic patients are at higher risk to present a re-infection if compared with healthy controls. The test-and-treat strategy does not appear useful in these patients and *H. pylori* eradication should be taken into account case by case by the physician.

Abstract no.: 11.09
Levofloxacin-based Rescue Regimens after *Helicobacter pylori* Treatment Failure: Systematic Review and Meta-Analysis

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Background. A quadruple therapy has been generally recommended as rescue regimen for *Helicobacter pylori* eradication failures.
Objective. To systematically review the efficacy and tolerance of levofloxacin-based rescue regimen and to conduct a meta-analysis of studies comparing it with the quadruple therapy for eradication failures.
Methods. Selection of studies: Levofloxacin-based rescue regimens. For the meta-analysis, randomized controlled trials comparing levofloxacin-based and quadruple regimens were selected. Search strategy: Electronic and manual bibliographic searches. Study quality: Independently assessed by two reviewers. Data synthesis: "Intention-to-treat" *H. pylori* eradication rate.
Results. Mean eradication rate with levofloxacin was 80%. Ten-day regimens were more effective than 7-day combinations (81% versus 73%; $p < .01$). The meta-analysis showed better results with levofloxacin than with the quadruple combination (81% versus 70%; OR = 1.80; 95% CI = 0.94–3.46). This difference reached statistical significance, and heterogeneity markedly decreased when a single outlier study was excluded or when only high-quality studies were considered. Incidence of adverse effects, and severe adverse effects in particular with levofloxacin was 18% and 3%, respectively. Levofloxacin had less adverse effects (19% versus 44%; OR = 0.27; 95% CI = 0.16–0.46) and less severe adverse effects (0.8% versus 8.4%; OR = 0.20; 95% CI = 0.06–0.67) than the quadruple regimen.
Conclusion. After *H. pylori* eradication failure, levofloxacin-based rescue regimen is more effective and better tolerated than the generally recommended quadruple therapy. A 10-day combination of levofloxacin-amoxicillin proton pump inhibitor constitutes an encouraging second-line alternative.

Abstract no.: 11.10
Clinical Application of He-Ne Laser for Eradication of *Helicobacter pylori*

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The irradiation of low-power laser is followed by significant reduction of the bacterial viability. Resistance of *Helicobacter pylori* to metronidazole promotes enhanced sensibility to laser irradiation. The purpose of the study was to eradicate *H. pylori* by the laser irradiation after failure of standard drug therapies and in patients with allergy to antibiotics.
Methods. Thirty patients with proven *H. pylori* infection were selected for photodynamic therapy. Fifteen of them had clinical improvement after courses of triple and/or quadruple therapies, but had not eradication of *H. pylori*. Ten patients had not completed triple therapy because of allergy to antibiotics. Methylene blue was given to patients as photosensitizer an hour before endoscopy. The quartz light conductor was put through biopsy channel of the endoscope. Gastric mucosa was irradiated by He-Ne laser ($\lambda = 633$ nm) with 25 megawatts output power during 10–15 minutes. The course consisted of three procedures every other day. Gastric biopsy samples from each patient were sent for culture and histology.
Results. The metronidazole-resistant strains were found in 16 patients (53.3%). The laser irradiation does not alter gastric epithelial cells. All patients completed photodynamic therapy and underwent control endoscopy after 4 weeks. The therapy success was confirmed in 27 patients by histology and the rapid urease test.
Conclusion. The photodynamic therapy with He-Ne laser may be used as alternative therapy for *H. pylori* eradication in patients with allergy to antibiotics and after standard treatment failures.

Abstract no.: 11.11
Novel Approach to Eradication of *Helicobacter pylori*: Carbonic Anhydrase Inhibition Interferes with Acid Acclimatization, Studies In Vitro and In Vivo

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As *Helicobacter pylori* increasingly acquire resistance to antibiotics new eradication treatments are needed. Periplasmic alpha-carbonic anhydrase plays an important role in enabling acclimatization of *H. pylori* to an acidic milieu, prompting the question of targeting this mechanism with selective eradication therapy. This study aimed to determine the ability and conditions of Diamox, a carbonic anhydrase inhibitor, to interfere with survival of *H. pylori* and the feasibility of eradication treatment. In vitro methods included exposure of the wild type and an α-carbonic anhydrase knockout *H. pylori* organism to Diamox and assessment of survival. For in vivo studies, four groups of Mongolian gerbils were infected with *H. pylori;* group I was treated IP once daily with Diamox 50 mg/kg for 7 days, group II received Diamox 50 mg/kg by gavage once daily for 5 days, and two infected groups served as controls. In vitro Diamox impaired survival of *H. pylori* at pH = 2.0–2.5 by approximately 1–2 log scale depending on experimental conditions. Gerbils treated with Diamox IP showed reduced bacterial scores in antrum, fundus, and cardia and delayed positive CLO tests for *H. pylori,* which suggest compromised acid acclimatization of the organism. Eradication rate for this treatment regimen was low 12.5% (2 out of 16 gerbils). However, gerbils treated with Diamox by oral gavage had > 90% reduction in colony-forming unit of *H. pylori* recovered from the stomachs, suggesting effective eradication for this route of drug administration. In conclusion, the acidic milieu facilitates the antimicrobial properties of a carbonic anhydrase inhibitor for *H. pylori* offering opportunity for developing new eradication regimens.

Abstract no.: 11.12
The Effect of Novel and Current *Helicobacter pylori* Eradication Regimes on Gastric Emptying

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Background. Although antibiotic therapy is the first line of therapy for *Helicobacter pylori,* there is a global need for new therapies. However, the potential effect of current and new treatments on gastric motility is unknown.
Aim. To assess the effect of infection and treatment by current and novel regimes on gastric motility in *H. pylori* infected mice.
Methods. Fasted mice (n = 15) were gavaged 0.1 mL nutrient solution (Intralipid) containing 1 μL/mL ^{13}C-octanoic acid for the assessment of gastric emptying (GE). Breath samples were collected at intervals before and after ingestion of the solution and analyzed for $^{13}CO_2$ content. Gastric half emptying times ($t^1/_2$) were calculated from the resulting $^{13}CO_2$ excretion curves. The GE breath test was performed at pre-infection, 4 weeks after *H. pylori* infection and after 14 days of treatment. Treatment groups included amoxycillin, metronidazole, hyperimmune bovine colostrum (HBC)/neoadjuvant chemotherapy (NAC), HBC/NAC + amoxycillin, HBC/NAC + metronidazole, and triple therapy (amoxycillin, metronidazole, omeprazole).
Results. *H. pylori* infection did not alter GE. However, after 14 days of treatment, all treatments except metronidazole and HBC/NAC + metronidazole significantly slowed gastric emptying. (Table 1)
Conclusions. Four-week *H. pylori* infection does not affect gastric motility in mice. However, 14-day treatment does have an impact on GE. Further studies should assess this effect in patients and determine if the effect persists after cessation of *H. pylori* eradication therapy.

Table 1.

	Gastric half emptying times ($t^1/_2$) (min) median [IQR]		
Treatments	Pre-infection	4 weeks infection	Post-treatment
Amoxycillin	26 [20, 30]	27 [22, 31]	40 [36, 51]†‡
Metronidazole	27 [22, 30]	28 [23, 33]	36 [27, 40]
HBC/NAC	33 [30, 44]	29 [26, 33]	49 [39, 67]†‡
HBC/NAC+amox	27 [25, 32]	25 [23, 31]	36 [30, 42]†‡
HBC/NAC+metro	31 [24, 37]	38 [33, 43]	33 [28, 40]
Triple Therapy	27 [23, 35]	34 [33, 43]	55 [47, 65]†‡
Not infected	27 [18, 32]	30 [25, 38]	40 [34, 47]†

†$p < .05$ compared to pre-infection, ‡$p < .05$ compared to 4 weeks infection.

Abstract no.: 11.13
The Efficacy of One-week Low-dose Triple Therapy Containing Pantoprazole (40 mg b.i.d.), Amoxicillin (750 mg b.i.d.) and Clarithromycin (250 mg b.i.d.) for *Helicobacter pylori* Eradication in Korea

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Background/Aims. The recommended regimen of *Helicobacter pylori* eradication in Japan is standard dose proton pump inhibitor (PPI) b.i.d. + amoxicillin 750 mg b.i.d. + clarithromycin 200–400 mg b.i.d. for 7 days. In Korea, the recommended regimen of *H. pylori* eradication is standard-dose PPI b.i.d. + amoxicillin 1000 mg b.i.d. + clarithromycin 500 mg b.i.d. for 7 days. We could expect patients' good compliance, decrease of drug side effects, and cost reduction by using low-dose therapy. But the efficacy of low-dose therapy is questionable in Korea. In this study, we compared the efficacy of low-dose therapy with standard-dose therapy.
Methods. Four hundred eighty patients who visited Seoul National Unversity Bundang Hospital during January 2005 to April 2006 with documented *H. pylori* infection were enrolled. Seven patients were excluded because of malignancy and drug history. One hundred eighty-two patients received low-dose triple therapy (pantoprazole 40 mg b.i.d. + amoxicillin 750 mg b.i.d. + clarithromycin 250 mg b.i.d.) and 291 patients received standard-dose triple therapy (pantoprazole 40 mg b.i.d. + amoxicillin 1000 mg b.i.d. + clarithromycin 500 mg b.i.d.). Eradication was confirmed by UBT 1 month after eradication.
Results. The two groups were similar with regard to all clinical characteristics. The *H. pylori* eradication rates was 74.2% (135/182) in low-dose triple therapy group, and was 77.3% (225/291) in standard-dose triple therapy group. There was no significant difference of *H. pylori* eradication rates between these two groups ($p = .435$). There was no serious side effect in both group.
Conclusions. The efficacy of low-dose triple therapy is similar to standard-dose triple therapy. These findings suggest that low-dose triple therapy could be another effective regimen considering cost benefit in Korea.

Abstract no.: 11.14
Comparison of Two and Four Times a Day Amoxicillin with Proton Pump Inhibitor, Clarithromycin for *Helicobacter pylori* Infection: A Randomized Study

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Background. A proton pump inhibitor (PPI)-based triple therapy with clarithromycin and amoxicillin is now a standard regimen for *Helicobacter pylori* eradication therapy. Most *H. pylori* are

susceptible to amoxicillin, an important component of many combination therapies for *H. pylori* eradication. Amoxicillin has time-dependent bactericidal activity for against *H. pylori.*

Aim. To evaluate and compare the efficacy of two and four times daily amoxicillin regimens for treatment of *H. pylori* in a randomized study.

Methods. One hundred sixty-three patients with peptic ulcer and *H. pylori* infection confirmed by endoscopy and histology were eligible to this study. *H. pylori* infection was proved by histology or rapid urease test. Patients randomly assigned to one of the two regimens: amoxicillin 1.0 g b.i.d. (group A, n = 90) or amoxicillin 500 mg q.i.d. (group B, n = 73) with clarithromycin 500 mg b.i.d. and omeprazol 20 mg b.i.d. for 2 weeks. All patients were asked to return at the end of treatment to access compliance and adverse events. The eradication rates of *H. pylori* were evaluated by repeated endoscopy or ^{13}C-urea-breath test 4 weeks after completion of treatment.

Results. One hundred fifty-four patients completed the trial (86 group A, 68 group B). The eradication rates were 91.1% in group A, 89.0% in group B ($p > .05$). Compliances were fairly good in both groups. Side effects in two groups were generally mild and nine discontinued treatment because of adverse effects.

Conclusion. Both the two and the four times daily amoxicillin regimens are equally effective and safe for *H. pylori* eradication therapies.

Abstract no.: 11.15
Food and Nutrient Intakes in Functional Dyspepsia Before and After *Helicobacter pylori* Eradication.

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Background. In spite of the fact that functional dyspepsia is a common disease, the number of studies evaluating nutritional patterns in those patients is very limited. The dietary patterns before after *Helicobacter pylori* eradication were never studied. The aim of the study was to evaluate nutritional habits in the group of patients with functional dyspepsia and to test if *H. pylori* eradication changes them.

Material and Methods. Fifty-four patients with functional dyspepsia and present *H. pylori* infection were submitted to the study. All of them underwent *H. pylori* eradication.

The 3-day diet history was used to obtain dietary assessment. A dietary questionnaire was filled two times: before eradication treatment and about 1 month (4–6 weeks) after finishing the treatment.

Results. The patients' diet does not correspond to dietary recommendations. Low energy intake, carbohydrate, fiber, vitamins (thiamine, riboflavin, vitamin B6, folic acid), minerals (calcium, potassium, iron, zinc, and copper), and high fat consumption were found. No significant dietary changes after successful *H. pylori* eradication treatment were found. The only exception was a certain drop in polyunsaturated fatty acids.

Summary. Diet of patients with functional dyspepsia is a nutritionally imbalance diet that cannot respond to many nutritional recommendations.

After successful *H. pylori* eradication, no specific dietary changes were found. *H. pylori* eradication has got no influence on dietary habits in the group of dyspeptic patients.

Abstract no.: 11.16
Helicobacter pylori First-Line Treatment and Rescue Options in Patients Allergic to Penicillin

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Aim. To assess the efficacy and tolerability of *Helicobacter pylori* first-line treatment and rescue options in patients allergic to penicillin.

Methods. Patients: Prospective single-center study including 40 consecutive treatments administered to patients allergic to penicillin. Therapy regimens: First-line (12 patients): omeprazole, clarithromycin, and metronidazole for 7 days. Second-line (17 patients): ranitidine bismuth citrate, tetracycline, and metronidazole for 7 days. Third-line (9 patients): rifabutin, clarithromycin, and omeprazole for 10 days. Fourth-line (2 patients): levofloxacin, clarithromycin, and omeprazole for 10 days. Outcome variable: a negative ^{13}C-urea breath test 8 weeks after completion of treatment.

Results. Per-protocol/intention-to-treat eradication rates were first-line regimen, 64%/58%; second-line regimen (ranitidine-bismuth-citrate), 53%/47%; third-line regimen (rifabutin), 17%/11%; fourth-line regimen (levofloxacin), 100%/100%. Compliance with treatment was generally good, except with the rifabutin-based regimen, which presented adverse effects in 89% of the patients, including four cases of myelotoxicity.

Conclusion. *H. pylori*-infected patients allergic to penicillin may be treated with a first-line treatment combining PPI, clarithromycin, and metronidazole. Rescue options may include a regimen with ranitidine bismuth citrate, tetracycline, and metronidazole. A levofloxacin-based rescue regimen (with PPI and clarithromycin) may also represent an alternative, even when two or more consecutive eradication treatments have previously failed. However, rifabutin-clarithromycin-PPI regimen is ineffective and poorly tolerated.

Abstract no.: 11.17
The Prediction of Ulcer/Erosion Relapse after *Helicobacter pylori* Eradication: a One-Year Follow-up Study

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We estimated the long-term effects of successful *Helicobacter pylori* eradication on relapse of ulcers/erosions in duodenal ulcer patients. One hundred eight adult patients (71 men, 37 women, 16–75 years) were enrolled in the study. The endoscopy, rapid urease test (RUT), histology of gastric mucosa specimens according to the updated Sydney system standards were performed for determination the *H. pylori* status and healing of mucosa defects before treatment and at 4–6 weeks and 1 year after usual triple therapy.

The *H. pylori* eradication rate was 90.7% at 4–6 weeks after triple therapy; however, complete healing of ulcer and concurrent gastroduodenal erosions was in 67.5% cases. In a 1-year

prospective study 41 (41.8%), patients were free from *H. pylori* infection, among them were 13 (31.7%) cases of relapse of ulcers/erosions, predominantly without clinical symptoms. The higher grade of atrophy of fundal mucosa before treatment, the presence of residual ulcers or erosions, higher grade of antral mucosa infiltration by mononuclear cells, and lower grade of fundal mucosa infiltration by polymorphonuclear cells at 4–6 weeks after *H. pylori* eradication were associated with the presence of ulcers or erosions in the 1-year follow-up after *H. pylori* eradication ($p < .05$, Fisher exact test for contingency tables, Mann–Whitney U-test for semiquantitative characteristics). There were marginally significant association of disease duration > 5 years with relapse of gastroduodenal mucosal defects 1 year later ($p = .095$).

Recognized predictor factors witness the high risk of silent ulcer recurrence after successful treatment of *H. pylori,* the group of high risk needed the prolonged treatment (PPI, mucoprotectors) and endoscopy controls.

Abstract no.: 11.18
Intragastric Balloon Tolerance is Independent of *Helicobacter pylori* Status in Patients with Morbid Obesity

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Endoscopic intragastric balloon consists a method for weight loss. However, most patients experience poor balloon tolerance. It has not studied if the presence of *Helicobacter pylori* infection is a factor affecting tolerability.

Thirty-three patients (median body mass index, 38.1), 23 women, 10 men, age range 20–65 years were studied. In all patients, an intragastric balloon (INAMED, USA) was inserted endoscopically and was filled up to a median volume of 550 mL. *H. pylori* status was confirmed during screening endoscopy (rapid urease test plus histology). Exclusion criteria were peptic ulcer, severe gastritis, or chronic nonsteroidal anti-inflammatory drug use. All patients were followed up daily in the first 7 days and monthly thereafter up to removal (6 months later) by a standard questionnaire. Patients were allowed to on-demand H_2-RA/PPI's and/or prokinetics. Nausea and/or vomiting and/or crampy epigastric pain were characterized as mild to moderate (nausea, vomiting < 10/day, duration < 10 days and/or pain without necessitating further management) or severe (intractable nausea and/or vomiting > 10/day, duration > 10 days and/or severe pain necessitating further management and/or premature removal of the balloon).

Fourtenn patients were *H. pylori* positive (42.4%) whereas 19 were negative (57.6%). All patients, independently to their *H. pylori* status, experienced mild to moderate symptoms with a mean duration of 2 days. Seven (21%) experienced severe symptoms requiring further management (three *H. pylori* positive, four *H. pylori* negative) and in four of them (12%) the balloon had to be removed within 1 month (three *H. pylori* negative, one *H. pylori* positive). These findings were not statistically significant, against or for, *H. pylori* status.

Therefore, *H. pylori* eradication is not justified prior to balloon insertion.

Abstract no.: 11.19
Use of Bovine Antibodies-Based Oral Immunotherapy for Eradication of *Helicobacter pylori* in a Placebo-Controlled Clinical Trial

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Antibiotics-based regimens are frequently used for treatment of *Helicobacter pylori* infections. However, predominantly resulting from antimicrobial resistance and poor patient compliance, antibiotic-based eradication fails in 15–40% of patients. Passive immunization against *H. pylori* with orally administered bovine antibodies was successful in animal studies, and may thus serve as an alternative therapy in humans. In this study, its potential is investigated in a clinical trial.

Polyclonal antibodies (sIgA) were raised in milk of dairy cows by nasal and supra-mammary lymph node immunizations during lactation. Cows were immunized with a mix of clinical *H. pylori* isolates. Specific anti-*H. pylori* sIgA milk titers were measured by enzyme-linked immunosorbent assay. The milk was processed into a whey protein concentrate (WPC). These preparations were first tested for their ability to reduce adhesion of *H. pylori* to gastric biopsies.

To study the efficacy and safety of this WPC product, a double-blind, placebo-controlled randomized clinical trial was designed. In this study, 15 patients will be treated with the WPC product and 15 with placebo during 4 weeks. At this moment, 10 patients are included in this ongoing study. Preliminary observations showed no adverse effects of medication. The efficacy is evaluated as reduction in intragastric *H. pylori* colonization density as determined by UBT, histology, and culture. All outcome measures are evaluated on days 0 and 29 after start of treatment, and on day 56, UBT and blood tests are repeated.

Data from this study will contribute to the development of new therapeutic or preventive strategies for *H. pylori* infection, without the adverse effects of antibiotic treatment.

Abstract no.: 11.20
Triple Therapy for *Helicobacter pylori* Infection in Patients Presenting to a Tertiary Care Center in Pakistan

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Aim. To determine the eradication rate of *Helicobacter pylori* infection in patients presenting to a tertiary care center.
Methodology. Patients presenting with symptoms and having an endoscopy (EGD) were enrolled from January 2005 to March 2006. ^{14}C-urea breath test (^{14}C-UBT), rapid urease test (RUT), histopathology, culture, and sensitivity were performed. Antibiotic susceptibility was determined by disk diffusion test. Triple therapy with proton pump inhibitor 20 mg b.i.d., clarithromycin 500 mg b.i.d., and amoxicillin 1 g b.i.d. was prescribed for 10 days. Eradication of *H. pylori* infection was confirmed 4 weeks after therapy by ^{14}C-UBT.

Results. Of 80 patients, 56 (68%) were male, age range 15–76 years, and mean age 45 years. The presenting symptoms were abdominal pain in 54 (67%), vomiting 16 (20%), and nausea 10 (13%). EGD showed mucosal erythema 76 (95%) and duodenal ulcer in 4 (5%). Histopathology demonstrated that *H. pylori* associated moderate gastritis in 36 (45%) and mild gastritis in 44 (55%). Antibiotic susceptibility was determined in 45 patients 27 (60%) males. The positive cultures were from the antrum in 33 (73%) and body in 12 (27%). Fourteen (31%) were resistant to clarithromycin and three (7%) to amoxicillin. All patients completed treatment. Eighteen patients did not return for repeat ^{14}C-UBT to determine eradication status. Of the remaining 62 patients, 15 (24%) patients had a positive repeat ^{14}C-UBT.
Conclusion. There was a high resistance to clarithromycin. Triple therapy for 10 days was effective in two-third of the patients to eradicate *H. pylori*.

Abstract no.: 11.21
Role of Eradication of *Helicobacter pylori* Infection in the Treatment of Functional Dyspepsia

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Aim. To ascertain the effect of eradication of *Helicobacter pylori* infection on dyspeptic symptoms in patients with functional dyspepsia (FD).
Material and Methods. The study included 140 patients with a verified FD (according to the 1999 Rome Definition). Of 75 patients with *H. pylori* infection, eradication therapy was performed in 68 patients (90% eradication success: 7-day therapy with pantoprazole, amoxicillin, claritromycin), and 65 patients tested negatively for *H. pylori*. All patients received pantoprazole for 4 weeks. Severity of dyspepsia was evaluated with the Nepean Dyspepsia Index at baseline and after 1 month of treatment. Statistical analysis was performed using analysis of variance.
Results. All three groups of patients with FD (*H. pylori* positive eradicated, *H. pylori* positive noneradicated, and *H. pylori* negative) demonstrated statistically significant decrease in dyspeptic symptoms ($p < .001$). There was no statistically significant difference in decrease in dyspeptic symptoms between the groups ($p > .05$).
Conclusion. Eradication of *H. pylori* infection in patients with FD is not associated with a statistically significant decrease in dyspeptic symptoms; patients with eradicated and noneradicated *H. pylori* infection show a similar decrease. The presence of *H. pylori* infection has no statistically significant influence on decrease in dyspeptic symptoms in patients with FD, the patients with noneradicated *H. pylori* infection and *H. pylori*-negative patients report an approximately same decrease. Statistically significant symptoms decrease in all three groups of patients is the result of PPI therapy.

Abstract no.: 11.22
The Effectiveness of Bismuth and Eupatilin Along with Proton-Pump Inhibitor-Based Triple Regimen in Eradication of *Helicobacter pylori*

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Background. Tripotassium dicitrato bismuth (Denol®) is an oral bismuth agent used in quadruple regimen, and eupatilin (Stillen®) is the extract of *Artemisia asiatica Nakai*. Eupatilin shows anti-inflammatory and cytoprotective effect. We studied about adding bismuth and eupatilin in triple regimen and its additional effect in eradication of *Helicobacter pylori*.
Methods. This is a retrospective study about the eradication of *H. pylori* in Bundang Seoul National University Hospital between March 2005 and April 2006. *H. pylori* infection was confirmed by endoscopic biopsy and CLO test. The eradication was assessed by the ^{13}C-urea breath test at 4 weeks after the end of treatment.
Results. Five hundred sixty-three patients were included and total eradication rate is 76.7%. Two hundred ninety-six patients treated with 1-week triple therapy (pantoprazole 40 mg, amoxicillin 1000 mg, clarithromycin 500 mg, two times a day). Their eradication rate was 77.4%. One hundred eighty-four patients treated with lower-dose regimen (pantoprazole 40 mg, amoxicillin 750 mg, clarithromycin 250 mg, two times a day). This is a standard regimen in Japan. Their eradication rate was 74.5%. Between the two regimens, statistical significance did not exist ($p = .536$). Forty-seven patients treated with adding bismuth in lower-dose regimen. Their eradication rate was 74.5%. Thirty-six patients treated with adding eupatilin. Their eradication rate was 86.1%. The eradication rate is higher, but there was no statistical significance ($p = .132$)
Conclusion. Considering cost benefit and medication number, lower-dose regimen (by Japanese guideline) is acceptable treatment of *H.r pylori* in Korea. Eupatilin is a promising agent in *H. pylori* eradication but more study will be needed.

Abstract no.: 11.23
Do Proton Pump Inhibitor (PPI) Therapy before *Helicobacter pylori* Eradication Influence on the Eradication Rate? A Preliminary and Clinical study

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Introduction. The main factors affecting the outcome of treatments for *Helicobacter pylori* infection were reported to be antibiotic resistance and patient compliance. The other factors were associated with age, gender, smoking, omeprazole, or H2 blocker pre-treatment. But it was not yet satisfactory reports

about influence on eradication rate of the proton pump inhibitor (PPI) therapy before *H. pylori* eradication. The aim of this study was to determine influence on eradication rate of PPI therapy.
Methods. From March 2004 to March 2006, Fifty-four patients with peptic ulcer including ulcer scar at endoscopy and positive result at the rapid urease test were enrolled. All infected patients were given PPI-based 7-day regimen (omeprazole 20 mg b.i.d. or lansoprazole 30 mg b.i.d., amoxicillin 1 g b.i.d., clarithromycin 500 mg b.i.d.). We included only patients with good drug compliance. Eradication was assessed by urease breath test at 4 weeks after therapy.
Result. Fourty-two male and 12 female (mean age, 46 ± 14.5) patients were enrolled. No pre-PPI group was 12 (22%) and pre-PPI therapy group was 42 (78%). *H. pylori* eradication rate of no pre-PPI group was 91% on ITT analysis and pre-PPI group was 89.5%. There was no statistical significance in the two groups ($p = .80$).
Conclusion. There was no significant difference in the *H. pylori* eradication rate between no pre-PPI group and pre-PPI group. We suggest that the PPI therapy before *H. pylori* eradication does not influence on the eradication rate. More lager, randomized controlled study was necessary.

Abstract no.: 11.24
A New Curcumin-Based One-Week Triple Therapy for Eradication of *Helicobacter pylori* Infection: Something to Learn from a Failure?

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Background. Curcumin is the principal element of turmeric powder extract from *Curcuma longa*. Some studies showed actions of curcumin against *Helicobacter pylori* infection. N-acetylcystein and lactoferrin with mucolytic and antibacterial activities respectively, could play important roles in *H. pylori* eradication therapy.
Aim. To determine if a 7-day nonantibiotic therapy based on curcumin, lactoferrin, N-acetylcystein, and pantoprazole is effective on: (1) *H. pylori* eradication; (2) gastric inflammation assessed by means of serum pepsinogens; (3) symptoms relief.
Materials and Methods. Twenty-five consecutive *H. pylori*-positive patients (12 men, mean age 50 ± 12 years, range 31–76) with functional dyspepsia were enrolled. Patients were administered for 7 days: curcumin 30 mg b.i.d., bovine lactoferrin 100 mg b.i.d., N-acetylcystein 600 mg b.i.d., pantoprazole 20 mg b.i.d. *H. pylori* status and upper GI symptoms were assessed and scored by means of ^{13}C-urea breath test and a Likert scale (absent, mild, moderate, and severe) at baseline (T0) and after 2 months (T1), as well as two blood tests (at T0 and T1) for serum pepsinogens (sPGI, sPGII), gastrin-17 (G-17), and anti-*Helicobacter* IgG (IgG-*H. pylori*) were performed.
Results. Three out of 25 patients (12%) were cured from *H. pylori* infection. There was a significant decrease in the overall symptoms severity (T0: 6 ± 3; T1: 3 ± 2, $p < .001$), in sPGI (T0: 80 ± 26; T1: 74 ± 26, $p = .02$), and sPGII levels (T0: 19 ± 10; T1: 12 ± 7, $p < .001$). IgG and G-17 values did not significantly decrease after 2 months.
Conclusions. This novel therapy is not effective on *H. pylori* eradication, but despite bacterium persistence seems to improve dyspeptic symptoms as well as gastric inflammation.

Abstract no.: 11.25
Brazilian Green Propolis on *Helicobacter pylori* Infection. A Pilot Clinical Study

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Background. There is an increasing search for nonantibiotic-based anti-*Helicobacter pylori* therapy. Recent in vitro studies suggest that propolis and its phenolic components are able to inhibit *H. pylori* growth. There are no clinical studies by now.
Aims. A pilot study to evaluate the effect of Brazilian green propolis on *H. pylori*-infected individuals.
Patients and Methods. After informed consent, 11 (six women, mean age 45 years) participants naive of previous anti-*H. pylori* treatment were included. Before treatment, all participants were submitted to gastroscopy, and *H. pylori* infection were confirmed by histology, urease test, and ^{13}C-urea breath test (UBT) (IRIS, Wagner Analysen-Technik, Germany). Participants with UBT showing a delta over baseline (DOB) value higher than 4‰ were considered positive for *H. pylori* infection. Twenty drops from an alcoholic preparation of Brazilian green propolis (FUNED, Brazil) were administered three times a day for 7 days. Clinical evaluation and UBT were performed at 1–3 days and at 40 days after therapy to evaluate *H. pylori* suppression or eradication, respectively.
Results. All participants took all medication and completed the study. Only two participants referred mild nausea with the medication. One out 11 participants reached partial suppression immediately after therapy and another participant eradicated *H. pylori* infection 40 days after treatment.
Conclusions. Brazilian green propolis administered at popularly used dosis showed minimal effect on suppression or eradication of *H. pylori* infection. Further studies using larger dosis and longer duration are needed to define an eventual role of Brazilian green propolis on *H. pylori* therapy.

Abstract no.: 11.26
Efficacy of Esomeprazole and Rabeprazole for *Helicobacter pylori* Eradication in Patients with Peptic Ulcer

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Background/Aims. Esomeprazole, a new proton pump inhibitor that is the S-isomer of omeprazole and produces a greater inhibition of acid secretion than omeprazole, has recently been evaluated in the treatment of *Helicobacter pylori*. However, the clinical efficacy of esomeprazole-based triple therapy for Korean patients is not well known. Thus, we assessed the efficacy of esomeprazole-based triple therapy with *H. pylori* active peptic ulcer.
Methods. Four hundred twenty-six patients (300 men, 126 women) were enrolled retrospectively to receive either regimen EAC (esomeprazole 40 mg, clarithromycin 500 mg, amoxicilin 1 g, all twice daily) or RAC (rabeprazole 20 mg, clarithromycin 500 mg, amoxicilin 1 g, all twice daily) for 1 week. *H. pylori* infection was confirmed by histology (Giemsa stain) after endoscopic biopsy. *H. pylori* eradication rate was determined by urea breath test 4–6 weeks after completion of the treatment.
Result. The overall eradication rate was 76.8% (327/426). *H. pylori* eradication rate was 78.1% (178/228) in the EAC group and 75.3% (149/198) in the RAC group.
Conclusions. Esomeprazole-based triple therapy is effective for the eradication of *H. pylori* infection and offers comparable efficacy to rabeprazole-based therapy.

Abstract no.: 11.27
Norfloxacin-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. *Helicobacter pylori* infection results in an acute, then chronic, inflammation of the gastric mucosa. The inflammation regresses following antimicrobial treatment.

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.

Present work involved the synthesis of an organometallic complex of norfloxacin with bismuth. The previously mentioned complex was purified and characterized by various spectral techniques like ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), differential scanning calorimeter (DSC), and atomic absorption spectrototometry (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 (norfloxacin alone and also with the bismuth salt alone).

NSAIDs, COXIBs, ASA, and *Helicobacter pylori* Infection

Abstract no.: 12.01
Deregulation of SHP-2 Affecting Signal Transduction Switch by *Helicobacter pylori* Oncoprotein CagA

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Aims. *Helicobacter pylori* CagA is associated with gastric carcinoma. It has been shown that phosphorylated CagA binds SHP2 and affects signal transduction switch. In addition, it has been shown that gp130, IL6 cytokine coreceptor, having a bifunctional domain, leads to balanced signaling through SHP2/Erk and STAT1/3 pathways. Therefore, the aims of this study were to evaluate the effect of translocated CagA in the signal transduction switch of gp130 and the role of tyrosine phosphorylation status regarding signal talk between SHP2/Erk and STAT1/3 pathways.
Methods. We used a pair of naturally occurring *cagA* isogenic mutants 147C and 147A. CagA expression vectors with or without CagA tyrosine phosphorylation activities were used. We performed immunoprecipitation assay to assessed the interaction between CagA, SHP2, and/or gp130. We assessed activation of STAT3 or Erk pathways,effect of bax and bcl-2 expression according to *cagA* isogenic mutants.
Results. Phosphorylated CagA showed greater magnitude of SHP2 binding activity, and SHP2 was recruited to gp130.

Phosphorylated CagA induced greater phosphrylaton of Erk1/2, whereas the unphosphorylated showed preferential activation of STAT3. Pro-apoptotic molecule, Bax expression between Hp147A, and 147C infected AGS cells did not show significant difference, whereas bcl-2 expression was significantly decreased in Hp147C-infected AGS cells rather than Hp147A infected AGS cells.

Conclusions. We concluded that the translocated CagA could affect the signal switch between SHP2/Erk and STAT1/3 pathways possible thorough gp130 receptor. These results could be an experimental evidence of elucidating the mechanism explaining the role of *H. pylori* in ulcerogenesis and carcinogeneis in vivo.

Abstract no.: 12.02
Serum Pepsinogen II as A Marker of Both *Helicobacter pylori* and Acetyl Salicylic Acid-Related Gastritis

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Background. Both *Helicobacter pylori* and acetyl salicylic acid (ASA) are risk factors for peptic ulcer. Serum pepsinogen II (PGII) levels increase in the presence of *H. pylori*-related gastritis. A biomarker of severity of ASA gastritis should be useful to adopt considering the lack of symptoms in ASA induced mucosal injury.
Aim. To evaluate the effect of both ASA and *H. pylori* on PG II levels and gastric histology.
Methods. One hundred twenty-two consecutive dyspeptic patients (80 women, mean age: 64.1 years ± 12) of whom 52 with chronic assumption of low dose of ASA for prophylaxis of cardiovascular events (ASA positive) and 70 without assumption of ASA (ASA negative) were enrolled. Gastrointestinal endoscopy with biopsies and a blood sample for serological levels of PGII and antibodies IgG anti-*H. pylori* were performed.
Results. ASA-positive patients had significantly higher mean PGII levels (13.47 ± 4.5 SD μg/L) than ASA negative (8.35 ± 3.1 μg/L, $p < .001$), as well as *H. pylori* positive (13.41 ± 4.0 SD μg/L) in comparison to *H. pylori*-negative patients (8.30 ± 4.0 μg/L, $p < .001$). The significant highest mean value of PGII levels was found in ASA-positive and *H. pylori*-positive patients (15.96 μg/L). ASA-positive patients had significantly higher mean of severity chronic gastric inflammation both in corpus and antrum than ASA negative as well as considering *H. pylori* positive in comparison to *H. pylori*-negative patients.
Conclusion. Both ASA and *H. pylori* infection induce increased levels of sPGII. These factors seems to have an additional effects on serum PGII levels. High PGII levels could single out patients with high ASA induced gastropathy and mainly candidates for PPI gastroprotection.

Abstract no.: 12.03
Effect of Nitric Oxide (NO)-releasing Aspirin on *Helicobacter pylori* Infection in Mongolian Gerbils

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Both, aspirin (ASA) and nitric oxide-releasing aspirin (NO-ASA) inhibit cyclooxygenase and prostaglandin generation but their interaction with *Helicobacter pylori* infection remains unknown. We compared the effect of NO-ASA and ASA on gastric *H. pylori* infection (cagA+ vacA+, 5×10^6 colony-forming units/mL) in gerbils treated daily for the 5 weeks with (1) vehicle (saline); (2) ASA (50 mg/kg i.g.); and (3) NO-ASA (64 mg/kg i.g.). Area of gastric lesions, gastric blood flow (GBF), plasma level of gastrin, the malonyldialdehyde (MDA) concentration as an index of lipid peroxidation, and SOD acivity were determined. At 4, 12, 30 and 80 weeks upon gastric *H. pylori* inoculation, the morphological changes in glandular mucosa were assessed by histology and the density of *H. pylori* colonization was evaluated by counting of the number colonies per plate. By the end of study typical ademato us hyperplasia with atrophic gastritis and intestinal metaplasia and these effects were not significantly influenced by ASA but significantly attenuated by NO-ASA. Both ASA and NO-ASA inhibited growth of *H. pylori* in vitro in a dose-dependent manner. In *H. pylori*-infected gerbils ASA significantly increased the mucosal MDA content and produced a significant fall in the GBF and SOD activity but failed to influence plasma gastrin levels and these effects were markedly reduced by NO-ASA. We conclude that (1) NO-ASA attenuates the damage in *H. pylori*-infected gastric mucosa due bacteria growth inhibition and mucosal hyperemia mediated by NO that compensates for PG deficiency induced by ASA, and (2) *H. pylori* enhances lipid peroxidation and suppresses SOD activity and these effects are counteracted by NO released from NO-ASA.

Abstract no.: 12.04
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. Nonsteroidal aintiinflammatory drug (NSAID) use was not considered an exclusion criteria. *H. pylori* infection was confirmed by rapid urease test, histology or ^{13}C-urea breath test. Several therapies were used, mainly omeprazole or ranitidine-bismuth citrate-based regimens. Afterwards, an H_2-antagonist was administered until eradication was confirmed by ^{13}C-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 ^{13}C-urea breath test. NSAID use was not permitted during follow-up.
Results. Up to now, 291 patients have been followed up for at least 12 months, with a total of 517 patient years of follow-up. Mean age was 58 years, 73% men, and 37% were previous NSAID users. Seventy percent had duodenal ulcer, 24% gastric ulcer, and 6% pyloric ulcer. Recurrence of bleeding was demonstrated in two patients at 1 year (incidence: 0.39% per patient year of follow-up), which occurred after NSAID use in both cases.
Conclusion. Rebleeding does not occur in patients with complicated ulcers after *H. pylori* eradication. Maintenance anti-ulcer (antisecretory) therapy is not necessary if eradication is achieved. However, NSAID intake may cause rebleeding in *H. pylori*-eradicated patients.

Drug Resistance

Abstract no.: 13.01
Evaluation of the Novel *Helicobacter pylori* ClariRes Real-time PCR Assay for Detection and Clarithromycin Susceptibility Testing of *H. pylori* in Stool Specimens and Gastric Biopsies; Comparison with the Stool Antigen Test

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Helicobacter pylori ClariRes assay is a novel commercially available real-time polymerase chain reaction (PCR) test allowing for detection and clarithromycin susceptibility testing of *H. pylori*. This test was evaluated using gastric biopsies and in particular stool specimens.

Gastric biopsies and stool specimens of 108 symptomatic adults were examined by *H. pylori* ClariRes assay; stool specimes were analyzed also by the monoclonal antigen test Amplified-IDEIA-HpStAR. Furthermore, stool specimens of 214 symptomatic children were examined by both the PCR and the antigen test; in case of discrepancy, *H. pylori* status was determined by ^{13}C-UBT and serology.

Of the adult patients, 33 (30.56%) were infected and all were culture also positive. With respect to detection of *H. pylori*, the results of the real-time PCR were identical to those of the reference method in gastric biopsies. In stool specimens, values for sensitivity and specificity of the real-time PCR were 96.97% and 100%, respectively; those of the stool antigen test were 93.94% and 98.67%. Nineteen of the strains (57.6%) were resistant to clarithromycin by E-test; in one case PCR delivered a false sensitive result. Examination of the pediatric stool specimens by real-time PCR and antigen test, revealed discrepancy in 9 cases. One false negative result was shown by the PCR and two by the stool antigen test. However, six samples were false-positive by the latter resulting in a positive predictive value of 85%.

Thus, the novel *H. pylori* ClariRes assay showed excellent diagnostic accuracy and is superior to the stool antigen test in particular in pediatric patients.

Abstract no.: 13.02
Evidence for High-level Dual Resistance and the Emergence of Multi-resistant Strains of *Helicobacter pylori* in A Posteradication Patient Population in the UK

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Background. Resistance, particularly to metronidazole and to clarithromycin, is a key factor in failure of repeated *Helicobacter pylori* eradication therapy. Our specialist laboratory conducts sentinel surveillance to monitor antibiotic resistance in the pre-treatment population in the UK, and also provides a susceptibility testing service for "problem" cases from numerous referral centres across the UK.
Aims. To compare antibiotic resistance rates in *H. pylori* recovered from pretreatment populations and from document treatment failure patients.
Methods. Antibiotic susceptibilities to clarithromycin, metronidazole, tetracycline, and amoxicillin were determined by E-test and/or disk diffusion in *H. pylori* recovered pretreatment (n = 1210) and post-treatment (n = 113).
Results. In the pretreatment population, metronidazole and clarithromycin resistance accounted for 34% (n = 409) and 11% (n = 131) of isolates, respectively. Dual resistance to both agents was observed in 7% (n = 80) of isolates. In contrast, resistance levels in the post-treatment group were significantly higher ($p < .0001$) to metronidazole (88%; n = 99) and clarithromycin (78%; n = 88), as was the level of dual resistance (73%; n = 83; $p < .0001$). This group contained two examples of multi-resistant

strains: one that was metronidazole, clarithromycin and tetracycline resistant and one that was metronidazole, clarithromycin, and amoxicillin resistant. Both strains were ciprofloxacin resistant also.
Conclusion. The fact that almost 75% of known treatment failures are infected by *H. pylori* with resistance to two of the antibiotics commonly used in eradication therapy highlights not only the need for continued monitoring of antibiotic resistance to advise on empirical treatment for problematic eradication failures, but also the need for continued monitoring of resistance trends in the community.

Abstract no.: 13.03
Implementation of a Novel Molecular Algorithm for Diagnosis of *Helicobacter pylori* Infection and Antibiotic Resistance

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Background. Culture from gastric biopsy is the gold standard approach for *Helicobacter pylori* antibiotic susceptibility testing. Test sensitivity may be compromised by the fastidiousness of *H. pylori*, by effects of therapy, and by loss of viability and/or overgrowth of contaminating microorganisms. In previous studies, we demonstrated the excellent sensitivity and specificity of a multiplex polymerase chain reaction (PCR) assay when applied to gastric biopsies for detection of *H. pylori* and/or *Helicobacter heilmannii*-like organisms (HHLOs), and of two real-time probe hybridization assays for clarithromycin and tetracycline susceptibility testing.
Aim. To implement and evaluate a specialist molecular service for detection and susceptibility testing of *Helicobacters* from gastric biopsies.
Results. From 2003 to 2005, gastric biopsies from 171 patients, 56% of whom were known treatment failures, were tested. None was HHLOs polymerase chain reaction (PCR) positive. Of the 71 (42%) *H. pylori* culture-positive biopsies, 70 were PCR positive (99% sensitive). PCR identified an additional 29 *H. pylori*-positive patients. In spite of the failure to obtain culture from these, susceptibilities to clarithromycin (21 resistant, 7 sensitive) and tetracycline (27 sensitive, 2 resistant) could be determined in 28 biopsies by real-time PCR.
Conclusion. Molecular testing is an invaluable adjunct to culture methods for diagnosis and drug resistance determination of *H. pylori* infection, particularly in instances where the specimen was contaminated or underwent severe transport delays. Of the 100 *H. pylori*-positive results, 29% was determined by PCR alone. Furthermore, molecular identification of antibiotic resistance provided information in 17% of all patients that otherwise could only be obtained by repeat endoscopy.

Abstract no.: 13.04
Rates of Ciprofloxacin and Rifampicin Resistance are Low in Persistent *Helicobacter pylori* Infections in the UK

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Background. The failure of current *Helicobacter pylori* eradication regimes in some patients has led to evaluation of "rescue therapies" containing alternative agents, including fluoroquinolones and rifamycins. Little is known about the levels of resistance to these antibiotics, particularly in populations that would benefit from such therapy (treatment failures and/or multi-resistant infections).
Aim. To determine the rates and molecular basis of resistance to ciprofloxacin and rifampicin in patients with refractive *H. pylori* infection in the UK.
Methods. Ciprofloxacin and rifampicin susceptibilities were determined by E-test in 106 dually resistant (to metronidazole and clarithromycin) isolates from dyspeptic patients, 67% of whom were known treatment failures. The molecular basis for ciprofloxacin and rifampicin resistance was determined by sequencing of *gyrA* and *rpoB,* respectively.
Results. Ciprofloxacin resistance [minimal inhibitory concentrations (MICs) ranging from 2 mg/L to > 32 mg/L] was observed in 7.5% (n = 8) of isolates. Disk diffusion inhibition zones were 0 mm for MIC > 16 mg/L and < 20 mm for lower MICs (> 2 mg/L < 16 mg/L). The *gyrA* of resistant isolates contained mutations Asp91Gly (n = 5), Asp91Asn (n = 1), and Asn87Lys (n = 2), whereas Asn87Thr was observed in 2/21 ciprofloxacin sensitive sequences. Rifampicin resistance was not observed. Testing an additional 21 isolates, recovered pre-treatment, demonstrated marginally lower ciprofloxacin resistance 4.8% (n = 1) and no rifampicin resistance.
Conclusion. Ciprofloxacin and rifampicin resistance is low in patients that failed eradication or were infected with a dually resistant strain. Few other studies have specifically examined the population that would benefit from alternative "rescue therapies" and ours is the first study in the UK to have surveyed resistance to these agents.

Abstract no.: 13.05
ResiNet – A German Nationwide Sentinel Study on *Helicobacter pylori* Resistance – An Update

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The antimicrobial treatment of *Helicobacter pylori* infections is jeopardized if *H. pylori* is resistant against anti-infectives in use, particularly metronidazole (MTZ) and clarithromycin (CLA). The aim of this nationwide study ResiNet, which was launched by the German National Reference Centre (NRZ) for *H. pylori* in 2001, is to investigate risk factors for the development of antimicrobial resistance in *H. pylori*. For this purpose the NRZ involved 16 microbiological centers (MC), each collaborating with three to

seven gastroenterologists (GE) in clinical practice. During "study weeks," GEs enroll consecutive patients into the study without preselection, sending gastric biopsies for microbiological investigation and completing a questionnaire. All MCs use identical culture media lots and standardized operation procedures during each study week.

At end of April 2006 a total of 669 patients were completely investigated. Overall, 37.5% and 18.7% of isolates were resistant against MTZ or CLA, respectively, and 14.1% showed double resistance against both drugs. The frequencies of primary resistance (n = 473) were 27.1% (MTZ), 5.7% (CLA), and 3.4% (MTZ and CLA), compared to 58.7% (MTZ), 47.6% (CLA), and 31.7% (MTZ and CLA) in patients pretreated once (n = 63). Repeated pretreatment (n = 61) was associated with an increase of double resistances up to 78.7%, clearly indicating that a significant increase in resistance to MTZ and CLA already occurs after the first treatment failure, reaching dramatically high resistances after repeated empirical eradication therapies. In conclusion, these results underline that development of resistance in *H. pylori* in contrast to other pathogens is nearly exclusively dependent on treatment strategies in the individual patient.

Abstract no.: 13.06
Evaluation of In Vitro Antimicrobial Effectiveness of Novel Nitroimidazole and Nitrofurane Derivatives Against *Helicobacter pylori*

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Background. Metronidazole resistance is the major reason of failure in treatment of *Helicobacter pylori* infection. Thus, design of novel antimicrobials as a substitute for metronidazole in therapeutic regimens appears to be of great value. In this study, 204 synthetic nitroimidazole and nitrofurane derivatives were screened for their antibacterial activity against *H. pylori* isolates from dyspeptic patients.

Methods. Disk diffusion method was recruited to screen 204 nitroimidazole and nitrofurane synthetic compounds. Serial dilutions (32, 16, 8.4 μg/mL) of each compound were prepared. Bacterial suspensions were prepared from fresh cultures of two *H. pylori* isolates, one susceptible and one resistant to metronidazole. Blank disks were deposited on the surface-inoculated *Brucella* blood agar and impregnated with different dilutions of each compound. After 2–3 days of microaerobic incubation, the diameter of inhibition zones was recorded and minimum inhibitory concentrations determined. Those compounds that produced inhibition zones of ≥ 20 mm were considered as inhibitory against *H. pylori*.

Results. Among 204 synthetic compounds, 33 were active against *H. pylori*. Of these 33, 15 were nitroimidazole and 18 were nitrofurane. Both metronidazole resistant and susceptible strains were inhibited by these compounds. Seventeen of these compounds produced inhibition zones of > 20 mm at MIC of 8 μg/mL, thus considered as highly effective.

Discussion. Two hundred four synthetic nitroimidazole derivatives were screened for antibacterial activity against *H. pylori*. Thirty-three compounds, 15 from nitroimidazole, and 18 from nitrofurane family exhibited considerable activity against *H. pylori*. It was concluded that nitroimidazole and nitrofurane compounds could be further studied as appropriate substitutes for metronidazole in therapeutic regimens.

Abstract no.: 13.07
Increasing Quinolone Resistance in *Helicobacter pylori* in Germany

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Resistance to first-line antibiotics such as clarithromycin is an important clinical problem, which jeopardizes eradication therapies of *Helicobacter pylori*. Thus, quinolone-based regimens are increasingly applied in drug-resistant *H. pylori* infection. In order to establish evidence-based recommendations for the use of quinolones, it is necessary to update actual data on quinolone resistance in *H. pylori*. We assessed the current status of quinolone resistance by testing the antimicrobial susceptibility to ciprofloxacin of 931 *H. pylori* isolates obtained over a 5-year period during routine diagnostics. The overall rate of quinolone-resistant *H. pylori* isolates increased from 9.7% in 2001 up to 22.1% in 2005. In parallel, the proportion of triple resistant isolates, characterized by resistance to quinolones, metronidazole, and clarithromycin rose from 6.2% up to 14.5%. Triple-resistant strains were detected in pretreated patients only, indicating a negative impact of unsuccessful eradication attempts on resistance development also to quinolones. Genetic analysis of a random selection of 60 resistant isolates confirmed that single mutations in the gyrA gene play the crucial role in quinolone resistance.

These results demonstrate that quinolone resistance and the proportion of triple-resistant strains have reached an alarming level in *H. pylori* infections, which jeopardizes the success rate of eradication therapies. Furthermore, data clearly indicate that the use of quinolones requires prior antimicrobial susceptibility testing especially in patients that have already undergone unsuccessful treatment. Finally, the data underline the need for regular surveillance studies in order to detect antibiotic resistance of *H. pylori* and to define relevant risk factors.

Abstract no.: 13.08
A Five Year Follow-up of *Helicobacter pylori* Resistance to Fluoroquinolones in Bordeaux, France

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Helicobacter pylori resistance to clarithromycin is the main cause of *H. pylori* eradication therapy failure. Among the antibiotics proposed in rescue therapies, the new fluoroquinolones are promising drugs. Our aim was to study the antimicrobial susceptibility of *H. pylori* to fluoroquinolones and other antibiotics during the period 2001–2005 and to look for the *gyr*A mutations associated with this resistance.

A disk diffusion method was used in order to test susceptibility to ciprofloxacin as well as to clarithromycin, amoxicillin, tetracycline, and rifampicin. Metronidazole was tested by inoculating a plate containing 8 mg/L of this drug.

Results from the first strain obtained from each patient were recorded. A total of 417 *H. pylori* strains were studied. Primary resistance to ciprofloxacin was 12.4% (CI 95%: 9.3–15.6) with a slight but no significant increase (2001–2002, 9.8%; 2003–2004, 11.6%; 2005, 16.4%). Only one strain was resistant to rifamycin and none to amoxicillin and tetracycline. Resistance to clarithromycin (31.8%) and metronidazole (47.7%) was high but represented a mixture of primary and secondary resistance.

The QRDR of the *gyr*A gene of 37 resistant strains was sequenced: 12 strains had a mutation in position 87 (Asn87Lys eight times; Asn87Ile, four times), 17 strains had a mutation in position 91 (Asp91Asn, eight times; Asp91Gly, six times; and Asp91Try, three times). Eight strains did not exhibit any mutation in these positions.

In conclusion, there is a trend to an increase in resistance to fluoroquinolones and these antibiotics should be used cautiously, and only after susceptibility testing.

Abstract no.: 13.09
Helicobacter pylori Resistance to Rifamycin is Connected to *rpoB* Mutations

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Background. Resistance to rifamycin in *Helicobacter pylori* is assumed to be of low rate as only one clinical strain has been described on a genetic basis so far. However, rifamycins (rifampicin and rifabutin) are becoming drugs of choice for treatment of cases because of clarithromycin- and metronidazole-resistant strains.
Aim. To determine the proportion of rifamycin resistant strains and to study their genotype, in order to correlate mutations in *rpoB* gene and rifamycin resistance.
Methods. One hundred twenty-six clinical strains consecutively isolated between 2004 and 2005 in our hospital were studied. Minimal inhibitory concentrations (MIC) were determined by the agar-dilution method. Two regions of the *rpoB* gene (from nucleotides 127 to 743 and from 1529 to 1839) known to be involved in rifamycin resistance were amplified and sequenced for the resistant strains.
Results. Eight (6.4%) resistant strains were found with rifampicin (Rif) MIC from 12 to 256 mg/L. *rpoB* sequencing revealed mutations at various positions in the two domains, whereas no mutations were found in these regions for 13 susceptible strains studied as controls.
Conclusion. In our hospital, the proportion of rifamycin-resistant strains is higher (6.4 %) than expected (less than 1% in the literature). According to our results, resistance to rifamycin is related to mutation(s) in *rpoB* in 5/8 (63%) of the strains studied. The two regions studied seem to be involved.

Strains	Rif MIC (mg/L)	Region 1 (70 to 220)	Nucleotide	Region 2 (515 to 605)	Nucleotide
167	> 256	/	/	D530G	A1589G
170	> 256	/	/	L547F	C1539T
239	> 256	/	/	D530V	A1589T
274	> 256	/	/	/	/
287	> 256	/	/	/	/
311	> 256	S111G	A331G	D530N	G1588A
319	12	/	/	/	/
320	32	V149G	T446G	/	/

Abstract no.: 13.10
Detection of *Helicobacter pylori* and Clarithromycin Resistance by Fluorescence In Situ Hybridization (FISH) Method in Turkish Dyspeptic Patients

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Clarithromycin resistance in *Helicobacter pylori* strains is increasing, and eradication therapy of this bacterium is becoming more difficult. Molecular methods have frequently been applied besides phenotypic methods for susceptibility testing to detect clarithromycin resistance as a result of mutations in the 2143 and 2144 positions of the 23S rRNA gene. We applied fluorescence in situ hybridization (FISH) method for the detection of *H. pylori* and determine the clarithromycin resistance in paraffin embedded tissue sections retrospectively. Fifty-four dyspeptic patients (17 men, 37 women; mean age, 46.48 ± 13.12 years) were studied between May to November 2003. Two antrum and corpus biopsies were taken from each patient. Rapid urease test (RUT) and histopathology were applied to all patients as gold standard methods for the diagnosis of *H. pylori* infection. A total of 108 paraffin embedded biopsy specimens were examined by FISH (SeaPro, Netherlands) method. Fourty-five of 54 patients (83.3%) were diagnosed as positive and 9 (16.7%) were negative by gold standard methods. Forty-five patients (83.3%) were *H. pylori* positive and 9 (16.7%) were negative by FISH method. Fourteen (31.1%) of 45 positive patients were sensitive, 4 (8.9%) were

resistant, and 27 (57.5%) were both wild type and resistant strains to clarithromycin. Clarithromycin-resistant strains were found often and may show treatment failure. The sensitivity, specificity, positive and negative predictive values of FISH method were 95.6, 77.8, 95.6, and 77.8%, respectively. Statistical difference was not found when compared to the gold standard methods ($p = 1.00$, kappa = 0.73). FISH method in paraffin-embedded tissues is a rapid, accurate, and cost-effective method for the detection of *H. pylori* infection and to determine clarithromycin resistance within three hours according to the gold standards.

Abstract no.: 13.11
Evaluation of Differentially Expressed Genes in Response to Amoxicillin Exposure by RNA Arbitrarily Primed PCR

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Helicobacter pylori is a common human pathogen that is strongly associated with the development of active gastritis and gastric cancer. The anti-*H. pylori* therapies consist of a proton pump inhibitor and/or bismuth component in combination with two antibiotics. Among the antibiotics, amoxicillin is one of the most widely used. The molecular mechanism of amoxicillin resistance seems to be partially explained by several mutational changes in the *pbp1A* gene. The aim of the present study was to evaluate the gene expression in response to amoxicillin in the Amx^R Hardenberg strain using RNA arbitrarily primed polymerase chain reaction (RAP-PCR). In the experiments, approximately one hundred differentially expressed cDNAs, in the presence and absence of amoxicillin, were identified using five arbitrary primers. The cDNAs that presented the highest levels of induction or repression were cloned, sequenced and the sequences were compared with those present in databases (Tigr – http://www.tigr.org/) using the BLAST search algorithm. The preliminary results showed that amoxicillin alters the expression of 4 cDNAs involved in biosynthesis; 2 involved with pathogenesis; 4 related to cell envelope formation; 2 involved in cellular processes; 3 related with transport and binding proteins; 1 involved with protein degradation; 1 involved with energy metabolism; and 7 hypothetical proteins. Further analysis of these cDNAs differentially expressed in the presence of amoxicillin will allow a better comprehension of both: the molecular mechanism(s) of amoxicillin resistance and the adaptative mechanism(s) used by *H. pylori* in the presence of this antibiotic.

Abstract no.: 13.12
Identification of *Helicobacter pylori* Proteins Involved In Metronidazole Resistance by Comparative Proteomic Analysis

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Background. The nature of metronidazole (MTZ) resistance, which is an increasing problem worldwide, still remains unclear. We aimed to investigate the biochemical and molecular processes associated with the selection of MTZ resistant strains, in course of MTZ treatment of *Helicobacter pylori* infection, by means of a comparative proteomic analysis.
Materials and methods. We examined six *H. pylori* strains isolated from two patients. Two strains, one MTZ susceptible and one MTZ resistant, were isolated from each patient before treatment; two strains after unsuccessful treatment (thus MTZ resistant). Bacterial proteins were resolved by two-dimensional electrophoresis; then, pre- and post-therapy protein patterns were compared using dedicated software. Differentially expressed proteins, presumably involved in MTZ resistance, were subsequently identified through MALDI-TOF mass spectrometry.
Results. We identified several proteins involved in MTZ resistance, particularly the pyruvate : ferredoxin oxidoreductase (*porA*), the fumarate reductase iron-sulfur protein (*frdB*), the isocitrate dehydrogenase (*icd*), and the nonheme iron-containing ferritin (*pfr*) (that inhibit drug activation). We also observed two proteins whose synthesis was altered in similar way in the two patients: the nonheme iron-containing ferritin (*pfr*) and the DNA-binding protein HU (*hup*). Finally, we detected a low expression level for the protein encoded by the gene *recA*.
Conclusions. Unlike the results of other studies, our findings do not support the hypothesis that MTZ-resistant strains possess a more active DNA reparation mechanism mediated by recombinase A. We hypothesise that MTZ-resistance is mediated, at least in some cases, by the differential expression of several protein molecular species, which could therefore be considered generic markers of MTZ resistance.

Abstract no.: 13.13
Novel Real Time PCR for Detection of Clarithromycin Resistance in *Helicobacter pylori* Clinical Isolates

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Purpose. To test the usefulness of MutaREAL® *Helicobacter pylori* (Clarithromycin resistance) real-time PCR (polymerase chain reaction) Kit (manufactured by Immundiagnostik AG and

distributed in Spain by Laboratorios LETI SL) in a set of 57 *H. pylori* strains studied by agar dilution (AD) and molecular methods.
Material and methods. Strains were grown form gastric biopsies. Clarithromycin (CLA) resistance was determined by an AD method. MIC ≤ 0.25 mg/L was considered susceptible (S), minimum inhibitory concentration (MIC) = 0.5 mg/L intermediate (I), and MIC ≥ 1 mg/L resistant (R). A2142G and A2143G mutations were determined by PCR-restriction fragment-length polymorphism and A2142C by 3′ mismatched PCR. MutaREAL® Kit was used according to manufacturer recommendations in a LightCycler® (Roche). Melting temperature (Tm) of the amplified fragment was determined: Tm in channel F2 was 64.5 °C for wild type and 60.5 °C for mutated strains and in F3 was 66.5 °C for mutated strains, and 59.5 °C for wild type.
Results. Twenty-one strains were CLA-S by AD (MICs: 0.008–0.25 mg/L); 20 showed a wild type Tm and 1 a mutated Tm (MIC = 0.25 mg/L). 36 strains were CLA-R (MIC, 1.5–64 mg/L); 22 with A2143G, 5 with A2142C, 2 with A2142G, and 7 not determined. Results with MutaREAL were 34 strains showed mutated Tm and 2 wild type Tm (2 strains with A2142C mutation, MIC = 1–2 mg/L, and negative by other molecular methods). MutaREAL showed a sensitivity of 94.4% and specificity of 95.2% compared with AD for CLA-R detection.
Conclusions. The new MutaREAL® Kit was able to detect CLA-R in *H. pylori* with high sensitivity and specificity. This method is quicker than AD or E-test and could be performed in 1 hour after DNA extraction.

Abstract no.: 13.14
Presence of Active Efflux in Multidrug Resistant Strains of *Helicobacter pylori* Isolated from Children

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Background. Efflux of compounds protects bacteria from toxic effects of metabolite-accumulation and can be mediated through specific pumps, or those that transfer a broad range of substance. In many pathogenic bacteria, active efflux of antibiotics is an important mechanism of resistance, but little is known about its association with resistance to antibiotics in *Helicobacter pylori*.
Aim. To study the efflux phenomenon in clinical *H. pylori* isolates and to evaluate its association with multidrug resistance (MDR) by comparison of MDR strains with the susceptible ones.
Methods. Five MDR-strains resistant to amoxicillin, metronidazole, tetracycline, and erythromycin were selected for this study. Ethidium bromide was used to detect the accumulation in cells and to observe the active efflux, as its accumulation can be easily detected using fluorescence. Minimum inhibitory concentration for ethidium bromide was determined for MDR strains using disk diffusion method. Exponentially grown cells were resuspended in *Brucella* broth to an OD_{600}-5.0 and ethidium bromide was added to a final concentration of 20 μg/mL. Fluorescence was measured using excitation at 544 nm and emission at 590 nm. To collapse the proton motive force, carbonyl cyanide m-chlorophenylhydrazone (CCCP) was added (200 μmol/L). Genomic DNA were amplified for two efflux genes HP1181, and HP1184 identified in *H. pylori*
Results. Accumulation of ethidium bromide was increased to the level of susceptible strains upon addition of the uncoupler CCCP and MIC for ethidium bromide was decreased by two- to threefold. Multidrug-resistant strains showed amplification for HP1181, HP1184.
Conclusion. Active efflux in *H. pylori* strains may contribute in multiresistance to structurally nonrelated antibiotics.

Abstract no.: 13.15
Resistance to Metronidazole, Clarithromycin, and Amoxicillin of *Helicobacter pylori* Strains Isolated Before and After Eradication in Children

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Introduction. In recent years, an increase of resistance to used drugs of *Helicobacter pylori* is observed.
Objectives. The objective of the work was estimation of the resistance frequency of *H. pylori* to metronidazole (MZ), claritrhomycin (CL), and amoxycllin (AMX).
Material and Methods. The study comprised 868 *H. pylori* strains isolated before and 126 *H. pylori* strains isolated after eradication from the children in years 1998–2004.
Results. The study showed an increase in resistance of *H. pylori* to clarithromycin before treatment from 5.3% in 1998 to 29% in 2004, high resistance to metronidazole – 32.9% in 1998 and 43% in 2004, increase in resistance to combined treatment with MZ and CL from 6.8% to 23.4%. After eradication an increase of resistance was observed: to MZ from 51.5% to 65%, to CL from 5.1% to 35.0% and to combined MZ and CL from 9.1% to 30.0%. An increase in resistance to amoxycillin was not observed.
Conclusions. Our study confirms increasing, in recent years, resistance to MZ and CL both before and after eradication.

Abstract no.: 13.16
High-Level Resistance to Antimicrobial Agents in *Helicobacter pylori* Isolated from Children at Pediatric Medical Center of Tehran

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Background. There are many reports indicating that the rate and the pattern of primary antibiotic resistance in developing regions differed from those of developed regions. Epidemiological data of *Helicobacter pylori* antimicrobial-resistance are essential to optimize the choices of effective treatment strategies.
Aim. To compare the rate and level of resistance to commonly used antimicrobial in *H. pylori* strains isolated in 1998, 2001, and 2005–2006 at Pediatric Medical Center of Tehran.
Method. *H. pylori* was cultured from biopsies taken from symptomatic children (aged 4–16 years) admitted to Pediatric Medical Center of Tehran during routine endoscopies in 1997–2006.

Resistance to metronidazole (Mtz), amoxicillin (Amx), ampicillin (Am), erythromycin (Ery), and tetracycline (Tet) was tested using agar-dilution method. For this purpose, 5 µL of fresh bacterial suspension (equivalent to N° 3 of MacFarland) was cultured in plates (Mueller-Hinton plus 7% sheep blood) containing various concentration of antibiotics, and incubated for 2–3 days. Resistance cut-off used were (in µg/mL): Mtz = 8, Am and Amx = 8, Ery = 8, Tet = 4.

Results. The rate of resistance to Mtz, Am, Amx, Ery, and Tet was 44, 39, 59, 47, and 23%, respectively. Their level of resistance ranged from low to very high as follow:

Mtz: 47% (4–8 µg/mL), 34% (16–32 µg/mL), 10% (> 32 g/mL).
Am: 33% (0.5 µg/mL), 28% (4–8 µg/mL), 39% (16–32 µg/mL)
Amx: 30% (0.5–2 µg/mL), 11% (4–8 µg/mL), 59% (16–32 µg/mL).
Ery: 12% (2–4 µg/mL), 41% (8 µg/mL), 47% (16–32 µg/mL).
Tet: 61% (0.5–1), 32% (4–8 µg/mL), 7% (16–32 µg/mL).

Conclusion. *H. pylori* isolated from children were highly resistant to traditionally used antibiotics. This study is ongoing and will include additional cases while determining the resistance by the other methods.

Abstract no.: 13.17
Sensitivity of *Helicobacter pylori* Diagnostic Tests Before and After Eradication

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Introduction. Data on sensitivity of *Helicobacter pylori* diagnostic tests before and after eradication are lacking.

Aim. To evaluate sensitivity diagnostic tests before and after *H. pylori* eradication treatment.

Methods. From 2000 to 2006, 200 consecutive *H. pylori* positive adult patients (gastritis, 149; DU, 51; 65 men; mean age, 33.6 years; age range, 16–75 years) suffering from dyspepsia and not using nonsteroidal anti-inflammatory drugs were enrolled. Before and after eradication treatment, 10–12 gastric biopsies were performed for histology, rapid urease test (RUT), and culture. Stool antigens (HpSA) and ^{13}C-urea breath test (UBT) were also practiced. *H. pylori* infection was defined if culture and/or histology and a third test were positive. Patients were treated by different tritherapies. *H. pylori* eradication was assessed on the negativity of all tests. Mean delay of control was 10 weeks. UBT sensitivity was established versus histology + RUT and those of other tests versus UBT.

Results. Sensitivity of tests before treatment for UBT, histology, RUT, culture, HpSA was 100, 98; 98; 66; 63%, respectively. After treatment, sensitivity was 100, 78; 76; 52; 59%, respectively.

Conclusion. Sensitivity of *H. pylori* tests is seriously decreased after *H. pylori* eradication treatment except for UBT. The choice of diagnostic eradication tests depends of their sensitivity but also of their availability.

Abstract no.: 13.18
Antibiotic Resistance in Clinical Isolates of *Helicobacter pylori* in Germany

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Background. The issue of antibiotic resistance in *Helicobcater pylori* is of particular concern and has become an important factor leading to eradication failure.

Aim. To evaluate the prevalence of primary clarithromycin (CLA), metronidazole (MET), and amoxicillin (AM) resistance among *H. pylori* isolates in the northeastern part of Germany.

Methods. *H. pylori* was cultured from biopsies taken during routine endoscopies from patients not pretreated for *H. pylori* infection in 1995 to 2005. The minimum inhibitory concentrations were obtained using the E-test (cut-off CLA 1 µg/mL, AM 2 µg/mL; MET 32 µg/mL).

Results. A total of 2,820 *H. pylori* isolates were investigated. The overall rates of primary resistance to MET and CLA were 27.5% and 2.8%, respectively (see Table 1). No strain was found to be resistant to AM.

Conclusion. Primary resistance rates for CLA and MET were stable over the last 11 years in our population. The prevalence of MET resistance is similar to the median rates in Europe, and the rate of CLA resistance is still low compared with Southern European countries. According to the Maastricht Consensus Report CLA-based triple therapy can still be recommended as first-line therapy for *H. pylori* eradication. On the basis of less than 40% prevalence of MET resistance in our population a combination with MET is preferable.

Table 1 Antibiotic resistance rates of *H. pylori* (%)

	1995	2000	2005
CLA	2.0	3.4	3.0
MET	25.1	24.3	26.3

Abstract no.: 13.19
In vitro Efficiency of Ciprofloxacin and Rifampicin as Potential Second-Line Treatment in Spanish *Helicobacter pylori* Clinical Isolates

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Objective. To characterize the efficiency of first- and second-line treatments, a multicentric study was carried out to determine the activity of amoxicillin, tetracycline, metronidazole, clarithromycin, ciprofloxacin, and rifampicin in *Helicobacter pylori* clinical isolates.

Methods. Samples were collected from gastric biopsies of symptomatic patients (September 2002–April 2006). In vitro activity was determined by E-test using 5% sheep blood agar (3–5 days incubation at 37 °C in 10% CO_2). Minimum inhibitory concentration (MIC, mg/L) was the lowest concentration of drug able to inhibit visible growth. Based on the Clinical Laboratory

Standards Institute (CLSI, formerly NCCLS) and other previously published data, strains were resistant if MIC = 2 for amoxicillin, = 4 for tetracycline, = 8 for metronidazole, = 1 for clarithromycin, = 4 for ciprofloxacin, = 32 for rifampicin, and intermediate if MIC = 0.5 for clarithromycin, = 2 for ciprofloxacin.
Results. Sixty-nine samples were collected. Adults and children were equally represented, with female predominance (63%). All the strains were susceptible to amoxicillin and tetracycline; 42% were resistant to metronidazole, 43% to clarithromycin, 6% to ciprofloxacin, 0% to rifampicin; 2% were intermediate to clarithromycin. Among adults, resistance to metronidazole (53%) and ciprofloxacin (12%) were higher than among children (31% and 0% respectively). Among women, resistance to metronidazole (49%) and ciprofloxacin (10%) were higher than among men (27% and 0% respectively) but resistance to clarithromycin was lower (39% versus 54%).
Conclusions. Amoxicillin and tetracycline are still to be considered as first-line antibiotics and ciprofloxacin and rifampicin as second-line treatment. The high resistance to metronidazole and clarithromycin is critical and its association with age and sex is to be further characterized.

Abstract no.: 13.20
Antimicrobial Activity of Rabeprazol Alone and in Combination with Chlaritromycin or Levofloxacin upon Clinical Isolates of *Helicobacter pylori*: Synergist or Antagonist effect?

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Background. Failing in antimicrobial treatment as a result of antibiotic resistance emergency among clinical isolates of *Helicobacter pylori* is big challenge at present. The search for new compounds with antibacterial activity and the maintenance of antibiotic resistance surveillance is crucial today for successfully manage of clinical infections. The aim of this work was to evaluate the antibacterial activity of a proton pump inhibitor (PPI), rabeprazole, alone and in combination with both clarythromicin or levofloxacin.
Methods. Thirty-nine clinical isolates of *H. pylori* were included in this study. Antibacterial activity was evaluated by an agar dilution test using Müeller–Hinton agar plus 7% horse blood and dent supplement, when needed. Incubation was carried out at 37 °C under microaerophile atmosphere.
Results. Forty-one percent and 90% of the strains were susceptible to clarithromycin and levofloxacin, respectively. Rabeprazole showed also antibacterial activity when assayed alone (36.2% of the strains with MIC < 1 mg/L), whereas only 8/39 strains had a MIC > 8 mg/L. The combination of rabeprazole and levofloxacin was generally indifferent (47.8%) or antagonic (30.4%) but synergy was observed when rabeprazole was combined with clarithromycin (48%).
Conclusion. Antimicrobial properties of PPIs selected for anti-*H. pylori* therapy should be considered as antagonic effect has been observed in vitro, which might influence the successfulness of the eradication therapy selected.

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Abstract no.: 13.21
Emergence of a Tetracycline-resistant *Helicobacter pylori* Clinical Isolate in Germany

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Tetracycline-resistant *Helicobacter pylori* isolates are extremely rare and have never been described for Germany so far. Here, we report the first tetracycline-resistant *H. pylori* strain isolated in Germany from a 70-year-old woman suffering from antral gastritis, chronic obstructive pulmonary disease, and an adult-onset diabetes mellitus. Because of relapsing bacterial lung infections, repeated antibiotic treatments were required. Between 2000 and 2005, she received multiple courses of cefuroxime, ciprofloxacin, roxithromycin, levofloxacin, and doxycycline. In March 2005, her gastroenterologist diagnosed an *H. pylori*-associated antrum gastritis by urea breath test and endoscopy. Both, a first-line therapy consisting of amoxicillin, clarithromycin and a proton pump inhibitor (PPI), and a second-line therapy were administered, but the patient was still complaining about dyspepsia and severe gastric pain. Because of the assumption of therapy failures, the gastroenterologist rescoped and initiated a microbiological investigation in our routine laboratory. Antimicrobial susceptibility showed sensitivity to amoxicillin and rifabutin, but resistance to metronidazole, clarithromycin, ciprofloxacin, and tetracycline. Agar dilution experiments verified a clinical relevant tetracycline resistance. Genetic analysis of the 16S rRNA encoding genes revealed a homozygous nucleotide exchange at position 926, thus underlining the pivotal role of mutations at the tetracycline-binding pocket of the 16S rRNA. This clinical case demonstrates the importance of antimicrobial susceptibility testing in *H. pylori*-associated infections in order to avoid further treatment failures and to reduce costs. Furthermore, it supports the hypothesis that repeated application of antimicrobial drugs resulting from other disease might contribute to the development of antibiotic resistance in *H. pylori*.

Abstract no.: 13.22
An Investigation of Antibiotic Resistance of *Helicobacter pylori* Species

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Helicobacter pylori causes gastroduodenal diseases varying from nonulcer dyspepsia to gastric adenocarcinoma. In recent years, it has been observed that success in treatments have decreased as a result of primary and secondary resistance to first propose antibiotics.

In this study, 386 (90.4%) isolates was isolated from gastric biopsy samples of 427 patients between the years 2002 and 2005, have been investigated by agar dilution methods (AD) for clarithromycine, rifampin, tetracycline, metronidazole, and amoxicillin resistances. Besides, A2142G-C and A2143G-C mutations in 23S rDNA, which cause resistance to clarithromycin had been investigated via the application of polymerase chain

reaction-restriction fragment-length polymorphism (PCR-RFLP) method.

Although no resistance to tetracycline, rifampin, and amoxicillin had been observed, resistance to metronidazole had been detected from 2002 to 2004 isolates in the ratios of 34/114 (34%), 54/145 (37.24%) and 43/124 (33.8%), respectively. The ratios of isolates resistant to clarithromycin had been detected respectively as 17/114 (14.9%), 26/145 (17.9%) and 23/127 (18.1%).

H. pylori isolates were conducted PCR-RFLP method in order to detect mutations that cause to clarithromycin resistance. We identified A2142G and A2143G mutations in 2002 isolates in the ratios 12 (70.5%) and 3 (17.6%), respectively. These ratios had been identified respectively as 21 (80.8%) and 3 (11.5%) in 2003 isolates, and 20 (86.9%) and 3 (13%) in 2004 isolates. When AD and PCR-RFLP results had been compared, we have detected that resistance developed in the 2002 isolate which is resistant in the ratio of 2 (11.7%), and in the 2003 isolate that is resistant in the ratio of 2 (7.26%) with other reasons than the previously known mutations.

Abstract no.: 13.23
Evolution of Antimicrobial Resistance in *Helicobacter pylori* Clinical Isolates during 4 Years in A Hospital in Madrid, Spain

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Objective. To determine the resistance to amoxicillin, tetracycline, clarithromycin, and metronidazole in 118 *Helicobacter pylori* clinical isolates, from January 2002 to December 2005.

Methods. *H. pylori* strains were isolated from 118 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 the presence of oxidase, catalase, and urease. The in vitro activity of amoxicillin and tetracycline was determined by disk diffusion, and for clatrithromycin and metronidazole by E-test, using Muller-Hinton agar suplemented with 7% horse blood. Minimum inhibitory concentration (MIC) was determined as the point of complete inhibition of growth after 3–5 days of incubation. Strains were considered resistant to clarithromycin when MIC ≥ 1 mg/L, intermediate when MIC = 0.5 mg/L, and susceptible when MIC ≤ 0.25 mg/L. To metronidazole, strains were considered resistant when MIC ≥ 8 mg/L and susceptible when MIC ≤ 4 mg/L.

Results. All strains were susceptible to amoxicillin and tetracycline. Global resistance to clarithromycin was 60% and to metronidazole 55%. The year 2002 resistance to clarithromycin was 56.25% and to metronidazole 64.58%. The year 2003 resistance were 70.59% and 58.82% respectively. The year 2004, 55% and 45% respectively. And the year 2005, 60.61% and 45.45% respectively.

Conclusions. All strains were susceptible to amoxicillin and tetracycline. Resistance to clarithromycin and metronidazole showed a high percentage.

Immunity, Animal Models and Vaccines

Abstract no.: 14.01
Th1-associated Cytokines, IL-10 and TLR4 are Increased in the Gastric Mucosa of Children with *Helicobacter pylori* Infection

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Aim. A better understanding of the gastric immune response against *Helicobacter pylori* could help to develop novel therapeutic strategies. We compared mRNA levels of Th1/Th2-associated molecules in gastric mucosa of *H. Pylori*-positive children, children with celiac disease (CD) and gastroesophageal reflux disease (GERD).

Methods. Fifteen patients (median 11 years, m = 7) with *H. pylori* infection (^{13}C-UBT, rapid urease test, culture, and histology), 11 children with newly diagnosed CD ("disease controls," 4 years, m = 4) and 13 children with GERD but normal gastric histology ("healthy controls," 2 years, m = 6) were included. No child took proton pump inhibitors, corticosteroids, or antibiotics within 4 weeks before endoscopy. Gastric CXCR3, IFN-γ, IL-12 (Th1), CCR4, CRTH2 (Th2), IL-10, TLR2, and TLR4 mRNA levels were quantified by real-time RT-PCR (iCycler, Biorad). Group differences were tested with MW*U*-test (SPSS version 12) and related to the histological Sydney classification.

Results. Compared to children with CD and GERD, *H. pylori*-positive children had higher levels of IL-12 ($p < .003$; $p < .002$),

CCR4 ($p < .03$; 0.04) IL-10 ($p < .005$; ns), and TLR4 (0.056; $p < .02$), but lower levels of CXCR3 ($p < .2$; $p < .005$). IFN-γ tended to higher levels in *H. pylori*-positive patients (ns). TLR2 and CRTH2 levels did not differ.

Conclusion. Similar to adults, gastric IFN-γ and IL-12 mRNA levels are increased in *H. pylori*-positive children. The higher levels of CCR4 indicate the presence of a concomitant Th2-response. The presence of IL-10 point toward the presence of regulatory T cells, which may limit the inflammatory response. We speculate that TLR4 plays a role for the recognition of *H. pylori*-derived LPS.

Abstract no.: 14.02
The Role of Sero Reactive Antigens of *Helicobacter pylori* in Reflecting Histopathological Changes in Gastric Cancer Patients

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Introduction. *Helicobacter pylori* is one of the most prevalent infectious agents in developing countries such as Iran and is indicated as a type I carcinogen. Therefore, identification of screening markers for *H. pylori* can be crucial for early detection of gastric cancer.

Methods and Materials. Seventy-nine gastric cancer patients undergoing gastrectomy were studied for the following histopathologic criteria: *H. pylori* load, chronic gastritis, activity, dysplasia, atrophy, intestinal metaplasia, lymphoid follicle formation, and type of tumor. Western blotting was performed to identify various immunogenic proteins including 19.5, 30 (UreA), 35, 37, 89 (VacA), and 116 kDa (CagA) proteins.

Results. This study revealed that anti-VacA antibodies were present in 78.5% of total GC cases, whereas sero-reactivity toward CagA was observed in 97.5% of the two studied groups.

In cases with cardia cancers, antibodies to 35 kDa antigen had a significant association with the presence of atrophy ($p < .05$). Whereas antibodies to VacA were associated with the intensity of *H. pylori* colonization and inversely associated with dysplasia. In noncardia cancers, antibodies to 30 kDa protein revealed an inverse association with the presence of atrophy.

Among low molecular weight antigens, 19.5 and 37 kDa proteins showed no significant association with histopathologic findings in either of the studied groups.

	Sero-reactivity toward *H. pylori* dominant proteins (%)					
Groups of patients	19.5 kDa	30 kDa (UreA)	35 kDa	37 kDa	89 kDa (VacA)	116 kDa (CagA)
Cardia	75	70	22.5	37.5	75	95
Noncardia	64.1	74.4	25.6	41	82.1	100
Total	69.6	72.2	24.1	39.2	78.5	97.5

Conclusions. This study is proposing UreA and 35kDa antigens as potential indicators of atrophy and VacA as a predictive marker for *H. pylori* load in cardia cancer patients.

Abstract no.: 14.03
The Effect of Blood Interluekin-4 Production and Interleukin-4 Genetic Polymorphism on *Helicobacter pylori* Eradication

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Background and Aim. Impaired host immunity can be a cause of eradication failure of *Helicobacter.* We aimed to evaluate the effect of Th2 immune system on eradication failure using blood interleukin 4 and genetic polymorphism of IL-4 and IL-4 receptor antagonist (Ra).

Methods. One hundred three dyspeptic patients who received endoscopy were enrolled. *Helicobacter pylori* antigens were stimulated and IL-4 were measured in the whole blood using enzyme-linked immunosorbent assay. Genetic polymorphism of IL-4 and IL-4RA were evaluated using polymerase chain reaction. Then, *H. pylori*-positive patients received 7-day proton pump inhibitor-based eradication therapy, and blood IL-4 was measured again after eradication therapy in them.

Results. Forty-five patients who were *Helicobacter* positive and followed up after eradication therapy and 13 *Helicobacter*-negative patients were included. Baseline IL-4 was not differed between *Helicobacter*-positive and -negative groups (20.3 ± 13.3 pg/mL versus 15.5 ± 8.0 pg/mol, $p = .10$). Before eradication, blood IL-4 was higher in the eradicated group than noneradicated group (21.8 ± 14.2 pg/mL versus 13.3 ± 2.1 pg/mL, $p = .03$). But after eradication, there was no difference of it between those groups (18.2 ± 11.9 pg/mL versus 12.6 ± 2.6 pg/mL, $p = .09$) In the eradicated group, there was a trend toward the decrease of Il-4 after eradication ($p = .087$). IL-4 and IL-4RA genetic polymorphism had no influence on blood IL-4 production, the infection status and the result of eradication.

Conclusion. The IL-4 increased in the eradicated group before the eradication therapy. It suggests that increased blood IL-4 production before eradication may contribute to the successful eradication of *H. pylori.*

Abstract no.: 14.04
Potent Neutralization of Vacuolating Cytotoxin of *Helicobacter pylori* by Immunoglobulins against the Recombinant Counterpart

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Aims. To gain further insights into the pathologic activities of VacA in gastric epithelial cells and elucidate the potential as a vaccine when it retains native conformation.
Methods. The antisera raised in a rabbit by intradermal injections of the pool of soluble expressed recombinant vacuolating cytotoxin (rVacA). The possibility of rVacA as the oral vaccine with CpG-oligonucleotide (ODN) adjuvant was assessed in the C57BL/6 mice.
Results. Anti-rVacA antibody reacted with both s1/m1 VacA and s2/m2 VacA in an immunoblot with a 88-kDa and a 92-kDa protein, respective, whereas this immunoglobulin detected only a s1/m1 VacA toxin in an enzyme-linked immunosorbent assay. This immunoglobulin inhibited the cell death partially and phosphorylation of ERK1/2 (extracellular signal-regulated kinase 1/2) induced by the supernatant of *Helicobacter pylori* (CFS) carrying s1/m1 *vacA* gene in AGS cells. The ERK1/2 activation and cell death induced by CFS were inhibited in the presence of BafilomycinA_1 as well. Separately, recombinant VacA plus CpG-ODN vaccine stimulated mucosal immune response rather than systemic immune response.
Conclusion. This recombinant VacA antigen can elicit neutralizing antibody against VacA activity and has a potential as a vaccine component to eradicate *H. pylori* from an infected host and a diagnostic kit recognizing homologous *vacA* types strains.

Probiotics

Abstract no.: 15.01
Effect of Butyric Acid and Inulin on Side Effects of anti-*Helicobacter pylori* Antibiotic Therapy: Preliminary Data

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Background. *Helicobacter pylori* eradication fails in about 20% of patients for occurrence of antibiotic resistance and gastrointestinal side effects. Prebiotics and probiotics could be a valid therapeutic option to reduce gastrointestinal antibiotic related side effect increasing patients' compliance. The aim of our study was to evaluate the effect of a preparation of the butyric acid and inulin on antibiotic-associated side effects during anti-*H. pylori* therapy.
Methods. Thirty *H. pylori*-positive patients were randomly assigned to receive: a standard triple-eradication therapy based on rabeprazole, clarithromycin, and amoxicillin (RCA) for 7 days with or without butyric acid and inulin for 30 days. Side effects (taste disturbance, nausea, vomiting, diarrhea, constipation) were assessed using a questionnaire for 4 weeks from the beginning of therapy. *H. pylori* eradication was evaluated by means of ^{13}C-urea breath test.
Results. No statistical difference in eradication rate was found between groups. During the first week of treatment, butyric acid and inulin supplementation significantly reduced the incidence of bloating (44% verus 64%, $p = .045$); and diarrhea (9.3% versus 30.08%, $p = .006$) in ITT analysis.
Conclusion. The association of butyric acid and inulin was able to reduce the incidence of side effects related to anti-*H. pylori* therapy compared to standard treatment. Prebotics can reduce the incidence of antibiotic side effect such as diarrhea and bloating.

Abstract no.: 15.02
Decrease in *Helicobacter pylori* Urease Activity after Interaction with Different Probiotic and Nonprobiotic Microorganisms

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Objective. To determine the effect of 21 probiotic and nonprobiotic microorganisms over the urease activity of three *Helicobacter pylori* clinical isolates.
Methods. *H. pylori* strains were isolated from three gastric biopsies and processed by standard microbiological methodology. Twenty-one probiotic and nonprobiotic microorganisms were obtained from commercial products and clinical specimens. Nine *Staphylococcus* spp. were identified by Gram stain and MicroScan (Dade-Behring). Four *Streptococcus* spp., 2 *Lactococcus* spp., 3 *Lactobacillus* spp., and 2 *Bacillus* spp. by Gram stain and Api (Biomérieux) and 1 *Saccharomyces cerevisae* by AuxaColor (Bio-Rad). A modified red phenol method was used to determine the effect of the microorganisms against *H. pylori* urease activity. One hundred microliters of brain heart infusion (BHI) suplemented with fetal calf serum and yeast extract containing *H. pylori* suspension and the microorganism, were added to 100 µL of urease buffer at the times 0, 3, 20, and 24 hours. After 2 hours of incubation, lecture was made by measuring absorbance at 550 nm.

Results. Urease activity of *H. pylori* strain A decreased between 22.96–99.76% with 20 of the 21 microorganisms tested, of strain B between 35.7% and 99.98% with 17 microorganisms and of strain C between 61.54–99.85% with 20 microorganisms. *St. auricularis* did not show effect over any of the three strains, and *St. warneri*, *St. hominis*, and *Sa. cerevisae* did not do so over strain B.
Conclusions. Probiotic and nonprobiotic microorganisms are able to decrease *H. pylori* urease activity. This effect may explain the coadjuvant action of probiotics in *H. pylori* treatment.

Abstract no.: 15.03
Viability of *Helicobacter pylori* in Presence of Lactic Acid Bacteria Isolated From Foods

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Recently, interaction between probiotic lactobacilli and *Helicobacter pylori* has become a matter of great interest, as it could be used in association with conventional antibiotic therapy. Several authors have reported the inhibitory activity of certain probiotic bacteria as *Lactobacillus acidophilus* and *Lactobacillus casei* against *H. pylori* both in vitro and in vivo. Lactic acid bacteria are widely used in the production of fermented foods, dairy products, and pharmaceutical preparations. Lactobacilli bacteria, present in fermented products, have been reported to exhibit antimicrobial activities against human pathogens.
Objetives. The study of the effects of lactobacilli isolated from commercial fermented probiotic and nonprobiotic products on the survival of *H. pylori* strains.
Methods. *Lactobacillus* strains were isolated from 20 commercial food samples: 9 probiotics, 2 lyophilized pharmaceutical preparations, and 9 dairy products. Two reference and two *H. pylori* clinical strains were used for inhibition assays. Inhibitory activity was assayed by agar diffusion tests. Clarithromycin resistance, urease activity, culture, and morphology changes of *H. pylori* was monitored after lactobacilli contact.
Results. The different isolates belonging to *L. casei*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *L. acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus Rhamnosus*, and *Lactobacillus pentosus* species presented antimicrobial activity against all *H. pylori* strains. Urease activity, antibiotic resistance, and culturability were affected by lactobacilli presence. Increase of coccoid and transition forms of *H. pylori* was simultaneous with decrease in growth capacity.

Abstract no.: 15.04
Inhibitory Effect of Different Gram-Negative Bacteria against *Helicobacter pylori* Clinical Isolates

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Objective. To determine the effect of nine gram-negative microorganisms against 25 *Helicobacter pylori* clinical isolates.
Methods. *H. pylori* strains were isolated from 25 gastric biopsies and processed by standard microbiological methodology. Several gram-negative bacteria were obtained from blood cultures. Two *Escherichia coli*, 2 *Klebsiella* spp., 1 *Pseudomonas aeruginosa*, 1 *Salmonella GDF9*, 1 *Acinobacter baumannii*, 1 *Eneterobacter cloacae*, and 1 *Stenotrophomonas maltophilia* were identified by Gram stain and MicroScan (Dade-Behring). To determine the effect of these microorganisms on *H. pylori*, we used the "drop" method. A blood agar plate was completely inoculated with *H. pylori* and a drop was deposited containing 10 µL of the microorganism after 0.5 McFarland concentration. Plates were incubated at 37 °C under microaerobic atmosphere for 3–5 days. Lecture was made by the diameter of the inhibition zone observed after incubation.
Results. One *E. coli* strain was able to inhibit the growth of 1 *H. pylori* clinical isolate. *Klebsiella pneumoniae*, *A. baumannii*, and *Salmonella GDF9* were able to inhibit the growth of four *H. pylori* isolates each. *E. cloacae* and *S. maltophilia* were able to inhibit the growth of 14 *H. pylori* isolates each. On the contrary, one *E. coli* strain, *P. aeruginosa*, and *Klebsiella oxytoca* were not able to inhibit the growth of any of the 25 *H. pylori* isolates.
Conclusions. Some gram-negative bacteria are able to inhibit the growth of *Helicobacter pylori* clinical isolates. Further studies are needed in order to know the clinical implications of these results.

Abstract no.: 15.05
Therapeutic Effect of *Lactobaccilus gasseri* and Plaunotol on the Triple Therapy for *Helicobacter pylori* Eradication

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Proton pump inhibitor-based triple therapy has been widely applied to patients with *Helicobacter pylori* infection. However, rate of treatment failure as a result of antibiotic resistance are increasing. Probiotics are microorganisms with beneficial properties for the host. Furthermore, plaunotol, an acyclic diterpene alcohol extracted from leaves of the plau-noi tree in Thailand, has been used in Japan as a unique anti-ulcer agent for patients with gastric ulcer. We have demonstrated that combination therapy with plaunotol and *Lactobacillus gasseri* improved histologic findings of gastritis. To determine whether the supplementation with *L. gasseri* with plaunotol could improve the efficacy of standard triple therapy, we conducted a pilot study in patients with *H. pylori* infection. The 53 *H. pylori* positive patients were divided into three groups: a group receiving of a 7-day triple therapy alone (LAC group), or the same regimen supplemented with 120 g yogurt containing *L. gasseri* twice daily for 8 weeks (LG group), or the same regimen supplemented with 120 g yogurt containing *L. gasseri* and plaunotol for 8 weeks (LG-P group). Four weeks later, patients were assessed for the success of *H. pylori* eradication. The eradication rate in the LAC, LG, and LG-P groups were 78.9, 80, and 89.4%, respectively by intention to treat ($p = .6434$). However, diarrhea and bloating were significantly less frequent in LG or LG-P groups than LAC group. These findings suggest that *L.gasseri* supplementation beneficially affects *H. pylori* therapy-related side effects for patients with *H. pylori* infection.

Other *Helicobacters*

Abstract no.: 16.01
A Novel Enterohepatic *Helicobacter* Species Leads to Ulcerative Colitis-Like Inflammatory Bowel Disease in Interleukin 10 Knockout Mice

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Interleukin 10 knockout (IL-10 KO) mice are reported to develop Crohn's disease-like intestinal inflammation if colonized by a complex flora. Individual components of the intestinal flora critical for disease initiation are not clear. IL-10 KO mice and wild-type controls were investigated for gastrointestinal inflammation and composition of the intestinal flora. A subgroup of the mice was infected with a novel enterohepatic *Helicobacter* species. Intestinal flora was examined using t-RFLP and a group-specific PCR for Helicobacteraceae. At 14 months of age, mice were sacrificed and the entire gastrointestinal tract was processed for histopathology. All mice were colonized by a complex intestinal flora. 83.3% of the *Helicobacter*-exposed IL-10 KO mice developed colitis compared to 12.5% of the unexposed IL-10 KO mice ($p < .001$). None of the unexposed and exposed wild-type animals developed inflammatory bowel disease ($p < .001$). In infected IL-10 KO mice, inflammation was localized in the colorectum, limited to the mucosa, and was characterized by severe acute and chronic inflammation through widespread infiltration with inflammatory cells, crypt hyperplasia, distortion of architecture, and the presence of ulcers and crypt abscesses similar to human ulcerative colitis. Furthermore, focal colitis-associated intraepithelial neoplasia was frequently observed in the inflamed distal colorectum. Infection of IL-10 KO mice with the novel enterohepatic *Helicobacter* results in an inflammatory bowel disease (IBD) with histological features closely resembling human ulcerative colitis. It appears that host genotype sets susceptibility to disease, whereas composition of colonizing flora determines if IBD develops and also whether the type of inflammation is ulcerative colitis-like or Crohn's disease-like.

Abstract no.: 16.02
Helicobacter equorum sp. nov., A Urease-Negative *Helicobacter* Species Isolated from the Feces of Two Horses

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Two bacterial isolates (EqF1^T and EqF2) were retrieved from fecal samples of two asymptomatic horses at the Faculty of Veterinary Medicine, Ghent University, Belgium. They consisted of gram-negative, curved, motile bacteria with a single, monopolar, sheathed flagellum. The bacteria grew at 37 °C under microaerobic conditions, were positive for oxidase, catalase, and alkaline phosphatase and negative for urease production. They reduced nitrate to nitrite, but no gamma-glutamyltranspeptidase activity was detected. Isolates did not grow at 42 °C nor on media containing 1% glycine. They were resistant to cephalotin and nalidixic acid and susceptible to metronidazole. Analysis of the 16S and 23S rDNA sequences of the two strains identified them as belonging to a single species, within the genus *Helicobacter.* In terms of 16S rDNA sequence similarity, *Helicobacter pullorum* and *Helicobacter canadensis* were the most closely related species (98% similarity). 23S rRNA gene analysis too classified EqF1^T and EqF2 within the enterohepatic division of the genus *Helicobacter*, but only 94% similarity was detected with *H. pullorum* and *H. canadensis*, which are helicobacters with unsheathed flagella. The most closely related species in terms of 23S rDNA sequence similarity was *H. canis* (95%). Numerical analysis of whole-cell protein extracts by SDS-PAGE was carried out and the new isolates were clearly differentiated from *H. pullorum*, *H. canadenis*, *H. canis*, and other *Helicobacter* species. On the basis of these genetic, biochemical and protein data, these isolates were classified in a new species, for which the name *Helicobacter equorum* sp. nov. is proposed.

Abstract no.: 16.03
Characterization of *Helicobacter cynogastricus*, A Novel *Helicobacter* Species Isolated from the Stomach of A Dog

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A gram-negative, microaerophilic helical-shaped rod, isolated from the gastric mucosa of a dog and designated JKM4^T, was subjected to a polyphasic taxonomic study. Electron microscopy showed an organism measuring 10 to 18 µm long and up to 1 µm wide, with multiple sheathed flagella at both ends and a periplasmic fibril running along the external side of the helix. Strain JKM4^T preferably grew on biphasic culture plates. Coccoid forms predominated in cultures older than 4 days, as well as in growth obtained on dry agar plates. The strain, growing at 30 and 37 °C but not at 25 or 42 °C, exhibited urease, oxidase, and catalase activity. On the basis of 16S rRNA gene sequence analysis, the isolate presented > 97% similarity with *Helicobacter felis*, *Helicobacter bizzozeronii*, and *Helicobacter salomonis*, all previously isolated from the canine gastric mucosa. Protein profiling by sodium dodecyl polyacrylamide gel electrophoresis showed a pattern different from those of other *Helicobacter* species of mammalian gastric origin and from *H. canis.* Additionally, the urease gene of JKM4^T differs from the urease gene of *H. felis*, *H. bizzozeronii*, *H. salomonis* and "*Candidatus H.* heilmannii." It is thus

proposed that strain JKM4^T (= LMG23188^T) represents a novel species within this genus, *Helicobacter cynogastricus* sp. nov.

Abstract no.: 16.04
Frequency of Colonization of the Colon of Patients with Inflammatory Bowel Disease by *Helicobacter* and *Campylobacter* Species

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Whether *Helicobacter* species are implicated in the pathogenesis of inflammatory bowel disease (IBD) is controversial. We looked for the presence of *Helicobacter* spp. in colonic biopsies of 29 IBD (Crohn's disease (CD), 26; ulcerative colitis, 3 and 6 control patients by culture and real-time polymerase chain reaction (PCR) with the *Taq*Man technology. Specimens were cultured on a selective medium (agar base 2 (Oxoid), 10% horse blood, Skirrow supplement) and, after filtration, on the same medium without Skirrow supplement. Plates were incubated at 37 °C under microaerophilic or anaerobic conditions for 15 days. Primers and an internal *Taq*Man probe were chosen in order to amplify a 192-bp fragment of the 16S rRNA gene specific of the *Helicobacter* genus. One strain of *H. pylori* was detected by culture in a control patient, but gastric biopsies from this patient were also *H. pylori* positive. No strains of *Helicobacter* were cultured from IBD patients. However, one *Campylobacter coli* and one *Campylobacter concisus* were cultured from two CD patients. The "genus *Helicobacter*" PCR was positive for the *H. pylori*-positive control patient as well as for two other CD patients. We identified by DNA sequencing the species implicated. As expected, *H. pylori* was identified for the control patient. The two other species implicated were *Helicobacter pylori* and *Helicobacter canis*. *Helicobacter* species do not seem to frequently colonize the colon of IBD patients. *H. canis* had never been associated with CD. As two CD patients were found to be *Campylobacter* positive by culture, it could be interesting to investigate this aspect.

Abstract no.: 16.05
Response of Hep-2 Cells to *Helicobacter hepaticus*

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Introduction. *Helicobacter hepaticus* infects the bowel and biliary tree of several animals producing inflammation. Colonization of mouse livers can induce hepatocellular carcinomas. The aim of the study was to investigate the effects of *H. hepaticus* on the proliferation and global protein expression of human HEp-2 cells.
Methods and Results. Human HEp-2 cells were grown for three days under a microaerobic atmosphere in Dulbecco's Modified Eagle's Medium or under the same conditions in cocultures with *H. hepaticus* at various inoculum densities. Cell morphology was examined by inverted light microscopy. Staining by trypan blue and counting the number of live and dead cells in an improved Neubauer cytometer were used to assess cell viability. Enlargement, distension and elongation of HEp-2 cells were observed in cocultures with *H. hepaticus.* The number of live cells declined by only an order of magnitude at bacterial inocula of approximately 10^8 cfu/mL, but were completely killed at inocula of about 10^{10} cfu/mL. Protein expression by HEp-2 cells was investigated employing two-dimensional gel electrophoresis. Seventeen differentially expressed proteins were identified by tandem mass spectrometry. These proteins perform several biological functions including amino acid metabolism, cell growth and proliferation, stress response, protein translation, and modification, etc.
Conclusions. The onset of a catastrophic killing of HEp-2 cells at a specific bacterial density suggested a multimodal action for *H. hepaticus* infection. The modulation of the expression of proteins related to different biological functions showed that the presence of *H. hepaticus* had broad effects on the physiology of HEp-2 cells.

Abstract no.: 16.06
Phylogenetic Analysis of "*Helicobacter heilmannii*" Isolates from Different Japanese Patients Based on DNA Sequences of 16S rRNA and Urease Genes

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Background. Although *Helicobacter heilmannii* infection is less common than *Helicobacter pylori* infection in humans, it is of medical importance because of its association with gastritis and mucosa-associated lymphoid tissue lymphoma of the stomach. *H. heilmannii* has been classified into types 1 and 2. Despite the inability of its cultivation, phylogenetic analysis of the 16S rRNA and urease genes have disclosed the occurrence of the types of *H. heilmannii*. However, to the best of our knowledge, no report has been documented concerning such investigation among Japanese *H. heilmannii* isolates. We have made an attempt to demonstrate the present state of the incidence of the types of *H. heilmannii* strains in Japan.
Methods. Prior to the examination, mice were successfully infected with the *H. heilmannii* strains derived from the four patients from different district in Japan. The genomic DNA was extracted from the infected mice, amplified by using the 16S rRNA and urease primers, and then sequenced. The sequence data were then analyzed by BLAST search and CLUSTAL W.
Results. Phylogenetic analysis of the sequences of 16S rRNA and urease genes retrieved from BLAST revealed that all of the four strains studied were *H. heilmannii* type 1, demonstrating 99% similarities of the sequences within the four strains tested.
Conclusions. All of the four Japanese isolates were found to be classified as *H. heilmannii* type 1, and should be assigned to *Candidatus Helicobacter suis*, according to the recent publications. It is noteworthy that none of the *H. heilmannii* type 2 strain was detectable.

Abstract no.: 16.07
Prevalence of *Helicobacter equorum* in Fecal Samples from Horses and Humans, Belgium

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Recently, a new enterohepatic *Helicobacter* species (EHS), *Helicobacter equorum*, was isolated from fecal samples of two clinically healthy horses. At the onset of this study, nothing was known about the prevalence of this organism in horses, nor was there any information available on the possible zoonotic character of this agent, a feature that has been ascribed to other EHS. This study aimed at determining the prevalence of *H. equorum* in fecal samples of human and equine origin by means of polymerase chain reaction (PCR).

Therefore, fecal samples of 120 privately owned horses, 227 riding-school horses, and 239 hospitalized horses were screened for *H. equorum* DNA by means of PCR, amplifying a 1075-bp sequence of the 23S rRNA gene, specific to *H. equorum*. Fecal material of 531 humans suffering from gastrointestinal disease and 100 clinically healthy humans was likewise examined.

H. equorum-DNA was demonstrated in the feces from 0.8% of the privately owned horses (1/120), 3.1% of the riding-school horses (7/227), and 7.9% of the hospitalized horses (19/239). The prevalence of *H. equorum* was significantly higher in hospitalized than in privately owned horses. The species could not be detected in any of the fecal samples from human origin.

In conclusion, the prevalence of *H. equorum* in horses seems fairly low on average, so one may consider the possible existence of a primary host species other than the horse. Moreover, the prevalence in horses appears to increase with crowding and/or stress. Finally, we assume that this microorganism does not commonly spread toward humans.

Abstract no.: 16.08
Helicobacter heilmannii Infection in Gastroduodenal Diseases in Children

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Introduction. *Helicobacter heilmannii* is a spiral-shaped bacterium, larger than *Helicobacter pylori*, and living in the stomach of humans and animals. Infection with *H. heilmannii* is known to cause the diseases of the stomach and duodenum.
Objective of the study. Evaluation of *H. heilmannii* infection in children and its influence on the occurrence of the diseases of the stomach and duodenum was the objective of our study.
Material and methods. Analysis comprised 12 children (7 boys and 5 girls) aged 9 to 17 years, treated in our clinic as a result of recurrent abdominal pain. In all children infection with *H. heilmannii* was diagnosed by endoscopic study. The diagnosis was made on the grounds of typical morphology of the bacteria in direct microscopic picture of gastric mucosa specimens, and negative culture. Infection with *H. pylori* was diagnosed by the means of positive culture.
Results. In all children, *H. heilmannii* infection was diagnosed, and in three, infection with *H. pylori* has also been found. Endoscopic study revealed in all patients chronic gastritis, in four cases duodenitis and duodenal ulcer in one case. Inflammation of gastric and duodenal mucosa was confirmed by histopathologic studies. Bismuth salts, omeprazole, amoxicillin, clarithromycin, or metronidazole were used in the treatment, causing eradication of bacteria and subsidence of the ailments.
Conclusion. Our observation confirms the contribution of *H. heilmannii* infection in the ethiopathogenesis of gastric and duodenal diseases.

Abstract no.: 16.09
Helicobacter pullorum-induced IL-8 Expression by Epithelial Cells Originating from the Digestive Tract Involves NF-κB Signaling

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Helicobacter pullorum is an enterohepatic bacteria isolated from patients suffering from digestive and hepatic diseases. Even though *H. pullorum* is clinically associated with enterohepatic inflammatory diseases, our knowledge of the pathogenicity of this bacteria is limited and only includes a cytolethal distending toxin (Cdt), which blocks the cell cycle in G2-M, and its lipopolysaccharide (LPS).

The aim of this study was to determine the pathogenicity of *H. pullorum* on its enterohepatic target cells in vitro, particularly in terms of pro-inflammatory response, and to elucidate the cellular signal transduction pathways involved and the bacterial factor responsible for the effects observed. For this purpose, we looked at the IL-8 secretion on human gastric or intestinal epithelial cell lines and on human hepatocytes after coculture with different *H. pullorum* strains from human and avian origin.

We found that *H. pullorum* stimulates IL-8 production by both AGS gastric cells and Caco2 and HT29 intestinal cell lines as well as by human HepG2 hepatocytes. Using pharmacological inhibitors, we demonstrated that the signalling pathways used by the bacteria to regulate IL-8 expression involve the NF-κB transcription factor. This result was confirmed by immunofluorescent cellular staining showing rapid nuclear translocation of NFκB p65 subunit after *H. pullorum* stimulation. A mutant strain inactivated for the Cdt gene has been prepared and will be tested in order to determine if this toxin is involved in NF-κB activation and IL-8 expression.

Abstract no.: 16.10
Detection of *Helicobacter* spp. Infection in Canine Gastric Mucosa Using Different Diagnostic Methods

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Background. *Helicobacter* species have been described in stomach of many animals. However their role in pathogenesis of gastric pathological changes in dogs and cats remains still unexplained. The frequency of colonization of canine gastric mucosa by *Helicobacters* represents actual problem.
Aim of the study. The aim was to evaluate using different methods the occurrence of *Helicobacter* spp. infection in dogs.
Materials and methods. We studied 55 dogs of different breed and sex, aged from 2 to 13 years with dyspeptic symptoms (chronic vomiting, lack of appetite, abdominal discomfort). Biopsy samples of animals were taken during endoscopy from gastric antrum and the corpus. The samples were analyzed by urease test, direct microscopy, culture, and polymerase chain reaction (PCR).
Results. The rapid urease test was positive in 46 dogs (83.6%). Direct microscopy showed the presence of spiral, corkscrew-shaped, gram-negative bacteria in corpus of stomach in 43 dogs (78.2%) whereas in gastric antrum, spiral microorganisms were seen in 38 dogs (69%). Culture of samples was positive for *Helicobacter felis* in nine animals (16.4 %). The presence of genus *Helicobacter* spp. was founded by PCR in 54 subjects (98.2%), whereas the most frequently identified organism was *H. heilmannii*, rarely *H. felis*. *Helicobacter pylori* was detected in two dogs.
Conclusions. PCR is very sensitive method for detection of *Helicobacter* spp. infection in dogs. The most commonly isolated organism belonging to the genus *Helicobacter* in canine gastric mucosa is *H. heilmannii*. *Helicobacter pylori* colonizes the stomach of dogs.
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Abstract no.: 16.11
Detection of the Genus *Helicobacter* in Racehorses Gastric Mucosa

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The equine gastric ulcer syndrome (EGUS) includes the inflammatory and ulcered mucosa diseases (acute lesions of gastric mucosa, chronic gastritis, chronic-peptic ulceration, and gastropathy). EGUS has long been identified postmortem, yet its etiology is unknown. In mammals, different *Helicobacter* species have been detected and associated with gastrointestinal pathologies. In horses, *Helicobacter* spp. is still poorly documented. The aim of this study was to detect the presence of *Helicobacter* infection in thoroughbred racehorses and its correlation with EGUS. Samples were collected from 20 horse's stomachs postmortem and were examined by histopathology and using *Helicobacter* genus-specific and *H. pylori*-specific primers by polymerase chain reaction (PCR). In the histopatologic studies we found 7/20 gastric ulceration, 5/20 gastritis and 6/20 with both pathologies. *Helicobacter* spp. were detected by PCR in 2/7 ulcers, 3/5 gastritis, 5/6 with both pathologies, and 1/2 with normal mucosa. All samples that were positive for Wharthing–Starry stain, were also positive for genus *Helicobacter* by PCR, with exception of one sample. Five of 20 of *Helicobacter* positive samples shared more than 98% sequence identity with *H. pylori* 399 bp fragment of the 16S rRNA gene. In contrast, none of gastric samples were positive for *H. pylori* specific PCR.

The 91% of thoroughbred racehorses infected with *Helicobacter* spp. had pathologies as ulcers and gastritis. These results supports that *Helicobacter* species are an important cause of EGUS in racehorses. Culture and subsequent bacterial identification are still required to identify these *Helicobacter* species.

Abstract no.: 16.12
Presence of *Helicobacter cetorum* and Candidatus *Wolinella africanus* in Gastric Juice of Asymptomatic Venezuelan Subjects

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The spectrum of human non-pylori *Helicobacter* infections is expanding. *Helicobacter pylori* is known to be indigenous to the human stomach, and the presence of *Helicobacter heilmannii* and *Helicobacter felis* has been associated with different clinical disorders. The aim of this study was to investigate the presence of *Helicobacter* in asymptomatic subjects. Using polymerase chain reaction-denaturing gradient gel elektrophoresis (DGGE), a sensitive molecular diagnostic tool, we evaluated the prevalence of Helicobacteraceae family in human gastric juice of 91 subjects (aged 18 to 68, 41 women, 50 men) from two cities of Venezuela. *H. pylori* was detected in 76% of the studied group, whereas, *Helicobacter cetorum* and Candidatus *Wolinella africanus* were detected in 16% and 15% of the subjects, respectively. Mixed colonization was observed in several cases: *H. cetorum* and Candidatus *Wolinella africanus* (4%), *H. pylori* and Candidatus *Wolinella africanus* (4%), and *H. pylori* and *H. cetorum* (2%). Our results constitute the first evidence of the presence of *H. cetorum* and Candidatus *Wolinella africanus* in the human stomach.

Abstract no.: 16.13
The Prevalence of *Helicobacter species* in the Stomach of Cats in Poland from Lower Silesia Area

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Background. Different species belonging to the genus *Helicobacter*, namely *Helicobacter heilmannii*, *Helicobacter felis*, *Helicobacter pylori*, and *Helicobacter bizzozeroni* are considered common inhabitants of feline gastric mucosa.
Aim. The aim of the study was to determine the presence of *Helicobacter* species in gastric mucosa in cats from Lower Silesia area.
Material and Methods. Thirty-five cats of European breed and different sex, aged from 1 to 10 years were studied. Animals were divided into two groups: control group concerning 10 cats without clinical signs and the second one consist of 25 cats with dyspeptic symptoms (chronic nausea, fetor ex ore, lack of appetite, abdominal pain). Gastric biopsy samples taken from animals during endoscopy were analyzed by polymerase chain reaction (PCR).
Results. In control group *Helicobacter* spp. infection was identified by PCR in seven cats (70%). Infection caused by *H. heilmannii* was observed in three animals whereas mixed infection with two species: *H. heilmannii* and *H. felis* in four cats (57.1%) respectively.
In the second group, *Helicobacter* spp. infection was found in 18 animals (72%). *H. heilmannii* was present in five subjects (27.8%). Mixed infections with two species: *H. heilmannii* and *H. felis* or *H. heilmannii* and other species not identified by PCR were observed in 13 cats (72.2%).
Conclusions. *H. heilmannii* is the most frequently detected organism belonging to the genus *Helicobacter* in stomach of cats. Different *Helicobacter species* are present in the gastric mucosa of both healthy cats and cats with various dyspeptic symptoms.
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Abstract no.: 16.14
Helicobacter heilmannii in a Child, Dog and Cat

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Background. *Helicobacter heilmannii,* is a species of genus *Helicobacter* that is found both in humans and animals and like *Helicobacter pylori* is associated with a range of gastrointestinal symptoms. Investigators speculate that *H. heilmannii* infection in humans may represent zoonosis and may be acquired from domestic animals.
Aim. The aim of the study was to investigate the epidemiologic features of gastric mucosa infection caused by *H. heilmannii* in a child and domestic animals.
Materials and methods. The project concerning 14-year-old girl who had been suffering from epigastric pain for 3 months. Gastrointestinal endoscopy revealed chronic inflammation in gastric mucosa with neutrophil infiltration. The gastric biopsy was also performed in domestic animals: dog and cat. Multiple biopsies were taken from the gastric antrum and the corpus of the child, dog, and cat. Samples were analyzed by histologic and bacteriologic examination using direct microscopy, culture, urease test, and polymerase chain reaction (PCR). Specimens were cultured on Columbia agar media with 7% horse blood and different selective supplements in microaerophilic atmosphere for 7 days.
Results. The rapid urease test for all samples was positive. Direct microscopy of all samples showed the presence of spiral (4–8 tight spirals), corkscrew-shaped, gram-negative bacteria, morphologically resembling *H. heilmannii*. Culture of all specimens was negative for *H. pylori* and *H. heilmannii*. The presence of *H. heilmannii* in animals' specimens was confirmed by nested PCR assay. The eradication treatment was performed in child and in animals. The girl was successfully treated with amoxicilin and clarithromycin.
Conclusions. *Helicobacter heilmannii* infection may have zoonotic origin.

Hepatobiliary Diseases

Abstract no.: 17.01
Urease-Induced Calcium Precipitation by Bile-Resistant *Helicobacter* Species may Initiate Gallstone Formation

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Background. *Helicobacter* species can colonize the gastrointestinal and hepatobiliary tract of many mammals, and often cause inflammation-associated diseases. Recently, infection with *Helicobacter bilis* and *Helicobacter hepaticus* has been linked to the formation of cholesterol gallstones in mice, but the mechanism underlying this phenomenon was not described. Gallstones are crystalline bodies formed by accretion or concretion of bile components. Bacteria are able to initiate stone formation through calcium precipitation via the activity of the enzyme urease. In this study we have investigated whether *Helicobacter* urease activity may initiate gallstone formation via calcium precipitation.
Methods. A precipitation agar was developed which allowed for both the growth of *Helicobacter* species, and the testing of their ability to precipitate calcium. Bile acid-resistance was tested by

growing *H. hepaticus* and *Helicobacter pylori* in media supplemented with a range of bile acids.
Results. Four urease-positive *Helicobacter* species (*H. hepaticus, H. pylori, H. mustelae,* and *H. bilis*) were capable of precipitating calcium in our assay. In contrast, isogenic *ureB* urease-negative mutants of *H. hepaticus, H. pylori,* and *H. mustelae,* and the urease-negative *Helicobacter* species *H. cinaedi* and *H. pullorum* were unable to do so. *H. hepaticus* was more resistant to deoxycholic acid and cholic acid than *H. pylori,* whereas their resistance to chenodeoxycholic acid did not differ.
Conclusion. Urease-positive *Helicobacter* species capable of colonizing the bile ducts may initiate the formation of gallstones via their urease activity. This provides a possible mechanism for thelink between hepatobiliary colonization with urease-positive *Helicobacter* species and gallstone formation.

Abstract no.: 17.02
Analysis of the Risk Factors Helicobacter Infection, Overweight, Sex, and Age in Gallstone Disease and Gallbladder Carcinoma in Germany

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Helicobacter infection of the hepatobiliary system has been proposed as a novel risk factor in the pathogenesis of gallstone disease (GSD) and gallbladder carcinoma (GBC). Because there seem to be differences in the incidences of *Helicobacter* infection in various populations, we investigated whether *Helicobacter* infection of the biliary tract is present in Germany, a region with a high incidence of GSD, but with a low incidence of GBC. Gallbladder tissue from 99 patients who had undergone cholecystectomy were investigated: 57 patients with GSD, 20 cases with GBC, and 22 control patients. The presence of *Helicobacter* spp. was investigated by culture, immunohistochemistry, and a group-specific polymerase chain reaction (PCR) targeting the 16S rRNA and detecting all currently known Helicobacteraceae. Of the 99 cases investigated, only one patient with GSD was PCR-positive for Helicobacteraceae. In this subject, sequence analysis of the 16S rRNA showed closest homology to the 16S rRNA sequence of *Helicobacter ganmani*. Helicobacteraceae were not detected by culture or immunohistochemistry. There was a higher body mass index in patients with GSD compared to controls ($p < .05$). Mean age of patients with GBC was significant higher than for GSD ($p < .01$) or control patients ($p < .005$), whereas there was no difference between GSD and controls. These data suggest that Helicobacteraceae plays no predominant role in the pathogenesis of GSD and GBC in the German population. The low prevalence of Helicobacteraceae in the gallbladder mucosa of German patients could be a possible explanation for the relatively low prevalence of GBC although GSD is frequent.

Abstract no.: 17.03
Usefulness of Two New Monoclonal Stool-Antigen Tests for Diagnosing *Helicobacter pylori* Infection in Cirrhosis

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Background. Because *Helicobacter pylori* infection is the major cause of peptic lesions in patients with cirrhosis, causing upper intestinal hemorrhage and significant morbidity, it is very important to find an accurate diagnostic noninvasive method for *H. pylori* detection in these patients.
Objective. To evaluate the usefulness of two new tests in diagnosing *H. pylori* infection in cirrhotic patients: amplified IDEIA™ *H. pylori* StAR™ and *RAPID H. pylori* StAR™, an enzyme-linked immunosorbent assay and a rapid immunochromatographic test respectively, that use monoclonal antibodies (both Dako-Cytomation, Denmark).
Methods. *H. pylori* infection was determined in 73 patients with cirrhosis by concordance of histology and urea breath test. Fecal tests were performed according to the specifications of the manufacturer. Sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively) were calculated for both tests. Spearman test and Kappa statistics estimated correlations between *RAPID H. pylori* StAR readings and concordance between both kits, respectively.
Results. According to the reference method sensitivity (%), specificity (%), PPV (%), and NPV (%) were 77–84, 55–66, 74–77, and 66–70 for *RAPID H. pylori* StAR™; and 77, 93, 94 and 73, for amplified IDEIA™ *H. pylori* StAR. Readings from both readers were highly correlated; Spearman correlation test was 0.936 ($p < .001$). Concordance between tests showed an acceptable correlation. Kappa statistics were 0.563 for observer 1 and 0.509 for observer 2.
Conclusions. Fecal tests could be an option to detect *H. pylori* infection in patients with cirrhosis. Unfortunately, in this study, the sensitivities and specificities obtained from the tests recent developed remain non optimal for these patients.

Abstract no.: 17.04
Helicobacter Species rDNA in Livers of Children with Inflammatory Liver/bowel Disease or with Intrahepatic Cholestasis

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Aim. To investigate the prevalence of *Helicobacter* species in the liver of children and adolescents with chronic liver- and/or inflammatory bowel disease or intrahepatic cholestasis.

Methods. We investigated 79 patients (43 girls, mean age 15, range 1.4–21.2 years) with the diagnoses autoimmune hepatitis (AIH) (n = 36), AIH and ulcerative colitis (UC) (n = 4), UC (n = 2), primary sclerosing cholangitis (PSC) (n = 3), PSC and UC (n = 18), PSC and Crohn's disease (CD) (n = 5), progressive familiar intrahepatic cholestasis (PFIC) (n = 10), and indeterminate colitis and unspecific liver disease (n = 1). Liver tissue specimens were analyzed using a semi-nested *Helicobacter*-specific 16S rDNA polymerase chain reaction (PCR) assay. PCR products were characterized by DNA-sequence analysis.
Results. The *Helicobacter* genus-specific PCR assay was positive in 3 of 18 (17%) liver specimens from the PSC-UC patients, in 1 of 4 (25%) livers from the AIH-UC patients, in 2 of 10 (20%) livers from PFIC patients, and in 1 of 36 (3%) livers from patients with AIH. PCR products of all positive samples were sequenced and subjected to a BLASTn analysis in GenBank. The sequenced specimens were similar to the 16S rDNA of *Helicobacter hepaticus* (n = 2), *H. pylori* (n = 2), *Helicobacter pullorum*, *Helicobacter muridarum*, and *Helicobacter canis*.
Conclusions. Enterohepatic *Helicobacter* species, as well as *H. pylori*, were detected in livers of patients with ulcerative colitis and a simultaneous liver disease (PSC and AIH). The relevance of these findings needs further elucidation by serological analysis and immunohistochemistry studies.

Abstract no.: 17.05
Prevalence Helicobacter and Other Bacteria in Bile and Gallbladders of Kosovan Patients with Chronic Cholecystitis in Correlation to Age, Gender, and Urban-rural Differences

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The aim of this study was to investigate the presence of *Helicobacter* DNA in bile and gallbladder specimens of Kosovan patients with and without chronic cholecystitis. DNA was extracted from 84 gallbladder and 52 bile specimens of patients with chronic cholecystitis, as well as from 44 bile specimens of patients without cholecystitis (control group). All specimens were examined for the presence of Helicobacter and other bacterial species by genus- and group-specific polymerase chain reaction assays. *Helicobacter* DNA was detected in 19 of 51 and 26 of 84 bile and gallbladder specimens of patients with cholecystitis, respectively, compared to 2 of 44 of a control group bile specimens ($p < .05$). DGGE analysis and DNA sequencing identified DNA of *Helicobacter pylori*, *Helicobacter pullorum*, *Helicobacter cholecystus*, *Helicobacter muridarum*, *Helicobacter pametensis*, and *Helicobacter hepaticus* in bile specimens and *H. pullorum*, *H. pylori*, *H. cholecystus*, and *Helicobacter bilis* and *H. pametensis* in gallbladders of patients with cholecystitis. On the other hand, Enterobacteriaceae and *Lactobacillus* DNA was detected in 4% and 8%, and 7% and 5% of bile and gallbladder specimens of patients with cholecystitis, and in 9% and 11% of the control group bile, respectively. There was an increase of *Helicobacter* prevalence in bile and gallbladder specimens with an increase of patients' age, which was maximum at the age range 50–60 years. In addition, a higher prevalence of *Helicobacter* in bile and gallbladder specimens collected from patients living in rural areas compared to urban areas ($p < .05$). These results highlight the possible role of *Helicobacter* species in chronic cholecystitis.

Abstract no.: 17.06
Efficacy of Proton Pump Inhibitor-Based Triple Therapy for Eradicating *Helicobacter pylori* in Patients with Chronic Liver Disease and Peptic Ulcer

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Background. Peptic ulcer occurs more commonly in patients with liver cirrhosis (LC). *Helicobacter pylori* is well recognized as the most important etiology in the pathogenesis of peptic ulcer.
Aims. To determine the efficacy and safety of proton pump inhibitor (PPI)-based triple therapy with clarithromycin and amoxicillin in patients with chronic liver disease and peptic ulcer.
Methods. One hundred sixty-three patients with LC (n = 104; LC group) or chronic hepatitis (n = 59; CH group) were studied when they had peptic ulcer and proven *Helicobacter pylori* infection. The combination of omeprazole, 20 mg twice daily; amoxicillin, 1 g twice daily; and clarithromycin, 500 mg twice daily, was administered for 1 or 2 weeks. *H. pylori* status was determined by rapid urease test, histology, or ^{13}C-urea breath test before starting and at least 4 weeks after completing treatment.
Results. Among the 104 patients of LC group, 70 patients had compensated LC and 34 patients had decompensated LC. The eradication rate of *H. pylori* was similar between the LC and the CH groups (79.8% and 84.7%, respectively, $p = .434$). Also, there were no significant differences in eradication rates between the patients with decompensated LC and CH group (76.5% in patients with decompensated LC, $p = .320$). Side effects in each group were generally mild and all patients completed the entire course of therapy.
Conclusions. The PPI-based triple therapy achieves a high eradication rate of *H. pylori* infection in patients with LC, similar to chronic hepatitis. The regimen is well tolerated.

Abstract no.: 17.07
High Antibody Responses to *Helicobacter pullorum* and *Helicobacter pylori* in Hepatitis C Virus Infected Patients

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Background. Some recent studies suggest that a co-infection with *Helicobacter* species occur in patients infected with hepatitis C

(HCV) and could be associated with the development of severe chronic liver diseases. The aim of this study was to analyze the serum antibody responses to three enterohepatic *Helicobacter* species and to *H. pylori* in sera from HCV infected patients and clinically healthy persons.

Material and Methods. Sera from 38 HCV infected patients and 40 blood donors were initially tested by enzyme-linked immunosorbent assay (ELISA), with high sensitivity, using cell surface proteins (CSPs) of *Helicobacter pullorum, Helicobacter bilis, Helicobacter Hepaticus,* and *Helicobacter pylori* as antigen. To minimize serological cross reactivity between the enteric *Helicobacter* species and *H. pylori,* sera were absorbed with a whole cell lysate of *H. pylori* prior to testing. Sera positive by ELISA were analyzed by immunoblot (IB), and antibody reactivity to specific immunogenic CSPs of each of the species were interpreted.

Results. Significant differences between the two groups were found for *H. pylori* and *H. pullorum* ($p > .01$) and to a lesser extent to *H. bilis* ($p = .07$).

Conclusions. Immunodiagnosis for infections with bile-tolerant *Helicobacter* species are important to establish as these microbes are extremely difficult to culture and liver biopsy sampling is not possible in many patients as a result of the high risk of bleeding. Infections with some *Helicobacter* species could be a co-risk factor in human HCV liver disease. Further studies are needed on host and regional factors to understand the progression of a *Helicobacter* infection in HCV-infected patients.

Abstract no.: 17.08
The State of Gastro-Esophageal Mucous and *Helicobacter pylori* Infection in Patients with Cholelithiasis

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Introduction. The states of gastroesophageal mucous and prevalence *Helicobacter pylori* infection in patients with cholelithiasis are not well known.

Aims. To evaluate frequency dyspeptic symptoms, state of gastroesophageal mucous, and prevalence of *H. pylori* infection in patients with cholelithiasis.

Methods. One hundred ten patients with cholelithiasis (18 men and 92 women, at middle age 48.2 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 histology and breath test (PY test 14C).

Results. The presence *H. pylori* infection defined in 87 (79.1%) of the 110 researched patients. The dyspeptic symptoms, i.e., heartburn, nausea, bilious vomiting, had 32, 31, and 36.8; and 13, 13, and 8.7% of *H. pylori*-positive and *H. pylori*-negative patients, respectively ($p < .05$). The RE grade A occurred in 2.35% and 4.55%, RE grade B in 1.18% and 0% of *H. pylori*-positive and *H. pylori*-negative patients, respectively ($p < .05$). Antrum and/or corpus gastritis revealed in 61.6% and 59.1%, gastric erosions occurred in 9.4% and 4.55% of *H. pylori*-positive and *H. pylori*-negative patients, respectively ($p < .05$).

Conclusions. The prevalence *H. pylori* infection was high in patients with cholelithiasis. The dyspeptic symptoms occurs much more often in *H. pylori*-positive patients than in *H. pylori* negative patients. The frequency and severity of gastroesophageal lesions was not high both in *H. pylori* positive and in *H. pylori* negative patients with cholelithiasis.

