Helicobacter

European Helicobacter Study Group

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Contents

Workshop Presentations

WS 1 Clinical Challenges

Abstract no.: W1.1
Search for a Grade A therapy for *H. pylori* eradication: 14-day sequential or sequential-concomitant hybrid therapy

P. Hsu[1], D. Wu[2], J. Wu[2] and D. Graham[3]
[1]Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; [2]Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [3]Michael E. DeBakey Veterans Affairs Medical Center and Baylor College of Medicine, Houston, TX, United States

Background & Aims: Typically, sequential therapy with a proton pump inhibitor (PPI) and amoxicillin followed by a PPI, clarithromycin, and an imidazole achieves *H. pylori* eradication rates of < 95% (Grade B result) by per-protocol (PP) analysis. We tested whether prolonging treatment duration or continuing amoxicillin throughout the treatment period would produce a ≥ 95% (Grade A) result.
Methods: These were two prospective, randomized, pilot studies where *H. pylori* infected patients received either a 14-day sequential therapy (esomeprazole and amoxicillin for 7 days followed by esomeprazole, clarithromycin, and metronidazole for 7 days) or a sequential-concomitant hybrid therapy (esomeprazole and amoxicillin for 7 days followed by all 4 drugs for 7 days). *H. pylori* status was examined 8 weeks after therapy. Success was defined as achieving a Grade A result (≥ 95% by PP analysis).
Results: 240 patients were randomized (123 to sequential and 117 to hybrid therapy). Eradication rates by intention-to-treat analysis were 91.9% (95% CI; 87.1–96.7%) with sequential therapy and 97.4% (95% CI; 94.5–100%) with hybrid therapy. Eradication rate by per-protocol analysis were 93.9% (Grade B result) and 99.1% (Grade A result), respectively. Both treatments exhibited similar frequencies of adverse events (21.1% *vs.* 14.5%) and drug compliance (95.9% *vs.* 94.9%).
Conclusions: Extending sequential therapy to 14-days did not result in a Grade A result. However, 14-day sequential-concomitant hybrid therapy achieved a Grade A success. Studies in different regions are needed to confirm these findings. Studies are underway to determine whether Grade A success is maintained with hybrid therapy shorter than 14 days.

Abstract no.: W1.2
High dose Amoxicillin-based first line regimen is equivalent to sequential therapy in the eradication of *H. pylori* infection

F. Franceschi, M. Campanale, R. Finizio, F. Barbaro, A. Tortora, G. Gigante, V. Cesario, S. Calcinaro, D. Marsiliani, A. Carroccia, N. Gentiloni Silveri, D. Currò, G. Cammarota, G. Gasbarrini and A. Gasbarrini
Internal Medicine, Catholic University of Rome, Italy., Rome, Italy

Background: It is known that *H. pylori* eradication rates with standard triple therapy have declined to unacceptable levels. On the other hand, sequential therapy has been shown to increase eradication rate in first-line therapy.
Aim: To compare the efficacy of a high-dose amoxicillin based first line regimen with that obtained by sequential therapy.
Methods: 150 sex and age matched patients were randomized into 3 different therapeutic schemes: (1) standard LCA, lansoprazole 15 mg bid, clarithromycin 500 mg bid and amoxicillin 1000 mg bid for 7 days; (2) high dose LCA (HD-LCA), lansoprazole 15 mg bid, clarithromycin 500 mg bid and amoxicillin 1000 mg tid for 7 days; (3) sequential LACT, lansoprazole 15 mg bid plus amoxicillin 1000 mg bid for 5 days, followed by lansoprazole 15 mg bid, clarithromycin 500 mg bid and tinidazole 500 mg bid for 5 days. Eradication was confirmed by ^{13}C-urea breath test. Compliance and occurrence of adverse effects were assessed by a validated questionnaire.
Results: Eradication rates were: LCA (50% PP, 48% ITT), HD-LCA (74% PP, 72% ITT), LACT (74% PP; 72% ITT). Eradication rates were higher in HD-LCA group compared to LCA ($p < 0.01$), while no significant differences were observed in HD-LCA group compared to LACT (p = ns). Compliance and occurrence of adverse effects was similar among groups.
Conclusions: High dose amoxicillin based eradicating treatment is superior to standard triple therapy and equivalent to sequential therapy; compared to the latter, the shorter duration may represent an advantage.

Abstract no.: W1.3
Sequential Levofloxacin modified therapy is superior to quadruple therapy but equivalent to standard Levofloxacin based second line treatment in patients with *H. pylori* infection

F. Franceschi, R. Finizio, M. Campanale, F. Barbaro, G. Gigante, V. Cesario, A. Tortora, S. Calcinaro, D. Marsiliani, A. Carroccia, N. Gentiloni Silveri, D. Currò, G. Cammarota, G. Gasbarrini and A. Gasbarrini
Internal Medicine, Catholic University of Rome, Italy., Rome, Italy

Background: Quadruple therapy is recommended in *H. pylori*-positive patients in whom first line treatment failed. Levofloxacin is another option, either as triple therapy or in a sequential way; whether the sequential is superior to standard administration is not known.
Aim: To compare the efficacy of a sequential levofloxacin-based second line therapy with standard levofloxacin based eradicating scheme and quadruple therapy.
Methods: 150 sex and age matched *H. pylori* positive patients were randomized into 3 different therapeutic schemes: (1)

standard LAL, lansoprazole 15 mg bid, amoxicillin 1000 mg bid and levofloxacin 750 mg for 10 days; (2) sequential LALT, lansoprazole 15 mg bid plus amoxicillin 1000 mg bid for 5 days, followed by lansoprazole 15 mg bid, levofloxacin 500 mg and tinidazole 500 mg bid for 5 days; (3) quadruple TMBL, tetracycline 500 mg qid., metronidazole 500 mg tid., bismuth salt 120 mg qid., lansoprazole 15 mg bid for 7 days. Eradication was confirmed by ^{13}C-urea breath test. Occurrence of adverse effects was also assessed.
Results: Eradication rates were: LAL (90% PP, 88% ITT), LALT (88% PP, 86% ITT), TMBL (70% PP; 68% ITT). Eradication rates were higher in LAL and LALT compared to TMBL ($p < 0.01$), while no significant differences were observed between LAL and LALT (p = ns). Occurrence of adverse effects was increased in TMBL group.
Conclusions: Sequential levofloxacin-based second line treatment is superior to quadruple therapy in *H. pylori* eradication, with a lower occurrence of side effects. However, the way of administration of levofloxacin does not influence its efficacy.

Abstract no.: W1.4
Nationwide survey antibiotic resistant strains of *H. pylori* infection in Thailand

R. Vilaichone[1], P. Gamarai[2] and V. Mahachai[3]
[1]Gastroenterology Unit, Department of Medicine, Thammasat Univesity Hospital, Pathumthani, Thailand; [2]Department of Biochemistry, Thammasat University Hospital, Pathumthani, Thailand; [3]Division of Gastroenterology, Department of Medicine, Chulalongkorn University Hospital, Thailand

Objective: The aim of this study was to national survey the antibiotic resistant pattern of *H. pylori* infection in different geographical locations in Thailand between January 2005 and January 2010 and to compare the antibiotic resistant strains among the patients with gastritis and peptic ulcer diseases.
Methods: A total of 2,648 dyspeptic patients who underwent upper endoscopy from different regions (North, Northeastern, Central and Southern) of Thailand during January 2005-January 2010 were enrolled in this study. Two antral gastric biopsies were obtained for culture and susceptibility tests were performed using the E-test.
Results: 882 patients (30%) were infected with *H. pylori* identified by rapid urease test. E-test for all 4 antibitiotics was successfully in 228 isolations (95 male, 133 female, mean age 52 years). The endoscopic findings demonstrated 167 gastritis patients and 61peptic ulcer patients. The prevalence of antibiotic-resistant *H. pylori* was amoxycillin 7%, tetracyclin 3.1%, clarithromycin 3.1%, metronidazole 38.6%, and multidrugs 4.4%. Age, gender and endoscopic findings were not statistical different between patients with resistant and sensitive strains.
Conclusion: Prevalence of *H. pylori* infection has decreased in all regions of Thailand. The prevalence of metronidazole resistant strain was high and remains the most common antibiotic resistant strains in Thailand whereas clarithromycin resistance has markedly declined in recent years. The reason for such a decline is likely due to the wide use of other newer antibiotics in place of clarithromycin.

Abstract no.: W1.5
Do members of the Helicobacteraceae play a role in Crohn's disease?

N. O. Kaakoush[1], J. Holmes[1], S. Octavia[1], S. Man[1], L. Zhang[1], N. Castano-Rodriguez[1], A. Day[2], S. T. Leach[1], D. A. Lemberg[3], S. Dutt[4], M. Storman[4], E. V. O'Laughlin[4], A. Magoffin[4] and H. M. Mitchell[1]
[1]University of New South Wales, Sydney, Australia; [2]University of Otago, Christchurch, New Zealand; [3]Sydney Children's Hospital, Sydney, Australia; [4]Children's Hospital, Westmead, Sydney, Australia

Background: Given that members of Helicobacteraceae colonize the intestinal mucus layer, we hypothesized that they may play a role in Crohn's disease (CD). The aim of this study was to investigate the presence of Helicobacteraceae DNA in biopsies from children with CD and controls and to identify the species present.
Methods: Intestinal biopsies were collected from 179 children undergoing colonoscopy of whom 77 were newly diagnosed CD and 102 controls. Helicobacteraceae DNA was detected using a nested Helicobacteraceae-specific PCR. DNA sequencing was conducted on positive samples and the sequences identified using BLASTn searches.
Results: Thirty-two children with CD (41.5%) were positive for Helicobacteraceae DNA which was significantly higher (P = 0.0062) that that in the controls (22.5%). Sequencing identified *H. pylori* as well a range of EHS including H. ganmani, H. bilis, H. canis, H. hepaticus and H. trogontum, as well as W. succinogenes. The prevalence of non-pylori Helicobacteraceae was significantly higher (P = 0.04) in patients than controls. As all children were proven negative for gastric *H. pylori* we undertook a phylogenetic analysis of *H. pylori* 16S rRNA gene sequences obtained from a subset of CD patients. This showed the CD *H. pylori* strains to cluster with other *H. pylori* strains detected in the intestine, gall bladder and liver, rather than with gastric *H. pylori*.

Abstract no.: W1.6
H. pylori eradication for prevention and healing of gastric and duodenal ulcers in patients requiring non-steroidal anti-inflammatory drugs (NSAID) therapy: A systematic review

M. Venerito and P. Malfertheiner
Dept. of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Germany

Objectives: To perform a systematic review of randomized controlled trials on the impact of *H. pylori*-eradication on prevention and healing of gastric (GU) and duodenal ulcers (DU) in patients requiring NSAID-therapy.
Methods: The search strategy consisted of combining medical words from three categories: *H. pylori* (term inserted: ''*pylori*''), prevention strategy (term inserted: ''eradication'') and drugs (terms inserted: ''NSAIDs'', ''aspirin'', ''cyclooxygenase inhibitors'') using PubMed. Studies were stratified according to the

type of outcome (primary or secondary prevention of GU and DU, ulcer healing) and intervention (*H. pylori-eradication* vs. no treatment/placebo or vs. PPI).
Results: A total of 71 references were identified. Eight papers met our inclusion and exclusion criteria. Major findings were: 1. For primary ulcer prevention *H. pylori-eradication* before starting an NSAID-therapy reduces the risk of NSAID-induced GU and virtually abolishes the risk of DU. 2. *H. pylori*-eradication alone is not sufficient for secondary prevention of NSAID-induced GU and DU. 3. *H. pylori*-infection appears to further increase the protective effects of proton pump inhibitors (PPI) to reduce the risk of ulcer relapse. 4. *H. pylori*-eradication does not influence the healing of both GU and DU if NSAID-intake is discontinued.
Conclusion: *H. pylori-eradication* is beneficial in primary prevention of GU as well as DU in patients requiring NSAID-therapy. In NSAID-users PPI therapy is mandatory for secondary prevention of gastroduodenal ulcers, and appears to further reduce the risk of ulcer relapse in those with persisting *H. pylori* infection. Eradication of *H. pylori* does not delay ulcer healing if NSAID-intake is discontinued.

Abstract no.: W1.7
H. pylori (HP) infection and osteoporosis: A population based study

N. Figura, L. Gennari, D. Merlotti, M. Campagna, B. Franci, A. Avanzati, B. Lucani, A. Calabrò, S. Lardiello and R. Nuti
Department of Internal Medicine, University of Siena, Siena, Italy

Introduction: We reported an increased risk of osteoporosis in males infected by CagA+ HP. At present, we investigate the influence of HP infection on the bone mineral density (BMD) and bone markers in a large epidemiological cohort.
Patients and Methods: 1118 individuals were examined (age range 50–87 yrs): 183 males (age, 65. 9 ± 6 yrs) and 935 females (age, 62.5 ± 6 yrs). 2). The HP and CagA status were determined serologically by commercial ELISA kits.
Results: 53. 0% of males (no., 97) and 42.9% of females (no., 402) (P = 0.015) were infected. Anti-CagA antibodies were detected in 27.8% of males (no., 51) and 25.9% of females (no., 243). The prevalence of the overall HP infection among subjects with normal BMD (43.9%) did not differ from that of patients with osteoporosis (41.5%) and osteopenia (46.2%). However, the prevalence of CagA+ HP infection in osteoporotic (30%) and osteopenic (26%) patients was significantly higher than that in subjects with normal BMD (21%) (P = 0.01). The anti-CagA antibody titer was significantly and negatively associated with BMD at different sites in males as well as in females. Above the median anti-CagA antibody level, only 14% of males and 30% of females had normal BMD. Finally, prevalent symptomatic fractures of the hip and the vertebral bodies were reported in 4% of CagA+ and 2% of CagA- patients and 0.8% of uninfected subjects (P < 0.05, CagA+ vs. uninfected individuals).
Conclusions: CagA+ HP infection may be considered a risk factor for osteoporosis and fractures in males as well as in females.

Abstract no.: W1.8
Meta-analysis on the relationships between *H. pylori (H. pylori)* infection and colon cancer

T. Rokkas, P. Sechopoulos, D. Pistiolas, G. Margantinis and G. Koukoulis
Gastroenterology Unit, Henry Dunant Hospital, Athens, Greece

H. pylori is an important causative factor in gastric carcinogenesis. However its role in colon cancer is controversial.
Aim: The main aim of this study was to explore the relationship between *H. pylori* infection and this malignancy 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 March 2010, 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 = 0.062. There was no publication bias (Begg and Mazumdar adjusted rank correlation test p two tailed value 0.7, Egger's regression test p value 0.09).
Conclusion: The results of this study showed a statistically significant relationship between *H. pylori* infection and colon cancer.

WS 2 Inflammation and Host Response

Abstract no.: W2.1
Immune response to the gastric pathogen *H. pylori*: microarray analysis of gastric CD2$^+$ cells

A. P. Moran[1], Y. O'Donoghue[1,2], K. Mashayekhi[1], A. Moshfegh[3], S. A. Zulquernain[1,4] and J. O'Keeffe[5]

[1]Microbiology, School of Natural Sciences, National University of Ireland, Galway, Ireland; [2]Biochemistry, School of Natural Sciences, National University of Ireland, Galway, Ireland; [3]Dept. of Oncology and Pathology, Cancer Center Karolinska, University Hospital, Stockholm, Sweden; [4]Dept. of Gastroenterology, University Hospital, Galway, Ireland; [5]Life and Physical Sciences, School of Science, Galway-Mayo Institute of Technology, Galway, Ireland

H. pylori induces an immune response involving classical T cells but potentially also unconventional T cells and natural killer cells (collectively CD2$^+$ cells). To further understand the host-pathogen interaction in the gastric mucosa following *H. pylori* infection, transcriptomic analysis using cDNA microarrays was performed to determine gene expression of CD2$^+$ cells in infected versus uninfected mucosae. Single-cell suspensions from the epithelial (EP) and lamina propria (LP) layers of gastric biopsies from *H. pylori*-positive (*HP*$^+$) and -negative (*HP*$^-$) individuals were prepared, CD2$^+$ cells were positively selected, and RNA isolated from each of the four groups (EP*HP*$^-$, EP*HP*$^+$, LP*HP*$^-$ and LP*HP*$^+$) for microarray experiments. After bioinformatic analysis, 54,675 responsive genes were identified: 12,311 were significantly up-regulated in EP*HP*$^+$ (compared with EP*HP*$^-$); 3,536 genes were up-regulated in LP*HP*$^+$ (compared with LP*HP*$^-$); 4,863 were down-regulated in EP*HP*$^+$ and 1,079 down-regulated in LP*HP*$^+$. Noteworthy changes in gene expression of markers of cytotoxicity, cell proliferation, cytokines, cellular receptors, CD1 and signal transduction were observed. Compared with the respective layer of uninfected individuals, markers of cytotoxicity, *e.g.* granzyme A, cytotoxic T-lymphocyte associated protein 4 and killer cell lectin-like receptor subfamily C were among the most significantly up-regulated (≥179-fold) in EP*HP*$^+$, while integrin, gastrokine and several unattributed genes were among the significantly down-regulated (≥10-fold) in LP*HP*$^+$. Comparison between EP*HP*$^+$ and LP*HP*$^+$ showed significant up-regulation of the chemokine CXCL14 (83-fold) but down-regulation of CD160, killer cell lectin-like receptor, gastrokine and granzyme (96-; 73-; 31-; 22-fold, respectively). Thus, significant responsiveness of gastric CD2$^+$ cells to *H. pylori* infection was observed.

Abstract no.: W2.2
Attenuation of *H. pylori*-induced gastric pathology in C57BL/6 mice by co-infection with enterohepatic helicobacters is *Helicobacter* species-dependent

Z. Ge, Y. Feng, S. Muthupalani, L. Lemke, M. Whary and J. Fox

Massachusetts Institute of Technology, Cambridge, MA, United States

To investigate how concurrent infection with an enterohepatic helicobacter species (EHS) affects *H. pylori* (Hp)-induced gastric pathology, C57BL/6 mice were inoculated with *H. hepaticus* (Hh), or *H. muridarum* (Hm), followed by infection with Hp. Compared to Hp-infected mice, HmHp-infected mice at 6 and 11 months postinoculation (mpi) had markedly attenuated histopathologic activity index (HAI) scores ($P < 0.0001$). By contrast, HhHp-infected mice had more severe HAI scores ($P = 0.01$) at 6 mpi and had similar HAI scores ($P = 0.8$) at 11 mpi when compared to Hp-infected mice. Hm-mediated attenuated pathology was associated with significant down-regulation of proinflammatory Th1 (IL1, IFN[[Unsupported Character - ]] and TNF) and Th17 (IL-17A) cytokine mRNA levels in stomachs when compared to the Hp-infected mice. Although co-infection with Hh suppressed Hp-induced elevation of gastric Th1 cytokines, Th17 cytokine mRNA levels were increased. Colonization levels of gastric Hp were increased in both HhHp- and HmHp-infected mice compared to mono-Hp-infected mice. The Hp levels correlated with the mRNA levels of the gastric proinflammatory Th1 cytokines. Furthermore, the mRNA levels of IL17A were positively correlated with the severity of helicobacter-induced gastric pathology (HhHp>Hp> HmHp). Our data suggest: (1) host Th1 responses plays a major role in limiting Hp colonization; (2) EHS-mediated attenuation of the Hp-induced gastric pathology depends on the ability of the individual EHS to suppress both Th1 and IL17 proinflammatory pathways.

Abstract no.: W2.3
Telomerase reverse transcriptase (TERT) gene expression is downregulated by *H. pylori* infection: a mechanism involving DNA methylation

F. Bussière[1,2], V. Michel[2], P. Avé[2], M. Huerre[2] and E. Touati[2]

[1]INRA - IASP 213 (Infectiologie Animale et Santé Publique), Tours, France; [2]Institut Pasteur, Paris, France

H. pylori infection is associated with gastric cancer development. The chronic inflammation associated to the infection contributes to the development of malignancy Telomerase is responsible for maintaining telomeres length, essential for

chromosome stability. Telomerase reverse transcriptase (TERT) is the major component of telomerase activity. In this study the consequences of *H. pylori* infection on TERT gene expression and telomerase activity were first investigated on human gastric epithelial cells and in the mouse model. In the presence of infection, hTERT mRNA levels were half those in the control associated to a lower telomerase activity than non-infected cells. Gene transcription can be inhibited by epigenetic mechanisms as DNA methylation. A restoration of hTERT gene expression levels was observed when cells were treated with an inhibitor of DNA methylation, 5′-azacytidine, prior infection. As revealed by immunohistochemistry in mice infected with *H. pylori* SS1 for 12 and 18 months, the number of TERT positive gastric epithelial cells was 3 times lower than in non-infected associated with the presence of large follicles of lymphocytes, suggesting a role of inflammatory cells in the TERT decrease. Accordingly, DNA methylation levels were higher in the TERT gene promoter region for all the infected mice compared to non-infected.

Our results demonstrate a decrease of TERT levels and telomerase activity due to *H. pylori* infection, directly correlated with chronic inflammation and higher level of DNA methylation in the TERT gene promoter region. This mechanism should play a role in driving early steps of gastric carcinogenesis during *H. pylori* infection.

Abstract no.: W2.4
H. pylori disturbs gastric epithelial tight junctions in an inflammation-independent manner

A. C. Costa[1,2], M. Leite[1], M. L. Pinto[1], R. M. Ferreira[1,2], A. M. Costa[1,2], J. C. Machado[1,2], J. C. Atherton[3] and C. Figueiredo[1,2]

[1]IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; [2]Medical Faculty of the University of Porto, Porto, Portugal; [3]NDDC BRU – Nottingham Digestive Diseases Centre Biomedical Research Unit, University of Nottingham, Nottingham, United Kingdom

H. pylori interferes with epithelial tight junction integrity by altering the localization of junctional proteins, compromising the barrier function. *H. pylori*-induced gastritis includes the production of pro-inflammatory molecules known to be implicated in deregulation of paracellular permeability in intestinal cell lines. Therefore, the aim of this study was to clarify the contribution of the direct effects of *H. pylori* versus the indirect effects of the host inflammatory response on tight junction alterations.

Gastric cell lines NCI-N87 and AGSEcad, and short-term cultures of primary gastric epithelial cells were infected with *H. pylori*. In parallel, cell lines were treated with different concentrations of IL-1β and TNF-α. Cellular localization and expression of transmembrane tight junction proteins were evaluated by immunocytochemistry and western blot.

In gastric cell monolayers we confirmed that *H. pylori* alters the status of transmembrane junction proteins, leading to significant changes in transepithelial electrical resistance and in dextran permeability, suggestive of decreased barrier function. Similar results were obtained in *H. pylori*-infected short-term cultures of human gastric epithelial cells. Treatment of cell monolayers with IL-1β and TNF-α did neither alter the localization nor the protein levels of transmembrane tight junction proteins.

Our results so far indicate that the tight junction alterations observed in the context of *H. pylori* infection are a direct consequence of *H. pylori* rather than a consequence of the pro-inflammatory cytokines elicited to the infection.

Abstract no.: W2.5
Olfactomedin 4 down-regulates innate immunity against *H. pylori* infection

W. Liu, M. Yan, Y. Liu, R. Wang, C. Li, C. Deng, A. Singh, W. G. Coleman Jr and G. P. Rodgers

NIDDK/NIH, Bethesda, MD, United States

Olfactomedin 4 (OLFM4) is a glycoprotein that has been found to be up-regulated in inflammatory bowel diseases and *H. pylori (H. pylori)* infected patients. However, the mechanism of its role in biological processes such as inflammation or other immune response is not known. In this study, we generated *OLFM4*$^{-/-}$ mice to investigate potential role(s) of OLFM4 in gastric mucosal responses to *H. pylori* infection. *H. pylori* colonization in the gastric mucosa of *OLFM4*$^{-/-}$ mice was significantly lower compared with wild-type littermates. The reduced bacterial load was associated with enhanced infiltration of inflammatory cells in gastric mucosa. Production and expression of proinflammatory cytokines/chemokines such as IL-1β, IL-5, IL-12 p70, and MIP-1α was increased in *OLFM4*$^{-/-}$ mice compared with infected controls. Furthermore, we found that OLFM4 is a target gene of NF-κB pathway and has a negative feedback effect on NF-κB activation induced by *H. pylori* infection through a direct association with nucleotide oligomerization domain-1 (NOD1) and -2 (NOD2). Together these observations indicate that OLFM4 exerts considerable influence on the host defense against *H. pylori* infection acting through NOD1 and NOD2 mediated NF-κB activation and subsequent cytokines and chemokines production, which in turn inhibit host immune response and contribute to persistence of *H. pylori* colonization.

Abstract no.: W2.6
Involvement of Toll-like receptors in Dendritic cell activation

C. Prinz[1], R. Käbisch[2], F. Anderl[2] and M. Gerhard[2]

[1]University of Witten/Herdecke gGmbH, Wuppertal, Germany; [2]Klinikum rechts der Isar, München, Germany

H. pylori (*H. pylori*) induces a strong Th1 response, but it is still unclear how this response is triggerd by Dendritic cells (DC), specifically which components of *H. pylori* are involved and which pattern recognition receptors and signalling pathways in DCs are activated. In response to a *H. pylori* infection human

Monocytes-derived Dendritic cells (MoDCs) express not only MHC class II, CD80 and CD86 - necessary for the antigen presentation and co-stimulation of T cells, but also CD11c and CD83 that correlates with their activation. Furthermore, these Antigen-presenting cells release the inflammatory cytokines IL-6 and IL-8, in order to attract other leukocytes. Coculture of human NK cells as well as naïve T cells with *H. pylori* infected MoDCs should imitate the natural setting. *H. pylori*-pulsed DCs induce an IFN-γ release in NK cells. A higher IFN-γ amount could be measured when naïve T cells were cocultured with *H. pylori*-infected DCs showing that *H. pylori* induces a cellular immunity. Interstingly, this effect was more pronounced with single recombinant antigens rather than LPS. We further aim to determine which Toll-like receptors are involved in this response to *H. pylori* antigens, which might be important for vaccine development. The ability of *H. pylori* to induce the maturation of MoDC and enable them to stimulate MoDC activation of Th1 cells and NK cells implicates that DCs as initiators of the immune response to *H. pylori*, and indicates new ways for adjuvant selection during immunization.

WS 3 Virulence Factors

Abstract no.: W3.1
H. pylori gamma-glutamyl transpeptidase (GGT) causes cell damage

S. S. L. Ling[1], M. Gong[1], K. G. Yeoh[2], S. Y. Lui[1] and B. Ho[1]

[1]Dept Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; [2]Dept Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

The indelible link between *H. pylori* and gastroduodenal diseases, exacerbated by the high prevalence of gastric cancer and the emergence of multiple-antibiotic-resistant strains, poses major public health concern. Amongst the known pathogenic factors of *H. pylori*, gamma-glutamyl transpeptidase (GGT) has been reported to be a colonizing factor that induces apoptosis in human gastric epithelial cells. However, GGT-induced pathogenic mechanism remains unclear.

Of the 98 *H. pylori* strains examined, we found that strains possessing high GGT activity proliferate more aggressively in culture and display a close association with patients having peptic ulcer disease ($p < 0.001$). Using native GGT purified to >90% homogeneity, we show that GGT dose-dependently generates H2O2 in gastric epithelial cells (AGS and Kato III) resulting in the activation of NF-kB and upregulation of IL-8, suggesting inflammatory response. However, pre-incubating these cells with NAC (an antioxidant), ST638 (a tyrosine kinase inhibitor) and MG132 (an NF-kB inhibitor) blocked IL-8 production. Furthermore, GGT also increases the level of 8-hydroxy-2-deoxyguanosine indicative of oxidative DNA damage. Similar effects were also observed when GGT interacted with primary human gastric cells and macrophages. These results were further complemented with study using *H. pylori* wild-type and various isogenic mutants (Δggt, ΔcagA, ΔcagPAI and Δggt/cagA).

Our study shows that GGT-induced IL-8 production is initiated by hydrogen peroxide generation, and that tyrosine kinase possibly plays a role in signal transmission towards the efficient activation of GGT-induced NF-kB activity, resulting in IL-8 generation. Our findings provide insights into how *H. pylori* GGT induces gastric epithelial cell damage during host-pathogen interaction.

Abstract no.: W3.2
PYK2 interaction with CagA protein is concomitant with PYK2 increased tyrosine phosphorylation and reduced paxillin expression in *H. pylori*-infected gastric epithelial cells

E. G. Panayotopoulou[1], D. N. Sgouras[1], K. Papadakos[1], C. Tzathas[2] and A. Mentis[1]

[1]Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece; [2]Gastroenterology Department, Piraeus General Hospital ''Tzaneio'', Piraeus, Greece

The calcium-dependent proline-rich tyrosine kinase-2 (Pyk2/CAKβ/RAFTK), closely related to FAK, has been recently implicated with cytoskeletal remodeling, proliferation and motility of epithelial cells and fibroblasts. Our aim was to investigate whether PYK2 may be implicated in CagA-induced alterations of signalling involved in cytoskeletal homeostasis.

Gastric epithelial cells (AGS) were infected with isogenic CagA-positive strains harbouring variable number of EPIYA-C motifs and the respective Δ*cagA* strains in the presence or absence of serum. Potential physical interactions of CagA with FAK, PYK2, p130Cas, Paxillin, Crk-L, SHP2, c-Src were investigated following immunoprecipitation with anti-CagA antibody and western blot analysis. Tyrosine phosphorylation levels of all proteins were detected in total cell lysates following anti-phosphotyrosine immunoprecipitation. Intracellular localization was investigated utilizing confocal laser scanning microscopy.

We observed an interaction between CagA and PYK2 with maximum levels at 60 min post infection, under our experimental conditions. Total PYK2 tyrosine phosphorylation levels were increased, with a concomitant reduction in tyrosine phosphorylated FAK, although PYK2 or FAK expression remained unchanged. Paxillin expression was reduced in a CagA-dependent manner, whereas p130Cas, Crk-L and SHP2 levels remained unaffected. In serum deprived cells, increased expression of PYK2 was observed only in loosely attached cells at

earlier time points, upon infection with strains harbouring more EPIYA-C repeats in CagA.
Our data suggest a CagA-PYK2 interaction that may depend on the number of EPIYA-C repeats and is accompanied by increased levels of PYK2 tyrosine phosphorylation and concomitant reduction of tyrosine phosphorylated FAK along with perturbation of Paxillin expression in *H. pylori*-infected cells.

Abstract no.: W3.3
H. pylori selectively disrupts SHP-2 dependent EGFR signaling to evade the antimicrobial impact of the human beta defensin hBD3

B. Bauer, C. Holland, S. Bartfeld, M. Kessler and T. F. Meyer
Department of Molecular Biology; Max Planck Institute for Infection Biology, Berlin, Germany

Defensins are key elements of innate host defence against infection. These endogenous antimicrobial peptides exert microbicidal activities against a range of bacterial, fungal and viral pathogens. Recently, the human beta defensin 3 (hBD3) was shown to effectively kill the human gastric pathogen *H. pylori* in vitro. *H. pylori* type I strains persistently colonize the human stomach, causing severe diseases such as ulcer and gastric cancer. Whereas most bacteria are free swimming organisms in the gastric mucosa, a small percentage adhere to epithelial cells. Adherence is followed by translocation of the effector protein CagA into the host cell, dramatically altering cellular signaling pathways. The mechanisms of how *H. pylori* establishes a protective biological niche remain poorly understood. We investigated, therefore, whether and how *H. pylori* escapes from the antimicrobial impact of hBD3 to enable persistent colonization. Intriguingly, we found that initially induced hBD3 expression vanishes over time resulting in the extracellular survival of *H. pylori* over longer infection periods. Moreover, we unraveled the underlying molecular mechanism that directly leads to *H. pylori*-mediated blockage of hBD3: Both the bacterial effector protein CagA as well as the cellular tyrosine phosphatase SHP-2 play essential roles in manipulating EGFR signaling, thereby allowing long-term survival of *H. pylori*. Our data provide new mechanistic insights into how *H. pylori* controls complex host cell pathways to ensure its survival. Furthermore, our data lead to the hypothesis that attachment of free-swimming bacteria facilitates the establishment of a protective biological environment.

Abstract no.: W3.4
In vitro effects of human beta-defensins on planktonic cells, bacterial adhesion and biofilm formation of the two sequenced strains of *H. pylori*

E. I. Kazakos[1,2], B. Alhaj-Hussein[3,1], M. Bajaj-Elliott[4] and N. Dorrell[1]
[1]London School of Hygiene & Tropical Medicine, London, United Kingdom; [2]AHEPA University Hospital, Aristotle University Medical School, Thessaloniki, Greece; [3]National Guard Health Affairs, Riyadh, Saudi Arabia; [4]Institute of Child Health, London, United Kingdom

Objectives: (a) Investigate the bactericidal potential of the human beta defensins (hbDs) 1,2,3 and 4 against planktonic *H. pylori* (HP) cells and (b) examine their ability to influence initial bacterial adhesion and subsequent development of a biofilm providing thus, some insights into the bacterium's adaptation mechanisms in the gastric niche.
Materials & Methods: HP strains 26695 and J99 were grown planktonically and as adherent to rectangular thermanox® cover slips to determine the effect of the biofilm phenotype on the level of susceptibility following short exposure to various concentrations of recombinant hbDs. Transmission electron microscopy was engaged to demonstrate possible structural changes. Hp cells pre-exposed to sub-inhibitory concentrations of the above peptides, were tested for their capacity to form a biofilm.
Results: In planktonic cells hbDs were highly susceptible over a 30 min period, however a significant decrease in the bactericidal effect of all four hbDs was evident for cells in biofilms indicating the presence of certain persister cells. Interestingly, sub-lethal concentrations of hbDs appear to induce the bacterium's ability to biofilm formation favoring mainly adherence.
Conclusion: This study provides evidence of the activities of hbDs against *Hp* biofilms and supports the hypothesis that the biofilm phenotype significantly affects the level of resistance to those peptides facilitating *H. pylori* persistence and survival.

Abstract no.: W3.5
Clinical relevance and diversity of two homologous genes encoding glycosyltransferases in *H. pylori*

M. Oleastro[1,2], A. Santos[1], R. Cordeiro[1], B. Nunes[3], F. Mégraud[2,4] and A. Ménard[2,4]
[1]Departamento de Doenças Infecciosas, Instituto Nacional Saúde Dr Ricardo Jorge, Lisboa, Portugal; [2]INSERM U853, 33076 Bordeaux, France; [3]Departamento de Epidemiologia, Instituto Nacional Saúde Dr Ricardo Jorge, Lisboa, Portugal; [4]Université Victor Segalen Bordeaux 2, 33076 Bordeaux, France

H. pylori is known to be a major cause of peptic ulceration. The *jhp0562* gene, encoding for a glycosyltransferase involved in the synthesis of the lipopolysaccharide, was associated with peptic ulcer disease (PUD) in children. The β-*(1,3)galT* gene (*jhp0563*), involved in Lewis (Le) antigen expression, is highly similar to *jhp0562*. The clinical significance and diversity of both genes were examined by PCR and sequencing among clinical strains (n = 117) isolated from children with PUD (n = 57) and non-ulcer dyspepsia (NUD) (n = 60). The *jhp0562* gene was significantly more prevalent in strains with a more virulent profile (strains positive for *cag* pathogenicity island (PAI), *vacA* s1 type, *babA*, *homB*, *oipA* ''on'' and *hopQ* I allele). The distribution of genotypes according to clinical outcome showed that the presence of *jhp0562* presented one of the highest risks for the development of PUD. Moreover, the triple-positive genotype for *cag* PAI, *jhp0562* and *homB* provided the best discriminatory model for separating outcomes of PUD and NUD.
Sequence and *in vitro* expression analysis of *jhp*0562 showed the presence of a complete open reading frame, while β-*(1,3)galT* was shown to be a phase-variable gene. The regular presence of *jhp0562* in strains with a truncated β-*(1,3)galT* suggests that

jhp0562 may also be implicated in the regulation of Le antigen expression.
Overall, the results of this study suggest that the *jhp0562* gene is of high clinical relevance, being a useful co-marker for severe *H. pylori*-related disease and contributing to host adaptation.

Abstract no.: W3.6
H. pylori outer membrane vesicles modulate proinflammatory and other signalling pathways in gastric and non-gastric cells

P. Brown[1], S. Smith[1], C. Botting[2], D. Kelleher[1] and H. Windle[1]
[1]Trinity College Dublin, Dublin, Ireland; [2]University of St Andrews, St Andrews, United Kingdom

Introduction: *H. pylori* releases outer membrane vesicles (OMV) in vivo and elicit diverse host immune responses due to the presence of PAMPs and OMV-bound virulence factors. Recent murine studies demonstrated proinflammatory responses to these structures, mediated in part by NOD1. The aim of this study was to monitor changes to the host cell phosphoproteome upon exposure to *H. pylori* OMV as a means of identifying proteins likely involved in signalling pathways and to undertake functional studies.
Methods: Phosphoproteins from control/OMV-treated AGS were affinity purified and subjected to 2D SDS-PAGE and phospho-specific staining followed by MS/MS analysis. Western blotting was used to confirm MS data. TLR2 transfected and non-transfected HEK cells were used to measure IL-8/chemokine mRNA and NF-κB activity.
Results: Infection of AGS cells with OMV resulted in a temporal and dose-dependent modulation of the gastric cell phosphoproteome. MS analysis of differentially phosphorylated proteins identified molecules known to be involved signalling pathways and adaptive stress responses. Functional studies demonstrated sustained MAP kinase activation in AGS cells and enhanced IL-8, CXCL1,2,3 and CCL20 mRNA in TLR2-transfected HEK cells.
Conclusions: *H. pylori* OMV induce several signalling events in gastric cells in a time and dose dependent manner. The proinflammatory activity observed in gastric cells can also be elicited in other cell types via both TLR2-dependent and independent mechanisms, partially modulated by *H. pylori* LPS. As OMV are not restricted to the gastric niche, these vesicles could induce sustained inflammation in non-gastric tissue also.

WS 4 Gastric Cancer: Premalignant lesions

Abstract no.: W4.1
Prevalence of premalignant changes in the stomach of patients undergoing routine colonoscopy; a cohort study

C. M. den Hoed, L. G. Capelle, B. C. van Eijck, P. D. Siersema and E. J. Kuipers
Erasmus MC University Medical Center, department of Gastroenterology and Hepatology, Rotterdam, Netherlands

H. pylori (*Hp*) initiates the pathway of gastric carcinogenesis which follows from gastritis through atrophic gastritis (AG), to intestinal metaplasia (IM), dysplasia (DYS) and malignancy. The presence of these lesions in the general population is a predictor for gastric cancer incidence in the coming decades. Most subjects with *Hp* infection and premalignant gastric lesions are asymptomatic. Prevalence data are mostly obtained from endoscopy data in symptomatic patients or serology. Therefore a need exists for histological prevalence data in asymptomatic subjects.
383 patients, (F/M: 192 /191; mean age 53.1; range 17–86) undergoing routine, colonoscopy, underwent esophagogastroduodenoscopy prior to colonoscopy. Biopsies were taken from the antrum and corpus and visible abnormalities.
Hp infection was present in 22%, ranging from 14% in subjects <40 to 33% in subjects >50 yrs. Non-Caucasian subjects had a higher rate of *Hp* infection 54% vs. 22% ($p < 0.001$). AG and IM and DYS were found in 9.3% of subjects; 0.8% had AG, 7.1% IM and 1.4% had DYS. Subjects with *Hp* infection or AG, IM or DYS were significantly older than subjects with normal gastric mucosa; mean age 53.1 yrs in normal gastric mucosa vs 56.1 yrs in *Hp* ($p = 0.025$), mean age 60 yrs in AG, IM or DYS ($p = 0.03$). No association was found between gender, GI symptoms, lifestyle and medication use between subjects with or without premalignant gastric lesions or *Hp*.
There is a considerable prevalence of premalignant gastric lesions in asymptomatic subjects. This means that gastric cancer will remain a prevalent disease in western countries.

Abstract no.: W4.2
H. pylori cagA and vacA genotypes as predictors of progression of gastric preneoplastic lesions: a long term follow up in a high- risk area in Spain

C. A. Gonzalez[1], C. Figueiredo[2], R. M. Ferreira[2], M. Pardo[3], J. Ruiz-Liso[3], P. Alonso[3], C. Bonet[1], N. Sala[1], G. Capellá[1]and J. Sanz-Anquela[3]
[1]Catalan Institute of Oncology, Barcelona, Spain; [2]Ipatimup and Medical Faculty of the University of Porto, Porto, Portugal; [3]Complejo Hospitalario de Soria, Soria, Spain

There are no established markers of predictor of progression of gastric preneoplastic (GPL) lesions. The aim of this study was to analyze the relationship between *H. pylori* cagA and vacA genotypes with progression of GPL. A follow-up study was

carried-out in a province with one of the highest risks of gastric cancer in Spain. 312 patients who underwent gastric biopsy in 1988–1994 with diagnoses of normal mucosa, non-atrophic gastritis (NAG), non metaplastic multifocal atrophic gastritis (MAG) and complete or incomplete intestinal metaplasia (IM) and accepted to undergo a new biopsy during 2005–2007 or had an end-point during follow up were included in the study. Inter- and intra-observer variability of histological diagnosis was assessed. Detection and characterization of *H. pylori* cagA and vacA genotypes was performed directly in baseline paraffin-embedded gastric biopsy specimens by polymerase chain reaction followed by reverse hybridization onto a line probe assay. Analysis was done using unconditional logistic regression. Patient mean age was 48.5 years (45% males) and mean of follow-up was 12.8 years. The presence of cagA and vacA s1m1 genotypes were strongly linked and associated with more advanced GPL. Comparing with s2m2 and cagA negative genotypes, s1m1 and cagA positive genotypes were associated with progression of GPL (multivariate OR = 3.38; 95% CI 1.34–8.53 and OR = 2.28; 95% CI 1.13–4.58, respectively). *H. pylori* genotyping may be useful for identification of patients at high risk of progression of GPL and that need more intensive surveillance.

Abstract no.: W4.2
The Expression of VEGFR1 and VEGFR2 in *H. pylori* Infected Intestinal Metaplasia and Gastric Cancer

N. Nakajima, T. Nakagawa, N. Kunoki, S. Ohta, S. Kobayashi, R. Nishiyama, Y. Akai and T. Ohtani, M. Mitshuhiko

Dept. of Gastroenterology and Hepatology, Nihon University School of Medicine, Tokyo, Japan

H. pylori (*HP*) infection causes chronic gastritis (CG), gastroduodenal ulceration and gastric cancer (GC). GC arises through a multistep process from CG to intestinal metaplasia (IM) and then finally to invasive GC. The overexpression of VEGF receptors, including VEGFR1 and VEGFR2, is thought to play a central role in cancer growth. IL-16 is involved in the pathophysiological process of chronic inflammatory diseases. The aim of this study is to determine the changes in the expression of VEGFR1 and VEGFR2 in CG, IM and GC as well as the effect of *HP* infection and IL-16 on epithelial cell proliferation and VEGFR1&2 expressions in gastric cells *in vitro*.

Methods: Gastric biopsies were classified by histological findings as CG; either with (CG+) or without *HP* infection (CG-), IM; either with (IM+) or without *HP* (IM-) and GC with *HP* (GC+). For *in vitro* studies, AGS cells were incubated with combinations of *HP* and IL-16. Cell proliferation was studied by BrdU uptake. The expression of VEGFR1 and VEGFR2 was studied by ABC, ELISA and RT-PCR.

Results: IL-16 expression was detected in all *HP* infected gastric mucosa. In CG, there was no significant difference in VEGFR1 and VEGFR2 expression between biopsies with and without *HP* infection (VEGFR1; CG+:2.43 ± 2.90% vs. CG-:1.37 ± 2.22%, VEGFR2; CG+:0.88 ± 0.93% vs. CG-:0.62 ± 0.65%). In *HP* infected mucosa, VEGFR1 expression was significantly higher in IM+ than CG+ (VEGFR1; IM+:4.23 ± 5.06% vs. CG+:2.43 ± 2.90%, $p < 0.01$). VEGFR1&2 expressions were higher in GC+ (VEGFR1; 12.14 ± 7.28%, VEGFR2; 9.53 ± 6.24%, $p < 0.01$) than in CG+ and IM+. *In vitro* studies: *HP* infection alone significantly decreased cell proliferation. Administration of IL-16 increased BrdU uptake and VEGFR1&2 expressions in AGS cells which had been decreased by *HP*. Administration of IL-16 increased the expression of VEGFR1&2 mRNA and protein in *HP* infected AGS cells.

Conclusions: The expression of VEGFR1&2 in long-term *HP* infected gastric mucosa may indicate an early stage of carcinogenesis because it appears before a definitive diagnosis of GC. The expression of IL-16 by *HP* infection may be a trigger for the expression of VEGFR1&2 in gastric mucosa, and it may also be a factor for development of gastric carcinogenesis.

% of AGS only	*HP* 10^5cfu/ml	*HP*+IL-16 10^{-10}M	*HP*+IL-16 10^{-9}M
BrdU uptake	84.79 ± 5.85*	99.76 ± 6.78#	98.59 ± 5.77#
VEGFR1 protein	86.06 ± 9.59*	98.79 ± 8.23#	98.37 ± 6.68#
VEGFR2 protein	76.37 ± 11.07*	88.43 ± 10.56#	88.52 ± 11.64#
VEGFR1 mRNA	77.90 ± 26.64	136.93 ± 108.34#	103.32 ± 60.34
VEGFR2 mRNA	70.31 ± 2.40*	82.11 ± 15.23	100.27 ± 21.25#

*= significant difference from AGS cells only
#=significant difference between with and without IL-16 on AGS cells
$p < 0.01$ was taken as statistically significant

Abstract no.: W4.3
Eradication of *H. pylori* improves gastric atrophy identified by serum pepsinogen levels in healthy middle-aged and elderly subjects – a 4-year follow up study

T. Shimoyama, A. Nakamura, M. Matsuzaka, K. Danjo, S. Nakaji and S. Fukuda

Hirosaki University Graduate School of Medicine, Hirosaki, Japan

Background: *H. pylori* infection has been associated with the development of gastric cancer according to the extent of chronic atrophic gastritis. Serum pepsinogen (PG) levels have been used to define chronic atrophic gastritis.

Aims: The aim of this study was to investigate effects of *H. pylori* eradication on the levels of PG in healthy middle-aged and elderly subjects with mild to severe atrophic gastritis.

Methods: Healthy subjects (born before 1960) who attended mass survey in 2005 and 2009 were tested by *H. pylori* stool antigen test and serum level of PG I, II and anti-*H. pylori* IgG antibodies were measured. Subjects with *H. pylori* infection were asked to receive eradication therapy. Level of PGs was considered as positive for atrophic gastritis when both a PG I level of <70 ng/ml and a PGI/II level of <3.0 were observed. Severe atrophic gastritis was positive when the levels of PGI and PGI/II were <50 ng/ml and <2.0.

Results: 267 subjects were examined by all the tests in both 2005 and 2009. Among 46 subjects who were eradicated *H. pylori* infection, 32 subjects were defined to have atrophic gastritis (8 severe atrophy) in 2005 while only 2 subjects had atrophic gastritis in 2009 ($p < 0.01$). In 121 infected subjects who had not received eradication therapy, the number of atro-

phic gastritis was 78 (17 severe atrophy) in 2005 and 81 (22 severe atrophy) in 2009.
Conclusion: Eradication of *H. pylori* improved atrophic gastritis even in elderly subjects with severe atrophy.

Abstract no.: W4.4
Interleukin-1B and IL1 receptor antagonist gene polymorphisms are not associated with premalignant gastric conditions: a combined haplotype analysis

L. Kupcinskas[1], T. Wex[2], J. Kupcinskas[1], G. Kiudelis[1], L. Jonaitis[1], D. Janciauskas[1], A. Ivanauskas[1], A. Sudraba[3], K. Funka[3], H. Chiu[4], M. Leja[5], J. Lin[4] and P. Malfertheiner[2]

[1]Department of Gastroenterology, Kaunas University of Medicine, Kaunas, Lithuania; [2]Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Germany; [3]Faculty of Medicine, University of Latvia, Riga, Latvia; [4]National Taiwan University Hospital, Taipej, Taiwan; [5]Faculty of Medicine, University of Latvia, Kaunas, Latvia

Objective: Contradictory results have been reported about the role of interleukin-1B (IL1B) and IL1 receptor antagonist (IL1RN) alleles in gastric carcinogenesis. Here, IL1B and IL1RN polymorphisms were analyzed as geno- and haplotypes in relation to the presence of atrophic gastritis (AG) and intestinal metaplasia (IM) in the stomach.
Methods: Two-hundred-seventy-eight patients (212 Caucasians and 66 Asians) aged 50 years and older, referred for upper endoscopy due to dyspeptic symptoms, were included. Gastric biopsies were histologically assessed according to the updated Sydney classification. Genomic DNA was typed for polymorphisms at position -3737, -1464, -511, -31 for the IL1B gene and the allele 2 of IL1RN using restriction fragment length polymorphism of amplified PCR fragments and intron-spanning PCR analysis, respectively.
Results: IL1B-1464-C/C genotype was associated with higher presence of AG in antrum of the stomach in Caucasians [OR: 4. 8 (95% CI = 1.7–14.3); p = 0.028]. IL1B-1464-G/C genotype was associated with lower incidence of AG in corpus of the stomach in Asians [OR: 0.7 (95% CI = 0.5–0.8); p = 0.02]. IL1RN*2 allele was not linked with AG or IM in all parts of the stomach both among Asians and Caucasians. Overall, data demonstrate that none of the major four IL1B polymorphisms (IL1B-3737C>T, -1464G>C, -511C>T, -31T>C) as well as the IL1RN*2 is individually or in its haplotype configuration linked to the presence of premalignant lesions in Caucasians.
Conclusion: The determination of these IL1-related loci does not have any predictive value for stratification of subgroups with respect to gastric cancer risk.

Abstract no.: W4.5
Serum Pepsinogen I and II in Dyspeptic Patients in Thailand - Is it Useful?

V. Mahachai[1], T. Thongbai[2], T. Ratanachu-ek[3], T. Khuhaprema[4] and R. Vilaichone[5]

[1]Division of Gastroenterology, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand; [2]Gastroenterology Unit, Department of Medicine, Central Hospital, Bangkok, Thailand; [3]Department of Surgery, Rajvithi Hospital, Bangkok, Thailand; [4]Director, National Cancer Institute, Bangkok, Thailand; [5]Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Bangkok, Thailand

We correlated pepsinogen (PG) I and II, PGI / PGII measured by gastropanel (Biohit) with *H. pylori* status and pathological findings in dyspeptic patients over 40 years in the community endoscopic survey. Total of 394 patients (M:F = 135: 259, mean age = 53.9 years) were included. The most common endoscopic finding was gastritis 288 (73.1%). Peptic ulcer was found in 31 (7.8%) with only 1 case (0.3%) of gastric cancer. Overall *H. pylori* infection was 32.4%, highest in the North and Northeastern and lowest in the Southern region. Pathological study revealed intestinal metaplasia (IM) in 29 cases (7.5%) but no gastric atrophy was found. Majority of patients (92.3%) has chronic gastritis. PGI; PGII; PG_I / $_{II}$ were not different between patients with gastritis and IM. The PGI / PGII in gastric cancer patients was 5.5 mg/L. PG_I was similar in *H. pylori* positive and negative group. However, PG_{II} level was significantly higher in *H. pylori* positive than *H. pylori* negative group.
Conclusion: The incidence of gastric cancer, IM and gastric atrophy are low in Thailand in spite of high *H. pylori* infection rate. PGI and PGII and PG_I / PG_{II} cannot be used to screen the presence of IM or gastric atrophy due to low incidence of premalignant conditions. Further study is underway to study biomarkers in high risk group including IM, atrophy and gastric cancer. Higher PG_{II} in *H. pylori* positive group suggests that PG_{II} may have a potential as a marker for *H. pylori* associated gastric inflammation.

Abstract no.: W4.6
Transcriptional progression of atrophic gastritis development in humans

S. Lundin[1], I. Nookaew[2], K. Worah[2], S. Wang[3], S. Pettersson[3], H. Sjövall[4] and J. Nielsen[2]

[1]Dept. of Microbiology and Immunology, Gothenburg, Sweden; [2]Chalmers University of Technology, Gothenburg, Sweden; [3]Karolinska Institute, Stockholm, Sweden; [4]Dept. of Medicine, Gothenburg, Sweden

Gastric cancer is the second most common cause of cancer death in the world, and is mostly affecting low- and middle income countries. Understanding the mechanisms of gastric carcinogenesis is important for further discovery of new prognosis markers. The majority of gastric cancer cases are believed to be caused by chronic infection with the gastric bacterium *H. pylori*. This infection in some cases lead to development of atrophic gastritis in the corpus mucosa, which is an important predisposing condition to gastric cancer development.

In this study human biopsies from different stages of pre-cancer development were taken from both antrum and corpus region of the stomach. These included *H. pylori* un-infected (Hp-), *H. pylori*-infected without corpus atrophy (Hp+) and *H. pylori*-infected with atrophic gastritis (Atr). Genome-wide expression analysis was performed for all samples using an oligonucleotide microarray. The transcriptome data was then analyzed by different methods including clustering and advanced integrated analysis. These systemic approaches enabled us to underline the genes that were related with development of atrophic corpus gastritis.

In atrophy patients, we found firm evidence for development of antralization of the corpus mucosa. Antralization was largely associated with diminished expression of corpus-related gene groups, such as genes associated with acid production and energy metabolism. In parallel with antralization, corpus atrophy was also associated with increased expression of gene groups related to inflammation and cell signaling.

Abstract no.: W4.7
Serological markers of atrophic gastritis may determine the risk of gastric cancer in a population-based study

S. Kurilovich[1], O. Reshetnikov[1], M. Voevoda[1], G. Simonova[1], T. Openko[1], Y. Ragino[1], S. Krotov[2] and V. Krotova[2]

[1]Institute of Internal Medicine, Novosibirsk, Russian Federation; [2]Vector-Best, Novosibirsk, Russian Federation

Chronic atrophic gastritis (AG) is considered as one of the most important predictors of gastric cancer (GC). A set of noninvasive tests named ''Gastropanel'' (Biohit, Finland) has recently been designed including three various markers of gastric atrophy pepsinogen I (PGI), pepsinogen II (PGII) and gastrin-17 (G-17), as well as antibodies to *H. pylori*.

Objective: To assess the value of biomarkers of AG tested with ''Gastropanel'' for early detection of GC.

Methods: 10,000 subjects aged 45–69 were studied from the general population of Novosibirsk in 2003–2005. Population Cancer Registry identified 25 novel cases of GC until 2009. For each case of GC, two control cases were selected matching on sex and age.

Results: Indicators of gastric atrophy (OR; 95% CI) were associated with GC for PGI (3.75; 1.36–10.32), PGII (6.83; 1.26–37.16) and PGI/PGII ratio (4.31; 1.49–12.45), but nor with G-17 (1.65, 0.57–4.75), neither with the presence of antibodies to *H. pylori* (1.76; 0.56–5.59). *H. pylori* antibodies were equally distributed between both groups with high prevalence of 74%. Multivariate regression analysis including sex and age showed a low level of PGI (4.82; 1.61–14.48) as the only significant indicator in the model.

Conclusions: In a retrospective cohort study the use of a set of serological tests showed that low levels of PGI significantly increase the risk of GC. However, testing for *H. pylori* is necessary for identification the etiology of gastritis and further eradication of the microorganism.

WS 5 Other Helicobacters

Abstract no.: W5.1
An experimental *Helicobacter suis* infection reduces daily weight gain in pigs

S. Kumar[1], K. Chiers[1], F. Pasmans[1], B. Flahou[1], J. Dewulf[2], F. Haesebrouck[1] and R. Ducatelle[1]

[1]Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine,Ghent University, Merelbeke, Belgium; [2]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine,Ghent University, Merelbeke, Belgium

Helicobacter suis (*H. suis*) is a Gram negative, long, spiral-shaped bacterium colonizing the stomach of more than 60% of pigs at slaughter age[2]. The prevalence is very low in sucklings, increases from the time of weaning and is highest in adult animals. This bacterium is considered to be one of the risk factors associated with gastric ulcers in pigs[1]. The effect of experimental inoculation with *H. suis* on the daily weight gain over the period of inoculation to euthanasia of 44 medicated early weaned piglets from 5 different experimental set-ups was compared to the sham-inoculated negative control animals (N = 29) using a multivariable linear regression model including all variables that varied between the experiments as co-variables to correct for their effect (SPSS 17). There was a significant reduction ($p < 0.05$) of approximately 20 g/day (5%) in the daily weight gain of experimentally inoculated animals compared to the non-infected control animals. Results of this study indicate that an *H. suis* infection reduces daily weight gain in pigs and thus may result in substantial economic losses. Clearance of infection with this bacterium may therefore have an economically beneficial effect.

1. Haesebrouck et. al., 2009. Clin. Microbiol. Rev. 22, 202–223.
2. Hellemans et. al., 2007. Vet. Rec. 161, 189–192.

Abstract no.: W5.2
Study of *Helicobacter hepaticus* infection in a transgenic mouse model expressing the hepatitis C virus polyprotein

E. Goglin[1], C. Asencio[1], P. Dubus[2], M. Jutand[3], J. Rosenbaum[4] and F. Mégraud[1]

[1]Laboratoire de Bactériologie, INSERM U853, Université Victor Segalen Bordeaux 2, Bordeaux, France; [2]Histologie et Pathologie moléculaires des tumeurs, EA2406, Université Victor Segalen Bordeaux 2, Bordeaux, France; [3]ISPED, Université Victor Segalen Bordeaux 2, Bordeaux, France; [4]Fibrose hépatique et cancer du Foie, INSERM U889, Université Victor Segalen Bordeaux 2, Bordeaux, France

The heterogeneity of hepatitis C virus (HCV) infections cannot always be explained by HCV genotypes or host genetic factors, raising the issue of possible co-factors. A new form of hepatitis leading to liver cancer was discovered in 1992 in mice, due to an infection by *Helicobacter hepaticus*. Moreover, several studies showed an association between the presence of HCV and DNA from a Helicobacter in the liver of patients with severe liver diseases. All of these data suggest a possible synergism between the two pathogens.

The aim of our project is to study this potential synergism in a transgenic mouse model expressing the HCV polyprotein. We studied a colony of about 200 male mice of a C3H majority genetic background, distributed in 4 groups (transgenic or not, infected or not by *H. hepaticus*). Fifteen months post infection, mice were sacrificed and liver assessed for lesions.

Infection by *H. hepaticus* promoted the initiation of preneoplastic and neoplastic liver foci, regardless of the inflammation process (hepatitis). HCV transgene tended to promote multiplicity of preneoplastic and neoplastic liver foci, and steatosis. Statistical models with addition or interaction are not statistically significant so far.

In conclusion, we showed that genetic susceptibility may be a more important factor than expected. Further analyses are on their way to refine these results and confirm that *H. hepaticus* can act without leaving the large bowel. The synergism between HCV and *H. hepaticus* infection may greatly depend on their specific interaction with the host.

Abstract no.: W5.3
Differential expression of genes involved in carcinogenesis induced in hepatic cells by Helicobacter pullorum

G. L. Mendz[1] and Q. V. Tu[2]

[1]The University of Notre Dame Australia, Darlinghurst, Australia; [2]The University of New South Wales, Sydney, Australia

Chronic viral infections of the liver are risk factors for the development of cancer. Bacterial infections that induce chronic inflammation could similarly act as carcinogens by triggering the aberrant expression of genes involved in central cellular functions. Several studies have shown associations between the presence of Helicobacter spp. DNA in liver samples of various types of cancers, but to date there are no studies on the transcriptional response of hepatocytes to the presence of Helicobacter spp. The effects of Helicobacter pullorum on the expression of genes previously identified to be dysregulated in hepatocellular carcinoma (HCC) in Huh-7 liver cells was investigated using qRT-PCR, and the results were compared to microarrays data.

The expression of 40 genes related to carcinogenesis in HCC whose products are responsible for angiogenesis and metastasis, apoptosis, cell proliferation, tumour suppression and oncogenesis was analysed using qRT-PCR. Twenty-seven Huh-7 genes were shown to be significantly differentially expressed in the presence of H. pullorum. Importantly, the expression of only 8 out of these 27 genes was correlated to the data of microarray analyses conducted on the same cell line.

The results supported the hypothesis that chronic H. pullorum could induced the physiological changes leading to liver carcinoma. In addition, the comparison between the qRT-PCR and microarray results showed that the latter technique is less sensitive to detect changes in gene expression in these liver cells. Microarray raw data of global responses are subject to stringent statistical analyses which may be responsible for the loss of significant biological information.

Abstract no.: W5.4
Detection of non-pylori Helicobacter species from IBD, IBS and healthy subjects: preliminary results

E. Rimbara[1], R. J. Shulman[2], B. P. Abraham[2], S. Sabounchi[1], T. A. Attumi[1], A. R. Opekun[2] and D. Y. Graham[1], Baylor IBD Study Group

[1]VA Medical Center, Houston, TX, United States; [2]Baylor College of Medicine, Houston, TX, United States

Background: Although, non-pylori Helicobacter species are proven to cause IBD-like colitis in animals, their role in human diseases is unclear. We investigated the prevalence of non-pylori Helicobacter species is stool samples from inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and non-IBD controls by PCR and culture.

Methods: DNA was extracted and PCR was performed using Helicobacter species-specific primers for both 16S rRNA and 23S rRNA genes. PCR products were cloned and multiple clones were sequenced for identification. Culture was performed using three types of Helicobacter-selective plates.

Results: Forty-seven stool samples (15 non-IBD controls, 17 IBS, 5 UC, and 10 CD) have been analyzed so far for the 23S rRNA gene. Culture was negative for all samples. The 23S rRNA gene of non-pylori Helicobacter species was detected from 10 samples. To date, non-pylori Helicobacter have been detected in 2 of 15 (13%) non-IBD control subjects, 4 of 17 (24%) IBS patients, 1 of 5 (20%) UC patients and 3 of 10 (33%) CD patients. Species identification is underway.

Conclusion: Our preliminary results suggest that non-pylori Helicobacter species can be detected in 20–30% of IBS and IBD patients, a prevalence that appears only slightly higher than in non-IBD controls. Whether there are disease-specific non-pylori associations remains to be determined.

Abstract no.: W5.5
Unique structure of *Helicobacter bizzozeronii* lipopolysaccharide

A. P. Moran[1], J. A. Ferris[1], N. A. Kocharova[2,3], Y. A. Knirel[2,3], G. Widmalm[4], L. P. Andersen[5], H. Annuk[1], A. M. Brennan[1] and P. E. Jansson[3]

[1]Microbiology, School of Natural Sciences, National University of Ireland, Galway, Ireland; [2]N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation; [3]Karolinska Institute, Clinical Research Centre, Huddinge University Hospital, Huddinge, Sweden; [4]Dept. of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden; [5]Dept. of Infection Control, Rigshospitalet, Copenhagen, Denmark

Preliminary studies have indicated that the lipopolysaccharide (LPS) of gastric-colonizing *Helicobacter bizzozeronii* contains fucose like Lewis antigen-mimicking *H. pylori* (*Hp*) LPS. Since further information is lacking the present study was undertaken to examine the structure of *Hb* LPS using electrophoretic, serological and structural analyses. Electrophoresis (SDS-PAGE) of *Hb* LPS with silver staining showed production of predominantly low-molecular-mass LPS, although a less intensely staining, high-molecular-mass O-chain band was observed also. Unlike *H. pylori* LPS, no reactivity of anti-Lewis or anti-blood group antibodies was observed with *Hb* LPS in immunoblotting indicating the absence of such molecular mimicry. Gel chromatography of acid-liberated saccharides confirmed the predominance of a core oligosaccharide (OS), rather than O-chain, in *Hb* LPS consistent with low-molecular-mass LPS. This isolated core OS was subjected to detailed structural determination using NMR spectroscopy, mass spectrometry, and classical sugar methylation analysis. The established structure of the *Hb* core, which contains fucose, differs notably from *Hp* LPS outer core, with only minor resemblance to *Hp* inner core. Only 1 of 7 polyclonal antisera against the LPS core of different *Hp* strains reacted with *Hb* LPS in serodot analysis, but not in Western blotting, reflecting the importance of test format for inner core epitope recognition. An antiserum that was raised against heat-treated *Hb* whole cells in rabbits agglutinated *Hb* but not *Hp* bacteria, and reacted with *Hb* LPS but not with other *Helicobacter* spp. LPSs in passive haemagglutination and Western blotting. Thus, unique structures occur in *Hb* LPS of pathogenic and diagnostic potential.

Abstract no.: W5.6
Helicobacter cinaedi Induced Cholecystitis in IL10 Deficient Mice

Z. Shen, B. Rickman, Y. Feng, M. W. Mobley, A. F. Potter and J. G. Fox

Massachusetts Institute of Technology, Cambridge, MA, United States

Helicobacter cinaedi is an important human pathogen causing a wide range of diseases especially in immunocompromised patients. In our previous study, cytolethal distending toxin promoted *H. cinaedi*-associated typhlocolitis in C57BL/6 IL10$^{-/-}$ mice. In this study, the ability of *H. cinaedi* to induce cholecystitis in IL10$^{-/-}$ mice was evaluated. Thirty B6.129P2-IL10$^{TM/Cgn}$ mice, divided into 3 groups, were infected orally with 1). *H. cinaedi* CCUG 18818; 2). *cdt*B mutant; 3). Sham dosed as controls. Liver, gallbladder and gastrointestinal pathology of the mice were analyzed 12 weeks post infection. *H. cinaedi* colonized the cecum and colon, but not the liver, of infected mice based on bacterial culture and quantitative PCR analysis. Wild-type *H. cinaedi* and *cdtB* mutants colonized IL-10$^{-/-}$ mice to a similar extent, but infection with *cdtB* mutant resulted in attenuated typhlocolitis and hyperplasia compared to infection with wild-type *H. cinaedi* ($P < 0.03$). WT-Hc infected IL-10$^{-/-}$ mice also had moderate, multifocal to diffuse cholecystitis compared to uninfected IL-10$^{-/-}$ mice. Inflammation was predominantly in the submucosa with occasional mucosal disruption, consisting of multifocal to coalescing areas with moderate numbers of neutrophils. *cdt*B-Hc infection in IL-10$^{-/-}$ mice was associated with less severe inflammation of the gallbladder; mild inflammation with focal areas of inflammation were noted. Our results indicate that *H. cinaedi* not only can cause typhlocolitis in IL 10$^{-/-}$ mice but also can induce cholecystitis. This model can be used to study the pathogenic mechanisms of inflammatory gall bladder diseases in humans.

Abstract no.: W5.7
Multilocus sequence typing (MLST) of *Helicobacter suis*

S. Kumar, A. Smet, B. Flahou, M. Vermoote, K. Van Deun, F. Pasmans, K. Chiers, R. Ducatelle and F. Haesebrouck

Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

A multilocus sequence typing (MLST) scheme was developed for *Helicobacter suis* (*H. suis*). This bacterium is considered to be one of the risk factors associated with gastric ulcers in pigs and is also of zoonotic significance[2]. Recently *H. suis* has been successfully cultured *in vitro*[1] and genome annotations of two isolates have been completed. The genetic diversity in 9 isolates, all obtained from the stomach of pigs, was studied by examination of allelic variation in 7 housekeeping genes *atpA, efp, mutY, ppa, trpC, ureI, vhpC* by MLST. These genes have also been used in the MLST scheme of *H. pylori*[3]. The *ppa* gene showed 100% homogeneity among the 9 isolates and does not seem to be useful in a MLST scheme for *H. suis*. Results obtained with the 6 other genes showed that the different isolates belonged to different sequence types, indicating that the present MLST scheme shows enough discriminatory power. MLST may be applied for strain typing directly in tissue samples and may allow to determine whether certain genotypes are more often associated with gastric disease in pigs and humans than other genotypes.

1. Baele et.al., 2008. Int. J. Syst. Evol. Microbiol. 58, 1350–1358.
2. Haesebrouck et. al., 2009. Clin. Microbiol. Rev. 22, 202–223.
3. *H. pylori* Multilocus Sequence Typing website (http://pubmlst.org/helicobacter)

Abstract no.: W5.8
Detection by culture and PCR of Helicobacters and Campylobacters in digestive biopsies and stools from patients with inflammatory bowel disease (IBD)

J. Tankovic[1], R. Dahoumane[1], D. Lamarque[2] and J. Delchier[3]

[1]Service de Bactériologie-Virologie, Hôpital Saint-Antoine, Paris, France; [2]Service de Gastroentérologie, Hôpital Cochin, Paris, France; [3]Service de Gastroentérologie, Hôpital Henri-Mondor, Créteil, France

Some *Helicobacter* and *Campylobacter* species have been implicated in IBD. We looked for their presence in digestive biopsies and diarhheic stools from adult IBD patients and controls by culture and PCR. Samples were grown microaerobically at 37°C on selective (Skirrow) blood agar. DNA extracts were tested with a *Helicobacter*-specific PCR. Extracts from stools were also tested with additional PCRs: *H. pylori* PCR, *Campylobacter*-specific PCR and *Campylobacter concisus* PCR. Biopsies from 38 patients (Crohn's disease (CD), 28; ulcerative colitis (UC), 4; controls 6) and stools from 26 patients (CD, 9; UC, 11; controls, 6) were tested. Cultures of biopsies from 2 CD patients yielded *Campylobacter coli* and *C. concisus*. Biopsies from 1 control grew *H. pylori*. *Helicobacter* PCR was positive for the above-mentioned control and for 2 culture-negative CD patients. The genera implicated were *H. pylori* for the control and one CD patient, and *Helicobacter canis* for the other. Concerning stools, *Helicobacter* PCR was positive only for 1 UC patient but the genus was not identified. *H. pylori* PCR was always negative. *Campylobacter* and *C. concisus* PCRs were positive for the same 4 patients, 1 with CD and 3 with UC. 2 of the 4 patients were also *C. concisus* positive by culture. These results suggest that *C. concisus* could be associated with IBD. This is in agreement with the report of Zhang et al. (J Clin Microbiol 2009; 47: 453–5). We also describe the first isolation of *H. canis* in chronic digestive lesions from a CD patient.

WS 6 Gastric Cancer: Pathogenesis

Abstract no.: W6.1
Eradication therapy and subsequent gastric and other common malignancies

T. U. Kosunen[1], E. Pukkala[2,3], S. Sarna[4] and H. Rautelin[1,5,6]

[1]Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland; [2]Finnish Cancer Registry, Helsinki, Finland; [3]School of Public Health, University of Tampere, Tampere, Finland; [4]Department of Public Health, University of Helsinki, Helsinki, Finland; [5]Helsinki University Central Hospital Laboratory, Helsinki, Finland; [6]Department of Medical Sciences, University of Uppsala, Uppsala, Sweden

Background: The combinations of 2–3 antimicrobials and an acid-suppressing drug, required to cure *H. pylori* infection, are rarely ordered for other indications. Due to the lack of specific symptoms for gastritis, it is possible that also subjects with symptoms associated with malignancies will be treated.
Methods: A cohort of 217,580 subjects (120,351 women and 97,229 men), who had received reimbursement for the drug combinations used in *H. pylori* eradication therapy in 1994–2004, was identified from the prescription registry of the national Social Insurance Institution and followed for cancer incidence via the Finnish Cancer Registry until the end of 2008 (1.9 million person years at risk). Numbers of observed cancer cases were compared with expected numbers derived from the average population rates and expressed as standardized incidence ratios (SIRs).
Results: A total of 22,400 malignancies were identified. Amongst common malignancies, there were 1055 stomach, 1678 colon, 751 rectum, 954 pancreas and 2275 lung cancers. During the first follow-up year the SIRs of these cancers were significantly elevated [from 1.7 (lung) to 7 (stomach)]. During the years 2–5 the SIR fell for all these cancers (stomach 1.4, colon 1.3, rectum 0.9, pancreas 1, lung 1.1). During the years 6–14, also the SIRs of colon and stomach cancers were at unity.
Conclusions: After eradication therapy recommended for *H. pylori* infection, the SIRs of several cancers remained significantly elevated for several years. This indicates that patients treated for *H. pylori* infection still have a considerable risk for serious diseases, including gastric cancer.

Abstract no.: W6.2
Determining the CCAAT/enhancer binding protein β (C/EBPβ) partnership profile in gastric carcinogenesis

C. Resende[1,2,3], G. Regalo[1], R. Seruca[1,2] and J. C. Machado[1,2]

[1]Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal; [2]Faculdade de Medicina da Universidade do Porto, Porto, Portugal; [3]Instituto de Ciências Biomédicas da Universidade do Porto (ICBAS), Porto, Portugal

Transcription factors from the CCAAT/enhancer-binding protein (C/EBP) family play fundamental roles in the control of differentiation and proliferation of many adult tissues. We have previously found that C/EBPα is downregulated in gastric carcinoma (GC), and that C/EBPβ is overexpressed in dysplastic lesions and GC. In this work our aim was to evaluate the functional consequences of these expression changes in a GC cell

line model. We transfected GC cell lines with a full-length C/EBPα protein, and we observed a significant decrease in cell proliferation, accompanied by a decrease in Cyclin D1, an increase in P27 expression, and an increase in expression of the gastric differentiation marker trefoil factor 1 (TFF1). Noteworthy, C/EBP members form transcriptional protein-complexes with other transcriptional partners, being cell-phenotype determination directly dependent on the proteins that are part of the transcriptional complex. Knowing that, we sought to identify novel C/EBPβ interacting proteins *in vitro* through co-immunoprecipitation using mass spectrometry-based proteomics techniques (MALDI-TOF/TOF). We identified members of the heterogeneous nuclear ribonucleoproteins (hnRNP) family, as interacting proteins of C/EBPβ transcriptional complexes in our screen. We confirmed the *in vitro* interaction of C/EBPβ with hnRNPs by reverse immunoprecipitations. hnRNPs are nuclear ribonucleoprotein-complexes involved in gene transcription and subsequent post-transcriptional modification of the newly synthesized pre-mRNAs. Our results suggest that the hnRNP-C/EBPβ interactions may have an important role in the regulation of expression of specific genes that are involved in the control of cellular differentiation and proliferation.

Abstract no.: W6.3
Global methylation profiles of genes in gastric cancer

M. Alvarez and M. L. Ribeiro

Clinical Pharmacology and Gastroenterology Unit, Sao Francisco University Medical School, Bragança P, Braganca Paulista, Brazil

Gastric carcinogenesis is a multistep process triggered by *H. pylori* and characterized by accumulation of molecular alterations. It has been shown that gastric cancer is manly an epigenetic disease, and *H. pylori* acting through inflammatory mediators may play a key role in the development of such molecular alterations. Thus, the aim of the present study was to evaluate the effect of *H. pylori infection* on the methylation profile. The methylation status was characterized in biopsies samples from the tumor and adjacent normal mucosa from a *H. pylori*-positive patient with gastric cancer, a biopsy from a patient with gastritis infected by the bacteria, and from a non-infected patient. The methylation pattern was analyzed using Panomics gene array system. Methylation binding protein-purified methylated DNA was hybridized on the membrane and detected by chemioluminiscence method. Our results demonstrate that the normal mucosa and the non-infected patient tissues shared similar DNA methylation patterns. We found that 17% of the genes were hypermethylated in gastric cancer mucosa and 14% were hypermethylated in mucosa from the infected patient. The analysis of the methylation pattern from the gastric cancer and *H. pylori* infected patient showed that 7% of gene were hypermethylated in both. Among these the following genes were considered to be biologically relevant such as GPC-3 (cell cycle), TFF1 (tumor suppressor), GATA 3 (transcription factor) and MGMT and MLH1 (DNA repair proteins). In summary, our preliminary data presented putative candidate genes that could be involved with *H. pylori*-induced gastric carcinogenesis.

Abstract no.: W6.4
Host genetic polymorphisms and gastric cancer

N. Castano-Rodriguez[1], N. O. Kaakoush[1], H. Schmidt[1], D. Forman[2], K. Goh[3], K. Fock[4], P. Sutton[5] and H. M. Mitchell[1]

[1]University of New South Wales, Sydney, Australia; [2]International Agency for Research on Cancer, Lyon, France; [3]University of Malaya, Kuala Lumpur, Malaysia; [4]Changi General Hospital, Singapore, Singapore; [5]University of Melbourne, Melbourne, Australia

Background: While more than half the world is infected with *H. pylori* only a small percentage progress to gastric cancer (GC). Previous studies suggest that in addition to *H. pylori* infection and dietary factors, host genetic factors contribute to GC. TIRAP, CD14 and PAR-1 are genes involved in innate immunity which may be implicated in GC pathogenesis.
Aims: To determine the prevalence of TIRAP S180L, TIRAP D96N, CD14–260 C/T and PAR-1 IVSn-14 A/T polymorphisms in *H. pylori*-related GC in a high-risk Chinese population.
Methods: This case-control study included 284 Chinese individuals, resident in Malaysia and Singapore, comprising 70 GC cases and 214 controls with functional dyspepsia. DNA was extracted from peripheral blood samples, and genotyping performed by means of PCR-restriction fragment length polymorphism and real-time PCR techniques.
Results: As expected, *H. pylori* infection is a risk factor for GC in this population (OR:2.39, 95% CI:1.25–4.56). A possible association between PAR-1 IVSn-14 A allele and GC was found (OR:0.71, 95% CI:0.49–1.05). The prevalence of TIRAP S180L in this population was extremely low (0.01) which may explain the non-significant results obtained. To date, real-time PCR methods have been developed and optimized for TIRAP D96N and CD14–260 C/T and analysis is being undertaken.
Conclusion: PAR-1 IVSn-14 A may be a protective allele against *H. pylori*-related GC in a Chinese population. The risk of GC may be decreased in those individuals due to higher PAR-1 expression on the epithelial cell surface that would limit the PAR-2-related inflammatory pathway more efficiently as previously published.

Abstract no.: W6.5
Genotype of the infecting *H. pylori* (HP) strains and clinical outcome of gastric carcinoma (GC)

D. Marrelli[1], N. Figura[2], L. Garosi[1], V. Pascale[1], C. Vindigni[1] and F. Roviello[1]

[1]Dipartimento di Patologia Umana e Oncologia, Siena, Italy; [2]Department of Internal Medicine, University of Siena, Siena, Italy

Introduction: GC is more prevalent in patients infected by cagA+ HP strains. Genes upstream cagA encode for a conjugative apparatus through which the oncoprotein CagA is injected into the mucocytes. The lack of some of these genes affects the cancerogenic potential of HP strains. We aim at determining the impact of different cag genotypes on the outcome of GC and survival of patients.

Patients and Methods: The presence of HP was determined by PCR in non-neoplastic gastric mucosa collected from 302 patients with primary GC, operated between 1990 and 2005. PCR was performed using primers specific for the vacA subtypes s1, s2, m1 and m2. In positive cases, the presence of cagA, cagE, and virB10 was investigated by PCR. The association with clinicopathological variables was studied by means of univariate and multivariate analysis.
Results: 202 patients were infected (66.8%), 169 of whom (83.6%) were cagA+, 168 (83.1%) cagE+, and 135 (66.8%) virB10+. We observed a significant correlation between cagE and virB10 positivity (p = 0.028) and between virB10 positivity and GC diffuse/mixed histotype (81% vs. 60% of the intestinal type, p = 0.004). After an overall mean follow-up period of 50 ± 47 months (77 ± 52 months for survivors) the cagE- cases had a better prognosis than the cagE+ cases (10-yr survival after radical resection: 74% vs. 47%, p = 0.085).
Conclusions: The genotype of the infecting HP organisms seems to influence the clinical outcome of GC. This correlation needs further confirmation in a larger group of patients.

Abstract no.: W6.6
Urokinase plasminogen activator receptor (uPAR) expression in gastric adenocarcinomas and non-neoplastic mucosa

W. Alpizar-Alpizar[1,2], B. S. Nielsen[3], R. Sierra[2], M. Illemann[1], J. A. Ramirez[4], A. Skarstein[5] and K. Ovrebo[5] and O. Laerum[1,6]

[1]Finsen Laboratory, Copenhagen University Hospital, Denmark, Copenhagen, Denmark; [2]Cancer Research Program, INISA, University of Costa Rica, San Jose, Costa Rica; [3]Exiqon A/S, Vedbæk, Copenhagen, Denmark; [4]Department of Pathology, Calderón Guardia Hospital, San Jose, Costa Rica; [5]Department of Surgical Sciences, University of Bergen and Department of Surgery, Haukeland University Hospital, Bergen, Norway; [6]The Gade Institute, University of Bergen and Department of Pathology, Haukeland University Hospital, Bergen, Norway

Gastric cancer is the second cancer causing death worldwide, and *H. pylori* infection is the most recognized risk factor. The receptor for urokinase plasminogen activator (uPAR), which mediates cell surface associated plasminogen activation, is upregulated in areas of inflammation and in association with cancer invasion. The presence of uPAR in bone marrow-disseminated gastric cancer cells is linked to poor prognosis.
We examined the expression and localization of uPAR in gastric cancer by immunohistochemistry, and compared its expression between countries with high- and low-risk of this malignancy (Costa Rica and Norway, respectively). In neoplastic tissue, uPAR was expressed by macrophages and tumor cells located at the invasive front of the malignant growth. uPAR-positive cancer cells were found in 54% and 56% of the Costa Rican and Norwegian cases, respectively. We also evaluated the prognostic significance of uPAR in gastric cancer. Multivariate analysis revealed uPAR-immunoreactivity in invasive tumor cells as an independent prognostic factor for overall survival in gastric cancer (HR = 2.83; 95%CI: 1.34–5.99; $p = 0.006$). In non-neoplastic gastric mucosa, uPAR was more frequently seen in surface epithelial cells in *H. pylori*-infected than in non-infected cases ($p = 0.033$; $\chi^2 = 4.54$).
In conclusion, the expression of uPAR in gastric cancer cells does not contribute to explain the difference in mortality between Costa Rica and Norway; however it is of prognostic relevance in gastric cancer. The upregulation of uPAR in epithelial cells in connection to *H. pylori* infection represents a novel finding and we are currently investigating on experimental mouse models the potential implications in gastric cancer development.

WS 7 Molecular genetics

Abstract no.: W7.1
Chronological evolution in *H. pylori*: lessons from whole genome sequencing of the three serial isolates obtained a decade apart from a single patient

N. Ahmed[1], T. Taylor[2] and F. Mégraud[3]

[1]University of Hyderabad, Hyderabad, India; [2]RIKEN Advanced Science Institute, Yokohama, Japan; [3]INSERM - U853, Laboratoire de Bacteriologie - C.H.U. Pellegrin, Bordeaux, France

The survival, persistence and evolution of *H. pylori* strains in the niches of the stomach in the aftermath of the eradication therapy are not fully understood. We earlier performed genetic fingerprinting, comprehensive genotyping and complete sequencing of the two genomic islands, cagPAI(40 kb) and tfs3 (16 kb) from the isolates obtained at inclusion (one sub clone) and after a 10-year period (two sub clones) from a patient who suffered duodenal ulceration at two occasions and has undergone eradication therapy for *H. pylori*. We report analyses of whole genome comparative genomic hybridization (array CGH) followed by whole genome sequencing using Solexa (Illumina) platform. Our observations reveal that the serial isolates represented a single parent strain and were almost identical. Microevolution, however, was evident in several important genes. Interestingly, no significant correlation between genetic rearrangements and the severity of gastroduodenal pathology was seen. This observation points to an obvious difficulty in correlating the continuously evolving virulence factors with disease characteristics that appear to remain stable. Further, our results suggest that *H. pylori* has achieved a great deal of ability to survive and reemerge as a microevolved strain post-eradication. This necessitates the requirement for follow-up of patients even after successful completion of the therapy. Annotation of the

three genomes, comparative genomics and characteristics of the *H. pylori* pan- genome in the aftermath of the three new genomes described herein shall be presented.

Abstract no.: W7.2
Genome rearrangements revealed through comparison of four complete genomes of Japanese *H. pylori*

Y. Furuta[1], M. Kawai[2], K. Yahara[3], T. Tsuru[1], N. Takahashi[1], N. Handa[1], K. Oshima[4], M. Hattori[4], M. Yoshida[5], T. Azuma[5], I. Uchiyama[2] and I. Kobayashi[1,6]

[1]Department of Medical Genome Sciences, Graduate School for Frontier Sciences, University of Tokyo, Minato-ku, Tokyo, Japan; [2]National Insisute for Basic Biology, Okazaki, Aichi, Japan; [3]School of Medicine, Kurume University, Kurume, Fukuoka, Japan; [4]Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Chiba, Japan; [5]Graduate School of Medicine, Kobe University, Kobe, Hyogo, Japan; [6]Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan

A clinical study in Japan strongly suggested that *H. pylori* is responsible for recurrence of gastric cancer. There have been hypotheses linking the genotype of East Asian *H. pylori* to the high incidence of gastric cancer in East Asia. In order to fully understand the East Asian strains, our group determined entire genome sequences of 4 *H. pylori* strains from Japanese patients by Sanger sequencing and compared them with the 6 complete genome sequences. We analyzed ortholog relationships, phylogenies, horizontal transfer, gene gain and loss, and genome rearrangements.

Potential macro-regional genome rearrangements were analyzed and rearrangement pathways were reconstructed by combination of regional inversion events. Even within the closely-related Japanese isolates, many genome inversions have occurred at various positions. Their recombination points were frequently linked to restriction-modification (RM) genes, which suggest their involvement. We also detected RM systems and genomic islands specific to the Asian strains. In a Japanese strain, we found a genomic island with features of putative prophages. This may be the first report of prophage-like region in *H. pylori*. Analysis of the other plastic regions and genes will also be reported.

Abstract no.: W7.3
Bacteriophage and *H. pylori*: an underestimated phenomena

F. Vale[1], J. Guegueniat[2], S. Lacomme[3], E. Gontier[3], A. Alves Matos[4], L. Engstrand[5], F. Mégraud[2] and P. Lehours[2]

[1]Faculty of Engineering, Catholic University of Portugal, Lisbon, Portugal; [2]Université Bordeaux 2, INSERM U853, Bordeaux, France; [3]Université Bordeaux 2, Pôle Imagerie Electronique-Bordeaux Imaging Center, Bordeaux, France; [4]CESAM, City Aveiro and Hospital Curry Cabral, Lisbon, Portugal; [5]Institute, Department of Microbiology, Tumor and Cell Biology (MTC), Stockholm, Sweden

The recently sequenced strain B45 isolated from a gastric MALT lymphoma patient has a prophage sequence in its genome with 25.4 kb, highly similar to *Helicobacter acinonychis* prophage II. Until now *H. pylori* has been described has a species without prophage with the exception of the strain B38 which contains some prophage remnant sequences. Moreover, the number of reports of *H. pylori* phages is sparse in the literature.

The aim of our study was (1) to test the inducibility of the B45 prophage and using mitomycin C or UV, (2) to investigate the prevalence of *H. pylori*-prophage carrying isolates.

No significant lysis plaque was observed after mitomycin or UV induction. However, phage DNA was recovered after extraction and amplified by PCR. Some phage particles having a structure compatible with the *Siphoviridae* phage family were observed by transmission electron microscopy.

Using a PCR strategy, based on degenerated primers designed on the B38, B45 and *H. acinonychis* prophage II aligned bacteriophage integrase genes, we have screened until now 310 *H. pylori* strains isolated from different geographic regions and pathologies. The prevalence of the integrase sequence was 21.5%, suggesting that this is a much frequent phenomena that initially estimated. Integrase gene prevalence is similar in different pathologies. Phylogenie analysis shows a trend for a geographical distribution. This study shows for the first time that bacteriophage can play a significant role in *H. pylori* genetic diversity. It also opens new exciting ways in the comprehension of the interaction between the bacteria within its environment.

Abstract no.: W7.4
Prophage carriage among *H. pylori* clinical isolates

E. Aznar, T. Alarcón, S. Agudo, Á. Somodevilla and M. López-Brea

H.U. La Princesa, Madrid, Spain

Introduction: Prophages are involved in DNA transfer among bacteria. Virulence factors or resistance determinants may be encoded by prohages. Very little is known about prophage carriage among *H. pylori* strains.

Objective: To detect the presence of prophages by the induction of its lytic cycle after culturing clinical isolates in the presence of low concentrations of mitomycin C.

Methods: 17 strains of *H. pylori* were isolated from gastric biopsies by standard culture methods. TIGR 26695 and the 17 clinical isolates were subcultured on recently prepared blood agar plates containing 5 ng/ml Mitomycin C, 10 mg/l vancomycin and 5 mg/l amphotericyn B. Induced phages, that were detected by the presence of inhibition plaques, were extracted by suspension on 3ml of BHI centrifugation and filtration. 10 μl of this extract was tested against the 18 strains cultured on Mitomycin C free blood agar plates.

Results: Inhibition plaques were observed in 6 out of 18 strains (not in TIGR). The extract obtained from these strains produced growth inhibition of different *H. pylori* strains. 1 extract in 15

strains, 1 in 12 strains, 1 in 5 strains, 1 in 3 strains and 2 in one strain each one.
Conclusion: Mitomycin C containing blood agar plates are an easy and reproducible method to detect prophage carriage among *H. pylori* strains.
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Abstract no.: W7.5
Polyphosphate Binds to the Principal Sigma Factor of RNA Polymerase during Starvation Response in H. pylori

Z. Yang, Y. Zhou and D. Jin
National Cancer Institute, National Institutes of Health, Frederick, MD, United States

H. pylori persists deep in the human gastric mucus layer in a harsh, nutrient-poor environment. Survival under these conditions depends on the ability of this human pathogen to invoke starvation/stress responses when needed. Unlike many bacteria, *H. pylori* lacks starvation/stress-responding alternative sigma factors, suggesting an additional mechanism might have evolved in this bacterium. *H. pylori* produces polyphosphate, however, the role and target of polyphosphate during starvation/stress have not been identified. Here we show that polyphosphate accumulated during nutrient starvation directly targets transcriptional machinery by binding to the principal sigma factor in *H. pylori*, uncovering a novel mechanism in microbial stress response. A positively-charged-Lys-rich region at the NTD of the major sigma factor is identified as the binding region for polyphosphate (region P) *in vivo* and *in vitro*, revealing a new element in sigma 70 family proteins. This interaction is biologically significant because mutant strains defective in the interaction undergo premature cell death during starvation. We suggested that polyphosphate is a second messenger employed by *H. pylori* to mediate gene expression during starvation/stress. The putative ''region P'' is present in sigma factors of other human pathogens including *Bordetella pertussis* and *Coxiella burnetii*, suggesting that the uncovered interaction might be a general strategy employed by other pathogens.

Abstract no.: W7.6
A functional difH/XerH system is required for DNA repair in *H. pylori*

A. W. Debowski[1], C. Carnoy[2], B. J. Marshall[1] and M. Benghezal[1]
[1]Marshall Centre for Infectious Disease Research, M502, University of Western Australia, Perth, Australia; [2]Faculté de Sciences Biologiques et Pharmaceutiques de Lille, Inserm U801, Institut de Biologie de Lille, Lille, France

Bacterial chromosome dimer resolution is mediated at the *dif* site by the action of the Xer recombinases during replication. Bioinformatics identified the dif_H/XerH system in ϵ-proteobacteria. Although another Xer-like recombinase x*erT* was identified in Helicobacter genomes, *H. pylori xerH* was the only ubiquitous Xer recombinase.
Here we show that *xerH* but not *xerT* is required for recombination between two direct repeated dif_H sites introduced in *H. pylori* genome. Single base pair mutations of the highly conserved dif_H sequence revealed that both the palindromic and non-palindromic sequences were critical for XerH activity. Surprisingly, *xerH* mutation led to UV sensitivity suggesting a close functional relationship between DNA replication and DNA repair in *H. pylori*.
Our results demonstrate that the dif_H/XerH system is functional and based on a single recombinase. Importantly, in *H. pylori* which is an organism that is missing several repair proteins, the conserved cellular function of chromosome dimer resolution participates in DNA repair, possibly through resolution of dimers formed by the replication of Holliday junctions.

POSTERS

P1 Microbiology, Molecular genetics

Poster no.: P1.01
Low-pH adaptation in *H. pylori*

H. Toledo, M. Valenzuela and S. Soto
Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile

Bacterial gene expression can be modified by environmental conditions. *H. pylori* colonizes the acid environment of human gastric mucosa. We have explored the physiological basis of the acid adaptation response in *H. pylori,* the function of the global transcriptional regulator Fur in the acid adaptation response, and the expression of the arginine descarboxilase, *speA* gene, as an alternative urease independent mechanism that could protect the bacterium against acid stress in presence of arginine. Using 2-D gels and RT-PCR we analyzed gene expression of *H. pylori* 26695 and 43504 strains and the *fur* negative isogenic mutants when bacteria were exposed to pH 7.0, pH 6.0, and pH 5.5. Like other enteric bacterial pathogens that must survive brief exposure to acid environment, *H. pylori* displays a rapid response to subtle changes in pH that confers an increased ability to survive more extreme acid conditions. Proteomic analysis of phenotypes of otherwise isogenic wild-type and *fur* mutant strains show that subtle pH changes may elicit dramatic changes in the pattern of protein synthesis and that Fur regulates the expression of a number of *H. pylori* proteins, a subset of which are involved in the acid tolerance response. Also, expression of the *speA* gen is induced by acid pH and depends on the Fur transcriptional regulator.
In *H. pylori,* acid tolerance response requires *de novo* protein synthesis, is dependent on the function of the global regulatory protein Fur, and the *speA* gene is over expression when the bacteria is exposed to acid pH.
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Poster no.: P1.02
Photodynamic therapy applied to the inactivation of *H. pylori*

M. Calvino-Fernández[1,2], D. García-Fresnadillo[3], J. P.Gisbert[4,2], A. McNicholl[4,2] and T. Parra-Cid[1,2]
[1]Unidad de Investigación. Hospital Universitario de Guadalajara., Guadalajara, Spain; [2]CIBEREHD, Madrid, Spain; [3]Departamento de Química Orgánica. Facultad de Química. Universidad Complutense, Madrid, Spain; [4]Hospital Universitario de La Princesa., Madrid, Spain

Introduction: Photodynamic therapy is based on the fact that a non-toxic dye can be activated by low doses of visible light to generate singlet oxygen and free radicals in a process called sensitization. These molecules are highly citotoxic and reactive with nucleids acids, lipids and proteins, so they can lead microorganisms such as bacteria or viruses to their death.
Increasing development of antibiotic resistance and the existence of nonresponsive patients suggest that alternative strategies for *H. pylori* eradication need to be sought.
Objectives: To analyse if *H. pylori* is susceptible to photodynamic inactivation employing two different polymer(P)-sensitizer conjugates(PS).
Methods: Two strains of *H. pylori* [Cag A(-)/(+)] isolated from biopsies specimens were grown (TSB/10% FBS) under microaerophilic conditions at 37°C.
Assays were performed by duplicate, in 96-well plates at bacterial concentrations of $5x10^4$ and10^5CFU/mL in TSB and by continuous shaking.
Each strain was placed in three wells: A:bacteria, B:bacteria+P(1–3mg) and C:bacteria+PS(1–3mg) and was incubated in the dark or illuminated with a blue LED (20–25mW).
Aliquots were taken each 30'until 2h, cultured onto TSA for 2–3 days and colonies were counted.
Results: All results of plates incubated in the dark were identical. C plates exposed to light showed a 90–95% decrease in the number of colonies compared to plates A and B. This decrease was independent of the incubation time, strains and PS used.
Conclusions: The strains of *H. pylori* mixed with PS conjugates were susceptible to the action of visible light. Our results suggest that a novel phototherapy approach could be applied to cure *H. pylori* infection.

Poster no.: P1.03
In vitro susceptibility of *H. pylori* to cationic photosensitisers

M. G. Strakhovskaya[1], Z. S. Plieva[2] and V. G. Zhukhovitsky[3]
[1]Lomonosov Moscow State University, Moscow, Russian Federation; [2]Centre of Theoretical Problems in Pharmacology RAN, Moscow, Russian Federation; [3]Botkin Hospital, Moscow, Russian Federation

Introduction: Not very high efficacy of *H. pylori* conventional eradication therapy calls for search of techniques alternative to antibiotics. The aim of this study was to investigate whether cationic photosensitisers can be used as anti-*H. pylori* resource.
Methods: Two hydrophilic photosensitisers were used: tetraiodide tetrakis 5, 10, 15, 20-(4-trimethylaminophenyl)porphyrin (TAP) and hydrazide cycloimide bacteriochlorin (HCB). Antibacterial activity of TAP and HCB against *H. pylori* NCTC 11639 and four freshly isolated under chronic gastritis *H. pylori* strains was evaluated. Genetically engineered strain *Escherichia coli* TG1 with cloned lux-operon from naturally luminescent *Photorhabdus luminescens* ZM1 was used as biosensor. Mercury-varop lamp

DRS-500 was used as a source of white light with intensity 10 mW/cm2. Photosensitivity of bacterial cultures (1/D50) was calculated as value reverse to dose of light (J/cm2) necessary for achievement of 50% survival (for *H. pylori*) or suppression of bioluminescence (for *E. coli* TG1).

Results: Both cationic photosensitisers killed *H. pylori* strains in dose-dependent manner. Survival of *H. pylori* NCTC 11639 decreased during 5 minutes from $3{,}0 \times 107$ CFU/ml to $4{,}0 \times 105$ CFU/ml and to $2{,}0 \times 102$ CFU/ml under 1 mcg/ml and 2 mcg/ml TAP, respectively ($p < 0.05$). The similar data for HCB were $6{,}0 \times 105$ CFU/ml and $8{,}0 \times 102$ CFU/ml, respectively. All freshly isolated *H. pylori* strains demonstrated the equivalent results. Photosensitivity of *H. pylori* NCTC 11639 was 0,42 cm2/J, *E. coli* TG1 - 0,52 cm2/J.

Conclusions: Obtained results may be useful for development of principles of photodynamic therapy of *H. pylori*-associated diseases.

Poster no.: P1.04
An improved method for purifying alkyl hydroperoxide reductase from *H. pylori*

T. Mohammadian[1], S. Sohaily[2] and M. Paknejadi[3]

[1]Department of Microbiology,School of Basic Sciences,Islamic Azad University, shahr-e Qods-Branch, Qods city, Iran, Islamic Republic of; [2]Department of Sport Sciences,School of Human Sciences,Islamic Azad University, shahr-e Qods-Branch, Qods city, Iran, Islamic Republic of; [3]Department of Microbiology, School of Basic Sciences,Islamic Azad University, shahr-e Qods-Branch, Qods city, Iran, Islamic Republic of

Alkyl hydroperoxide reductase (AhpC) of *Helicobacter* has been described as a specific and unique enzyme for *H. pylori* and therefore, both *H. pylori* AhpC and anti-AhpC could be useful in the development of serologic and stool antigen tests,for detecting and monitoring *H. pylori* infection. In this study, an improved method has been used to purifying AhpC from *H. pylori.*

In order to whole cell protein extraction, the bacterial cells were ruptured by octly-β-D glucopyranoside. AhpC from *H. pylori* isolated and purified by techniques including ammonium sulfate precipitation, dialysis, preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroelution and in any step, enzymatic activity of AhpC was determined. AhpC was purified 100-fold with an overall recovery of 60% from clinical isolates of *H. pylori*. The details of enzyme purification have been shown in Table 1.

The present method is simple, rapid and makes it possible to preparation AhpC from *H. pylori*.

Table 1 Details of alkyl hydroperoxide reductase (AhpC) purification from *H. pylori* and evaluation of its enzymatic activity

Purification step	Protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Yield (%)	Fold purification
Crude homogenate (50 mL)	150	26210	174	100	1
35,000 × g supernatant (43 mL)	95	24797	261	95	1.5
$(NH_4)_2 SO_4$ fraction (0–50%) (5 mL)	70	23142	330	88	1.9
After dialysis (6 mL)	44	21437	487	82	2.8
Electroelution (3 mL)	0.9	15726	17473	60	100.4

Poster no.: P1.05
H. pylori strains associated with pediatric peptic ulcer disease: proteome analysis and evaluation of its biological significance

I. Vitoriano[1], K. Saraiva-Pava[1], A. Santos[2], A. Rocha-Gonsalves[3], C. Calado[1], A. Lopes[4], M. Oleastro[2] and M. Roxo-Rosa[1]

[1]Faculdade de Engenharia, Universidade Católica Portuguesa, Rio de Mouro, Portugal; [2]Departamento de Doenças Infecciosas, Instituto Nacional Saúde Dr. Ricardo Jorge, Lisboa, Portugal; [3]Chymiotechnon, Departamento de Química, Universidade de Coimbra, Coimbra, Portugal; [4]Hospital de Santa Maria, Lisboa, Portugal

Peptic ulcer disease (PUD) is in general a pathology of the adulthoods which arises as a consequence of a chronic inflammation caused by *H. pylori* (HP) infection acquired during childhood. The development of PUD in children suggests more virulent strains and proteome analysis of these is useful to search for bacterial risk factors.

Accordingly, we compared the proteome of 10 HP clinical strains, 5 isolated from children with PUD and 5 with non-ulcer dyspepsia (NUD), by two-dimensional gel electrophoresis followed by mass spectrometry. A group of 10 proteins was found to be up-regulated in PUD-strains, including proteins associated with bacterial colonization, pathogenesis and protein folding. Additionally, 10 proteins were found to be down-regulated in PUD-strains, including proteins involved in cell detoxification system, amino-acid biosynthesis and citric-acid cycle, and translation/transcription mechanisms. Our data indicate that the PUD-associated strains included in the study share a characteristic proteome profile which may underlie their more aggressive phenotype.

We are now conducting *in vitro* studies using the NCI-N87 gastric cell line (ATCC CRL-5822), to better understand the differences in virulence of the PUD-related strains compared with the NUD-associated strains. For that, after infection of the gastric-epithelial cells with a pool of those 5 PUD-strains versus a pool of the 5 NUD-strains, we evaluate the following parameters: changes in the gastric cells' phenotype (light microscopy); rearrangements of the cells' cytoskeleton and of cell-cell adherent junctions (immunocytochemistry); and differences in the cellular viability.

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Poster no.: P1.06
H. pylori isolates with faster growth under aerobic and microaerobic conditions

F. Siavoshi, P. Saniee and M. Atabakhsh
Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran, Islamic Republic of

Introduction: *H. pylori* the causative agent of gastritis, peptic ulcer and gastric cancer is defined as a microaerophilic bacterium which is sensitive to atmospheric O2 and needs CO_2 to grow within 2–3 days . In this study we describe two unusual *H. pylori* strains.
Methods: During studies on 200 strains of *H. pylori,* we observed two strains with distinct colonies. Isolates were subcultured on selective Brucella agar containing 5% blood and incubated at 37°C under both aerobic and microaerophilic conditions. After observing the colonies, bacterial strains were examined for urease, oxidase and catalase activities. Genomic DNAs were recruited for amplification of *H. pylori 16S rDNA*. A PCR-confirmed *H. pylori* was used as a control.
Results: The two *H. pylori* strains exhibited confluent growth after 24 hr incubation under both aerobic and microaerophilic conditions. When compared with the typical distinct pinpoint colonies of *H. pylori* under microaerophilic condition, these bacterial strains tended to form mucoid colonies after aerobic incubation. Light microscopy showed no difference in morphology between the isolates cultured aerobically and those cultured microaerobically. They were oxidase and catalase positive similar to the control but their urease activity was reduced. The *H. pylori 16S rDNA* gene was amplified from both strains.
Discussion: Two strains of *H. pylori* with the potential to grow under aerobic conditions were identified. Compared with control they grew faster under aerobic and microaerobic conditions but developed mucoid colonies under aerobic conditions. Both strains had reduced urease activities. Results indicate that mucoid aggregates could protect bacteria from O2, meanwhile reduce the release of urease.

Poster no.: P1.07
Detection of *H. pylori* and morphological changes in the gallbladder mucous membrane in gallstone disease

J. V. Valeeva[1], O. K. Pozdeev[1], A. M. Khromova[2], E. R. Abuzarova[3] and A. P. Kirshin[4]
[1]Kazan State Medical University, Kazan, Russian Federation; [2]SCPHE ''Republican Bureau of Forensic Medical Examination of the Ministry of Public Health of Republic of Tatarstan'', Kazan, Russian Federation; [3]Kazan State Medical Academy, Kazan, Russian Federation; [4]Kazan Institute of Biochemistry and Biophysics og Kazan Research Center of Russian Academy of Sciences (RAS), Kazan, Russian Federation

Aim of this study was to investigate morphological changes of the gallbladder mucous membrane in gallstone disease and ascertain the fact of *H. pylori* infection of the bile passages.
32 specimens of the gallbladder mucous membrane taken in the course of cholecystectomy were examined. The isolation of the *H. pylori* culture was performed on an erythrite-blood agar supplemented with B amphotericin. The cultivation was carried out in microaerophilic conditions with subsequent determination of the culture belonging to *H. pylori.*
A ''Chelicopol'' commercial kit (''Litech'', Russia) was applied for isolating of DNA from biopsy specimens of the gallbladder mucous membrane.
Histological sections were prepared on the sledge microtome and stained with hemotoxily-eosins. The image analysis system was applied for specimen examination.
Results and their discussions: Bacteriological examination of specimens of the gallbladder mucous membrane revealed the presence of *H. pylori* in 68.75% (75% among males, 64.2% among females) of cases. In 16 (50%) specimens of the gallbladder *H. pylori* DNA was isolated.
The signs of inflammatory process of the gallbladder wall: hyperplastic (adenomatosis, adenomyomatosis, polyposis, the presence of deep immersion ducts in the gallbladder wall), hypo-, atrophic processes (relief flattening, thinning of the mucous membrane and muscular layer with considerable thinning and deformation of the gallbladder wall villi) were detected in the course of histological examination.
It is quite possible that a microbial factor plays a certain part in the development of such morphological changes.

Poster no.: P1.08
A natural antioxidant: *Trans*-resveratrol against *H. pylori*

D. Altıok[1], E. Demiray Gürbüz[2], N. Bekmen[2], F. Tıhmınlıoğlu[1] and Ö. Yılmaz[2]
[1]Izmir Institute of Technology, Department of Chemical Engineering, İzmir, Turkey; [2]Dokuz Eylül University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey

Aim: *Trans*-resveratrol, $C_{14}H_{12}O_3$, is a phytoalexin produced naturally by several plants such as grapes, raspberries, mulberries, blueberries, cranberries, peanuts and pine trees in response to microbial infection or stress. It has high antioxidant, anti-viral and anti-inflammatory activities. The aim of this study was to determine minimum inhibition concentrations (MICs) of *trans*-resveratrol and clarithromycin on *H. pylori* NCTC 11637 strain.
Methods: *H. pylori* strain was cultured on Columbia Blood Agar including 7% defibrinated horse blood and *H. pylori* selective supplement (DENT) under microaerophilic atmosphere at 37°C for 3 days. The agar dilution method was used to determine the MICs of trans-resveratrol and clarithromycin on *H. pylori* NCTC 11637. The stock solutions of clarithromycin (100 μg/ml) and *trans*-resveratrol (100 mg/ml) were prepared by dissolving in methanol and ethanol, respectively. Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood containing the two-fold serial dilutions of *trans*-resveratrol and clarithromycin as a susceptibility control, carried out with PBS Dulbecco (w/o Ca^{+2},Mg^{+2}) in duplicates, were prepared for the final concentrations in the ranges of 1000–4 μg/ml and 8–0.0037 μg/mL, respectively. The bacterial suspension in Brucella Broth was adjusted to McFarland 2 ($6x10^8$CFU/mL), 3 μl of it was inoculated onto the agar plates

and incubated at 37°C for 4 days in a microaerophilic environment.
Results: The MICs of *trans*-resveratrol and clarithromycin against *H. pylori* were determined as 32 and 0.125 μg/ml, respectively.
Conclusion: *Trans*-resveratrol would have a potential role as antioxidant and antimicrobial agent in the treatment for *H. pylori* infection, gastric tissue inflammation and gastric cancer.

Poster no.: P1.09
Antibacterial effect of antimalarials on *H. pylori* infection in vivo

A. Boschian[1,2], C. S. Jensen[1,2], L. Rasmussen[1,2] and L. P. Andersen[1]
[1]Dept. of Infection Control, Rigshospitalet, Copenhagen, Denmark; [2]Dept. of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

Aim: To investigate if antimalarial drugs with antibacterial effect on *H. pylori* in vitro have an antibacterial effect in vivo in *H. pylori* infected mice.
Materials and Methods: 6–8 weeks old female Balb/c mice were inoculated orally once with *H. pylori* SS1 bacteria suspension, and divided into four groups of eight mice in each group. Two groups were inoculated orally with one of the two antimalarial drugs mefloquine and artesunate, which have antibacterial effect on *H. pylori* in vitro, and two control groups were inoculated with the drug solvent. To estimate antibacterial effect, the bacterial load in the mice stomachs were examined by quantitative culturing and described as log (cfu/g stomach).
Results: Mean bacterial load for mefloquine group was 4,33 and for mefloquine control group 5,63 ($p < 0,05$). Mean bacterial load for artesunate group was 4,75 and for artesunate control group 4,72 ($p > 0,05$).
Conclusion and Discussion: Artesunate does not have an antibacterial effect on *H. pylori* infection in vivo, which can be explained by it's pharmacokinetic. Artesunate is rapidly degraded to its metabolite dihydroartemisinin, which display no antibacterial effect. There is a significant lower bacterial load in *H. pylori* infected mice treated with mefloquine than in the group treated with sterile water. Mefloquine might be a candidate for *H. pylori* eradication treatment. Further studies to investigate synergism/antagonism between mefloquine and other drugs used in *H. pylori* treatment are required. Mefloquine has some neuropsychiatric side effects that have to be taken into account.

Poster no.: P1.10
Genotypying of *H. pylori* strains isolated from inflammatory diseases of the gallbladder

J. V. Valeeva[1], E. R. Abuzarova[2], O. K. Pozdeev[1] and E. R. Fedorova[1]
[1]Kazan State Medical University, Kazan, Russian Federation; [2]Kazan Institute of Biochemistry and Biophysics og Kazan Research Center of Russian Academy of Sciences (RAS), Kazan, Russian Federation

The genotypes of different *H. pylori* strains from bile and gallbladder mucous membrane were studied. 24 biopsy specimens of the gallbladder mucous membrane from the patients with gallstone disease taken in the course of cholecystectomy; 24 specimens of C portion of bile obtained from the patients with noncalculous cholecystitis.
A commercial kit for isolation of DNA from bioassays (''Litech'' Russia) was used for isolating *H. pylori* DNA from bile of C portion. A ''Chelicopol'' commercial kit was applied for isolation of DNA from the biopsy specimens of the gallbladder mucous membrane. The isolation and indication of *H. pylori* DNA were performed according to the technique suggested by ''Litech''. The results were documented by means of a «DNA Analyzer» video system for gels recording. A kit of reagents produced by ''Litech'' for determining the presence of *cagA, vacA (s1, s2, m1, and m2* subtypes*), babA* genes in *H. pylori* strains was used. Bacteria with vacAs1 *H. pylori* genotype were revealed in 16 (66. 6%) specimens of the gallbladder wall, and the strains with vacAs1/m2 genotype were found in 45.8% of cases. *H. pylori* isolates with cagA and babA genotype were not detected. Bacteria with v*acAs1/m2* (12. 5%) genotype were revealed in three bile specimens of C portion. The marker of *H. pylori* cagA pathogenicity island is found in 6 specimens (25%). BabA *H. pylori* positive strains were detected in 3 (12.5%) patients. Combination of vacAs1/m2, cagA and babA genotypes was observed in three specimens obtained from the patients with chronic noncalculous cholecystitis.

Poster no.: P1.11
Antimicrobial Activity of Essential Oils Against *H. pylori*

D. Altıok[1], N. Bekmen[2], E. Demiray Gürbüz[2], F. Tıhmınlıoğlu[1] and Ö. Yılmaz[2]
[1]Izmir Institute of Technology, Department of Chemical Engineering, İzmir, Turkey; [2]Dokuz Eylül University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey

Aim: Essential oils are hydrophobic, strong smelling volatile liquids with antimicrobial and antioxidant activities due to their active components. Essential oils have been used in the treatment of some diseases without any indication of bacterial resistance. The aim of this study was to determine and compare the minimum inhibition concentrations(MICs) of lemongrass, cinnamon bark, clove leaf, lemon and thyme essential oils with clarithromycin against *H. pylori (H. pylori).*
Method: The MICs of five essential oils and clarithromycin against *H. pylori* NCTC 11637 were determined by agar dilution method. *H. pylori* strain was cultured on Columbia Blood Agar including 7% defibrinated horse blood and *H. pylori* selective supplement(DENT) under microaerophilic atmosphere at 37°C for 3 days.Each essential oil stock solutions was separately prepared at a concentration of 100 mg/ml by dissolving in ethanol and two-fold serial dilutions in duplicates were carried out with PBS Dulbecco (w/o Ca^{+2},Mg^{+2}). When each dilution was added to the Mueller-Hinton agar supplemented with 5% defibrinated sheep blood, the final concentrations of essential oils and clarithromycin as a susceptibility control were obtained in the ranges of 1000-1 μg/ml and 8–0.0037 μg/mL,respectively.

The agar plates containing these dilutions were inoculated with 3 μl of the bacterial suspension ($6x10^8$CFU/mL) in Brucella Broth and incubated at 37°C for 4 days in a microaerophilic condition.
Results: The MIC values of lemongrass, cinnamon bark, clove leaf, lemon and thyme essential oils and clarithromycin against *H. pylori* were determined as 62, 8, 125, 500, 62 and 0.125 μg/ml, respectively.
Conclusion: The use of cinnamon bark oil in *H. pylori* eradication as itself or by combining with antibiotics is a promising idea.

Poster no.: P1.12
Combination studies of Eucalyptus torelliana F. MUELL. leaf extract and clarithromycin on *H. pylori*

T. O. Lawal[1], B. A. Adeniyi[1], J. O. Moody[2] and G. B. Mahady[3]
[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria; [2]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria; [3]Department of Pharmacy Practice, PAHO/WHO Collaborating Centre for Traditional Medicine, College of Pharmacy, University of Illinois Chicago, Chicago, IL, United States

H. pylori has been implicated in the development of chronic active gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. *Eucalyptus torelliana* leaf extracts are used in Nigerian traditional medicine to treat gastrointestinal disorders.
The fractions and isolated compounds of *E. torelliana* leaf extracts with MIC values >100 μg/mL were combined with clarithromycin and the effect of such combinations was investigated by the agar dilution checkerboard assay and evaluated using fractional inhibitory concentration (FIC) index. Two typed strains ATCC 43629, ATCC43579 and four clinical isolates Hp Ed, Hp A2, Hp G1-1, Hp 5514 were used for the study.
While the extracts weakly inhibited the growth of HP strains, the addition of the fractions and isolated compounds of *E. torelliana* leaf enhanced the activity of clarithromycin. The MIC values of clarithromycin and the botanical compounds were reduced twofold (from 0.125 to 0.0625 μg/mL and >100 to 50 μg/mL respectively) in the combination study. The FIC index (ΣFIC) showed an additive effect (ΣFIC = 0.75-1.0) of the combination of fractions and isolated compounds from *Eucalyptus torelliana* leaf with clarithromycin.
The results of the investigation showed that the combination of botanical extracts/compounds and antibiotics may be beneficial in the treatment of *H. pylori* infections.

Poster no.: P1.13
H. pylori (HP) strains isolated from patients with gastric carcinoma (GC) show an increased susceptibility to resveratrol (Res) in vitro

S. Martini[1], C. Bonechi[1], N. Figura[2], P. Nardi[2] and A. Santucci[3], C. Rossi[1]
[1]Dipartimento Farmaco Chimico Tecnologico, University of Siena, Siena, Italy; [2]Department of Internal Medicine, University of Siena, Siena, Italy; [3]Department of Molecular Biology, University of Siena, Siena, Italy

Introduction: In normal stomachs, HP expresses an adaptive response to low pH levels through urease and several ionic pumps (IPs), which are essential for the maintenance of a proton gradient across membranes. We hypothesized that HP strains from GC may lose certain IPs that help helicobacters to resist to acid (as they live in a hypo-achlorhydric environment) and show a different susceptibility to the antimicrobial activity of anti-oxidants.
Methods: We tested the susceptibility to Res of 6 cagA+ HP strains from GC, and 7 cagA+ and 6 cagA- strains from controls. Res was diluted in broth; bacterial suspensions were added and incubated at 37 °C in microaerobic atmosphere overnight. After subculture on agar and incubation for 3–5 days, plates were examined. Tests were performed in triplicate and repeated twice. MBCs were the mean values, obtained in the various tests, of the lowest Res concentrations in broth whose subcultures on agar gave no growth. Differences were calculated by the t test for independent samples.
Results and Discussion: The MBCs (in μg/ml) of Res (SD) for GC cagA+ strains, and cagA+ and cagA- control strains were respectively 98 (56), 463 (158) and 245 (76) ($P < 0.001$ and $P = 0.003$; GC vs. cagA+ and vs. cagA- controls). The increased susceptibility to Res of the GC strains may be due to the reduced number/expression of IPs that enable the bacteria to maintain normal proton fluxes across membranes and putatively to resist to low pH levels and the antimicrobial activity of substances endowed with scavenging power.

Poster no.: P1.14
Allelic diversity and phylogeny of two *H. pylori* OMP-coding genes, *homC* and *homD*, in different geographical regions

R. Cordeiro and M. Oleastro
Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal

homC and *homD* genes encode *H. pylori* outer membrane proteins (OMP) but no specific function of these genes is known. They belong to the OMP *hom* family together with the recently studied *homB* and *homA* genes.
This study aims to investigate the allelic diversity and phylogeny of *homC* and *homD* genes, using a panel of 130 *H. pylori* clinical strains isolated in East Asian and Western countries, from patients presenting different gastric diseases. PCR, sequencing and bioinformatics analysis were used.

Phylogenetic reconstruction of *homC* revealed a geographic segregation, with two predominant clusters, East Asian and Western. *homC* sequence analysis showed the existence of seven allelic variants, localized in the middle region of the gene. One of the alleles was only detected among East Asian strains, while the other six were observed among Western strains, although with different prevalence.

homD sequence analysis indicated a high conservation of the gene among strains, corroborated by the phylogenetic analysis that showed no geographic separation. A rich repeat sequence, encoding lysine-proline (KP) motifs, was observed in the *homD* 5′-region, with a variable number of repeats among strains. *In silico* analysis, showed that this variable peptide presents a strong hidrophilicity and antigenicity and a high probability of being surface exposed.

These results suggest that *homC* and *homD* genes are good candidates to be implicated in virulence and/or persistence of *H. pylori*. In order to clarify their contribution, we pretend to evaluate the correlation between clinical outcome with both *homC* allelic variation and *homD* KP-motif repeats.

Poster no.: P1.15
A complexomic study of two *H. pylori* strains of two pathological origins: potential targets for vaccine development and new insight in bacteria metabolism

C. Bernarde[1,2], P. Lehours[1,2], J. Lasserre[1,2], M. Castroviejo[3], M. Bonneu[4], F. Mégraud[1,2] and A. Ménard[1,2]

[1]INSERM U853, 33076 Bordeaux, France; [2]Université Victor Segalen Bordeaux 2, Laboratoire de Bactériologie, 33076 Bordeaux, France; [3]Laboratoire de Microbiologie Cellulaire et Moléculaire et Pathogénicité, UMR CNRS 5234, 33076 Bordeaux, France; [4]Université Victor Segalen Bordeaux 2, Pôle Protéomique, Plateforme Génomique Fonctionnelle, 33076 Bordeaux, France

H. pylori infection plays a causal role in the development of gastric MALT lymphoma (LG-MALT) and duodenal ulcer (DU). While many virulence factors have been associated with DU, many questions remain unanswered regarding the evolution of the infection towards this exceptional event: LG-MALT.

The present study describes and compares the complexome of two *H. pylori* strains: strain J99 associated with DU and strain B38 associated with LG-MALT, using the 2D BN/SDS-PAGE method. It was possible to identify 90 different complexes (49 and 41 in the B38 and J99 strains, respectively); 12 of these complexes were common to both strains (7 and 5 in the membrane and cytoplasm, respectively) reflecting the variability of *H. pylori* strains. The 44 membrane complexes included numerous outer membrane proteins, such as the major adhesins BabA and SabA retrieved from a complex in the B38 strain, and also proteins from the *hor* family rarely studied. BabA and BabB adhesins were found to interact independently with HopM/N in the B38 and J99 strains, respectively. The 46 cytosolic complexes were essentially comprised of proteins involved in *H. pylori* physiology. Some orphan proteins were retrieved from hetero-rooligomeric complexes and a function could be proposed for a number of them via the identification of their partners such as JHP0119 which may be involved in the flagellar function. Overall, this study gave new insight into the membrane and cytoplasm structure, and those which could help in the design of molecules for vaccine and/or antimicrobial agent development are highlighted.

Poster no.: P1.16
Methyltranferases expression in *H. pylori* isolates: through geography to disease

F. Vale[1] and J. Vítor[2]

[1]Faculdade de Engenharia - Universidade Católica Portuguesa, Sintra, Portugal; [2]iMed. Faculdade de Farmácia - Universidade de Lisboa, Lisboa, Portugal

The genome methylation pattern of *H. pylori* is due to the expression of a high number of methyltransferases and allows its use as a strain typing method. Most methyltransferases belong to type II restriction and modification (RM) systems and their presence in high number is neither clear nor common in *Bacteria*. RM systems are likely to play a role in the defence of the integrity of the genome that might be highly exposed to alien DNA, since *H. pylori* is a natural competent bacterium. We have shown that methyltransferases are expressed according to their host geographic origin. Asian strains have the most dissimilar methylation profiles with increased expression of M.FokI ($p = 0.001$), M.DraI ($p < 0.001$), M.BstUI ($p = 0.006$) and M.FauI ($p = 0.004$). African strains are associated with increased expression of M.HpyCH4III ($p < 0.001$) and decreased expression of M.AseI ($p = 0.031$). As a consequence, dendrograms produced with Minimum Common Restriction and Modification (MCRM) algorithm show separate clusters of Asian and Africa strains, whereas European and American strains are more mixed. When we analyse the same strains, but considering host disease (gastritis or peptic ulcer) rather than host geographic origin, the strains do not cluster accordingly. This result suggests that MTases are not virulent factors, nor can be identified as virulence factors in early phases of disease progression.

P2 Treatment trials & other clinical studies

Poster no.: P2.01
Comparison of triple rabeprazole/metronidazole/sitafloxacin therapy versus triple rabeprazole/amoxicillin/sitafloxacin therapy as the 3rd rescue regimen for eradication of *H. pylori*

T. Furuta, M. Sugimoto, C. Kodaira, m. Nishino, M. Yamade and T. Uotani
Hamamatsu University School of Medicine, Hamamatsu, Japan

Backgrounds/Aims: Fluoroquinolone-based therapy has been used as the 3rd rescue regimen after failure of eradication of *H. pylori* with the triple therapy with proton pump inhibitor (PPI), amoxicillin and clarithromycin and that with PPI, metronidazole and amoxicillin. Sitafloxacin is one of fluoroquinolones and has the lower MIC for *H. pylori* in comparison with other quinolones, such as levofloxacin. However, which antimicrobial agent should be used with sitafloxacin has not been determined. Then, we tested which antimicrobial agent, amoxicillin or metronidazole had good combination with sitafloxacin in the 3rd rescue regimen for eradication of *H. pylori*.
Methods: 42 patients who failed in eradication of *H. pylori* after two (1st: PPI/amoxicillin/clarithromycin and 2nd: PPI/amoxicillin/metronidazole) or more regimens were enrolled to the study. They were treated with rabeprazole 10 mg bid (qid for CYP2C19 rapid metabolizers), sitafloxacin 100 mg bid and amoxicillin 500 mg qid or rabeprazole (rabeprazole) 10 mg bid (qid for CYP2C19 raid metabolizers), sitafloxacin 100 mg bid and metronidazole 250 mg bid for 1 weeks. At 4 weeks after the treatment, they underwent the [13C]-urea breath test (UBT). When the result of [13C]-UBT was negative, they underwent the endoscopy and the successful eradication was confirmed by RUT.
Results: All patients competed the treatment. The eradication rates in the rabeprazole/amoxicillin/sitafloxacin therapy and rabeprazole/metronidazole/sitafloxacin therapy were 95.5% (21/22, CI = 77.2% - 99.9%) and 95.0%, respectively (95% CI = 75.2% - 99.9%). Mild dierrhea was observed in 1 case in each treatment group during the study period.
Conclusions: Sitafloxacin 100 mg bid can attain the sufficient rescue eradication rates together with metronidazole (250 mg bid) or amoxicillin (500 mg qid) when used with rabeprazole 10 mg bid or qid. The sitafloxacin-based triple therapy with rabeprazole and amoxicillin or metronidazole could be the rescue regimen for the *H. pylori* infection refractory to the standard therapy with a PPI, amoxicillin and clarithromycin or metronidazole.

Poster no.: P2.02
Second-line rescue therapy with levofloxacin after *H. pylori* treatment failure. Time trends of eradication in a Spanish multicenter study of 742 patients

J. P. Gisbert
La Princesa University Hospital and CIBEREHD. On behalf of the H. pylori Study Group of the Asociación Española de Gastroenterología (AEG), Spain

Background and Aims: Second-line quadruple therapy is complex and can induce frequent adverse effects. A rescue levofloxacin-based regimen may represent an alternative; however, resistance to quinolones is rapidly increasing. Our aim was to evaluate the efficacy/tolerability of a triple second-line levofloxacin-based regimen, extending the experience of an ongoing multicenter study, and to assess whether its efficacy decreases with time.
Methods: Design: Prospective multicenter study. Patients: In whom a treatment with PPI-clarithromycin-amoxicillin had failed. Intervention: levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.) and omeprazole (20 mg b.i.d.) for 10 days. Outcome: Eradication was confirmed with ^{13}C-urea-breath test 4–8 weeks after therapy. Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.
Results: 742 consecutive patients were included (mean age 49 years, 43% males, 35% peptic ulcer/65% dyspepsia). 96% patients took all medications correctly. Adverse effects were reported in 18% of patients, most commonly nausea (8%), metallic taste (5%), myalgias (3.5%), and abdominal pain (3%), none of which were severe. Per-protocol and intention-to-treat (ITT) eradication rates were 76% (95%CI = 73–79%) and 74% (71–78%). Efficacy (ITT) was 77% in 2006, 68% in 2007, 72% in 2008, and 76% in 2009. In the multivariate analysis, none of the studied variables (including diagnosis and year of treatment) was associated with eradication success/failure.
Conclusion: Ten-day levofloxacin-based rescue therapy constitutes an encouraging second-line strategy representing a safe and simple alternative to quadruple therapy in patients with previous PPI-clarithromycin-amoxicillin failure. Efficacy of this regimen remains stable with time.

Poster no.: P2.03
Egg yolk antibodies suppress *H. pylori* infection and gastritis

Y. Yamashita[1,2], M. Okuda[1], N. Fukuda[1], S. S. Yun[2], Y. B. Noh[3], H. S. Yea[3], N. Yamamoto[1], H. Koizuka[1], K. Horie[2], D. Saito[1] and Y. Fukuda[1]

[1]Department of General Medicine and Community Health Science, Hyogo College of Medicine, Sasayama, Japan; [2]Pharma Foods International co., ltd, Kyoto, Japan; [3]Maeil Dairies co., ltd, Pyungtack, Korea, Republic of

Background: *H. pylori* (*H. pylori*) colonizes in the gastric mucosa with the urease, and secrets the VacA inducing an inflammation. To control *H. pylori* we develop two egg yolk antibodies (immunoglobulin Y, IgY), anti-urease or VacA IgY. In this report, we considered possibility of these antibodies as the alternative therapy of the antibiotic

Methods: Hens were immunized with purified urease or VacA. IgYs were purified from egg yolks. Experiment 1: VacA was added with anti-VacA IgY in AZ-521 gastric cell lines culture. Then, vacuolation, apoptosis, viability and inflammatory cytokines mRNA (IL-1β, -6, -8, TNF-α and IFN-γ) were measured. Mongolian gerbils infected with *H. pylori* were used for anti-VacA IgY administration test. Experiment 2: Volunteers had high UBT value enrolled in a clinical study to ascertain the *H. pylori* reduction efficacy of the anti-urease IgY yogurt.

Results: The anti-VacA IgY decreased VacA activities, vacuolation and apoptosis, in AZ-521 cells, thereby significantly raised their viability. Additionally, the inflammatory cytokines were suppressed. As a result of the administration of anti-VacA IgY to *H. pylori* infected gerbils, the inflammation of stomach was improved. Meanwhile, in human clinical study, the UBT value significantly decreased in anti-urease IgY yogurt group compared with control.

Conclusion: This study demonstrated the IgYs could effectively suppress *H. pylori* infection and inflammation. Therefore, IgY is a powerful food ingredient for suppression and prophylaxis against *H. pylori*.

Poster no.: P2.04
A large-scale nationwide observational study of triple therapy using rabeprazole, amoxicillin and clarithromycin for *H. pylori* eradication in Japan

T. Fujioka[1], K. Sugizaki[2], Y. Sakata[2] and T. Arai[2]

[1]Department of Gastroenterology, Faculty of Medicine, Oita University, Oita, Japan; [2]Eisai Co., Ltd., Tokyo, Japan

Background & Aim: The widespread use of clarithromycin in various treatments is considered to be associated with an increasing prevalence of clarithromycin-resistant *H. pylori*, potentially causing treatment failure in Japan. To elucidate the efficacy of first-line triple therapy using rabeprazole, amoxicillin and clarithromycin, a nationwide study was conducted.

Methods: This observational study involved 754 medical institutions. Subjects who were diagnosed with gastric ulcer or duodenal ulcer and were confirmed to be *H. pylori*-positive received one of the 2 triple therapies: rabeprazole 10 mg + amoxicillin 750 mg + clarithromycin 200 mg, b.i.d. for 7 days or rabeprazole 10 mg + amoxicillin 750 mg + clarithromycin 400 mg, b.i.d. for 7 days.

Results: A total of 3,162 subjects were included in the efficacy analysis. The eradication rate in this population was 80.7%. The eradication rates based on other factors were: 1) 81.4% in subjects without a history of eradication vs. 48.0% in subjects with the history ($p < 0.001$); 2) 81.0% for clarithromycin 400 mg/day vs. 80.0% for clarithromycin 800 mg/day; 3) 81.8% for <65 y.o. vs. 78.0% for ≥65 y.o. ($p < 0.05$); 4) 81.9% in subjects without a complication vs. 78.6% in subjects with a complication ($p < 0.05$).

Discussion: Despite the high prevalence of clarithromycin-resistant *H. pylori* in recent years, the eradication rate (80.7%) using the rabeprazole-based regimen is considered to be excellent. The advantage of rabeprazole is its ability to exhibit a rapid onset of increased pH from the first day of administration, providing a quick and favorable pH environment for antimicrobial activities.

Poster no.: P2.05
Relationship between Small Intestinal Bacterial Overgrowth and intestinal permeability in cirrhosis

E. Scarpellini, A. Cazzato, M. Gabrielli, M. E. Ainora, L. Di Maurizio, M. Campanale, G. Gasbarrini, V. Valenza and A. Gasbarrini

Catholic university of sacred heart, Rome, Italy

Introduction: Impaired intestinal permeability (IP) is associated with liver cirrhosis (LC), especially with ascites and spontaneous bacterial peritonitis (SBP). This study wants to assess small intestinal bacterial overgrowth (SIBO) prevalence and verify potential relationship with impaired IP in LC pts vs healthy controls.

Methods: SIBO was determined by glucose-hydrogen breath test (GBT). A basal breath-hydrogen >20 ppm or a rise by ≥12 ppm above baseline following glucose administration was considered as positive. IP was evaluated by the (51)Cr-EDTA permeability test. SIBO prevalence in LC was compared with healthy controls and correlated with LC severity and IP.

Results: Twenty-nine out of 56 LC patients had SIBO vs 2 of the 48 healthy controls (51. 8% vs 4%, $p < 0.0001$). SIBO prevalence correlated with the severity of LC (68.2% of Child C pts vs. 56.2% of Child B and 31.2% of Child A pts), with presence of ascites (66% in ascitic pts vs. 27% in non-ascitic pts), and with history of SBP (92% of patients with SBP vs. 48% of those without SBP).

A significant correlation was found between presence of SIBO and altered IP: it was weak for Child A but significant for Child B and C pts (R = 0.07, NS; R = 0.35, p = 0.005; R = 0.29, p = 0.03, respectively).

Conclusions: SIBO seems to have an higher prevalence in LC, correlates with LC severity and altered IP. This association suggests that SIBO may affect intestinal permeability of splancnic vessels and/or peritoneal membranes leading to SBP

outbreak in LC. Future interventional studies are needed to confirm these preliminary data.

Poster no.: P2.06
Fourth-line rescue therapy with rifabutin in patients with three *H. pylori* eradication failures

J. P. Gisbert[1], M. Castro-Fernandez[2], A. Perez-Aisa[3], J. L. Cabriada[4], A. Cosme[5], L. Rodrigo[6], J. L. Gisbert[1], E. Lamas[2], S. Marcos[1] and J. M. Pajares[7]

[1]La Princesa University Hospital and CIBEREHD, Madrid, Spain; [2]Valme Hospital and CIBEREHD, Sevilla, Spain; [3]Costa del Sol Hospital, Malaga, Spain; [4]Galdakao Hospital, Vizcaya, Spain; [5]Donostia Hospital and CIBEREHD, San Sebastian, Spain; [6]Central de Asturias Hospital, Oviedo, Spain; [7]La Princesa University Hospital, Madrid, Spain

Aim: In some cases, *H. pylori* infection persists even after 3 eradication treatments. Our aim was to evaluate the efficacy of an empirical forth-line rescue regimen with rifabutin in patients with 3 eradication failures, extending the experience of an ongoing multicenter study.
Methods: We prospectively included 51 patients in whom the following 3 eradication treatments had consecutively failed: 1st treatment: PPI-clarithromycin-amoxicillin; 2nd treatment: quadruple therapy (PPI-bismuth-tetracycline-metronidazole); 3rd treatment: PPI-amoxicillin-levofloxacin. In patients failing these 3 regimens, a 4th regimen with rifabutin (150 mg bid), amoxicillin (1 g bid) and omeprazole (20 mg bid) was prescribed for 10 days. Compliance with therapy was determined from interrogatory and recovery of empty envelopes of medications. Eradication was confirmed with 13C-urea-breath-test.
Results: Fifty-one patients (mean age 51 years, 43% males, 43% peptic ulcer/57% functional dyspepsia) were included. Compliance: 4 patients did not take correctly the medication (due to adverse effects): fever, myalgia, abdominal pain and diarrhoea (1 patient) and vomiting (3 patients). Per-protocol and intention-to-treat eradication rates were 51% (95%CI = 36–66%) and 49% (34–64%). Adverse effects were reported in 17 (33%) patients (none severe): nausea/vomiting (8 patients), diarrhea (2), fever (2), myalgia (2), hypertransaminasemia (2), leucopenia (<1,500 neutrophils) (2), abdominal pain (1), and thrombopenia (<150,000 platelets) (1). Myelotoxicity resolved spontaneously.
Conclusion: Even after 3 previous *H. pylori* eradication failures, an empirical fourth-line rescue treatment with rifabutin may be effective in approximately 50% of the cases. Therefore, rifabutin-based rescue therapy constitutes a valid strategy after multiple previous eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole, tetracycline, and levofloxacin.

Poster no.: P2.07
Seasonal variation of *H. pylori* eradication rates with standard triple therapies: a 10-year retrospective study

G. M. Buzás[1], J. Józan[2], B. Visnay[3], J. Nagy[1] and É. Szilágyi[1]

[1]Ferencváros Health Service Non-Profit Ltd, Budapest, Hungary; [2]Semmelweis University, Institute of Physiology, Budapest, Hungary; [3]2nd District Policlinic, Central Laboratory, Budapest, Hungary

Background: The seasonal periodicity of peptic ulcer was described in 1905 by Lord Moynihan and later recognised as a peculiar feature of patients living in temperate regions.
Aim: To analyse the seasonal changes of eradication rates of *H. pylori* in duodenal ulcer and functional dyspepsia patients.
Methods: 458 patients were included who had endoscopically proven duodenal ulcer, functional dyspepsia and *H. pylori* infection confirmed by rapid urease test and biopsy and who underwent a standard triple therapy (any of proton pump inhibitor + amoxicillin + clarithromycin b.i.d. for 7 days) between 1999 and 2009. Eradication control was performed with a ^{13}C-urea breath test 6 weeks after treatment. The seasonal and monthly eradication rates were calculated and statistically compared (ANOVA/Tukey post-hoc tests).
Results: Eradication rates were 65. 7 (95% confidence interval: 56.8.-74.2) in the spring, 79.5 (70.5- 88.3) in the summer ($p = 0.002$), 65.4 (57.4–73.4) in the autumn and 82.9 (75.9–89.3) during the winter ($p = 0.001$). The eradication rates peaked in December (83.8%, CI:71.3–95.2) and January (86.8%, CI: 75.6–97.3), and were lowest in September (54.1%, CI: 37.2–72.3) and March (66.7%, CI: 52.8–77.6). The seasonal variations were similar in duodenal ulcer and functional dyspepsia patients, the overall rates (70.6 and 76.6%, respectively) were not different ($p = 0.18$). Regression analysis showed that seasons influence the rates of eradication independently ($r = 0.009$).
Conclusions: Our results suggest for the first time that the success of eradication treatment undergoes seasonal variations. The reasons for this could be seasonal changes in bacterial pathogenecity/antimicrobial resistance, host immunity or environmental factors (climate, nutrition?).

Poster no.: P2.08
Comparing the Effect of first line *H. pylori* eradication therapy between 5-day quadruple ''Concomitant'' therapy regimen versus 7-day standard triple therapy regimen

S. Kim, S. Lee, S. Jung, B. Kwon, J. Kim, J. Kim, J. Koo, H. Yim and J. Choi

Division of gastroenterology, Department of internal medicine, Korea university, Seoul, Korea, Republic of

Background/Aims: Proton pump inhibitor (PPI)-containing triple therapy with clarithromycin and amoxicillin is a standard regimen for *H. pylori* eradication in Korea. However, many recent studies have reported lower eradication rate than before

due to increasing antibiotic resistance. Several studies showed superiority of concomitant quadruple therapy containing three antibiotics over triple therapy. The aim of this study was to compare concomitant therapy with standard triple therapy for the first line *H. pylori* eradication.
Methods: A total of 117 patients with proven *H. pylori* infection were randomly assigned to one of two regimens: amoxicillin 1000 mg with clarithromycin 500 mg and lansoprazole 30 mg twice daily for 7 days (triple therapy group, n = 61), or amoxicillin 1000 mg with clarithromycin 500 mg, metronidazole 500 mg and lansoprazole 30 mg twice daily for 5 days (concomitant therapy group, n = 56). The success of *H. pylori* eradication was evaluated 4–5 weeks after completing treatment.
Results: Eradication rates were 85.2% in the triple therapy group and 94.6% in the concomitant therapy group; there was no statically significant difference. Compliances were 100% in the triple therapy group and 96.4% in the concomitant therapy group. The side effects in each group were generally mild.
Conclusion: Five-day quadruple concomitant therapy is found to eradicate *H. pylori* in over 90% of patients. Therefore, concomitant therapy appears to be an effective alternative to triple therapy as the first line treatment regimen of *H. pylori* eradication.

Poster no.: P2.09
Antibiotic-Metal Complexes - An approach for Helicobacter Therapy

A. R. Shaikh[1], M. Yadav[2] and R. Giridhar[2]
[1]Department of pharmaceutical Chemistry MGVs Pharmacy College, Panchavati, Nashik, India; [2]Department of Pharmacy, The M.S. University of Baroda, India, Baroda, India

The present work incorporates the synthesis and characterization of metal complexes of antibiotics with metal ions like bismuth, zinc, copper, iron and manganese active against *H. pylori* (*H. pylori*. Drugs that specifically inhibit *H. pylori* can be included in the category of antiulcer agents. It was decided to synthesize novel organometallic compounds of some antibiotics with metals. These complexes could behave as antibacterial as well as antiulcerative, a dual function, which is hitherto unknown in the therapy.
The selected antibiotics were reacted with metal salt in an alkaline medium to get the desired compounds. The selected antibacterial drugs in the present study are fluoroquinolones.
These compounds were evaluated for antibacterial activity against some Gram-positive and Gram-negative mirco-organism. They were also evaluated for in vitro anti- *H. pylori* activity.
From the experimental work performed following conclusions were drawn,
1) These compounds were characterized was done by various spectral analysis (UV, IR, Mass), Thermal analysis (DSC, TGA) and chemical analysis (Elemental composition and Karl-Fisher aquametry).
2) Preliminary microbiological screening of the synthesized compounds showed excellent results.
3) In vitro anti-*H. pylori* activity was performed on 19 different strains of *H. pylori*. It was observed that the MIC values for the MFCs were less then the corresponding ligands. The results were statistically analyzed by Tukeys multiple comparison test (P values <0.05).
4) Some of these complexes also showed promising activity against antibiotic-resistant strains of H. pyori.

Poster no.: P2.10
Changes of Eradication rates of first-line and second-line therapy for *Helicoabacter pylori* infection

G. Kim, Y. Kim, J. Song and S. Park
The Catholic University of Korea College of Medicine, Youido St Mary's Hospital, Seoul, Korea, Republic of

Background/Aims: The aims of this study were to evaluate the recent 5-year changes of eradication rates and to investigate the efficacies of repeat first line regimens in first line Helicobacter eradication failure patients.
Methods: From January 2004 to May 2008, eradication rates in 782 patients with *H. pylori* infection who received the first-line therapy for 7 (PAC7) or 14 days (PAC14) was evaluated retrospectively. In patients who failed to the first-line therapy, 68 patients were prescribed same first line regimens for 14 days and 94 patients were received the second-line therapy for 7 or 14 days. The C^{13}urea breath test was performed 4–6 weeks after the completion of eradication therapy.
Results: The eradication rates of 7 day from 2004 to 2008 were decreased 87.4%, 89.1%, 81.9%, 76.6% and 70.5%, respectively. Fourteen days eradication rates were 89.0%, 91.1%, 85.6%, 80.7% and 74.4%, respectively. In first line treatment failure group, the eradication rates of repeated first-line regimens group was 66.7% and second line regimens were 70.6% in 7 days group and 86.7% in 14 days group.
Eradication rates of the PAC14 were higher than those of the PAC7 group (86% vs. 79.4%). There is a linear by linear association of decreasing trend of eradication rates during the past five years ($p < 0.001$).
Conclusions: Annual *H. pylori eradication* rates was significantly decreased. Eradication rates of 14-day PPI-containing triple therapy were superior than that of 7-day therapy. Repeated administration of same first-line treatment could be considered in progressive higher rate of eradication failure.

Poster no.: P2.11
Sequential Therapy with High Dose of Proton Pomp Inhibitors as Second-Line *H. pylori* Treatment Regimen in Crimean Peptic Ulcer Population

I. Klyaryts'ka, V. Kryvy
Crimean Medical University, Simferopol, Ukraine

Background: The eradication rate of *H. pylori* with proton pump inhibitor (PPI)-based triple regimen tended to decrease recently.
Aim: To evaluate the efficacy and safety of sequential therapy with high dose of PPI in patient with eradication failure.

Methods: Consecutive 90 *H. pylori*-positive patients with peptic ulcer were randomized into one of two groups with a 1:1 ratio. Group A was given a sequential regimen consisted of rabeprazole 40 mg and amoxicillin 1 g for 7 days, followed by rabeprazole 40 mg, clarithromycin 500 mg, and metronidazole 500 mg for the next 7 days, all given twice daily.
Group B was given standard treatment with rabeprazole, tetracycline, metronidazole and bismuthi subitras for 14 days. Metronidazole was given t.i.d., tetracycline q.i.d. and all other drugs b.i.d. An upper endoscopy with biopsy, rapid urease test (RUT) and 13C-Urea breath test (UBT) was performed before enrollment. Eradication was confirmed by UBT 4 weeks after the end of the treatment.
Results: The eradication rates were better in sequential groups than in the standard group. There was no significant difference in compliance and side-effects rates among the protocols.
The intention-to-treat (ITT) eradication rate was thus 71.1% (32/45) for sequential and 51.1% (23/45)for bismuth-based quadruple therapy. The per-protocol (PP) cure rate was 78.1% (32/41) for sequential and 59.0% (23/39) for bismuth-based quadruple therapy.
Conclusions: The sequential treatment achieved a better eradication rate of *H. pylori* compared to standard second-line regimen ($p < 0.01$). Sequential regimen may be an alternative to standard second-line treatment *H. pylori*.

Poster no.: P2.12
Influences of inflammatory cytokine polymorphisms on eradication rates of *H. pylori*

M. Sugimoto[1], Y. Yamaoka[2] and T. Furuta[1]
[1]Hamamatsu University School of Medicine, Hamamatsu, Japan; [2]Oita University, Yufu, Japan

Introduction: Pro-inflammatory and anti-inflammatory cytokines are produced in gastric mucosa from inflammatory cells activated by *H. pylori* infection. Especially, IL-1β and TNF-α have a potent inhibitive effect on gastric acid production. Polymorphisms in these genes are associated with individual differences in cytokine mRNA levels, which result in different gastric mucosal inflammation, different acid inhibition and different gastroduodenal disease risks. The aim of this study is to investigate whether inflammation-related cytokine polymorphisms would influence the eradication rates of *H. pylori*.
Methods: Influences of inflammatory cytokine polymorphisms on the eradication rates of *H. pylori* were analyzed by meta-analysis using previous reports.
Results: The IL-1B-511 polymorphism was related to eradication rate and the eradication rate in patients with the IL-1B-511 C/C genotype (77. 4%, 209/270), low IL-1β producer genotype, was lower than that of the IL-1B-511 C/T and T/T genotypes (87.2%, 631/724) (OR for eradication failure: 1.98, 95%CI: 1.38–2.84, $p = 0.0002$). Moreover, the OR of combined CYP2C19 rapid metabolizer-IL-1B-511 C/C type for eradication failure was 11.15 (5.23–23.78) times that of CYP2C19 poor metabolizer-IL-1B-511 non-C/C type. However, there was no positive data indicating what the role of other inflammatory cytokine polymorphisms (e.g., IL-1RN, TNF-A or IL-10) was for eradication therapy.
Discussion: Inflammatory cytokine polymorphisms, especially the IL-1B-511 T/T genotype, are the determinants of eradication by affecting gastric acid secretion and mucosal inflammation in meta-analysis. Therefore, the tailored eradication therapy, considering inflammatory cytokine polymorphisms, may be effective for the higher eradication rates.

Poster no.: P2.13
Action of novel copper compounds on the viability of *H. pylori*

F. Perna[1], T. Hall[2], S. Hickok[2], V. Gant[2], D. Vaira[1] and J. Holton[2]
[1]University of Bologna, Bologna, Italy; [2]Windeyer Institute of Medical Sciences, London, United Kingdom

Objectives: Owing to the increasing prevalence of antibiotic resistance in *H. pylori* novel antimicrobial agents are being actively sought. One novel biocide is a series of highly reductive copper complexes having broad antimicrobial activity. Copper is also recognized as angiogenic and may have healing effects on ulcers. We have investigated the action of two of these compounds on the viability of *H. pylori*.
Methods: Three Type and 53 clinical isolates were tested. For 5 of the isolates a kill curve was performed with an inoculum of 107–8 cfu/ml in sterile water at differing concentrations (0.5, 1.0, 5.0, and 12 ppm) of two copper compounds CuAL42 and CuPC33 for 15, 30, 60 and 120 minutes. At each time point samples were withdrawn, decimal diluted into ¼ strength Ringers lactate, plated and incubated. Subsequently all the isolates were tested at the same inoculum against 12 ppm for 60 and 120 mins. The plates were incubated for 5 days at 370 C in an atmosphere generated by CampyGen (Oxoid UK)
Results: CUAL42 was more active than CUPC33. At 5 ppm the viable count was reduced by 5–6 logs at 2 hrs whilst for CUAL42 at 1 hour and 2 hours respectively 20 and 40 isolates were completely killed compared to CUPC33, where 16 and 34 isolates were killed at 1 and 2 hours exposure. Neither Cag A status nor antibiotic sensitivity bore any relationship to the efficacy of the copper compounds
Conclusions: These novel copper compounds deserve further study in relation to eradication of *H. pylori*

Poster no.: P2.14
H. pylori levofloxacin-based rescue option in patients allergic to penicillin failing a previous treatment with clarithromycin and metronidazole

J. P. Gisbert[1], A. Perez-Aisa[2], M. Castro-Fernández[3], J. Barrio[4], L. Rodrigo[5], A. Cosme[6], J. L. Gisbert[1], S. Marcos[1] and R. Moreno-Otero[1]
[1]La Princesa University Hospital and CIBEREHD, Madrid, Spain; [2]Costa del Sol Hospital, Malaga, Spain; [3]Valme Hospital and CIBEREHD, Sevilla, Spain; [4]Rio Hortega Hospital, Valladolid, Spain; [5]Central de Asturias Hospital, Oviedo, Spain; [6]Donostia Hospital and CIBEREHD, San Sebastián, Spain

Background: *H. pylori* eradication is a challenge in patients allergic to penicillin, especially in those who have failed a first-eradication trial with the two key antibiotics clarithromycin and nitroimidazoles.
Aim: To assess the efficacy and safety of *H. pylori* levofloxacin-based rescue option in patients allergic to penicillin failing a previous treatment with a proton pump inhibitor (PPI), clarithromycin and metronidazole, extending the experience of an ongoing multicenter study.
Patients: Prospective multicenter study including consecutive patients allergic to penicillin who had failed a first eradication treatment with PPI, clarithromycin and metronidazole. Second-line treatment with omeprazole (20 mg b.i.d.), clarithromycin (500 mg b.i.d.) and levofloxacin (500 mg b.i.d.) was administered for 10 days. Compliance with therapy was determined from the interrogatory and the recovery of empty envelopes of medications. Incidence of adverse effects was evaluated by means of a specific questionnaire. Eradication was confirmed with ^{13}C-urea breath test 8 weeks after completion of treatment.
Results: 100 patients allergic to penicillin were included. Compliance: 1 patient did not take correctly the medication (due to adverse effects: abdominal pain). Per-protocol and intention-to-treat eradication rates were 66% (95%CI = 56–75%) and 65% (55–75%). Adverse effects were reported in 16 patients (16%): metallic taste (8 patients), mild nausea/vomiting (7 patients), asthenia (2 patients), abdominal pain (1 patient), diarrhea (1 patient), oral and vaginal candidiasis (1 patient), and myalgias/arthralgias (2 patients).
Conclusion: A levofloxacin-based regimen (together with a PPI and clarithromycin) represents a second-line alternative in the presence of penicillin allergy.

Poster no.: P2.15
H. pylori: Standard Triple Therapy is still highly effective in u nselected Swedish patients. A 6-year follow up in clinical practice

B. H. Jaup[1,2]
[1]Seffle Hospital, Dept. of Medicine, Seffle, Sweden; [2]Seffle Hospital, Seffle, Sweden

H. pylori. Standart Triple Therapy is still highly effective in unselected swedish patients. A 6-year follow up in clinical practice.
Background: In the litterature the effect of Standard Triple Therapy (STT) is often much lower in clinical practice compared to what is reported for selected cohorts. We have therefore analyzed the results of this therapy given in our unit for eradication of *H. pylori*.
Methods: We reviewed eradication records from january 2002 to january 2008. The patients have been treated according to Maastricht 2 guidelines with Nexium hp(esomeprazole 20 mg, amoxycillin 1 g, clarithromycin 500 mg) twice daily for 7 days. All patients underwent a C14 urea breath test after 5 weeks.
Results: Data from 400 consecutive pts. (214 female) have been reviewed.
In 383 pts eradication was achiewed (96%PP,95,2%ITT). Two pts.were lost for follow up.In 4 pts.with eradication failure we found resistance to macrolides.
Conclusion: STT is in Sweden still highly effecive even in daily practice. This might be due to the low resistance rate to macrolides and the low consumption rates of macrolides in Sweden due to the Swedish strategic programme against antibiotic resistance (STRAMA) Good compliance is supported by the use of a Therapy-Kit and the specialist setting. The eradication rate is almost identical with results ten years ago.

Poster no.: P2.16
Influence of Bismuth on Gastritis Healing and Effectiveness of *H. pylori* Eradication

L. B. Lazebnik, A. A. Masharova, D. S. Bordin and S. G. Khomeriki
Central Research Institute of Gastroenterology, Moscow, Russian Federation

Bismuthate Tripotassium Dicitrate (BTD) dispose of antibacterial effect as well as anti-inflammatory action, therefore it might improve the efficacy of eradication of *H. pylori* (HP) and reduce intensity of gastritis.
The aims of the study were to determine the ability of BTD to improve HP eradication and reduce intensity of gastritis.
Methods: Ninety patients with HP-associated chronic gastritis were randomized into three groups: 30 patients in the first group were treated with omeprazole, amoxicillin and clarithromicyn for 10 days; 30 patients in the second group took the same triple therapy plus BTD 240 mg b. i.d. during 10 days; for 30 patients in the third group BTD was prescribed for 28 days. Upper gastrointestinal endoscopy was performed for all the patients twice. Morphology and urea test were used to detect presence of HP. Grade of gastritis and stage of atrophy were evaluated by OLGA system.
Results: We found no intergroup difference for grade of gastritis (I-II) and stage of atrophy (0-II) before the treatment. One month after the therapy grade of gastritis reduced in 63.3% of patients in the first group, 70% in the second and 100% in the third and positive dynamics in stage of atrophy was shown in 3.3%, 6.6% and 16.6% patients. Eradication rate was 73.3% in the first, 93.3% in the second and 33.3% in the third group.
Conclusion: Our data confirms that bismuth increases the rate of HP eradication and suggests that including of BTD in treatment regimen may reduce intensity of gastritis.

Poster no.: P2.17
Efficacy of Minocycline Based Metronidazole, Bithmuth and PPI Quadrupple Second-Line Therapy for Eradication of *H. pylori*

B. S. Moon[1], Y. C. Lee[1], H. W. Jung[1], H. C. Lim[1], Y. J. Lee[2], S. H. Ahn[1], K. H. Hahn[1], J. B. Jung[1], C. Y. Cheon[1] and S. I. Lee[1]
[1]Division of Gastroenterology, Department of Internal Medicine, Medical College of Yonsei University, Seoul, Korea, Republic of; [2]Department of Family Medicine, Medical College of Yonsei University, Seoul, Korea, Republic of

Background: The treatment failure of second-line standard quadruple regimen was 5–35% and the efficacy of this regimen

is limited by poor patient's compliance due to side effects. Minocyclin(MNO) is tetracycline derivative and effective against clarithromycin resistant HP. We studied the effectiveness of MNO-based second- line therapy.
Methods: Patients with failed in the first-line therapy were randomly allocated into 2 groups. Group A received tetracycline(TC) 500 mg *qid*+lansoprazol(LPZ) 30 mg *bid*+tripotassium dicitrato bithmuthate(TDB) 600 mg *bid*+metronidazole(MTZ) 500 mg *tid* for 1 week. Group B received MNO 100 mg *bid*+ LPZ 30 mg *bid*+TDB 600 mg *bid*+MTZ 500 mg *tid* for 1 week. It was recorded the side effects profiles and eradication of HP was assessed by UBT 4–6 weeks after therapy.
Results: 90 patients were enrolled in each group. In group A eradication rate ITT was 32/37 (86.1%) and PP 32/36 (88.6%). In group B eradication rate ITT was 46/52 (88.5%) and PP 46/51 (90.2%). In group A the side effects occurred 14 cases (37.8%). Epigastric discomfort was in 7 cases (19.4%), nausea 3 cases (8.3%). In group B the side effects occurred 8 cases (15.8%). Epigastric pain was in 4 cases (7.7%), diarrhea 3 cases (5.8%).
Conclusion: MNO-based quadruple therapy regimen was effective for second-line treatment for HP. It coluld be a kind of therapeutic option as second line treatment.

Poster no.: P2.18
Ten-day sequential therapy as first-line treatment for *H. pylori* infection in Korea: A Prospective randomized study

H. S. Oh[1], D. H. Lee[2], N. Kim[2], Y. S. Park[2], H. C. Jung[1] and I. S. Song[1]

[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic of; [2]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Korea, Republic of

Background and Aims: The eradication rate of proton-pump inhibitor-based triple therapy for *H. pylori* infection is low due to increasing antibiotics resistance, especially clarithromycin. Recently, it was reported in Europe that a 10-day sequential strategy produced good outcomes. The aim of this prospective randomized study was to assess the efficacy of sequential therapy as first-line treatment for eradication of *H. pylori* in clinical practice in Korea.
Materials and Methods: A total 33 patients (mean age 56 years and male 20, female 12) with proven *H. pylori* infection received 10-day sequential therapy (20 mg of rabeprazole, and 1 g of amoxicillin, twice daily for the first 5 days, followed by 20 mg of rabeprazole, 500 mg of clarithromycin, and 500 mg of metronidazole, twice daily for the remaining 5 days). Eradication was evaluated 4 weeks later, after completion of treatment by 13^{c}-urea breath tasting. Compliance and adverse events were assessed in study group. Control group was a total 38 patients(mean age 49 years and male 23, female 15) received 7-day triple therapy(20 mg of rabeprazole, 500 mg of clarithromycin, and 1 g of amoxicillin, twice daily for 7 days).
Results: The eradication rate of sequential therapy was 84. 8% (28/33) by ITT and same result was reported by PP analysis(28/33), compared with 7-day triple therapy's result 71.0%(27/38) by ITT and PP. Mild adverse events happened frequently(24.2%-bitter taste, nausea, diarrhea, etc) but the treatment was well tolerable.
Conclusion: 10-day sequential therapy is found to effectively eradicate *H. pylori* infection as first-line treatment in Korea with high clarithromycin resistance rate.

Poster no.: P2.19
Efficacy of Rifabutin based triple therapy including PPI and high dose amoxicillin for third line rescue therapy of *H. pylori*

Y. Lee[1,2], B. Moon[1], H. Jung[1], S. Ahn[1], K. Han[1], J. Cheon[1], J. Chung[1] and S. Lee[1]

[1]Yonsei University College of Medicine, Seoul, Korea, Republic of; [2]Institute of Gastroenterology, Seoul, Korea, Republic of

Background: Rifabutin has been founded to be effective in multi-resistant patients after various treatment cycles for *H. pylori* (HP) infection. There were few trials of changing doses and duration of rifabutin based rescue therapy. The eradication rate was reportedly to be around 60–90%. We investigate the efficacy of rifabutin based rescue therapy for HP infection after the failure of first and second line treatment.
Patients and methods: From December 2007 to September 2009 32 patients was enrolled after eradication failure of first and second line therapy of HP. Patients were assigned to receive a 7 day eradication therapy with lansoprazole 30 mg bid, amoxicillin 1.0 g tid, rifabutin 150 mg bid. Urea breath test (UBT) was performed at 4 weeks after the end of treatment to evaluate the response of therapy.
Results: 32 patients were enrolled including 12 males and 20 female patients. The mean age was 55 years, ranging from 26 to 77 years. The eradication rates ITT were 78.1%(25/32) and per-protocol analysis 80.6% (25/31). Only 15.6% patients complained mild to moderate adverse events such as epigastric pain 3 cases (9.4%), abdominal discomfort was 3 cases (9.4%). Only one case was withdrawn due to severe abdominal pain and vomiting.
Conclusion: Rifabutin based triple therapy including PPI and high dose amoxicillin was effective and safe for third line rescue therapy of *H. pylori*. Large scaled randomized prospective study is mandatory to confirm above findings in the near future.
Keywords: rifabutin, triple, rescue therapy

Poster no.: P2.20
Effect of additional ecabet sodium on conventional proton-pump inhibitor-based triple therapy for *H. pylori* (*H. pylori*) eradication in Korea

J. Y. Kim[1], D. H. Lee[2], Y. S. Park[2], N. Kim[2], H. C. Jung[1] and I. S. Song[1]

[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic of; [2]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Korea, Republic of

Aims: The eradication rate of proton-pump inhibitor-based conventional triple therapy for *H. pylori* infection has decreased due to increasing antibiotics resistance. Ecabet sodium is known for bactericidal effect against *H. pylori*. It was reported that the supplement of ecabet sodium on conventional triple therapy met with good results in Asia, recently. Aim of this study was to ascertain the efficacy of additional ecabet sodium on conventional triple therapy for eradication of *H. pylori* in clinical practice.
Methods: One hundred eleven patients (Group A) with proven *H. pylori* infection received ecabet sodium with triple therapy (20 mg of rabeprazole, 1 g of amoxicillin, 500 mg of clarithromycin and 1 g of ecabet sodium, twice daily for 7 days). One hundred ninety patients (Group B) received PPI- based triple therapy (same as above, except ecabet sodium). Eradication was evaluated 4 weeks later after completion of treatment by 13^{C}-urea breath testing. The Side effects were also assessed.
Results: The eradication rates were 74. 8% (83/111) in group A and 71.1% (135/190) in group B by intention-to-treat ($p = 0.507$), and 75.2% (82/109) in group A and 71.4% (132/185) in group B by per protocol ($p = 0.500$). There were no significant differences in the side effects between two treatment groups.
Conclusions: The addition of ecabet sodium on conventional triple therapy is found to not effectively improve eradication rate of *H. pylori* in this study. The results imply ecabet sodium as additional agent could not overcome antibiotics resistance, which is the most important cause of failure of triple therapy.

Poster no.: P2.21
New variant of anti *H. pylori* sequential therapy

B. Starostin
Interdistrict Gastroenterological Centre No 1, Saint-Petersburg, Russian Federation

Introduction: In recent years, the steady growth of *H. pylori* resistance to clarithromycin and nitroimidazoles is determined in Russia, which requires the search for new variants of eradication of *H. pylori* (H.p.).
Aim: to determine effectiveness of the modified sequential therapy for eradication H. p. patients with diseases associated with H.p.
Materials: in open comparative study in outpatients, in which patients of study group (n = 79) with chronic gastritis (53) or peptic ulcer disease (26) with established H. p. took proton pump inhibitor (rabeprazole) 20 mg b.i.d. within 10 days, amoxicillin 1000 mg b.i.d. within 5 days, and the next 5 days clarithromycin 500 mg b.i.d. plus nifuratel (makmiror) 400 mg b.i.d. 5 days too, but in the control group (n = 77) patients with chronic gastritis (n = 55) and peptic ulcer disease (n = 22) received standard triple therapy. Control eradication H.p. carried out 6 weeks after cessation of treatment by the rapid urease test, histologically and the definition of antigen of H.p. in stool immunoassay.
Results: the eradication H. p. was observed in 74 of 79 intention to treat (ITT) - 94% and in 74 out of 77 per protocolum (PP) - 96% in the study group and the control group, respectively, in 63 of the 77 (ITT) intention to treat - 82% and in 63 out of 73 completed the study (PP) - 86%. Differences between groups are statistically significant.
Conclusions: The new anti-H. p. sequential therapy can be recommended for eradication H.p as the regime of first-line therapy.

Poster no.: P2.22
Eradication rate of Bismuth-containing quadruple therapy as a second-line treatment for *H. pylori* infection

S. R. Jee, S. H. Lee, E. U. Jung, K. S. Ok, J. H. Kim, J. S. Choi, Y. J. Lee and S. Y. Seol
Inje University Hospital, Busan, Korea, Republic of

Background & Aims: The eradication rate of first-line *H. pylori* (*H. pylori*) treatment has been decreasing due to antibiotic resistance in Korea. The aim of this study was to evaluate the efficacy of bismuth-containing quadruple therapy as second-line treatment for *H. pylori* infection.
Methods: Between Febrary 2007 and April 2010, patients who failed to respond to the initial proton pump inhibitor (PPI) based triple therapy, received second-line eradication with bismuth-containing quadruple regimen for seven days. Therapy was consisted of PPI standard dose b.i.d., tripotassium dicitrate bismuthate 300 mg q.i.d., metronidazole 500 mg t.i.d., and tetracycline 500 mg q.i.d. Urea-breath test or biopsy was performed to detect *H. pylori*.
Results: A total of 45 patients received second-line quadruple regimen. Of these, 41 patients had peptic ulcer disease (26 with gastric ulcers, 15 with duodenal ulcers). The eradication rate was 88.9% (40/45). There was no difference in the eradication rate between GU (96.2%, 25/26) and DU (86.7%, 13/15).
Conclusions: Bismuth-containing quadruple therapy as a second-line treatment for *H. pylori* Infection is an effective regimen in Korea.

Poster no.: P2.23
Docosahexaenoic acid inhibition of *H. pylori growth* is associated with decrease in its virulence and morphology

M. Correia[1,2], V. Michel[3], M. Bonnis[4], M. J. Oliveira[5], R. Ferreira[1,2], I. Boneca[4], M. C. Prévost[6], A. P. Matos[7], R. Seruca[1,8], C. Figueiredo[1,2], E. Touati[9] and J. C. Machado[1,8]
[1]Institute of Molecular Pathology and Immunology, Porto, Portugal; [2]Faculty of Medicine, Porto, Portugal; [3]Unité de Pathogenèse de Helicobacter, Institut Pasteur, paris, France; [4]G5 Biologie et Génétique de la Paroi Bactérienne, Institut Pasteur, Paris, France; [5]INEB - Instituto de Engenharia Biomédica, Porto, Portugal; [6]Plate-forme de Microscopie Ultrastructurale, Institut Pasteur, Paris, France; [7]Serviço de Microscopia Electrónica, Hospital Curry Cabral, Lisboa, Portugal; [8]Faculty of Medicine, Porto, Porto, Portugal; [9]Unité de Pathogenèse de Helicobacter, Institut Pasteur, Paris, France

H. pylori (Hp) infection is recognized as a major etiological factor in gastritis, ulcers and gastric cancer. Treatment of Hp infection has not being significantly changing. We have already demonstrated that Docosahexaenoic acid (DHA) posses an Hp growth inhibitory effect, in vitro and in vivo. Our main aim was to unravel how DHA is affecting Hp structure, morphology and virulence.

Hp, previously grown for 12 hours in the presence of DHA, was analysed in terms of cell wall morphology, structure, cell envelope protein composition and LPS. Hp adhesion to human epithelial gastric cells (AGS) was assessed with an ELISA-like assay. Expression of CagA protein, an important bacterial virulent factor, was detected by Western Blot analysis, and IL-8 production was measured by an ELISA kit.

Electron microscopy revealed differences in morphology of Hp when treated with DHA. Extracts from external and total membrane profile suggested differences in protein composition between controls and Hp DHA-treated. Silver staining of LPS extracted from Hp presented a rough LPS phenotype, whereas LPS from Hp previously treated with DHA had a smooth one. Treatment with DHA induced a decreased adhesion of Hp to AGS (* $p < 0,05$). Infected AGS cells by Hp previously treated with DHA, did not expressed CagA protein synthesis. Additionally, IL-8 production was also decrased in the presence of DHA (*$P < 0,05$).

The inhibitory effect previously demonstrated by DHA is associated with differences on bacteria cell envelope morphology and function. These observations may pave the way to evaluate DHA as a co-adjuvant agent in Hp eradication.

Poster no.: P2.24
Analysis of efficacy of different *H. pylori* Eradication regimens in Thailand

T. Thongbai[1], R. Vilaichone[2], S. Treeprasertsuk[3] and V. Mahachai[4]

[1]Gastroenterology Unit, Department of Medicine, Central Hospital, Bangkok, Thailand; [2]Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Oathumthani, Thailand; [3]Division of Gastroenterology, Department of Medicine, Chulongkorn University Hospital, Bangkok, Thailand; [4]Division of Gastroenterology, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand

Many regimens have been used as first-line therapy for *H. pylori* eradication in Thailand. The eradication rate of legacy triple therapy has decreased in recent years to only 70%. We collected the pooled data of 13_C-UBT performed at least one month after *H. pylori* eradication therapy. A total of 365 patients underwent 13_C-UBT after receiving different eradication regimens as first line therapy including: clarithromycin based triple therapy, bismuth based quadruple therapy, sequential therapy and levofloxacin based triple therapy. The commonly used clarithromycin based triple therapy resulted in eradication rate of 69.1% (67/97). Bismuth based quadruple therapy resulted in eradication rate of 83% (88/106) whereas sequential therapy resulted in the highest eradication rate of 95% (132/139). Levofloxacin based triple therapy used as first line result in low eradication rate of 69.6% (16/23) but when used as a second line therapy after failed standard triple, it yielded eradication rate of 76.2% (16/21). Alternative regimens are required to replace legacy triple therapy for *H. pylori* eradication and optimal second line therapy is required in the presence of failure to primary eradication therapy. The choices of eradication therapy need to be tailored based on data in each geographical location.

Poster no.: P2.25
Helicobacteriosis and *H. pylori*-infection therapy in out-patient conditions in Tajikistan

S. Satorov[1,2], K. Abdulloev[2] and G. Akhmedova[1,2]

[1]Avicenna Tajik State Medical University, Dushanbe, Tajikistan; [2]Tajik Scientific Research Institute of Preventive Medicine, Dushanbe, Tajikistan

Aim: Analysis of morbidity and study the effectiveness of treatment of *H. pylori* infection in the outpatient condition.

Materials and methods: Analysis of morbidity was performed according to the negotiability of patients to the doctors for medical help. The specimens for the study were stool of examined persons. Polymerase chain reaction (PCR) was used to detect *H. pylori*. Amplification and the registration of results were performed on MiniOpticon by ''BioRad'' in real time.

Results: The survey covered the whole of Tajikistan. Women (59,3%) and men (40,7%) were randomly included in this study. The detection of *H. pylori* from specimens of men and women was similar (22,2 and 22,5% consequently). *H. pylori* were isolated oftener from stool of 15–19 (25,2%) and 30–39 (24,1%) years of age. These figures were lower (20,5% and 22,4%) for population aged 20–29 and over 40 years. Eradication therapy was carried out according to the scheme recommended at Maastricht Conference (omeprazole, clarithromycin, amoxacillin within 7 days). Repeated analysis did not show Helicobacter in the stool of 97,4% of patients. The remaining patients were received therapy with four drugs: omeprazole, clarithromycin, amoxacillin and bismuth subcitron (De-nol). The control analyses were negative after treatment.

Conclusion: Our preliminary studies indicate a relatively high degree (22,5%) of contamination of tajik population by *H. pylori*. These microorganisms are often found in the group 15–19 years of age (25,2%). The scheme of therapy with De-nol can be recommended as an effective method of treatment of *H. pylori* infection in the outpatient conditions in Tajikistan.

Poster no.: P2.26
Optimization of the eradication treatment of *H. pylori* - associated ulcer disease

N. Zaharova[1], A. Sarsenbaeva[2], I. Brook[3] and S. N. Teplova[2]

[1]Hospital No 15, Snezhinsk, Russian Federation; [2]Chelyabinsk State Medical Academy, Chelyabinsk, Russian Federation; [3]Republic Diagnostic Center, Izhevsk, Russian Federation

Research objective is optimization of the eradication of *H. pylori* - associated ulcer disease.

Materials and methods: 80 patients with ulcer disease in an aggravation stage are surveyed. Middle age 47, 23 ± 0,74 years.

Control group made 25 clinically the healthy people comparable on sex and age. To *H. pylori* infection diagnostics applied: the ureasis respiratory Helic-test, morphological, serological markers of *H. pylori* infection, a method of polymerase chain reaction. By all patients it is spent endoscopy. Patients are divided into 2 groups: the group A received complex therapy (according to the Maastricht III, the traditional first-line treatment - PPI (twice daily), amoxicillin (1 g twice daily) and clarithromycin (500 mg twice daily) appointed within 7 days and Imudon appointed within 10 days), group B - the traditional first-line treatment.
Results: The percent successful eradication of *H. pylori* was above in group A - 82,5% (in group B-70%). In group A within a year of supervision is noted disease relapses (group B -10%), development of complications (group B 2,5%), is not present cases disable patients (group B-2,5%).
Conclusion: An effective complex treatment for ulcer disease which associated with *H. pylori* with inclusion in scheme immunology preparation Imudon was offered.

Poster no.: P2.27
The comparison of two eradicative regimens (*OCA*: Omeprazole,Clarithromycin,Amoxicillin & *OCM*: Omeprazole,Clarithromycin,Metronidazole) for *H. pylori* induced peptic ulcer diseases in Mashad, Iran

A. R. Khalighi[1], M. R. Khalighi[2], S. Kouhestani[1], N. Khalighi[2] and M. Ghasemi[2]
[1]Ghaem hospital, Mashad University of Medical Sciences (MUMS), Mashad, Iran, Islamic Republic of; [2]Islamic Azad University of Mashad, Mashad, Iran, Islamic Republic of

Introduction: PUD includes both gastric & duodenal ulcers. The comparison of two *H. pylori* eradicative regimens (OCA & OCM) to recognize the best one & enough treament duration had been studied in this research.
Materials & Methods: In this study (2006 - 2009),120 patients with proven PUD were divided into two groups (each: 60). For patients Urease Breath Test (UBT) had performed. Then, for mild & severe *H. pylori* infections one of eradicative regimens (OCA: Omeprazole 20 mg/bid, Clarithromycin 500 mg/q12h & Amoxicillin 1000 mg/q12h or OCM: Omeprazole & Clarithromycin with same doses & Metronidazole 500 mg/q12h) was started for one week, randomely. Eradication of *H. pylori* was confimed by negative UBT, one month later.
Results: In the first group, UBT result showed 95% severe & 5% mild infection before treatment & in the second group 91. 7% & 8.3%, respectively. After treatment, negative result of re-UBT one month later had been showed in 83.3% of the first group (whom received OCA) & 86.7% of the second one (whom received OCM). So, rate of success was acceptable & there were no meaningful differences between the two groups (P-value: 0.927).
Conclusion: For *H. pylori* induced PUD, a one week periscription of eradicative regimens of OCA or OCM is effective enough & there is no predominancy for their selection.

P3 Pathogenesis, host response & vaccines

Poster no.: P3.01
Loss-of-function analysis identifies new factors important for NF-kappaB activation by *H. pylori*

S. Bartfeld, C. Rechner, B. Bauer, J. Liu, S. Hess, A. Mäurer, N. Machuy and T. F. Meyer
Department of Molecular Biology; Max Planck Institute for Infection Biology, Berlin, Germany

In the promotion of *H. pylori* -induced pathology, the transcription factor NF-kappaB is believed to be crucial, because activation of this factor leads to transcription of an array of genes promoting inflammation, survival and anti-apoptosis. NF-kappaB is therefore thought to be a link between inflammation and cancer. Although several factors are known to be involved in NF-kappaB activation by *H. pylori* the knowledge about the host signaling pathways involved remains fragmentary.
To elucidate more of the host cell factors important for NF-kappaB activation, we have conducted an RNA-interference based screen. For this purpose, we have developed an assay for high throughput analysis of a human epithelial cell line stably expressing a p65-GFP-fusion construct. The nuclear translocation of p65-GFP can be quantified by automated microscopic analysis. Screening a library of siRNAs against 646 kinases and associated factors, we have identified new factors that are important in NF-kappaB signaling induced by *H. pylori*. These factors have not so far been linked to this pathway.
Furthermore, they are specific for *H. pylori* infection and not necessary for NF-kappaB activation by the cytokines TNF-alpha or IL-1beta. The screen has been expanded to a genome wide scale.

Poster no.: P3.02
Peculiarities of gastric mucosa epithelium ultrastructure in patients with *H. pylori*-associated gastric ulcer disease with different polymorphic loci of cytokine genes (IL-1 and IL-10) genotypes

E. Abuzarova[1], O. Chernova[1], G. Shaimardanova[1], O. Gorshkov[1], R. Abdulkhakov[2] and V. Chernov[1]
[1]Kazan Institute of Biochemistry and Biophisics, Kazan, Russian Federation; [2]Kazan State Medical University, Kazan, Russian Federation

Introduction: Initial effect of any factor includes elementary damage of the cells' structure. The clinical outcome of gastric mucosa damage by *H. pylori* depends on a variety of host and bacterial factors, such us variability of bacterial genotypes in respect to virulence factors (CagA, VacA etc.) and genes' polymorphism of host immune response. Polymorphism of cytokine genes (IL-1 and IL-10) determining the different level of the interleukins genes expression may influence the character of immune response and tissue damage, controlled by the genes products.
Aim: The aim of our study was comparative analysis of gastric mucosa ultrastructure in patients with different genotypes of polymorphic loci of cytokine genes (IL-1 and IL-10) infected with virulent (cagA+vacAs1+) *H. pylori* strains.
Methods: Gastric antrum mucosa tissue specimens received from 35 patients with gastric ulcer disease were used for DNA isolation. The presence of bacterial virulence genes was investigated by cagA, vacAs1/s2 PCR. PCR and RFLP were used for IL-1 and IL-10 genotyping in clinical samples. The specimens for transmission electron microscopy (TEM) assessment were fixed immediately after sampling in 2,5% glutaraldehyde for 2 hours, and postfixed in 1% osmium tetroxide. Ultrathin sections were contrasted with uranyl acetate and lead citrate.
Comparative analysis of samples was performed concerning pathologic changes of gastric mucosa epithelium ultrastructure, specific for gastric ulcer disease, associated with *H. pylori*: destroying of epithelial layer; absence of dense cell contacts and desmosomes; absence of microvilli on apical surface; absence of mucin granules. The severity of changes was estimated as slight (+), moderate (++) and highly expressed (+++).
Results: The profound alterations induced in the lamina propria by the virulent (cagA+vacAs1+) bacterial infection were observed in patients with genotypes responsible for normal level of inflammatory and anti-inflammatory cytokines expression (IL-1B-511*C/*C, IL-1B+3954*T/*C, IL-1RN*1/*1, IL-10-1082*A/*A). The least expressed changes were in gastric mucosa epithelium of patients with IL-1 and IL-10 genotypes determining high level of interleukin genes expression (IL-1B-511*T/*C, IL-1B+3954*C/*C, IL-1RN*1/*1, IL-10-1082*G/*G) which formes the adequate immune response against virulent acterial straines.
Conclusion: As a result of comparative analysis of TEM micrographs of gastric mucosa epithelium of patients with *H. pylori* -associated gastric ulcer disease we found that pathologic changes of ultrastructure of gastric mucosa epithelium vary among carriers of different IL-1 and IL-10 cytokine genotypes.

Poster no.: P3.03
H. pylori -Infection Changes Proepithelin Levels Independent of the Expression of Secretory Leukocyte Protease Inhibitor (SLPI) in AGS cells and gastric mucosa

T. Wex, D. Schindele, C. Schönberg and P. Malfertheiner
Otto-von-Guericke University Magdeburg, Magdeburg, Germany

Background: Recently, a downregulation of mucosal SLPI expression by *H. pylori* was reported. Proepithelin is an epithelial

growth factor that is proteolytically degraded into fragments by elastase (the main target of SLPI). After degradation, Epithelin-fragments act as proinflammatory mediators. Here, we analyzed whether the *H. pylori* -induced reduction of SLPI levels affects the expression of Proepithelin in AGS cells and in biopsies of *H. pylori* -infected subjects.
Methods: AGS cells were infected with *H. pylori* wildtype strain ATCC-43504. In addition, endogenous SLPI expression was reduced by siRNA technique. SLPI and Proepithelin expression in AGS cells and human biopsies of *H. pylori*-infected subjects were quantified by ELISA and real-time RT-PCR.
Results: Infection of AGS cells by *H. pylori* ATCC-43504 reduced cellular SLPI levels by 50-60% independent of direct cell-bacteria contact and SLPI-transcription. *H. pylori*-conditioned medium reduced SLPI expression similarly. This effect was abolished by boiling and reduced by freezing conditioned media implying protein/s as ''inducer'' of SLPI-downregulation. Proepithelin levels were affected by the *H. pylori* infection differentially. Cellular levels decreased by 15–30%, while corresponding levels in the supernatant increased up to 250% ($p < 0.05$). siRNA-mediated downregulation of SLPI (-70%, $p < 0.01$) did not affect Proepithelin expression of AGS cells. SLPI-siRNA approach in combination with *H. pylori* infection led to similar results as for the infection alone, a reduction of Proepithelin by 40% and increase up to 380% in lysate and supernatant, respectively. *Ex vivo* analysis of antral biopsies revealed significant upregulation of 50 and 150% of cellular Progranulin levels in *H. pylori*-infected compared to *H. pylori*-negative and -eradicated subjects, respectively ($p < 0.01$). Corresponding Progranulin levels of corpus mucosa and serum from same subjects did not differ with respect to *H. pylori* status.
Conclusions: *H. pylori*-secreted protein/s mediate the reduction of epithelial SLPI expression. Proepithelin levels are changed by *H. pylori*, but the underlying mechanism is independent of SLPI expression in AGS cells and gastric mucosa.

Poster no.: P3.04
Modulation of pellino 1 by *H. pylori* lipopolysaccharide enhances Toll-like receptor 2-mediated nuclear factor-kappa B activation and chemokine induction

S. M. Smith[1], A. P. Moran[2], P. N. Moynagh[3] and D. P. Kelleher[1]

[1]Trinity College Dublin, Dublin, Ireland; [2]National University of Ireland, Galway, Ireland; [3]National University of Ireland, Maynooth, Ireland

Background: Toll-like receptors (TLRs) play a key role in host defence against invading pathogens by functioning as primary sensors for conserved microbial structures, known as pathogen-associated molecular patterns (PAMPs). Recognition of PAMPs by a particular TLR results in the activation of transcription factors such as NF-κB, AP-1 and interferon regulatory factors, culminating in the induction of gene expression necessary for the co-ordination of the innate immune response.
Aim: This study set out to investigate the role of Pellino 1 (PELI1) during the TLR2-mediated response to *H. pylori* lipopolysaccharide (LPS). PELI1 has recently been suggested to interact with components of the TLR signalling pathway (IRAKs 1 & 4, TRAF6), thereby regulating NF-κB activation.
Methods & Results: Quantitative real-time PCR revealed that stimulation of both human embryonic kidney (HEK)-TLR2 cells and MKN45 gastric cells with either intact *H. pylori* or LPS resulted in a significant increase in PELI1 mRNA expression. PELI1 over-expression in HEK-TLR2 cells resulted in a dose-dependent increase in NF-κB activity in response to both the TLR2 ligand Pam_2CSK_4 and *H. pylori* LPS. Additionally, increased PELI1 expression led to transcriptional activation of the IL-8 and CCL20 promoters in *H. pylori* LPS-treated cells. Furthermore, PELI1 knock-down using siRNA inhibited the TLR2-mediated activating properties of Pam_2CSK_4 and *H. pylori* LPS.
Conclusions: PELI1 expression positively regulates TLR2-mediated signalling in response to *H. pylori* LPS, resulting in increased NF-κB activation and pro-inflammatory chemokine induction. Thus, modulation of PELI1 by *H. pylori* and/or its products may be an important mechanism during *H. pylori*-associated pathogenesis.

Poster no.: P3.05
Enhanced expression of CCL20 and CXCL13 in *H. pylori*-associated gastritis in humans

H. Isomoto[1], Y. Nakashima[1], K. Nakao[1] and T. Hirayama[2]

[1]Nagasaki University Hospital, Nagasaki, Japan; [2]Nagasaki University, Nagasaki, Japan

Aim: Accumulating evidence shows elevated expression of a variety of proinflammatory cytokines and chemokines in *H. pylori*-infected mucosa. However, there is little information on lymphoid chemokines such as CCL20 and CXCL13. CCL20 attracts CCR6-expressing cells, while CXCL-13 does CXCR5-expressing ones.
Methods: Using endoscopic biopsies from gastric antrum of 42 subjects infected and 42 uninfected with *H. pylori*, mucosal CCL20 and CXCL13 mRNA and protein levels were measured by real-time polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. Cellular expression of CCL20, CXCL13, CCR6, CXCR5, Fascin, CD20 and αSMA were assessed by immunohistochemistry. Chronic gastritis was evaluated by the updated Sydney system.
Results: The CCL20 and CXCL13 mRNA and protein levels were significantly elevated in *H. pylori*-positive patients compared to those in the uninfected ones. The CCL20 concentrations correlated with the degree of chronic gastritis. Immunohistochemistry revealed that CCL20 was principally expressed by gastric epithelium, while CXCL13 was substantially expressed in Fascin-positive dendritic cells and αSMA-expressing stromal cells. CCR6-expressing cells including CD45RO+ memory T lymphocytes and fascin+-CD1a+ immature dendritic cells infiltrated closely to the CCL20-expressing epithelial cells. On the other hand, CXCR5 was predominantly expressed in CD20-positive B cells.
Conclusion: The CCL20/CCR6 and CXCL13/CXCR5 interaction may be involved in the development of *H. pylori*-associated gastritis.

Poster no.: P3.06
H. pylori infection is associated with similar mucosal cytokine expression pattern in patients from Kenya and Germany

A. Kimang'a[1,2], M. Vivienne[2], G. Revathi[3], S. Sayed[4], S. Devani[5], S. Kariuki[6], D. Kuester[1], K. Mönkemüller[1], P. Malfertheiner[1] and T. Wex[1]

[1]Otto-von-Guericke University Magdeburg, Magdeburg, Germany; [2]Sub-Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya; [3]Department of Pathology, Aga Khan University Hospital,, Nairobi, Kenya; [4]Department of Pathology, Aga Khan University Hospital, Nairobi, Kenya; [5]Department of Internal Medicine, Aga Khan University Hospital, Nairobi, Kenya; [6]Centre for Microbiology Research (CMR)-Kenya Medical Research Institute, Nairobi, Kenya

Aim: To study the immunological responses of geographically unrelated patient groups in relation to *H. pylori* infection.
Methods: Eighty-five and 51 patients with reflux-related or dyspeptic symptoms were included and stratified according to their *H. pylori* status. Degree of gastritis and density of *H. pylori*-colonization were assessed histologically in accordance to the updated Sydney classification. Gene expression levels of cytokines *IL-1β, IL-8, IL18, IL-33, IL-17A, IL17F* and *IL-23* as well as *IL-23R* were analyzed by real-time RT-PCR.
Results: Histopathological evaluation of gastritis pattern revealed similar results in Kenyan and German patients. Within each group, *H. pylori*-infected subjects had significantly higher inflammatory scores for activity and chronicity than *H. pylori*-negative subjects (P values between 0.006 to <0.0001). In both ethnic groups, a significant induction of both *IL-17A* and *IL-17F* was noted in *H. pylori*-infected individuals. Almost all *IL-17F*-positive samples revealed co-expression of *IL-17A* (40/42, 95.2%). Analyzing *IL-17A* and *-F* transcript levels of these 40 ''double-positive'' samples, a significant positive correlation between both genes were identified. *IL-8* mRNA was induced about 6-fold in *H. pylori*-infected patients in Germany ($P < 0.05$), while Kenyans showed a similar tendency. The expression levels of *IL-1β, IL-18, IL-23, IL-33* and *IL-23R* did not differ with respect to the *H. pylori* status in both groups.
Conclusion: The immunological response towards *H. pylori* in infected patients from Kenya is similar to the pattern observed in *H. pylori*-positive patients from Germany.

Poster no.: P3.07
Diagnostic accuracy of serum pepsinogens and gastrin to follow-up *H. pylori* infection

F. Di Mario

University of Parma, Clinical Science, Chair Of Gastroenterology, Parma, Italy

13C-UBT and stool test are accurate non invasive tools to monitor *H. pylori* infection after treatment, but there is interest in whether changes in blood tests would provide an equally reliable but simplex determination

Aims: To assess the use of serum Pepsinogen I(sPGI), serum Pepsinogen II(sPGII),sPGI/sPGII,and serum gastrin 17(sG17) to diagnose *H. pylori* eradication 8 weeks after the end of the treatment.Prospective comparison study designed to fulfil the STARD recommendations. In 228 consecutive *H. pylori* infected patients 13C-UBT and blood samples were performed immediately prior to endoscopy. Patients were offered a 7-day triple therapy, and were asked to return 8 weeks after the end of treatment to obtain blood sample and perform a 13C-UBT.
175 patients completed the study, with an eradication rate of 67%. Percentage change in values before and after treatment of sPGII was the best predictor as a decrease in values of sPGII > 22.7% resulted in a sensitivity of 100% (95% CI: 96.8 to 100) and a specificity of 96.6%(95% CI: 91.5 to 99.1),with a LR+ve of 29.5(95% CI: 8.6 to 107), and LR-ve of 0(95% CI: 0 to 0.03). Spectrum analysis including sex, age, PUD, smoke and alcohol consumption did not found difference in term of accuracy. sPGI, sPGI/sPGII, and sG17 did not show an adequate accuracy to diagnosis eradication.
Percentage variation of sPGII seems to be accurate and reliable to follow-up *H. pylori* infection 8 weeks after the end of treatment. Further studies are needed to confirm the results of this report.

Poster no.: P3.08
The lectin cascade on killing of *H. pylori*

R. O. Mahony[1], C. Basset[2], I. Roitt[3] and J. Holton[4]

[1]NICE, London, United Kingdom; [2]University of Dijon, Dijon, France; [3]University of Middlesex, London, United Kingdom; [4]Windeyer Institute of Medical Sciences, London, United Kingdom

Introduction: *H. pylori* (HP) survives in the stomach despite a good systemic response but also manages to circumvent the innate immune defences by coating itself with host CD59 which is anticomplementary. The role of mannose binding lectin in killing HP has not been investigated. Thus we have studies the effect of serum components including MBL on HP survival using an in-vitro system.
Material & Methods: Six clinical isolates of HP and NCTC strain 11637 were used. Anti-HP antibody positive and negative normal serum, serum heated to 50^0 C or 56^0 C for 30 minutes, serum deficient in C2, C7 and 3 sera deficient in MBL were used. Purified MBL at 100 microg/ml was used. A suspension of HP (1.0×10^7 final concentration) was mixed with sera or sera supplemented with MBL and samples taken ever 15 minutes for 1 hour and dilutions plated on Colombia blood agar with 5% horse blood and incubated for 4 days at 37^0 C in CO_2.
Results: Five percent antibody positive and negative sera killed HP within 15 minutes. Sera heated to 50 or 56 and sera deficient in C2, C7 and MBL did not kill HP. MBL alone had no activity on the viability of HP. C2 and C7 deficient sera with added MBL did not kill HP. The 3 MBL deficient sera with added MBL did kill HP to the same level as the control sera.
Conclusion: HP activates the lectin cascade resulting in killing.

Poster no.: P3.09
Small intestinal bacterial overgrowth, an alternative candidate to *H. pylori*, in explaining delayed gastric emptying in idiopathic parkinsonism

O. Iguodala[1], J. Bowthorpe[1], I. T. Bjarnason[1], A. Charlett[2], A. J. Lawson[2], R. Dobbs[1], S. M. Dobbs[1], D. Taylor[1] and C. Weller[1]

[1]King's College London, London, United Kingdom; [2]Centre for Infections, Health Protection Agency, London, United Kingdom

Background: Delayed gastric-emptying is described in idiopathic parkinsonism (IP), improvement reported following *Helicobacter*-eradication.
Methods: Hydrogen-breath-tests for small-intestinal-bacterial-overgrowth (SIBO) were carried out, following 24-hour deprivation of dairy-products/medicinal lactulose and breakfast of 250 ml clear fluid. Breath-hydrogen was measured before 25G lactulose and after (15 minute-intervals for 4 hours): 326 tests in 76 subjects (54 IP-probands (P); 15 probands' spouses without IP (Ps); 7 with (P/Ps)). SIBO was treated using bulk/osmotic laxatives, and, where clinically-indicated, in *Helicobacter*-negative P+P/Ps, ciprofloxacin and metronidazole.
Results: Meter-manufacturer's diagnostic cut-point (20 ppm increment) was exceeded ≥ once within 120 minutes in 59% (P+P/Ps), 63% Ps. Temporal patterns showed no evidence of a cohort with two distinct peaks. Summary outcomes examined were (i) maximum-value between 15–240 minutes; (ii) time, and (iii) linear increase, to maximum; (iv) 120-minute-value. No differences were found between P+P/Ps and Ps, with age or *Helicobacter*-status (only 8/76 urea-breath-test- and/or stool-antigen-positive). Time-to-maximum (165 (95% CI: 156, 174) minutes) was unaffected by first antimicrobial-treatment (21 patients), decreasing (by 31 (10, 53) minutes) after second (in 8), tending to (29 (-5, 64)) after third (in 3) ($p = 0.7$, 0.004 & 0.1, respectively). Maximum-value was most sensitive to intervention, falling (from 71 (60, 82) ppm) with first antimicrobial-treatment (by 30 (20, 41)), with second (53 (36, 69)), with third (41 (15, 66)) ($p = 0.001$, 0.001 & 0.002). Reduction in maximum-value following first and second antimicrobial-treatments was annulled in presence of a higher neutrophil count ($p = 0.004$ & 0.001), not a higher lymphocyte.
Discussion: Reducing chronic inflammation due to SIBO may facilitate gastro-intestinal transit.

Poster no.: P3.10
The immunohistochemistry profile of lymphocyte immunophenotype in *H. pylori* associated chronic antral gastritis

T. Babic[1], B. Kocic[2], B. Miljković-Selimović[3] and P. Stojanović[4]

[1]Institute for Public Health Nis, Center for Microbiology, National Referents laboratory for Helicob, Nis, Serbia; [2]Institute for Public Health Nis, Center for Microbiology, National Referents laboratory for Helicob; Faculty of Medicine, University of Nis, Nis, Serbia; [3]Institute for Public Health Nis, Center for Microbiology, National Referents laboratory for Helicob; [2]Faculty of Medicine, University of Nis, Nis, Serbia; [4]Institute for Public Health Nis, Center for Microbiology, National Referents laboratory for Helicob2Faculty of Medicine, University of Nis, Nis, Serbia

Introduction: *H. pylori* cause chronic active inflammation of the gastric mucosa which persists over the life-time in association with gastric inflammatory response, implying that local mechanisms are ineffective in clearing the infection.
Aim: The aim of this study was to determine an immunophenotype of the lymphocyte infiltrates in the lamina propria by immunohistochemistry using a commercially available anti-*H. pylori* antibody (Dako, Denmark).
Materials and methods: The frozen sections of gastric antral mucosa showing chronic gastritis (30 cases) were processed immunoenzymatic by alkaline phosphatase anti-alkaline phosphatase (APAAP) method for the identification of *H. pylori*. To determine lymphoid aggregates, antral samples were assessed by immunohistochemical staining for CD3, CD4, CD8, CD20, CD30, CD45.
Results: The immunoenzymatic treated sections were carefully examined for the presence of *H. pylori* and for the immunotype of the lymphocyte cells. With anti - *H. pylori* antibody immunostaining, microorganisms were identified attached to the brush border of the gastric foveolar epithelial cells or within the superficial mucus. The lymphoid follicles were detected within lamina propria. Lymphocytes showed intense staining (red deposits) with CD3, CD4, CD8, CD45 antibodies; only small numbers of lymphocytes were CD20 positive. We found marked association between the density of bacterial colonization and intensity of T- lymphocytes infiltrates during *H. pylori* infection.
Conclusion: The marked increased of CD4+ and CD8+ T cells in the gastric mucosa of patients with *H. pylori* gastritis may provide further insights into the development and maintenance of chronic inflammation and associated diseases induced by *H. pylori*.

Poster no.: P3.11
The role of *H. pylori* infection on IL-1β and IL-8 expression in patients with chronic gastritis, GERD and gastric cancer

J. Santos, T. dos Santos, W. Bartchewsky Jr, M. Alvarez, J. Pedrazzoli Jr and M. L. Ribeiro

Clinical Pharmacology and Gastroenterology Unit, Sao Francisco University Medical School, Bragança P, Braganca Paulista, Brazil

Infection with *H. pylori* strains harboring determinants of pathogenicity may lead to a strong inflammatory response in gastric mucosa which is related with clinical outcome. Thus, the aim of the present study was to evaluate the influence of *H. pylori* as well as the *cagA* and *vacA* genotypes on IL-1β and IL-8 expression and correlated them with the clinical manifestations. Included in the study were 305 patients, of which 53 were uninfected; 49 were endoscopically normal and were *H. pylori*-positive, 82 were infected and had chronic gastritis (CG), 57 had gastroesophageal reflux disease (GERD) and were infected, and 64 had gastric cancer. Bacterial genotypes were evaluated by PCR, and the expression values were determined by quantitative real-time PCR. There was a strong association

between the genotype cagA-positive and gastric cancer. We also found an association between the infection with *cag*A, *vac*A s1m1 strains and CG, GERD and gastric cancer development. The higher levels of IL-8 and IL-1β were detected in gastric mucosa from infected patients with chronic gastritis, and they were also associated with the infection by *cag*A, *vac*A s1m1 strains. The IL-8 and IL-1β levels decrease significantly from chronic gastritis to gastric cancer. On the other hand, the relative expression remains unaltered amongst patients with GERD. Since inflammatory response to *H. pylori* infection plays an important role in cellular proliferation and gastric mucosal damage, the up-regulation of IL-1β and IL-8 in patients with chronic gastritis has an important clinical implication in gastric carcinogenesis.

Poster no.: P3.12
High prevalence of hepatic and lung metastasis of gastric MALT lymphoma in Helicobacter heilmannii-infected mice

M. Nakamura[1], H. Matsui[2], T. Takahashi[1], H. Serizawa[1], S. Takahashi[3] and K. Tsuchimoto[1]

[1]School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan; [2]Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan; [3]3rd Dept of Internal Medicine, Kyorin University, Mitaka, Japan

Background & Aims: We established the low-grade MALT lymphoma model in C57BL/6 mouse infection of Helicobacter heilmannii obtained from cynomolgus monkey (Infect. Immun. 75 (3): 1214–1222, 2007). After long term infection of this bacilli, we found the hepatic and pulmonary infiltration of lymphocytes. Thus, the present study was undertaken to clarify the pathological characteristics of this lesion.
Methods: We used an Helicobacter heilmannii isolated from the stomach of a cynomolgus monkey stomach and maintained in C57BL/6 mouse stomachs. Mucosal homogenates were used to inoculate C57BL/6 mice which were then examined over 24 months. Copy numbers of the bacterial 16S rRNA gene using a real-time quantitative PCR method. Macroscopic observations were carried out, and histochemical studies were performed at intervals over the observation period.
Results: Nine months after the infection, small lymphocyte aggregates mostly composed of B cells were observed in the portal area of the liver and the peripheral area of the lung as well as the gastric MALT lymphoma. These lymphocytes were small and showed lymphoepithelial lesion characteristic to the MALT lymphoma. Twelve and eighteen months after the infection, the size of the lymphocyte aggregates enlarged with the formation of the germinal center. PCR and in situ hybridization analysis showed the existence of Helicobacter heilmannii in the fundic mucosa and not in the lung and the liver.
Conclusion: Long term infection of Helicobacter heilmannii in C57BL/6 mouse induced the hepatic and pulmonary MALT lymphoma as well as fundic mucosa, suggesting the metastasis or homing of the B lymphoma cells.

Poster no.: P3.13
microRNA profiling in duodenal ulcer disease caused by *H. pylori* infection

S. Lario[1,2], M. J. Ramírez-Lázaro[1,2], A. M. Aransay[3], J. Álvarez[3], A. Montserrat[1,2], Á. Casalots[1], E. Brullet[1,2], F. Junquera[1,2], F. Segura[1], R. Campo[1,2] and X. Calvet[1,2]

[1]Digestive, Pathology and Infectious Services. Corporació Sanitària Parc Taulí, Sabadell, Spain; [2]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto de Salud Carlos III., Barcelona, Spain; [3]CIC bioGUNE, Bilbao, Spain

Background: MicroRNAs (miRNAs) are potent regulators of gene expression implicated in important biological processes. Although the connection of miRNA to some diseases is well established, their implication in chronic infections has received much less attention. The aim was to compare miRNA expression profiling in patients with duodenal ulcer disease (DUD) related to *H. pylori* infection to those of infected patients without DUD and uninfected patients.
Patients and Methods: Case-patients (n = 39) were divided in two groups according to endoscopic findings: DUD (n = 19) and normal endoscopy (n = 20). Nine non-infected patients with strictly normal gastric histology were also included. Antral biopsies were collected for histology, culture and molecular analyses. *H. pylori* isolates were tested for *cagA* status and genotyped for *vacA* alleles. miRNA expression profiling was determined with the Illumina DASL assay. TargetScanHuman and DAVID were used to identify targets for the deregulated miRNAs and to ascertain the enrichment of gene ontology terms, respectively.
Results: Normal and infected antrum expressed 441 miRNAs. Among them, 17 miRNAs were upregulated and 3 downregulated in the mucosa of *H. pylori*-infected patients. Individual miRNAs were associated to the grade of gastritis and virulent type I strains, but not to DUD. miRNA target predictions and analyses showed that the most enriched GO terms were for genes involved in the regulation of transcription.
Conclusion: We have identified several deregulated miRNAs in the antrum of *H. pylori*-infected patients. The miRNA profile described deserves further functional characterization and may lead to new approaches in the diagnosis and management of the disease.

Poster no.: P3.14
Markers of enhanced angiogenesis in gastric mucosa in patients with *H. pylori*-associated chronic gastritis

O. I. Kovaliova, S. G. Khomeriki and L. B. Lazebnik

Central Research Institute of Gastroenterology, Moscow, Russian Federation

Introduction: Generation of new blood vessels is stimulated by vascular endothelial growth factor (VEGF), the regulator of angiogenesis. Previously, we showed intensifying of neoangiogenesis in human gastric mucosa in patients with liver cirrhosis

and portal hypertension. But influence of *H. pylori* (HP) on this process is still unclear.
Aim: The aim of study was to investigate changes in gastric microvessels in patients with HP-associated gastritis, and regulatory factors influencing angioarchitecture of gastric mucosa.
Methods: Endoscopic biopsy of human gastric mucosa was performed in 56 patients. 30 of them (53,6%) were *H. pylori*-positive. The specimens were obtained from antrum and corpus. We determined expression and localization of Flt-1 receptor for VEGF in human gastric mucosa by immunohistochemistry, performed identification and localization of endothelial progenitor cells by immunostaining with CD34 antibodies and revealed proliferating endothelial cells by Ki-67 antibodies. Nikon CP 995 camera and digital image analyzing system (DMI-1) was used for evaluation of immunopositive cells.
Results: The number of vessels (per mm^2) in corpus mucosa was significantly higher in HP(+) patients (269 ± 25) ($P < 0,01$). The majority of the vessels were presented by newly formed small sized capillaries. Relative volume of vessels in gastric mucosa was also increased. Receptors for VEGF and Ki-67 positive endotheliocytes were revealed in HP(+) patients, predominantly in superficial regions of lamina propria. 2,1% of capillaries in corpus mucosa and 6,8% in antrum had Flt-1 receptor-positive endotheliocytes. The reaction was not observed in HP(-) patients.
Conclusion: These observations represent evidence for activation of angiogenesis in gastric mucosa in HP(+) patients via enhanced expression of VEGF R1.

Poster no.: P3.15
Alterations in tight junction proteins are associated with the density of bacterial colonization in *H. pylori*-induced gastritis

A. C. Costa[1,2], M. Leite[1], S. Relvas[3], M. L. Pinto[1], F. Carneiro[1,2,3] and C. Figueiredo[1,2]

[1]IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; [2]Medical Faculty of the University of Porto, Porto, Portugal; [3]Dept. Pathology, Hospital de S. João, Porto, Portugal

H. pylori infected individuals develop chronic gastritis, characterized by surface epithelial degeneration and infiltration of the gastric mucosa by acute and chronic inflammatory cells. Since gastric epithelial barrier function is compromised in *H. pylori*-associated gastritis, our aims were to characterize the expression patterns of tight junction proteins occludin and JAM-A in gastric biopsy specimens of *H. pylori* infected and uninfected individuals, and to evaluate if there was a relationship between tight junction proteins and the histopathological parameters of *H. pylori*-induced gastritis.
In epithelial cells, the normal expression pattern of occludin and JAM-A appeared as a continuous belt circumscribing the apical region. Sixteen (66.7%) cases showed tight junction alterations, including decrease of intensity and/or interruption of membrane staining with loss of the belt pattern. The large majority of cases with altered pattern of occludin also exhibited altered JAM-A, and conversely cases with normal expression of one tight junction protein also displayed a normal staining pattern for the other ($p = 0.0001$).
Higher frequency of occludin and JAM-A alterations were observed with increasing densities of *H. pylori* colonization ($p = 0.037$ and $p = 0.027$, respectively). Neither the intensity of lymphocytic infiltrate nor the presence of neutrophilic activity were associated with alterations in the expression patterns of occludin or JAM-A.
These results suggest that tight junction alterations in the context of *H. pylori*-associated gastritis are associated with the density of *H. pylori* infection rather than with the immune host response.

Poster no.: P3.16
Gastric biomarkers in *H. pylori* positive and negative patients

A. Sudraba[1,2], K. Funka[1], I. Tolmanis[3], A. Vanags[3], I. Daugule[1], D. Janciauskas[4], L. Jonaitis[4], L. Kupcinskas[4], A. Ivanauskas[4], J. Lin[5], H. Chiu[5] and M. Leja[1,3,2]

[1]University of Latvia, Riga, Latvia; [2]Riga East Clinical University Hospital, Riga, Latvia; [3]Digestive Diseases Centre GASTRO, Riga, Latvia; [4]Kaunas University of Medicine, Kaunas, Lithuania; [5]National Taiwan University Hospital, Taipei, Taiwan

Introduction: Non-invasive methods for evaluation of gastric mucosal atrophy are proposed. However, presence of *H. pylori* (HP) infection could influence the results.
Aim: To analyze levels of Pepsinogen I (PgI), Pepsinogen II (PgII), Pg I/II ratio, fasting gastrin-17 (G-17) and stimulated gastrin-17 (sG-17) in HP positive and negative patients.
Patients and Methods: In total 269 prospective dyspeptic patients (mean age 65) referred for upper endoscopy were included. Patients with gastric cancer, peptic ulcer, previous gastric surgery or eradication therapy were excluded. PgI, PgII, G-17, sG-17 were determined by ELISA method (Biohit, Plc., Finland). Patients were considered HP positive if two out of three tests (rapid urease test, histology and serology) were positive. The mean values were compared by one-way Anova test.
Results: The mean values (+/−SE) of each biomarker parameter are shown in the table.
Conclusions: Among dyspeptic patients the mean values of PgI and PgII were significantly higher while the mean values of PgI/II, G-17 and sG-17 were significantly lower in HP positive patients. Presence of HP infection could influence the diagnosis of gastric atrophy made by biomarkers.

Table The mean values (+/−SE) of different biomarkers in HP positive and negative dyspeptic patients

	HP+	HP−	P value
PgI	88.1 (3.1)	62.2 (4.8)	<0.001
PgI	16.1 (0.5)	10.6 (0.98)	<0.001
PgI/II	6.0 (0.2)	7.0 (0.42)	0.02
G-17	14.9 (1.45)	33.5 (6.5)	<0.001
sG-17	37.9 (2.9)	71.0 (8.9)	<0.001

Poster no.: P3.17
H. pylori eradication improves serological diagnosis of atrophic gastritis in patients with duodenal ulcer

S. I. Pimanov, E. V. Makarenko and E. V. Ruselik
Vitebsk State Medical University, Vitebsk, Belarus

The aim of this study was to estimate the accuracy of serological diagnosis of atrophic gastritis before and after *H. pylori* eradication in patients with duodenal ulcer.
Patients and methods: A total of 44 patients with *H. pylori*-associated duodenal ulcer were examined. Levels of pepsinogen I and II, gastrin-17 and anti-*H. pylori* antibodies were determined in serum samples by means of Gastropanel (Biohit plc, Helsinki, Finland). The standard serological diagnostic algorithm was used. For histological examination the degree of gastritis was assessed according to the updated Sydney system. All patients underwent serological and morphological examination for diagnosis of gastric atrophy before eradication of *H. pylori* and one year after it.
Results: According to the results of serological diagnosis of antral atrophy before treatment the accuracy was 59. 9%, sensitivity made up 82.9%. After treatment the accuracy constituted 68.2%, sensitivity made up 70,4% ($p > 0,1$). Differences between groups with successful and failed eradication were statistically nonsignificant. In total the accuracy of serological diagnosis of corpus atrophy before eradication was 61.4%. After treatment it slightly increased up to 72.7% ($\chi^2 = 0.82$, $p = 0.364$). In patients with corpus atrophy after successful eradication serological diagnosis accuracy was higher (88.5%) than that in patients with failed treatment (50.0%, $\chi^2 = 6.11$, $p = 0.013$).
Conclusion: Accuracy of serological diagnosis of corpus atrophy in patients with duodenal ulcer after successful eradication is higher than that in a case of unsuccessful treatment.

Poster no.: P3.18
Chronic gastritis associated-*H. pylori* infection. Exist histopathological differences between Wayuu ethnia and Mestizos patients in an urban area of Venezuela? Preliminary results

G. Arismendi-Morillo, I. Hernandez, E. Mengual, A. Fuenmayor, N. Abreu, G. Romero and M. Lizarzabal
University of Zulia, Maracaibo, Venezuela, Bolivarian Republic of

Introduction: Exist clinical and pathological diversity in gastritis associated *H. pylori* between different countries, geographic regions and endemic groups. The aim was to compare the histopathological features of gastritis associated with active infection with Hp (IAHP) among the Wayuu Indians and Mestizos of Maracaibo-Venezuela.
Patients and Methods: Mestizos and Wayuu symptomatic patients underwent esophageal-gastro-duodenoscopy and diagnostic tests to IAHP (urease test, microbiological culture, histopathologic examination with hematoxylin-eosin and immunoblot for strains CagA + and VacA + Hp) selecting 21 mestizos and 23 Wayuu Hp +. Statistical significance of differences was determined by X2 test.
Results: The comparison between mestizos and Wayuu, revealed: a) antrum biopsy, atrophic gastritis, 86% vs. 64% ($p = 0.02$), moderate gastritis, 71.4% vs. 40% ($p = 0.03$), mild gastritis, 24% vs. 55% ($p = 0.03$), b) body biopsy: atrophic gastritis, 93% vs. 39% ($p = 0.03$), mild gastritis, 75% vs. 58.3% ($p = 0.01$), c) anti-CagA/VacA Hp IgG +, 87.5% vs. 53% ($p = 0.02$), IgG + Hp anti-CagA/VacA atrophy in patients with stage 0 - I - II, 64% vs. 88% ($p = 0.01$) and in patients with stage III-IV, 36% vs. 12% ($p = 0.01$).
Conclusion: Chronic gastritis was significantly associated with more severe IAHP in mestizos than in the Wayuu, associated with increased risk of developing gastric neoplastic diseases (stages III - IV). The differences could be linked to genetic, environmental and socio-cultural characteristics of each ethnic group, as well as variability in the virulence of Hp strains circulating among them.

Poster no.: P3.19
Helicobacter species and common gut bacterial DNA in Meckel's diverticulum and appendix

U. Stenram[1], P. Karagin[2], T. Wadström[2] and Å. Ljungh[2]
[1]Department of Pathology, Lund, Sweden; [2]Department of Medical Microbiology, Lund University, Sweden

Background: *Helicobacter*-like bacteria in Meckel's diverticulum has been reported by histological methods. However there is no study by PCR on *Helicobacter* DNA in Meckel's samples. There are also doubts about the presence of *Helicobacter* in appendix.
Objective: To analyse the association between *Helicobacter* genus and some common gut bacteria in Meckel's diverticulum and in appendix.
Material and Methods: A nested-PCR, specific to 16S rRNA of *Helicobacter* genus, was performed on paraffin embedded samples of 33 cases of Meckel's diverticulum with gastric metaplasia, 50 of normal appendix and 50 of acute appendicitis. *Helicobacter* genus positive samples were sequenced for species identification. The samples were also analysed for some common gut bacteria by PCR.
Results: *Helicobacter pullorum* DNA was found in one and *E. coli* in two cases out of 33 samples of Meckel's diverticulum. *H. pylori* DNA was found in three, *Enterobacter* in 18 and *Bacteroides* in 19 out of 100 appendix samples by PCR. All the *H. pylori* positive cases were from normal appendix. *Enterococcus* was not found in any samples of Meckel's or appendix.
Conclusion: *Helicobacter pullorum* DNA in Meckel's diverticulum has been found for the first time in our study. Also for the first time *H. pylori* DNA was found in normal appendix.

Poster no.: P3.20
Correlation between the OLGA gastritis stage and *H. pylori* density by culture

A. Sudraba[1,2], D. Rudzite[1,2], I. Daugule[1], K. Funka[1], I. Tolmanis[3], A. Vanags[3], L. Engstrand[4], D. Janciauskas[5], L. Jonaitis[5], L. Kupcinskas[5], A. Ivanauskas[5] and M. Leja[1,2,3]

[1]University of Latvia, Riga, Latvia; [2]Riga East Clinical University Hospital, Riga, Latvia; [3]Digestive Diseases Centre GASTRO, Riga, Latvia; [4]Swedish Institute for Infectious Diseases Control, Stockholm, Sweden; [5]Kaunas University of Medicine, Kaunas, Lithuania

Introduction: *H. pylori* disappears in patients with progressing gastric atrophy, therefore *H. pylori* density should decrease along with increasing atrophy stage.
Aim: To analyze the correlation between OLGA gastritis stage and *H. pylori* density assessed by culture.
Patients and Methods: In total 133 prospective *H. pylori* positive patients (mean age 65) referred for upper endoscopy due to dyspeptic problems were included. Patients with gastric cancer, peptic ulcer, previous gastric surgery or eradication therapy were excluded. Gastric atrophy was assessed according to OLGA staging system. Biopsies from antrum and corpus were cultured separately; *H. pylori* density was expressed as low (<50 cfu/100 mkl) or high (>50 cfu/100 mkl).
Results: *H. pylori* from antrum was cultured in 119 patients, from corpus - in 128 patients. The proportion of patients with high density of *H. pylori* decreased significantly in relation to OLGA gastritis stage ($p < 0.05$) (table).
Conclusions: Patients with higher OLGA gastritis stages had lower number of colony forming units in *H. pylori* culture, supporting the data about the disappearance of *H. pylori* with increasing atrophy stage.

Table Number of patients with high and low *H. pylori* density by culture in different OLGA gastritis stages

	OLGA gastritis stage					
H. pylori density	0	I	II	III	IV	Total
High (antrum)	58	18	7	0	0	83
Low (antrum)	11	8	7	5	5	36
High (corpus)	57	29	12	4	1	103
Low (corpus)	14	3	4	1	3	25

Poster no.: P3.21
Gamma-glutamyltranspeptidase as promising vaccine candidate to treat *H. pylori* infection

F. Anderl[1], K. Goller[1], R. Semper[1], C. Bolz[1], B. Kalali[1], R. Schmid[1], D. H. Busch[2] and M. Gerhard[1]

[1]Department of Medicine II, Technische Universität München, Munich, Germany; [2]Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany

H. pylori (*H.p.*) is known as the most common bacterial infection worldwide with about 50% of the population chronically infected with this pathogen causing peptic ulcers and gastric cancer. Until now no vaccine has been developed in means of human therapy or prophylaxis although experimental approaches exhibited successful results. In our group we recently described *H.p.* gamma-glutmyltranspeptidase (HPgGT) as virulence factor that efficiently can inhibit T-cell proliferation but not affecting their activation. In two experimental animal models we used HPgGT and HpaA in combination for vaccination studies. We could induce strong T-cell and antibody responses whereas the anti HPgGT humoral response exhibited the potency to inhibit the enzymatic activity of the protein. Furthermore we could pinpoint the responsible peptide stretch of HPgGT showing this effect. Our results in challenge experiments suggest that the induction of this inhibitory antibodies lead to a liberation of the host immune T-cell response against the pathogen hence reducing the bacterial load in the vaccinated animals. We propose that blocking of HPgGT activity leads to the break of bacterial induced tolerance related to T-cell immunity and allow the host to establish an effective immune response against *H. pylori*. Thus HPgGT seems to be an important vaccine candidate for prophylaxis and therapy against *H.p.* infection.

Poster no.: P3.22
Evaluation of different routes and adjuvants for immunization against *H. pylori*

R. Semper[1], F. Anderl[1], K. Goller[1], C. Bolz[1], D. H. Busch[2] and M. Gerhard[1]

[1]Department of Medicine II, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; [2]Institute for Medical Microbiology, Immunology and Hygiene, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

H. pylori infects more than half of the global population, making it to one of the most widespread bacterial infection worldwide. In spite of strong humoral and cellular immune responses, this bacterium can persist in the host for decades and is regarded as the leading cause of peptic ulcer disease and gastric adenocarcinoma. Great efforts have been put to establish a vaccine against this pathogen, but so far none of these approaches was successful in humans. Some years ago we found that the virulence factor γ-glutamyltranspeptidase (HPgGT), a secreted enzyme, inhibits the proliferation of T-cells and thus avoids the generation of an effective cellular immune response. This antigen was used in an experimental mouse model for testing different routes, formulations and adjuvants for their immunogenicity. Vaccination with HPgGT and cholera toxin is able to induce inhibitory antibodies which block the enzymatic activity of the HPgGT and counteract the immune suppression. HPgGT neutralizing antibodies were induced by a systemic or nasal vaccination alone and in the combination of a mucosal and a systemic route, but not by an oral immunization alone. The cellular immune response, which seems to be crucial for protective immunity towards *H. pylori* infection, was stronger after a mucosal or mucosal/systemic immunization using cholera toxin compared to a protocol in which Alum was used for systemic routes. These results suggest that mucosal priming followed by a systemic boost using cholera toxin generates a promising immune reaction for an experimental, prophylactic vaccination against *H. pylori*.

Poster no.: P3.23
H. pylori bacterial ghost containing recombinant Omp18 as a putative vaccine

Y. Talebkhan, M. Bababeik, A. Oghalaie, M. Esmaili, P. Hassanpour, A. Farjaddoost and M. Mohammadi
Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of

Introduction: Rate of *H. pylori* (Hp) infection remains high in developing countries such as Iran. Due to several complications associated with antibiotic treatment, preventive and therapeutic vaccination approaches remain the ideal method of intervention.
Methods: omp18 gene was amplified from type I Hp strain and fused to tac promoter by soeing PCR. The amplified fragment was cloned into pHel2 shuttle expression vector and introduced into E. coli expression system. Hp bacterial ghosts (HPBG) were generated by the expression of PhiX174 lysis gene E and loaded with purified rOmp18 protein. Therapeutic immunization of Hp-infected C57BL/6 female mice was performed using r-Omp18 loaded-HPBG plus cholera toxin. Rapid urease test (RUT), Hp and Omp18-specific serologic assays were used for assessment.
Results: Recombinant Omp18 loaded-HPBG plus cholera toxin stimulated serum anti-Hp and rOmp18-specific antibodies in immunized mice. RUT analysis of resected stomachs also demonstrated a significant reduction in the rate of infection as well as the intensity of bacterial colonization ($P < 0.05$).
Conclusion: This study recommends application of Hp Omp18 as a therapeutic vaccine candidate in reducing the rate of infection as well as the bacterial load and Hp bacterial ghost as a suitable vector for gastric delivery of this and other Hp vaccine candidates.

Poster no.: P3.24
Effects of immunization by recombinant total and C-terminal fragments of UreC against *H. pylori* infection

M. Ameri[1], S. L. Mousavi[1], Z. vaise malekshahi[1] and J. Amani[2]
[1]Department of Biology, Faculty of Basic Science, Shahed University, Tehran, Iran, Islamic Republic of; [2]Applied Biotechnology & Environmental Research Center, Baqiyatallah Medical Science University, Tehran, Iran, Islamic Republic of

H. pylori is a Gram-negative spiral bacterium that colonizes on the human gastric mucosa and causes gastric infection. According to WHO about 50% of the adult population of the world is infected by this bacterium. There are several virulent and antigenic factors of which Urease is the most important factor of the bacterium and hence suitable target for immunization. In this research total and C-Terminal fragment of UreC were expressed in prokaryotic system. . Recombinant proteins were purified by Ni-NTA column and analyzed by SDS-page. Female c57bl6/j mice were injected with recombinant purified fragments (C-Terminal and Total fragment of UreC) on day 0 with complete feronds adjuvant, and on days 14, 28 and 42with incomplete feronds adjuvant.Control mice were received PBS along with adjuvant. Serums obtained from test group showed potent antibody production against UreC compaired to that of control group. Challenge was established by oral gavage of live Iranian *H. pylori* isolates. 10^9 CFU of bacteria was inoculated in immunized and control mice three times within five days. Six weeks after the last inoculation, the blood was collected and the serum antibody titer was estimated by ELISA. The result showed that the immune group prevented infection by *H. pylori* effectively. There was no significant difference in the level of protection in the mice immunized with either recombinant fragments.

Poster no.: P3.25
Studies on the effect of oral administration of UreC specific IgY of *H. pylori* on infection induced c57bl6/j mice

Z. vaise Malekshahi, S. Mousavi and M. Ameri
Department of Biology, Faculty of Basic Science, Shahed University, Tehran, Iran, Islamic Republic of

H. pylori is a gram-negative, motile, spiral and microaerophilic bacteria that colonizes on the gastric mucosa and causes gastric infection and is highly linked to the development of stomach cancer. Due to increasing antibiotic resistance, new approaches have focused on using specific antibodies against this bacterium. Recently egg yolk antibody was recognized to be useful against gastric infections.
In this study we evaluated the effect of the IgY obtained from egg yolk of hens immunized with recombinant UreC of *H. pylori* for treatment of *H. pylori* infection induced mice. Bacteria isolated from Iranian patient was cultured in brucella agar medium and was confirmed with catalase, urease and oxidase tests.10^9 CFU in BHI broth medium(400 μl) was inoculated into stomach of c57bl6/j mice three times within five days. Same amount of BHI broth medium (400 μl) was given to the control mice. Six week after the last inoculation, serum was obtained from the mice and assayed for the rate of infection by ELISA. The severity of gastritis was analyzed by a histopathologist.
Each infected mouse was treated orally with 60 mg of IgY dissolved in 500 μl PBS for 30 days.The result of ELISA showed contamination rate about 60% by *H. pylori*. photomicrograph of Giemsa-stained of gastric tissue sections demonstrating mucosal alterations in response to *H. pylori*. Pathological observation indicated prevention of *H. pylori* colonization after the treatment period. ELISA result showed that there was a significant reduction in the antibody titer of the infected mice orally administered with UreC specific IgY compared to the infected but untreated mice.

Poster no.: P3.26
H. pylori infection is associated with similar mucosal cytokine expression pattern in patients from Kenya and Germany

A. N. Kimang'a[1,2,3], R. Gunturu[3], S. Kariuki[4], S. Shahin[3], S. Devani[3], V. Matiru[1], D. Kuester[5], D. Kuester[6], K. Moenkemueller[6], P. Malfertheiner[7] and T. Wex[7]

[1]Jomo Kenyatta University of Agriculture and Technology, Sub-Department of Medical Microbiology, Nairobi, Kenya; [2]Otto-von-Guericke University, Department of Gastroenterology, Hepatology and Infectious Diseases,, Magdeburg, Germany; [3]The Aga Khan University Hospital, Department of Pathology, Nairobi, Kenya; [4]Kenya Medical Research Institute, Centre for Microbiology Research, Nairobi, Kenya; [5]Otto-von-Guericke University, Nairobi, Germany; [6]Otto-von-Guericke University, MAGDEBURG, Germany; [7]Otto-von-Guericke University, Department of Gastroenterology, Hepatology and Infectious Diseases, MAGDEBURG, Germany

Aim: To study the immunological responses of geographically unrelated patient groups in relation to *H. pylori* infection.
Methods: Eighty-five and 51 patients with reflux-related or dyspeptic symptoms were included and stratified according to their *H. pylori* status. Degree of gastritis and density of colonization were assessed histologically in accordance to the updated Sydney classification. Gene expression levels of cytokines IL-1β, IL-8, IL18, IL-33, IL-17A, IL17F and IL-23 as well as IL-23R were analyzed by real-time RT-PCR.
Results: Histopathological evaluation of gastritis pattern revealed similar results in Kenyan and German patients. Within each group, *H. pylori*-infected subjects had significantly higher inflammatory scores for activity and chronicity than *H. pylori*-negative subjects (P values between 0.006 to <0.0001). In both ethnic groups, a significant induction of both IL-17A and IL-17F was noted in *H. pylori*-infected individuals. Almost all IL-17F-positive samples revealed co-expression of IL-17A (40/42, 95.2%). Analyzing IL-17A and -F transcript levels of these 40 ''double-positive'' samples, a significant positive correlation between both genes were identified. IL-8 mRNA was induced about 6-fold in *H. pylori*-infected patients in Germany ($P < 0.05$), while Kenyans showed a similar tendency. The expression levels of IL-1β, IL-18, IL-23, IL-33 and IL-23R did not differ with respect to the *H. pylori* status in both groups.
Conclusion: The immunological response towards *H. pylori* in infected patients from Kenya is similar to the pattern observed in *H. pylori*-positive patients from Germany.

Poster no.: P3.27
H. pylori as a live vaccine vector: screening and characterisation of clinical isolates

A. Fulurija[1], M. Sehnal[1], H. O. Nilsson[1], W. Lu[1], R. Himbeck[1], M. Middleton[1], L. Engstrand[2], B. J. Marshall[1] and M. Benghezal[1]

[1]H. pylori Research Laboratory, Ondek Pty. Ltd. Microbiology and Immunology, Univ.of Western Australia, Nedlands, Australia; [2]Department of Microbiology, Tumour and Cell Biology, Karolinska Institutet, Stockholm, Sweden

H. pylori infection induces a robust, non-protective immune response despite the presence of circulating antibodies suggesting the bacteria have adapted to the host and can evade host defence mechanisms. The aim of this study was to screen fresh clinical isolates of *H. pylori* to identify those suitable for use as live vectors for vaccine delivery and pre-clinical studies. We assumed that *H. pylori* strains originating from asymptomatic patients would represent the ideal recipient strain for expression and delivery of foreign antigens to the gastric mucosa of the host. Thus, criteria for selection were based on transformability, colonisation in the mouse model and induction of specific immune responses.
Briefly, ~ 100 clinical isolates derived from mostly asymptomatic patients were screened for transformability and for colonisation of the stomach in the mouse model. We identified four *H. pylori* strains that without prior adaptation in the host were robust colonisers of the mouse gastric mucosa and induced specific immune response. Furthermore, two of the identified strains induced high titres of *H. pylori* specific antibodies. Additional *in vivo* studies confirmed only a few strains could achieve colonisation and persist in the host without adaptation. The data will be presented and discussed.
To conclude, suitable strains were selected for the development of the *H. pylori* platform technology (HPPT) for delivery based on their asymptomatic, low grade clinical pathology in humans, genetic manipulation and ability to colonise the host and elicit strong immune responses. These strains are currently part of a Phase I clinical trial for safety and infectivity in humans.

Poster no.: P3.28
The external features of polymorphism in genes IL-1B in patients with atrophic gastritis

E. S. Ageeva[1], O. V. Shtygasheva[1], N. N. Butorin[1] and N. V. Ryazantseva[2]

[1]Khakas State University, Abakan, Russian Federation; [2]Siberian State Medical University, Tomsk, Russian Federation

Aim of investigation: Possible associations between polymorphism gene interleukin-1B (IL-1B) and indications characterizing atrophic chronic gastritis in patients with *H. pylori* were investigated.
Objects of investigation: The screening included 23 patients with *H. pylori* (HP)-associated atrophic chronic gastritis.
Methods: endoscopy, gastric biopsy samples were investigated according to Sydney classification. Genomic DNA was typed for polymorphisms at position -+3953 in the IL-1B gene using RFLP analysis (Taq I). Analysis was performed by PCR and agarose gel electrophoresis.
Results: As the result of genotyping it was obtained that 62% of the patients with HP-associated atrophic chronic gastritis had genotype CC +3953 IL-1B, 24% - genotype CT +3953 IL-1B and 18% genotype TT +3953 IL-1B. The analysis between genotypes and atrophy quantitative characters allowed to reveal correlations between genotype CT +3953 IL-1B and glands density ($r = -0,77$, $p < 0,05$), internal glands diameter ($r = -0,82$, $p < 0,05$), height of the grandular epithelium ($r = -0,76$, $p < 0,05$). Genotype TT +3953 in the IL-1B gene correlated with the pits depth ($r = 0,72$, $p < 0,05$), glands density ($r = -0,77$, $p < 0,05$). Revelation of high and average level (strength) of the correlation dependence between definite genotypes and indexes of morphometry in patients with chronic gastritis give us the basis for wider investigation of the ways of atrophy development in patients with chronic gastritis.

P4 Diagnosis

Poster no.: P4.01
Are there VacA serotypes in *H. pylori*?

S. Wang[1,2], A. Michel[1], W. Zhang[2] and M. Pawlita[1]
[1]Division of Genome Modifications and Carcinogenesis (F020), German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Department of Pathology, Tangdu Hospital, the Fourth Military Medical University, Xi'an, China

Persistent *H. pylori* infection is causally associated with severe gastric disease including gastric cancer. This infection leads to strong antibody responses to many bacterial proteins that can be exploited as diagnostic markers for infection and potentially also for Helicobacter-associated diseases. We have recently shown (Michel et al., 2009, Helicobacter (6):525) strain specific differences in antibodies to the *H. pylori* pathogenicity factor VacA of strains 26695 and G27. To further analyse the extent of and the structural basis for strain specific VacA antibody responses, we recombinantly expressed an N-terminal fragment (aa: 34–331, reference strain 26695), a central fragment (aa: 291–440) containing the repetitive hydrophilic motif (RHM) and the proteolytic p37/p58 cleavage site and a C-terminal fragment (aa: 355–1009) of strains 11638 (uncharacterized Chinese isolate), J99, 26695, and G27 as GST fusion proteins. Serum collections from Asia (n = 773), South America (n = 830) and Europe (n = 1276) were analysed for antibodies to these VacA fragments by multiplex serology. The N-terminal fragment appears to be non-immunogenic, with seroprevalence below 3%. For the C-terminal fragments seroprevalences ranged from 67–73% and responses to proteins of the four strains were highly correlated (R^2: 0,79–0,91). For RHM seroprevalences ranged from 28–42% and differed vastly between strains (R^2: 0,08–0,15; pairwise aa identity: 80–84%) except for the closely related strains 11638 and G27 (R^2: 0,97; aa identity: 94%). In conclusion the variable RHM region is the target of strain specific VacA antibodies and could be useful for definition of VacA serotypes.

Poster no.: P4.02
Comparison of monoclonal antibody-based stool antigen tests to determine the results of *H. pylori* eradication therapy

K. Suzuki[1], T. Shimoyama[1], I. Kobayashi[2], C. Kato[3], M. Kodama[4] and Y. Fukuda[5]
[1]Hirosaki University Graduate School of Medicine, Hirosaki, Japan; [2]Toho University, Tokyo, Japan; [3]Toyama City Medical Association Health Care Center, Toyama, Japan; [4]Oita University School of Medicine, Oita, Japan; [5]Hyogo Collage of Medicine, Nishinomiya, Japan

Backgrounds: Stool antigen tests using monoclonal antibody are used to test the results of eradication therapy of *H. pylori*. A newly developed test using multiple monoclonal antibodies is considered to have higher sensitivity. Although UBT is widely used accurate test, UBT isn't still universally available.

Aims: The aim of this study was to examine whether monoclonal antibody-based stool antigen tests are equally applicable to determine the results of eradication therapy comparing with UBT.

Methods: Stool specimens obtained from 4 patients infected with *H. pylori* were diluted and tested by both Testmate pylori antigen EIA (TPAg EIA) and Premier Platinum HpSA PLUS (HpSA ELISA II). Reduction rate of OD value and the results of the tests were compared after dilution. 239 patients infected with *H. pylori* received eradication therapy and stool samples were tested by both tests and UBT 5–8 weeks after finishing the treatment.

Results: After x5 dilution, OD values of TPAg EIA was significantly reduced ($p < 0.05$) and 3 of 4 stool specimens were tested negative after x10 dilution. In contrast, 3 specimens were tested positive even after x100 dilution by HpSA ELISA II. In the determination of eradication therapy, accordance between the two tests was 95.8%. Accordance of TPAg EIA and HpSA ELISA II to UBT was 91.2% and 95.4%, respectively (NS).

Conclusion: HpSA ELISA II seems to have better sensitivity comparing with TPAg EIA. However, both TPAg EIA and HpSA ELISA II similarly determined the results of eradication therapy comparing with UBT.

Poster no.: P4.03
Comparison of routinely used invasive and non invasive approaches in diagnosis of *H. pylori* infection

Y. Talebkhan[1], M. Esmaili[1], P. Hassanpour[1], A. Morakabati[2], N. Rakhshani[3], M. Mohagheghi[4], M. Eshagh Hosseini[5] and M. Mohammadi[1]
[1]Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of; [2]Qom University of Medical Sciences, Qom, Iran, Islamic Republic of; [3]Gastrointestinal and Liver Diseases Research Center, Iran University of Medical Sciences, Tehran, Iran, Islamic Republic of; [4]Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; [5]Endoscopy Unit, Amiralam Hospital, Tehran, Iran, Islamic Republic of

Aim: To assess reliability of invasive biopsy-based tests including histology, culture and rapid urease test in addition to Hp IgG ELISA as a non invasive approach in detecting *H. pylori* infection in different GI patient populations.

Methods: By using a combination of methods as the gold standard in Hp detection, *H. pylori* infection was diagnosed in 649 dyspeptic patients including 502 NUD (nonulcer dyspeptic disease) and 147 GC (gastric cancer) patients. In reference to the results of defined gold standards, the sensitivity, specificity, positive and negative predictive values as well as accuracy of each test was independently calculated for each assay.

Results: Multiple testing as reported in the literature was set as the gold standard by which: Hp-positive cases are defined as: 1) Culture-positive, 2) RUT and Histology-positive and 3) RUT or

Histology-positive plus serology-positive. Accordingly Hp-negative cases are defined as: 1) Culture/RUT/Histology/Serology-negative, 2) RUT or Histology-negative plus Serology-negative and 3) Culture/RUT/Histology-negative plus Serology-positive. In reference to the mentioned criteria, the diagnostic indices for each individual assay were determined as follows:
Conclusions: This evaluation recommends multiple testing for accurate detection of Hp infection and caution should be practiced in interpretation of individual testing results.

	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Accuracy (%)
Multiple testing	100	100	100	100	100
Culture	74.5	100	100	60	81
RUT	80	99	99.5	65	85
Histology	77	94.4	97	64	82
Serology	95.3	65.3	87	86	87

Poster no.: P4.04
The performance of routine *H. pylori* tests in patients with atrophic gastritis

A. Sudraba[1,2], D. Rudzite[1,2], I. Daugule[2], I. Tolmanis[3], L. Engstrand[4], D. Janciauskas[5], L. Jonaitis[5], L. Kupcinskas[5], A. Ivanauskas[5], J. Lin[6], H. Chiu[6] and M. Leja[2,3,1]

[1]Riga East University hospital, Riga, Latvia; [2]University of Latvia, Riga, Latvia; [3]Digestive Diseases Centre GASTRO, Riga, Latvia; [4]Swedish Institute for Infectious Diseases Control, Stockholm, Sweden; [5]Kaunas University of Medicine, Kaunas, Lithuania; [6]National Taiwan University Hospital, Taipei, Taiwan

Introduction: The data for diagnosing *H. pylori* infection in the presence of gastric atrophy are inconclusive.
AimThe aim of the study was to evaluate accuracy of routinely *H. pylori* diagnostic tests in patients with atrophic gastritis.
Patients and Methods: Five *H. pylori* diagnostic tests were compared in a prospective study in altogether 159 patients aged 55 or above referred for upper endoscopy due to dyspeptic symptoms. Patients with gastric cancer, peptic ulcer as well as those having undergone gastric surgery or having received eradication therapy were excluded. *H. pylori* was diagnosed with five tests: rapid urease test (RUT), ^{13}C urease breath tests (^{13}C UBT), histology, *H. pylori* IgG/IgA antibody test (serology), and by microbiological *H. pylori* (culture) determination.
Results: Atrophy in gastric mucosa was detected in 28 (17. 6%) patients: corpus mucosa was present in 12 (7.5%) patients, antrum - 9 (5.7%), panatrophy - 8 (5%) patients. Incidence of *H. pylori* in the group with atrophy was 50%. Comparison of performance of diagnostic tests for *H. pylori* detection in atrophic group see in ^{13}C UBT and culture did not show any false positive result, RUT test showed the highest false positive (36%) and false negative (43%) results.
Conclusions: The best performing *H. pylori* test was culture. Our results did not support the consideration that serology should be the method of choice to diagnose *H. pylori* infection in gastric mucosa atrophy.

Table Nr.1.

	Sensitivity	Specificity	Overall accuracy
RUT	57%	64%	61%
Histology	86%	93%	89%
13C UBT	86%	100%	93%
Serology	93%	64%	79%
Culture	93%	100%	96%

Poster no.: P4.05
A prospective evaluation of new stool antigen test for detection of *H. pylori*, in comparison with histology, rapid urease test, ^{13}C-urea breath test, and serology

J. Choi, D. Kim and M. Park

Department of Internal Medicine and Healthcare Research Institute, Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Korea, Republic of Page 1

Aims: The aim of this prospective study was to evaluate a new polyclonal enzyme immunoassay (EIA) (EZ-STEP *H. pylori*, Dinona Inc.) for detection of *H. pylori* (*H. pylori*) antigen in stool.
Patients and methods: A total of 515 subjects (288 women, mean age 47. 8 ± 9.6 years) undergoing a routine health check-up were prospectively enrolled in this study. Participants who had been treated previously for *H. pylori* infection were excluded. *H. pylori* status was defined by results of histology, rapid urease test (CLO), ^{13}C urea breath test (UBT), and IgG serology, which was considered positive on at least two of four tests.
Results: *H. pylori* positivity was in 274 subjects (53.2%). The sensitivity, specificity, positive and negative predictive value, and accuracy of stool antigen test were 94.9%, 83.8%, 87.0%, 93.5%, and 89.7%, respectively. The accuracy of stool antigen test was higher than that of serology (89.7% vs. 88.4%, $P < 0.013$), while in contrast it was slightly lower than that of histology, UBT, and CLO test. But, if new cut-off value was used as stool antigen positivity, the specificity increased up to 94.6%, and sensitivity was 93.1%, which was comparable with other methods.
Conclusions: The performance of new polyclonal stool antigen EIA was comparable with other methods in diagnosing *H. pylori* infection in screening population if cut-off value was adjusted. Stool antigen test may be effective for detection of *H. pylori* in mass screening settings or primary care clinics because it is accurate, non-invasive, and it does not require expensive instruments.

Poster no.: P4.06
Gastrointestinal symptoms and gastritis: five year follow-up after *H. pylori* eradication therapy in peptic ulcer patients

H. Maaroos

University of Tartu, Tartu and Estonia

Background: When patients have clinical symptoms after *H. pylori* eradication therapy it is obligatory to support phy-

sicians and patients in the understanding a cause of complaints.
Aim: The current study was aimed at assessment of gastrointestinal symptoms, using the reliable Gastrointestinal Symptom Rating Scale (GSRS), and at correlating its results with different stages of gastritis and *H. pylori* colonization after eradication therapy.
Patients and Methods: Five years after treatment, 91 patients (68% of the initial treatment group of patients with peptic ulcer) completed the GSRS. Symptoms were grouped under three syndromes: dyspepsia (stomach pain, heartburn and reflux), bowel dysfunction (diarrhoea, loose stool, constipation and hard stool) and indigestion (bloating, regurgitation, and flatulents). Of them, 53 patients were endoscopied and specimens from the antrum and corpus mucosa were evaluated by the Sydney classification.
Statistics: Regression analysis was made for finding the association of symptoms with mucosal changes.
Results: Although almost all patients reported by GSRS some symptom, the relationship of symptoms with stage of gastritis and *H. pylori* colonization was commonly rare. Dyspepsia score was higher (mean score 3.36, $p = 0.035$) for patients with severe *H. pylori* colonization in the antral mucosa than for patients without *H. pylori* colonization (mean score 2.21). Bowel dysfunction was associated with activity of gastritis in the corpus mucosa ($p = 0.093$). Mean score of gastrointestinal symptoms was lower for patients who refused to be endoscopied.
Conclusion: Patients with peptic ulcer reported some gastrointestinal symptoms after *H. pylori* eradication therapy but it was rarely related to gastritis and *H. pylori* colonization.

Poster no.: P4.07
Accuracy of Ultra-Rapid Urease Test for the diagnosis of *H. pylori* infection

A. G. McNicholl[1,2], J. Ducons[3], J. Barrio[4], L. Bujanda[5,2], M. Forné-Bardera[6], R. Aparcero[7], J. Ponce[8,2], R. Rivera[9], J. M. Dedeu-Cuso[10], P. Garcia-Iglesias[11,2], M. Montoro[12], A. Bejerano[1], Y. Ber-Nieto[3], B. Madrigal[4], E. Zapata[5], C. Loras-Alastruey[6], M. Castro[7], A. Nevarez[8], I. Mendez[9], F. Bory-Ros[10], M. Miquel-Planas[11], J. Vera[12], O. P. Nyssen[1,2] and J. P. Gisbert[1,2]

[1]Hospital Universitario de la Princesa, Madrid, Spain; [2]CIBERehd, Barcelona, Spain; [3]Hospital Lozano Blesa, Zaragoza, Spain; [4]Hospital Rio Hortega, Valladolid, Spain; [5]Hospital de Donostia, San Sebastian, Spain; [6]Mutua de Tarrassa, Tarrassa, Spain; [7]Hospital de Valme, Sevilla, Spain; [8]Hospital de la Fe, Valencia, Spain; [9]Hospital Costa del Sol, Malaga, Spain; [10]Hospital del Mar, Barcelona, Spain; [11]Hospital de Sabadell, Barcelona, Spain; [12]Hospital de San Jorge, Huesca, Spain

Background: Rapid Urease Test (RUT) is a simple, cheap and relatively fast method to diagnose *H. pylori* infection. Therefore, it is the method of choice used in patients undergoing gastroscopy. Most kits require 24 h to give results. The new Ultra-Rapid Urease Test (URUT) kit by Biohit requires less than one hour.
Aim: To determine URUT's diagnostic accuracy.
Methods: Prospective, blind, multicenter study including dyspeptic patients. Three antrum and 1 corpus biopsies were obtained during endoscopy for standard histological analysis, RUT and URUT. URUT result was checked after 1′, 5′, 30′ and 60′ while RUT was checked out over 24 hours. Histology was used as gold standard.
Results: 144 patients were included, 68% female, mean age 49 years, 42% taking proton pump inhibitors (PPIs), 50% *H. pylori* positive. RUT and URUT diagnosis were correct in 85.9% and 90.0% of the cases respectively. Mean waiting time for positive RUT result was 6.2 h. Sensitivity, specificity, Positive and negative predictive values for RUT were, respectively, 82%, 90%, 89% and 84%. URUT's results were equivalent (85%, 94%, 94% and 87%). These figures were higher when no PPI treatment was being taken (RUT: 86%, 91%, 93% and 83%; URUT: 91%, 94%, 96% and 89%).
RUT and URUT distributions were not statistically different (McNemar Test, $p = 0.3$) but there was a tendency towards better URUT results.
Conclusion: Ultra-Rapid Urease Test is equivalent to (or even slightly better) traditional rapid urease tests in the diagnosis of *H. pylori* infection, and provides results in less than an hour.

Poster no.: P4.08
Accuracy of a triple molecular genetic test for *H. pylori* (HP) infection and its resistance to fluoroquinolones and clarithromycin: Preliminary results

N. Mathou[1], S. Likousi[2], K. D. Paraskeva[1], E. Andrikopoulos[1], K. Zografos[1], E. Platsouka[2] and J. A. Karagiannis[1]

[1]Gastroenterology Unit, ''Konstantopoulio'' Hospital, Athens, Greece; [2]Microbiology Department, ''Konstantopoulio'' Hospital, Athens, Greece

Background: Diagnosis and treatment of HP infection consists a very frequent and important task in routine clinical practice.
Aim: To assess the applicability, efficacy and accuracy of a triple molecular genetic test for identification of HP infection and its resistance to fluoroquinolones and/or clarithromycin.
Patients and Methods: Thirty-six consecutive patients undergone upper GI endoscopy due to various upper GI tract symptoms. HP infection was assessed by a RUT test (CLO), histology of gastric biopsies (2 antral and 2 corpus specimens) and the GenoType Helico DR [based on the DNA strip technology for the identification of HP and its resistance to fluoroquinolones and/or clarithromycin through the detection of the most significant mutations of the gyrA gene (codons 87 and 91) for fluoroquinolones and the examination of 23S gene (positions 2146 and 2147) for clarithromycin]. The biopsy samples for the GenoType Helico DR were analyzed into three steps which included DNA extraction, amplification and hybridization.
Results: Endoscopic biopsies were processed with the three HP detection methods. There was concordance of the three detection methods in 28/36 (78%) patients. In 4 patients histology was negative for HP, while both CLO test and GenoType Helico DR were positive and in another 4 patients CLO test was positive while histology and GenoType Helico DR were negative. Resistance to clarithromycin was detected in 4/24 (16,5%) and to fluoroquinolones in 4/24 (16,5%) of HP+ patients.

Conclusions: The triple molecular genetic test looks promising for both the detection of HP infection and its resistance to fluoroquinolones and/or clarithromycin.

Poster no.: P4.09
Evaluation of urine antibody test for *H. pylori* in Japanese children

M. Okuda[1], H. Oomatsu[2], J. Kotake[1], N. Fukuda[1], N. Yamamoto[1], N. Sakaedani[1], H. Yamada[1], J. Ono[1] and Y. Fukuda[1]

[1]Department of General Medicine and Community Health Science, Hyogo College of Medicine, Sasayama, Japan; [2]Department of Pediatrics, Wakayama Rosai Hospital, Wakayama, Japan

Introduction: Detection of *H. pylori* antibodies have been developed that performed well in adults with the sensitivity and specificity values higher than 90%. However, its usefulness for children remains controversial. To evaluate the accuracy of a urine-based ELISA (Urine-HpELISA) and Immunochromatography (Rapid Urine-HpAb) kit for anti-*H. pylori* IgG antibody in children, we compared its sensitivity and specificity in reference to 13C-urea-breath test (UBT) and *H. pylori* stool antigen test (HpSA).
Study design: One hundred and one Japanese children without significant upper-abdominal symptoms were included (mean age, 7. 1 years; range 2 to 15). UBT, HpSA, Urine-HpELISA and Rapid Urine-HpAb were performed.
Results: Of 101 children, 37 and 64 were judged *H. pylori*-positive and -negative, respectively, by UBT and HpSA. Equivocal results of HpSA were not obtained, and no discrepancy was observed between UBT and HpSA. Urine-HpELISA showed 91.9% sensitivity (34/37) and 96.9% specificity (62/64) with the accuracy of 95.0%. Rapid Urine-HpAb showed 78.4% sensitivity (29/37) and 100% specificity (64/64) with the accuracy of 92.1%. Antibody titers of Urine-HpELISA with pseudo-negative results of Rapid Urine-HpAb are lower than true-positives.
Discussion: Reports from France and Israel revealed that Urine-HpELISA is not useful for the diagnosis in children. But reports about Japanese children, they show good sensitivity. This suggests the difference in the performance could be attributed to the strain variation.
Conclusion: The urine antibody tests are non-invasive, inexpensive, reliable and easy-to-perform method for the diagnosis of *H. pylori* infection in Japanese children.

Poster no.: P4.10
Associations between the optical density values of *H. pylori* stool antigen and gastritis

M. Kobayakawa[1], N. Uemura[1], S. Nakajima[2], H. Kijima[3] and T. Shimbo[1]

[1]National Center for Global Health and Medicine, Tokyo, Japan; [2]Social Insurance Shiga Hospital, Ohtsu, Japan; [3]Hirosaki University Graduates School of Medicine, Hirosaki, Japan

Backgrounds and Aim: *H. pylori* (*H. pylori*) is classified as definite cartinogen of gastric cancer. Uemura et al indicated that corpus predominant gastritis and pangastritis was gastric cancer risk. Although Optical density (OD) values of *H. pylori* stool antigen (HpSA) are quantitative, associations between HpSA OD values and gastritis were not enough investigated. We conducted this study to investigate whether HpSA OD values could predict corpus predominant gastritis or pangastritis instead of invasive gastric biopsies.
Methods: A total of 112 consecutive subjects with positive HpSA were recruited from 5 endooscopy units in Japan. Gastric biopsies were taken from antrum and corpus. The histological scores (0–3) by the updated Sydney system (activity and chronic inflammation) were examined. Distribution of gastritis was classified to 3 groups (corpus predominant gastritis, pangastritis, and antrum predominant gastritis) according to the activity scores.
Results: HpSA OD values were significantly higher among the subjects with high histological activity scores in corpus ($p < 0.05$). But there were no associations between HpSA OD values and activity scores in antrum and between HpSA OD values and chronic inflammation scores both in antrum and corpus. HpSA OD values among subjects with corpus predominant gastritis and among subjects with pangastritis were significantly higher than among subjects with antrum predominant gatsritis (median OD: 1.89, 1.84, and 1.05, respectively; $p < 0.05$).
Conclusions: High HpSA OD values might be possible to predict pangastritis and corpus predominant gastritis.

Poster no.: P4.11
Indicator Medium for Culture and Identification of *H. pylori* Colonies Isolated from Gastric Biopsy Specimens

D. Davoudi Oskouei[1], Y. Doğan[1], G. Bengi[2], Ö. Sağol[3], M. Soytürk[2], H. Akpınar[2], I. Şimşek[2] and Ö. Yılmaz[1]

[1]Dokuz Eylül University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey; [2]Dokuz Eylül University, Faculty of Medicine, Department of Gastroenterology, İzmir, Turkey; [3]Dokuz Eylül University, Faculty of Medicine, Department of Pathology, İzmir, Turkey

Background: A culture medium having ability to facilitate presumptive identification of *H. pylori (H. pylori*) from other contaminant bacteria especially when the small number of *H. pylori* present in biopsy specimens is preferable.
Aim: To develope an indicator medium for culture and identification of *H. pylori* easily and confidently.
Methods: A novel tetrazolium salt supplemented culture medium was introduced by using Columbia blood agar (Oxoid) with 1% soluble hemoglobin powder (Oxoid), *H. pylori* selective supplement (Dent, Oxoid), 0.3% active charcoal (Galenik) and 0.004% 2,3,5-triphenyl tetrazolium chloride (TTC) (Applichem). Fourty-nine patients with gastroduodenal complaints were included. Rapid Urease Test (RUT), histopathology, real-time PCR and culture were used for each gastric biopsies of antrum and corpus. Biopsies were processed and inoculated onto indicator culture medium and were incubated at 37°C for 3–7 days under microaerophilic condition and 90% humidity. Culture medium without TTC was also used.NCTC11637 *H. pylori* standard strain was used as a control.

Results: *H. pylori* was isolated from twenty-four patients.RUT, histopathology and real-time PCR all together correlated with culture results.All the isolated *H. pylori* colonies showed golden appearance in the dark field of the TTC containing medium with minor differences in color tone between strains.The other contaminant bacteria showed pink or red color. In the medium without TTC, *H. pylori* colonies were translucent and colorless.The golden color of *H. pylori* colonies was intensified by raising the pH of medium to7.6, but adding 0.0025 mg/L $CuSo_4$ did not alter color intensity.
Conclusion: Detecting redox potential of live colonies of *H. pylori* by reductin of TTC to formazan, a compound responsible for unique golden color of colonies could be considered a reliable test for identification of *H. pylori.*

Poster no.: P4.12
Determining cut-off values for serodiagnosis of *Helicobacter hepaticus* infection by HRII-51-HH-15 capture enzyme-linked immunosorbent assay

R. Takahashi[1], T. Shimoyama*[2], D. Abe[1], N. Nakabayashi[1], M. Okuda[3] and Y. Fukuda[3]
[1]Sagami Reserch Lab. Wakamoto Pharmaceutical Co. Ltd., 378 Ohi-machi, Ashigarakami-gun, Kanagawa, Japan; [2]Department of Gastroenterology, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki, Japan; [3]Sasayama Medical Center, Hyogo College of Medicine, 5 Kurokawa, Sasayama, Hyougo, Japan

Background & Aim: In humans, conclusive evidence of *H. hepaticus* infection has not been elucidated, so that a negative control, which is used for a cut-off to define *H. hepaticus* seropositivity, has not been guaranteed. We prepared the negative control from serum of absorption with *H. hepaticus* whole cell lysate (HhWCL). *H. hepaticus, Helicobacter bilis* and *H. pylori* were intraperitoneally inoculated to mice, respectively. Sera from *H. hepaticus*-inoculated mice (n = 23) were absorbed with HhWCL and measured *H. hepaticus* antibody level by the HRII-51 antigen capture ELISA (Helicobacter. 2009; 14: 66–71).
Result: The ELISA value was 0. 260 ± 0.133 (mean ± SD) and the cut-off value was set at 0.526 of the mean ± 2SD. By using this cut-off, the ELISA positivity was 73.9% in sera from *H. hepaticus*-inoculated mice and no cross-reactivity was detected in sera from *H. bilis*-inoculated mice, *H. pylori*-inoculated mice and *Helicobacter* free mice. Serum from healthy donors (n = 34) was absorbed with the same manner of HhWCL, and the cut-off value was set at 0.223 (the mean ± 3SD). The ELISA positivity was 41.2% (14/34) and 2.9% (1/34, $p < 0.0001$) in pre- and post-absorption, respectively.
Conclusion: To establish cut-off value in the HRII-51- antigen capture ELISA, a negative control serum was prepared by absorption with *H. hepaticus* whole cell lysate. A cut-off value was set at the mean value plus 2 or 3 SD of the negative control population. The cut-off method would be useful for determining the seroprevalence of *H. hepaticus* infection.

Poster no.: P4.13
Is Antibiotic Supplemented Culture Medium Inhibits Recovery of *H. pylori* from Gastric Biopsy Specimens?

Y. Doğan[1], D. Davoudi Oskouei[1], Ö. Binicier[2], E. Demiray Gürbüz[1], M. Soytürk[2], H. Ellidokuz[3], H. Akpınar[2], I. Şimşek[2] and Ö. Yılmaz[1]
[1]Dokuz Eylül University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey; [2]Dokuz Eylül University, Faculty of Medicine, Department of Gastroenterology, İzmir, Turkey; [3]Dokuz Eylül University, Faculty of Medicine, Instıtute of Oncology, İzmir, Turkey

Background: Successful isolation of *H. pylori (H. pylori)* from gastric biopsy specimens needs some precautions in selecting or proper use of culture media. Culture of *H. pylori* is time-consuming and an expensive procedure. For this reason loosing even one strain of bacterium will be important.
Aim: To evaluate the use of antibiotic supplemented culture medium as well as medium without any antibiotics for culture and isolation of *H. pylori* from gastric biopsies.
Methods: Gastric biopsies of both antrum and corpus of 44 patients were studied for Rapid Urease test(RUT), PCR, histopathology as well as culture. For culture, both antrum and corpus biopsies were separately processed and equally inoculated onto Columbia blood agar (Oxoid) containing 1%soluble hemoglobin powder (Applichem), 0.3%active charcoal (Galenik) and *H. pylori* selective supplement(Dent,Oxoid) and without antibiotic supplement. The plates were incubated 3–7 days at 37°C under microaerophilic condition.
Results: Thirthy patients (68. 2%) were *H. pylori* positive by gold standard methods. Among 20 culture positive patients, *H. pylori* was isolated from 20 antrum (66.6%) and 12 corpus (40.0%)biopsies. The recovery rates for antrum and corpus were 75%, 66.6% in antibiotic supplemented and 90%, 100%, for non-supplemented media, respectively.In spite of high recovery rates for non-antibiotic supplemented medium, the contamination rate was also high.
Conclusion: The use of non-antibiotic supplemented medium could improve the isolation rate from 52. 2% to 68.2%.The reason for lower isolation rates in antibiotic supplemented medium migh indicate the susceptibility of *H. pylori* strains to antibiotics normally used as supplement. For more efficient isolation, the simultaneous use of antibiotic supplemented and non-supplemented culture medium is recommended.

Poster no.: P4.14
Utility of serum pepsinogens to identify chronic atrophic gastritis

F. Di Mario
University of Parma, Clinical Science, Chair Of Gastroenterology, Parma, Italy

Gastropanel® is a serological tool for assessing pepsinogens 1,2 (PG 1,PG 2), gastrin-17 (G-17)and antibodies anti-*H. pylori*, and it can identify chronic atrophic gastritis(CAG) ''serologically''.

Aims: to assess PG1,PG2 and G17 in a group of patients(group A)with a histological diagnosis of CAG; 2)to assess histological characteristics in a second group of patients (group B) with serological diagnosis of CAG. Patients underwent endoscopy with biopsies (Sydeny System). Blood samples were taken before EGDS, patients having fasted for a minimum of 6 hours.
In group A (16women/4men, mean age 65 years) according to histology there were 11 patients with CAG in gastric corpus, 4 with CAG in antrum, 4 with pan-atrophy, and 1 with normal histology; 7 were on PPI treatment. According to Gastropanel, there was an agreement of 91% for patients with CAG in gastric corpus, whilst there wasn't agreement for patients with atrophy in antrum and for those with pan-atrophy. In group B (18 women/12 men, mean age 66 years) patients had PG1 < normal limit. According to the histology, there were 22 patients (73%) with CAG of corpus, and 4 of these (13%) presented atrophy in the antrum. 8 patients (27%) didn't have atrophy. 4 out 30 patients were on PPI treatment. There was a significant association between low PG1 values, PGI/PGII values and CAG in corpus ($p < 0.05$).
Serum pepsinogens seem to be an accurate non invasive tool to identify patients with CAG and might be used to identify and follow-up patients with CAG to improve their management.

Poster no.: P4.15
Application of stool-PCR test for the diagnosis of *H. pylori* in Nigeria

S. I. Smith[1], M. A. Fowora[1], O. A. Lesi[2], E. Agbebaku[3], F. B. Abdulkareem[2], M. Contreras[4] and R. Haas[5]

[1]Nigerian Institute of Medical Research, Lagos, Nigeria; [2]College of Medicine, University of Lagos, Lagos, Nigeria; [3]University of Lagos, Lagos, Nigeria; [4]IVIC, Caracas, Venezuela, Bolivarian Republic of; [5]Max von Pettenkofer Institut, Munich, Germany

Aim: To evaluate the usefulness of stool-PCR test for the diagnosis of *H. pylori* in Nigeria.
Methods: Stool samples from 73 patients presenting with various gastro-duodenal disorders in LUTH were collected and screened for Helicobacter spp, using the stool-PCR and compared with the results from the urea breath test (UBT).
DNA from the stool samples were purified using the QIAamp® DNA Stool Mini kit, and detected using a PCR assay targeting a 399bp fragment of the 16S rRNA gene with two specific primers, HeliF and HeliR. PCR was performed using the Ready To-Go PCR beads kit. The positive control was *H. pylori* DNA and the negative control was a no-template PCR reaction.
The patients were screened for *H. pylori* positivity using UBT from Heliprobe.
Results: Out of 73 stool samples analysed, 33 (45.2%) were positive for Helicobacter spp using the 16s rRNA gene. Of the 33 positive for the 16s rRNA Helicobacter spp gene, 6 (18.8%) were positive for the cagA gene, while only 2 (33.3%) of the 6 were positive for glmM gene. The UBT results show that 42 (57.5%) were positive for *H. pylori*. Those simultaneously positive for stool-PCR using the 16s rRNA gene and UBT were 23 (54.8%) while 23.8% were negative for UBT and positive for stool-PCR.
Conclusion: *H. pylori* detection from stool using PCR is promising in our environment, most especially where the patients cannot afford to pay for high cost of endoscope. This is the first report done in Nigeria.
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Poster no.: P4.16
Application of immunoassay for detection of *H. pylori* antigens in saliva

K. Leszczyńska[1], D. B. Namiot[1], A. Namiot[1], M. Chilewicz[2], J. K. Leszczyńska[1], R. Bucki[3], A. Kemona[1] and Z. Namiot[1,2]

[1]Medical University of Białystok, Białystok, Poland; [2]District Hospital, Białystok, Poland; [3]University of Pennsylvania, Philadelphia, PA, United States

Background: The methods usually used to diagnose *H. pylori* in the stomach are not good enough for this aim in the oral cavity. Quite recently, an immunoassay for detection of bacterial antigens has been used to diagnose *H. pylori* infection in the dental plaque. The aim of the present study was to determine the utility of this method to detect *H. pylori* antigens in saliva.
Material and methods: The study was conducted on 124 patients; 76 with *H. pylori* infected and 48 with non-infected stomachs. Each subject collected 5 ml of unstimulated saliva before daily oral hygiene procedures and gastroscopic examination. The detection of *H. pylori* antigens in saliva was performed with the method used for *H. pylori* antigens detection in faces.
Results: It was found that bacterial concentration in saliva necessary to obtain a positive result in immunoassay was 100 cells/ml, for 400 μl of saliva sediment used. Pre-incubation of saliva in microaerophilic conditions for 72 hours with and without antibiotics did not increase the number of positive results in the test. The storage of saliva at -20^{o} C for 30 days decreased the optical density by 7% in subject with positive results of the test, but did not change the result from positive to negative in any subject. Using 400 μl of saliva sediment without sample pre-incubation in microaerophilic conditions, *H. pylori* antigens were detected in 42.1% of subjects with infected stomach while in none of non-infected one.
Conclusion: The immunoassay for detection of *H. pylori* infection in saliva is not good enough as a single test to diagnose stomach infection, but it may be useful as a supplementation of other more accurate tests, specifically those evaluating *H. pylori* infection in the oral cavity.

Poster no.: P4.17
Comparative evaluation of efficiency of rapid urease test ''HELPYL-test'' for diagnostics of H. pylori infection in gastric mucous and gastric metaplasia mucous of duodenum

M. R. Konorev, M. E. Matveenko, U. V. Krylov and T. V. Kovalenko

Medical University, Vitebsk, Belarus

Objectives: To determine the efficiency of ''HELPYL-test'' for diagnostics of *H. pylori* (Hp) in gastric and duodenal mucous at patients with dyspepsia syndrome.

Materials and Methods: Blind, diagnostic cross-section research (n = 107) with application of Latin square (table 2 x 2) for comparison of ''HELPYL-test'' (AMA, Russia) and ''Jatrox-H. p.-test'' (Rohm Pharma, Germany) is carried out. Examination of *H. pylori* infection in gastric and duodenal mucous by Giemsa used as comparison method. Regions of gastric metaplasia of duodenum were confirmed by periodic acid-Schiff and alcian blue staining (pH 1.0; 2.5). The estimation of efficiency was carried out at the same patients.
Results: Of estimation of efficiency rapid urease test ''HELPYL-test'' (gastric and duodenal mucous respectively): sensitivity (Se) - 0. 97; 0.91; specificity (Sp) - 0.98; 0.98; prevalence (P) - 0.54; 0.20; test accuracy (TA) - 0.97; 0.96; negative predictive value (-PV) - 0.96; 0.98; positive predictive value (+PV) - 0.98; 0.91; positive likelihood ratio (LR+) - 48.5; 39.3; negative likelihood ratio (LR-) - 0.04; 0.1. Results of estimation of efficiency rapid urease test ''Jatrox-H.p.-test'' (gastric and duodenal mucous respectively): Se - 0.98; 0.95; Sp - 0.98; 0.99; P - 0.54; 0.20; TA - 0.98; 0.98; -PV - 0.98; 0.99; +PV - 0.98; 0.95; LR+ - 49.0; 79.3; LR- - 0.02; 0.05.
Conclusion: ''HELPYL-test'' and ''Jatrox-H. p.-test'' have high clinical efficiency for diagnostics *H. pylori* in gastric and duodenal mucous. ''HELPYL-test'' in 480 times reduces time of diagnostics of *H. pylori* in comparison with ''Jatrox-H.p.-test'' and allows to use in further biopsy of gastric and duodenal mucous for morphological research.

Poster no.: P4.18
A Novel Convenient Culture Medium for Isolation of *H. pylori* from Gastric Biposy Specimens

D. Davoudi Oskuoei[1], Y. Doğan[1], N. Bekmen[1], M. Yalçın[2], Ö. Sağol[3], H. Ellidokuz[4], M. Soytürk[2] and I. Şimşek[2], Ö. Yılmaz[1]

[1]Dokuz Eylül University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey; [2]Dokuz Eylül University, Faculty of Medicine, Department of Gastroenterology, İzmir, Turkey; [3]Dokuz Eylül University, Faculty of Medicine, Department of Pathology, İzmir, Turkey; [4]Dokuz Eylül University, Faculty of Medicine, Institute of Oncology, İzmir, Turkey

Background: *H. pylori* is a Gram-negative spiral-shaped bacterium which has been associated with various gastroduodenal diseases. Due to the fastidious nature of the organism, the culture of *H. pylori* needs blood, serum or growth supplements. The successful isolation of *H. pylori* from biopsies strictly depends on the type of medium.
Aim: To develope a convenient culture medium which can provide the nutritional requirements for growth of *H. pylori* and optimal recovery of the organism from biopsies.
Methods: Fourty-nine patients, 42 with gastritis, six with gastric and duodenal ulcer and one with gastric cancer, were included. Rapid Urease Test(RUT), histopathology, real-time PCR and culture were used for each of both antrum and corpus gastric biopsies. A culture medium prepared by using Columbia blood agar (Oxoid) with 0.3% active charcoal (Galenik), *H. pylori* selective supplement (Dent, Oxoid) and 1% soluble hemoglobin powder (Oxoid). Antrum and corpus biopsies were processed and inoculated onto culture media. The plates were incubated 3–7 days at 37°C under microaerophilic condition and 90% humidity. *H. pylori* NCTC11637 was used as positive control.
Results: Thirty-five (71. 4%) patients were *H. pylori* positive by RUT, histopathology and real-time PCR all together. *H. pylori* was isolated from 24 (68.6%) patients. Results obtained from culture showed 81.4%, 64.7%, 77.7% consistency with RUT, histopathology, and real-time PCR, respectively.
Conclusion: The Columbia blood agar supplemented with 1% soluble hemoglobin and 0. 3% active charcoal was found suitable for growth and isolation of *H. pylori* from gastric biopsies providing better bacterial morphology than that of fresh human serum containing culture medium which we previously described.

Poster no.: P4.19
Identification of potential protein markers to detect *H. pylori* infection in different gastric pathologies in Malaysia

A. Santhanam[1,2], L. Chun Wei[1], A. Khalilpour[1], A. Mohammad[1], R. Noordin[1], S. Osman[1] and N. Velusamy[3]

[1]Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Pualu Pinang, Malaysia; [2]Madurai Kamaraj University, Madurai, India; [3]Hospital Seberang Jaya, Penang, Malaysia

H. pylori is one of the gastro intestinal pathogens which infects more than 50% of the adult population world wide. The diagnosis of this infection based on endoscopy is an invasive and expensive method. Attempts are thus being made in developing accurate non-invasive detection method that may replace endoscopy. There is a wide spectrum of clinical outcomes by *H. pylori,* ranging from ulceration to gastric cancer. The pathogenicity of the infection depends on the strain virulence, host susceptibility and environmental co-factors. Hence, there is an urgent need to identify potential infection markers from local *H. pylori* strain to facilitate development of specific and sensitive diagnostic test for Malaysian patients, which may also be applicable to patients from neighboring countries. *H. pylori* used in this study was isolated from biopsy sample of a peptic ulcer patient in Malaysia. Excretory-secretory protein of the bacteria was separated by SDS-PAGE and immunoblotted with serum samples from different gastric pathologies (19 chronic gastritis, 2 duodenal ulcer, 5 peptic ulcer). The results of this study showed that the most promising diagnostic candidates were three bands with approximate molecular weights of 60 KDa (sensitivity 86.6%), 25 KDa (sensitivity 86.6%) and 13 KDa (sensitivity 76.6%). The specificities of the three proteins were found to be >85%. Thus these three antigenic proteins are potential *H. pylori* infection markers that warrant further investigation.

Poster no.: P4.20
Influence of Ecabet sodium to Urea Breath Test in volunteers with *H. pylori* infection

M. Okuda, N. Sakaedani, N. Yamamoto, N. Fukuda, J. Kotake, M. Suda, Y. Hayashi, R. Takahashi and Y. Fukuda

Department of General Medicine and Community Health Science, Hyogo College of Medicine, Sasayama, Japan

Introduction: Ecabet sodium is reported to have protective effects on the gastric mucosa and show a bactericidal effect on *H. pylori* and inhibit urease activity in vitro. We investigated the inhibitive effect of ecabet sodium to urease activity in volunteers with *H. pylori* infection using 13C-urea breath test (UBT). Methods: Six male volunteers (mean age, 51.3; range 45–55 years) with *H. pylori* infection were enrolled and diagnosed *H. pylori* infection using UBT and stool antigen test. Volunteers were medicated ecabet sodium 1g t.i.d. for 4 weeks. UBT was performed total 10 times per person; before medication, every other day for 2 weeks and 3, 4 weeks.
Results: UBT value of pre-medication ranged from 10.7 to 50.9‰ and the end of medication (at 4 weeks) ranged from 5.8 to 44‰. Nobody became negative UBT (below 2.5‰) during medication. We calculated the average of UBT value during medication and compared before medication. In two volunteers the average of UBT values are lower than before, but in 4 volunteers showed higher UBT before medication. Discussion: Ecabet sodium is reported to inhibit urease in vitro, but in this study it didn't suppress the UBT values in volunteers. And during medication, it didn't influence the judgment of UBT.
Conclusions: In this study, ecabet sodium didn't influence the UBT in volunteers with *H. pylori* infection. So, even during taking of ecabet sodium we can perform UBT correctly.

Poster no.: P4.21
Influence of the background ammonia content on the results of the modified urease breath test

L. G. Bajenov[1], I. V. Kosnikova[1] and A. N. Aripov[2], Uzbek Helicobacter Study Group

[1]Republican Specialized Centre of Surgery, Tashkent, Uzbekistan; [2]Tashkent Institute for Postgraduate Medical Education, Tashkent, Uzbekistan

Background ammonia content (AC) in expired air at patients and healthy people at carrying out modified urease breath test (MUBT) was studied. 24 patients with duodenal ulcer (DU) were examined thereby MUBT. Control group was made of 26 healthy persons. We used sets of reactants for urea detection (Hospitex Diagnostics, Italy). Patients were proposed to drink 1% solution of carbamide (50 ml). In 30 minutes we obtained formed ammonia from expired air as its water solution. AC increase in expired air after carbamide taking in comparison with initial AC proved *H. pylori* (HP) persistence. HP presence was determined at 17 (70,8%) patients. Initial AC fluctuated at them from 0,05 to 27,2 mmole/l, divided into 3 groups: I - up to 1,0, II - from 1,0 up to 8,0, III - > 8 mmole/l. Average AC in II group made in relation to I group 120,8%, in III group it was 1,8 times more, than in II group ($P < 0,001$), and 2,2 times more, than in I group ($P < 0,001$). AC before carbamide taking at patients with negative results was 1,45 times more ($P < 0,05$) than at healthy people with negative results. At the same patients difference between initial AC and after test carrying out was 2,3 times more ($P < 0,001$), at healthy people this difference was statistically invalid. At patients with DU initial AC essentially exceeds that one of healthy persons. There is a correlation between initial AC, its level after carbamide taking and HP presence.

Poster no.: P4.22
Antibodies to CagA- *H. pylori* in ulcer disease

N. Zaharova[1], A. Sarsenbaeva[2], V. Nagaeva[3], V. Deryabin[1], N. Vladimirova[3] and I. Brook[4]

[1]Hospital 15,Snezinsk, Russia, Snezhinsk, Russian Federation; [2]Chelyabinsk State Medical Academy, Snezhinsk, Russian Federation; [3]Hospital 15,Snezhinsk, Russia, Snezhinsk, Russian Federation; [4]Republic Diagnostic Center, Izhevsk, Russian Federation

Aim: To study the count of antibodies to *H. pylori,* their role in diagnosis and prognosis in ulcer diseases.
Materials and Methods: 120 patients with ulcer disease in an aggravation stage are surveyed. Control group made 25 clinically the healthy people comparable on sex and age. To HP-infection diagnostics applied: the ureasis respiratory Helic-test, morphological. Enzyme immunoassay (kits from ''Vektor-Best'', Russia) was used to measure serum levels of (IgM+IgG+IgA) antibodies to CagA-HP. By all patients it was carried out endoscopy.
Results: It is shown that ulcer disease is characterized by a regular rise of antibodies CagA-HP, which are serological markers of *H. pylori* infection. Percentage and absolute value of (IgM+IgG+IgA) antibodies to CagA-HP (90%, titer 1:40 and more vs control 1:10). Direct correlation dependence between sizes titers of antibodies to Hp and change of a mucous membrane of a stomach and duodenum ($r = 0,95$, [[Unsupported Character - р]] $<0,001$).
Conclusion: High concentrations of antibodies to *H. pylori* are a diagnostic criterion of *H. pylori* infection. Long-term of elevated antibodies to CagA-HP concentrations is one of the indications of ulcer disease associated with *H. pylori* an aggravations stage and for application of antichelicobacter therapy.

Poster no.: P4.23
Comparison of Histopathological Study, Urease Test and, Microbiologic Culture for Diagnosis of Gastric-active-infection by *H. pylori* in a Venezuelan Hospital Setting

G. Arismendi-Morillo, I. Hernandez, E. Mengual, A. Fuenmayor, N. Abreu, G. Romero and M. Lizarzabal

University of Zulia, Maracaibo, Venezuela, Bolivarian Republic of

Introduction: Currently there is no optimal method for diagnosis of gastric active infection with *H. pylori* (HPai), since exist

advantages and disadvantages linked with clinical, technical difficulty, cost and its availability in the hospital setting.
Objective: The aim was to compare the histopathological study with hematoxylin-eosin staining (HS), the urease test (UT) and microbiological cultures (MC) to diagnose HPai at the Hospital Universitario of Maracaibo, Venezuela, that results in recommendations applicable to regular clinical experience.
Patients and Methods: Gastric biopsies for HS (body-antrum), UT in two hours (antrum) and MC (antrum) of 115 adult patients (85 [[Unsupported Character - ♀]] / 30 [[Unsupported Character - ♂]]), who underwent esophagus-gastro-duodenoscopy were evaluated. HPai was diagnosed with at least one positive test, and absence of HPai, when the three tests were negative.
Results: 66% had HPai, 34% no exhibited HPai. The Hpai was detected by means HS, UT and MC in 87%, 79% and 70% of cases, respectively. There were significant differences between HS and UT compared to MC ($p < 0.0001$ and $p < 0.001$, respectively). There was no significant difference between HS and UT ($p = 0.7$).
Conclusion: The HS and UT are the most beneficial to diagnose HPai in clinical setting studied. The HS is essential to determine the histopathology of gastric lesions, and the UT, by requiring immediate diagnosis. The MC is recommended only in cases of persistent or recurrent infection, which may warrant testing antimicrobial susceptibility. Selected cases may require a combination of several of these tests.

P5 Virulence factors

Poster no.: P5.01
Gamma-glutamyltranspeptidase of *H. pylori* induces apoptosis in human biliary cells via mitochondria pathway

W. Boonyanugomol[1], C. Chomvarin[1], S. Baik[2], J. Song[2], K. Kim[2], M. Cho[2], W. Lee[2], H. Kang[2] and K. Rhee[2], B. Sripa[3], C. Hahnvajanawong[1]

[1]Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [2]Department of Microbiology, School of Medicine, Gyeongsang National University, Jinju, Korea, Republic of; [3]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

The presence of *H. pylori* in patients with hepatobiliary diseases has been discovered. The possible role of this microorganism in hepatobiliary tract is still unknown. Apoptosis-inducing factors of *H. pylori* have been examined in a previous study. Gamma-glutamyltranspeptidase (GGT) is a novel protein of *H. pylori* which involved in the induction of apoptosis in gastric epithelial cells. However, the effect of *H. pylori*-GGT on the induction of apoptosis in biliary cells has not been shown. This study, recombinant GGT was prepared by using molecular cloning technique. Recombinant GGT of *H. pylori* was used to determine the induction of apoptosis in biliary cell (cholangiocarcinoma, KKU-100 cell line). Recombinant GGT suppressed cell growth, DNA synthesis, and induced apoptosis in KKU-100 cells. Up-regulation of iNOS gene is associated with increased *p*53 level due to recombinant GGT induced DNA damage in KKU-100 cells. Apoptosis mechanism was associated with up-regulation of Bax and down-regulation of Bcl-2, and Bcl-xL, which lead to a release of cytochrome *c* and activate caspase-9, and-3 in KKU-100 cells exposed with recombinant GGT. However, Fas and caspase-8 were not expressed in recombinant GGT-treated cells. These results indicated that recombinant GGT of *H. pylori* induces apoptosis through mitochondria-mediated pathway. *H. pylori*-GGT may play an important role in carcinogenesis of hepatobiliary tract.

Poster no.: P5.02
The prevalence of *dupA* gene of *H. pylori* strains in Polish children

M. Biernat[1], B. Iwańczak[2], J. Grabińska[1], A. Junka[1], L. Łaczmański[3] and G. Gościniak[1]

[1]Department of Microbiology, Medical University of Wrocław, Poland; [2]2nd Department and Clinic of Paediatrics, Gastroenterology and Nutrition, Medical University of Wrocław, Poland; [3]Department and Clinic of Endocrinology, Diabetology and Isotope Therapy, Medical University of Wrocław, Poland

A high diversity of *H. pylori* strains and their genomic variability may influence on clinical outcome in humans. Duodenal ulcer promoting (*dupA*) gene was reported to be associated with increased risk for duodenal ulcer.
The aim of present study was to evaluate the presence of *dupA* gene of *H. pylori* strains isolated from paediatric patients with different gastroduodenal diseases.
Materials and Methods: The study was performed on *H. pylori* strains (n = 137) collected in years 2007–2009. The strains were isolated from gastric biopsies of patients with chronic gastritis, duodenal ulcer (DU) and gastric ulcer (GU). The biopsy specimens taken from each patient during upper gastrduodenal endoscopy were evaluated histologically according to the updated Sydney System. The genes encoding specific virulence factors, such as: *cagA, vacA* (allelic subtypes *s1/s2, m1/m2*) and *dupA* were detected by PCR.
Results: In examined *H. pylori* strains *dupA* gene was present in 35% (48/137). No association was observed among the strains from children with gastritis (36%,p = 0,63), duodenal ulcer (31,25%,p = 0,73) and gastric ulcer (28,5%,p = 0,71). Genes encoding virulence factors: *cagA, vacAs1/m1, vacAs1/m2, vacAs2/m2* were detected in 63%, 33,5% vs.31% and 36% of strains, respectively. The triple positive genotype (*cagA, vacAs1/m1, dupA*) was detected in 14,6% (20/137) tested strains.
Conclusions: The prevalence of *dupA* gene in Polish children is frequent however its presence is not associated with clinical outcome.

Poster no.: P5.03
Co-ordinated regulation of gene expression by *H. pylori* in response to low pH and iron limiting growth conditions

A. P. Moran, A. Gupta, S. Ustinov and K. Mashayekhi
Microbiology, National University of Ireland, Galway, Ireland

Establishing a successful infection within the gastric environment and its varied and changing niches requires adaptation achieved through regulation of bacterial gene expression. In the gastric mucosa, in addition to the low pH *H. pylori* is required to adapt to conditions of iron limitation. The aim of this study was to identify genes whose expression was altered when the bacterium was grown under differing conditions of pH (5 versus 7) and iron limitation. Therefore, cDNA microarray analyses following exposure to different growth conditions were carried out for three *H. pylori* strains 26695 (gastritis-associated), J99 (ulcer-associated) and AG-1 (atrophic gastritis-associated). Genes were identified whose expression was acid pH-dependent in all the strains: 37 genes were down-regulated and 68 up-regulated, which included urease-related genes (*ureA, ureB, ureI, UreF*), amidase (*aimE*), flagellar synthesis genes (*flgB, fliM, flgE*), transport-related genes and genes encoding binding proteins (*glnQ, glnH, exbD*). At pH 7, iron limitation mainly induced motility-associated genes, whereas at pH 5, iron depletion, although inducing down-regulation of 12 genes, led to the increased expression of 21 genes including those coding for carbonic anhydrase and metal-ion transport proteins, instead of urease and amidase enzymes. Genes encoding virulence factors (*e.g., cagA* and *vacA*) and proteins involved in motility were over--expressed under all the stress conditions. Collectively, as effectors of the *H. pylori* stress response, this transcriptome analysis emphasizes the link between the bacterial response to acidity, metal metabolism and virulence. Moreover, this response was similar in *H. pylori* strains associated with different pathologies.

Poster no.: P5.04
H. pylori HP986 acts via TNFR1 to induce simultaneous proinflammatory and proapoptotic responses

N. Ahmed[1], S. A. Ansari[1], A. Alvi[2] and S. E. Hasnain[2]
[1]Pathogen Biology Laboratory, School of Life Sciences, University of Hyderabad, Hyderabad, India; [2]Institute of Life Sciences, University of Hyderabad Campus, Hyderabad, India

Several putative virulence encoding genes have been identified in *H. pylori* that possibly play crucial roles during chronic persistence of the bacterium and to provide survival advantage. Many of such genes are located in the so called 'plasticity region cluster' when multiple genomes from different *H. pylori* strains were compared. We studied the signalling pathways pertaining to proapoptotic and proinflammatory behavior of one such novel virulence factor, HP986, from *H. pylori*. In this study, proinflammatory role of HP986 was assessed through cytokine assays for IL-8 and TNF-α and further, we confirmed the proapoptotic role of the protein by measuring the Fas expression levels in PMA differentiated Thp1 cell lines. We did not find contributions from TLR2 and TLR4 induced signalling to our observed proinflammatory and proapoptotic effects. We confirmed the role of NF-κB (p65/p50) mediated pathway in the above responses that were triggered through the interaction of HP986 with TNFR1. Computational modeling and docking simulation studies were carried out to verify molecular interactions between HP986 and TNFR1 which were further validated through mobility shift assays. A kinetic affinity analysis by Biacore® surface plasmon resonance technique revealed a real-time, molecular interaction between HP986 and TNFR1. Functional validation in animal models will be our next strategy to explore if this novel protein could be harnessed in understanding its functional role in survival and persistence of *H. pylori* in different niches of the gut.

Poster no.: P5.05
Tyrosine-phosphorylation of stress-inducible ITAM-like proteins associated with *H. pylori* specific bacteriophages by various protein-tyrosine kinases

M. Asahi
Fukui Prefectural University, Fukui, Japan

Background: *H. pylori (Hp)* have the genetic and phenotypic diversities, and are associated with gastric adenocarcinoma. *Hp*-CagA protein has been known to be a bacterial oncoprotein that acts via CagA-tyrosine-phosphorylation in mammals. We have previously reported that CagA protein possesses anionic-region containing ITAM-like-motifs, and is phosphorylated at tyrosine-residues in this motif, and also that other ITAM-like-motifs containing microorganism include human herpesvirus-8, influenza virus, HIV-1 and Staph aureus phage phiP68. There are recent reports that magnetic-Viro-Adembeads can capture viruses (or phages) having anionic-structures.
Aim and Methods: We speculated that the genetic and phenotypic diversities of *Hp*-bacteria may derive from diverse-*Hp*-specific-bacteriophages (*Hp*-phages). Using Viro-Adembeads, we analyzed *Hp*-phage-associated-periplasmic-proteins by 2DE/MS and Western-Blot, and investigate the tyrosine-phosphorylation of *Hp*-phage-associated-periplasmic-proteins using *in vitro* [γ-^{32}P]ATP-labeled-kinase assay with various protein-tyrosine kinases (PTKs).
Results: We identified *Hp*-molecular-chaperonin-GroEL (HSP60), *Hp*-molecular-chaperone-DnaK(HSP70) and *Hp*-ureaseB-subunit (*Hp*-ureB) and CagA as *Hp*-phage-associated-proteins which can be bound with Viro-Adembeads, and found that these *Hp*-phage-associated-proteins have both anionic- and cationic-amino-acid-sequences and ITAM-like-amino-acid motifs similar to ITAM-like-CagA. Therefore, *Hp*-phage-associated-ITAM-like-HSP60– and -HSP70-family-heat-shock proteins and -CagA proteins as well as their ITAM-like-peptides were phosphorylated at tyrosine-residues in their ITAM-like-motifs by Src and/or Syk, Fyn, IGF-1R, Jak2, Abl, and PDGFR PTKs. Especially, high ionic-strength-stress of NaCl induced the protein-expression and tyrosine-phosphorylation of *Hp*-phage-associated-CagA and *Hp*-phage-associated-DnaK(HSP70) proteins by Syk PTK; meanwhile, stress-inducible-*Hp*-phage-associated-ureB-dependent-GroEL(HSP60)-family proteins were markedly tyrosine-phosphorylated by Fyn/Jak2 PTKs.

Conclusion: Stress-inducible-*Hp*-phage-associated-ITAM-like-proteins including *Hp*-GroEL(HSP60)-, *Hp*-DnaK(HSP70)-, *Hp*-UreB- and *Hp*-CagA-family proteins and their ITAM-like-peptides can be tyrosine-phosphorylated by various host-PTKs, which may act on *Hp*-gastric, non-gastric and systemic *Hp*-infectious diseases including cancers in tyrosine-phosphorylation-dependent fashion.

Poster no.: P5.06
The role of CagA subspecies in induction of cytokeratin-18 mediated apoptosis

M. Douraghi[1,2], S. Saberi Kashani[1], M. Shokrgozar[3], K. Azadmanesh[4], H. Zeraati[5], M. Esmaili[1], M. Eshagh Hosseini[6], M. Mohagheghi[7] and M. Mohammadi[1]

[1]Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of; [2]Department of Bacteriology, School of Public Health, Tehran University of Medical Sciences, Iran, Islamic Republic of; [3]National Cell Bank, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of; [4]Hepatitis and AIDS Department, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of; [5]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; [6]Endoscopy Unit, Amiralam Hospital, Tehran, Iran, Islamic Republic of; [7]Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of

Introduction: Polymorphic cytotoxin-associated gene A (cagA) of *H. pylori* (Hp) is of prime importance for targeting the signaling circuits involved in cytoskeleton rearrangement. The variable C-terminal region is categorized as EPIYA-A,-B,-C, or -D motifs based on the surrounding sequences. We have aimed to assess the impact of Hp strains and the polymorphic cagA variants on the CK18 cleavage process as a key apoptotic phenomenon.
Methods: Seventy six Iranian clinical strains consisted of following groups were studied: 1. A-B type (n = 6), 2. B-C type (n = 3), 3. A-B-C type (n = 31), 4. A-B-C-C type (n = 23), 5. A-B-B-B-C-C type (n = 3) and 6. cagA-negative (n = 10). Eight hours post co-culture of AGS cell line with Hp strains, flow cytometric analysis was performed using M30 CytoDEATH antibody.
Results: Different Hp strains varied in their ability to cleave CK18 ranging from 1.14% to 18.53% (Mean ± SEM; 5.82 ± 0.51). The strains bearing A-B-C-C type cagA variant gave rise to the highest level of CK18 cleavage (7.89 ± 1.06) as compared to A-B type strains (3.73 ± 1.32; $P < 0.05$) and A-B-B-B-C-C type (2.19 ± 0.82; $P < 0.05$). CK18 cleaved fragments increased by the number of EPIYA-C motifs. CK18 cleavage was inhibited by increasing numbers of EPIYA-B motifs.
Conclusion: It was thus demonstrated that increasing numbers of EPIYA-B motifs may attenuate the effect of CagA on apoptosis through inhibitory interaction with EPIYA-C motif. We provide the evidence for implication of CagA EPIYA motifs in induction or inhibition of apoptosis through CK18 cleavage.

Poster no.: P5.07
H. pylori BabA Expression, Gastric Mucosal Injury and Clinical Outcome

S. Saberi Kashani[1], Y. Talebkhan[1], F. Ebrahimzadeh[1], A. Oghalaie[1], N. Mohajerani[1], A. Nahvijou[2], M. Mohagheghi[2], M. Eshagh Hosseini[3] and M. Mohammadi[1]

[1]Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of; [2]Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; [3]Endoscopy Unit, Amiralam Hospital, Tehran, Iran, Islamic Republic of

Background: BabA is one of Hp outer membrane proteins, which attaches to human Lewis b surface epitopes and is mainly responsible for Hp-colonizing capacity. The aim of this study was to investigate the status of Hp babA/babB genes and their association with BabA protein expression in various disease groups.
Methods: Presence of babA and babB genes was investigated by PCR on 102 fresh single colonies isolated from Iranian patients (26 GC, 21 PUD and 55 NUD). The ability of published PCR-based methods to correctly identify babA2 sequence in reference to BabA expression by immunoblotting and Lewis b (Leb) binding functional assay was evaluated.
Results: The sensitivity and specificity of babA2 primers in reference to BabA protein were 65% and 35% respectively. These values were significantly enhanced in the four primer-PCR assay (94% sensitivity, 75% specificity). There was a ''substantial'' to ''Fair'' agreement between the presence of babA gene and expression of BabA protein ($K = 0.726$, $P = 0.0001$) and Leb binding ($K = 0.360$, $P = 0.0001$) respectively. Performing immunoblotting and Leb binding assays on 156 Hp strains revealed 3 levels of expression: 1) 30.4% as BabA high producers with Leb binding activity,, 2) 25.5% as BabA low producers, without Leb binding activity, and 3) 44.1% with no BabA production or Leb binding activity. A significant risk was inflicted upon patients infected with Bab-H ($p = 0.005$, OR = 11.4, 95%CI = 2.122–61.254) and Leb binding ($p = 0.005$, OR = 8.4, 95%CI = 1.927–36.618) in development of gastric atrophy. Whereas, infection with BabA-L strains increased the risk of duodenal ulcer development ($p = 0.0001$, OR = 14, 95%CI = 3.623–60.271).

Poster no.: P5.08
Variability in Lewis antigen expression in *H. pylori*-infected Greek children: preponderance of nontypeable and type 1 Lewis b antigen-positive strains

E. Altman[1], V. Chandan[1], B. A. Harrison[1], E. G. Panayotopoulou[2], E. Roma[2] and D. N. Sgouras[2], J. Li[1]

[1]Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, Canada; [2]Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece

H. pylori infection is acquired in childhood and can persist for life. Previous studies in adult patients from North America and Europe have shown that *H. pylori* lipopolysaccharides (LPSs) express predominantly type 2 Lewis x (Le^x) and Le^y epitopes, while LPSs from Asian strains have the capacity to express type 1 Le^a and Le^b structures.
In order to understand the influence of environmental and host factors on the expression of Le antigens we analyzed 49 Greek *H. pylori* isolates from symptomatic children. Both CagA-positive and -negative strains were evaluated. The isolates were characterized by whole-cell indirect enzyme-linked immunosorbent assay, gel electrophoresis and immunoblotting. It was found that pediatric isolates had the propensity to express type 1 Le^b blood group antigen, a feature relatively uncommon in *H. pylori* isolates from adult population. Additionally, one-third of the isolates were nontypeable. Combined chemical and mass spectrometric analyses carried out directly on bacterial cells revealed that the majority of nontypeable strains expressed the O-chain composed of partially fucosylated *N*-acetyllactosamine polysaccharide chains. This suggests that LPS of *H. pylori* is involved in host adaption of the bacterium and that fucosylation of the O-chain might be host-induced.

Poster no.: P5.09
Characterization of virulence factors CagA, VacA and Lewis Antigens in *H. pylori* strains isolated from children and lack of correlation with the severity of histological findings

E. G. Panayotopoulou[1], B. A. Harrison[2], K. Papadakos[1], C. van Vliet[3], A. Mentis[1], E. Roma[3], V. Chandan[2], D. N. Sgouras[1] and E. Altman[2]

[1]Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece; [2]Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, Canada; [3]First Department of Pediatrics, Athens University School of Medicine, Athens, Greece

Our aim was to characterize Lewis antigens and virulence factors CagA and vacA in *H. pylori* clinical strains isolated from children and correlate with histopathology.
Lewis antigens Le^x, Le^y, Le^a and Le^b were determined by whole cell ELISA in 49 clinical isolates from symptomatic children (10.4 ± 2.9 yo) of Greek origin. Presence of CagA EPIYA motifs and the vacA s, i, m isotypes were determined by PCR. Functionality of type IV secretion system (TFSS) was verified by detection of phosphorylated CagA protein. Histology was evaluated with the updated Sydney System. Non-parametric analysis and logistic regression were utilized for statistical analysis.
Type 2 (Le^x, Le^y) antigens were observed in the majority of strains. The predominant type was Le^y (35/49, 71%), with concomitant presence of Le^x in 24 isolates (49%). Le^b were observed in 11 (22%) cases, out of which 4 (8%) expressed Le^y as well as Le^x. Only one strain expressed Le^a. Simultaneous presence of Le^x and Le^y antigens with a functional TFSS and *vacA*s1 allele (OR: 4.433, 95%CI: 1.269–15.489) was observed. No correlation with the presence of increased numbers of EPIYA motifs in CagA was evident. Moreover a lack of correlation between all the aforementioned bacterial virulence characteristics with the histopathological observations was observed. We established that there is simultaneous presence of Le^x and Le^y antigens, a functional TFSS and VacAs1 allele in *H. pylori* isolates from symptomatic children, which is however, not correlated with the severity of histopathological lesions, but may confer advantageous conditions for the establishment of persistent infection.

Poster no.: P5.10
Detections and functions of *H. pylori* with its specific bacteriophages associated with diverse ITAM-like antigens

M. Asahi

Fukui Prefectural University, Fukui, Japan

Background: *H. pylori* (*Hp*) are human pathogen that is successful in evading the human innate-immune responses and is associated with the development of gastric cancers; however, the molecular basis remains unclear.
Aim and Methods: We focused on tyrosine-phosphorylation of *Hp*-bacteriophage (*Hp*-phage)-associated ITAM-like-antigens. To visualize *Hp*-bacteria with *Hp*-phage-associated- ITAM-like-antigens and to examine their functions in *Hp*-infection, we used electron microscopy, confocal- and time-lapse-immunofluorescence microscopies, Fluorescence Bioimaging Analyzer (FBA) with various anti-*Hp*-ITAM-like-antigens-Abs and FITC-, Alexa-405- and Alexa-633-conjugated anti-pY-ITAM-like-peptide-pAbs.
Results: We successfully visualized *Hp*-bacteria with *Hp*-bactetiophage-associated ITAM-like-antigens including CagA, (GroEL)HSP60, (DnaK)HSP70 existing on *Hp*-cell-surface and in the host-cells in IgG- and tyrosine-phosphorylation-dependent fashions, and also on the whole-blood agar plate in fibrinogen-dependent manner. Time-lapse-microscopy revealed that *Hp*-aggregated-*Hp*-organisms with pY-ITAM-like-antigens tyrosine-phosphorylated inside the cells are released from host cells and emit stronger fluorescence on the infected-cell-surface, and further showed the existence of the fluorescent *Hp*-phage-like- flying-object without *Hp*-bacteria during *Hp*-infection and of the fluorescent-pY-ITAM-like-antigens dissolved in the culture-medium following bursting of infected-cells. Furthermore, using electron-microscopy, we observed that many *Hp*-phages are released near intracellular-living-*Hp*-organisms and the partially-lysed-*Hp*-organisms and in *Hp*-megasomes, which are involved in escaping of *Hp*-bacteria from host's autophagic- and phagocytic-killing systems and in intracellular-surviving of *Hp*-organisms in Src-family-PTK-, *oip*A- and *cag*PAI-dependent-fashions. Additionally, *Hp*-phages releasing from the filamentous-pilus of type-IV-system were observed in *Hp*-infected cells.
Conclusion: *Hp*-organisms with *Hp*-phage-associated-ITAM-like-antigens can be visualized and are essentially involved in evading host's-innate-immune responses as ''Trojan Horse'' and in intracellular-*Hp*-surviving via stress-inducible *Hp*-phage-associated-pY-ITAM-like-antigens in *oip*A- and *cag*PAI- and tyrosine-phosphorylation-dependent-fashions, which may lead to gastric and non-gastric cancers.

Poster no.: P5.11
Clinical relevance of cagPAI intactness in *H. pylori* isolates from Vietnam

T. L. Nguyen, T. Uchida, Y. Tsukamoto, K. Murakami, Y. Yamaoka, T. Fujioka and M. Moriyama
Oita University, Yufu, Japan

The purpose of this paper is to investigate the relationship between clinical outcome and the intactness of cagPAI in *H. pylori* strains from Vietnam. The presence or absence of 30 cagPAI genes was investigated by polymerase chain reaction (PCR) and dot-blotting. *H. pylori*-induced interleukin-8 secretion and hummingbird phenotype, and *H. pylori* adhesion to gastric epithelial cells were examined. The serum concentration of pepsinogen 1, pepsinogen 2, and gastrin was also measured in all patients. cagPAI was present in all 103 Vietnamese *H. pylori* isolates, of which 91 had intact cagPAI and 12 contained only a part of cagPAI. Infection with the partial cagPAI strains was less likely to be associated with peptic ulcer and chronic gastric mucosal inflammation than infection with strains possessing intact cagPAI. The partial cagPAI strains lacked almost all ability to induce interleukin-8 secretion and the hummingbird phenotype in gastric cells. Their adhesion to epithelial cells was significantly decreased in comparison with intact cagPAI strains. Moreover, for the first time, we found an association between cagPAI status and the serum concentration of pepsinogens 1 and 2 in infected patients. *H. pylori* strains with internal deletion within cagPAI are less virulent and, thus, less likely to be associated with severe clinical outcomes.

Poster no.: P5.12
Characterization of *H. pylori* CagA EPIYA motifs in *H. pylori* strains of Turkish Origin

M. Güvenir[1], Ö. Yılmaz[1], A. Olivares[2], C. Gönen[3], S. Sarıoğlu[4], H. Ellidokuz[5], M. Soytürk[3], I. Şimşek[3] and G. I. Perez Perez[2]
[1]Dokuz Eylül University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey; [2]New York University, School of Medicine, Department of Medicine and Microbiology, NYUSM, New York, NY, United States; [3]Dokuz Eylül University, Faculty of Medicine, Department of Gastroenterology, İzmir, Turkey; [4]Dokuz Eylül University, Faculty of Medicine, Department of Pathology, İzmir, Turkey; [5]Dokuz Eylül University, Faculty of Medicine, Instıtute of Oncology, İzmir, Turkey

Aim: Tyrosine phosphorylation of CagA in *H. pylori* occurs at the EPIYA motif region, a five aminoacid sequence (Glu-Pro-Ile-Tyr-Ala) that is present in variable numbers in the C-terminal EPIYA repeat region of the CagA protein. The aim of the study was to characterize the 3′variable region of the *cag*A gene of *H. pylori* strains, determine their EPIYA motifs and correlate its presence with histopathological findings in patients' biopsies.
Method: A total of 124 patients with dispepsia referred for upper endoscopy were included. *cagA* status of *H. pylori* strains was established by PCR.*cag*A PCR products ranging between 370–570 bp were amplified and purified by QIAquickPCR kit and sent to Macrogen for sequencing.
Results: Sixty-two patients (50%) were positive by culture. Among *H. pylori*-culture positive patients;30(48.4%) were *cag*A positive. We found a statistical significant difference between scores for inflammation of the pits in antrum and corpus and atrophy in corpus between CagA-positive and CagA-negative patients; No differences were found between CagA positive and CagA negative patients among histopathological *H. pylori* scores, intestinal metaplasia, and inflammation of the lamina propria. Among the 30 *cag*A positive strains;21(70%) had three EPIYA motifs (EPIYA-ABC), seven (23.3%) had more than three EPIYA motifs(EPIYA-ABCC).However no association was found between histopathological features with EPIYAmotifs. Two patients (6.7%) were colonized with more than one strain(mix infection) both had EPIYA-ABC and-ABCC.*H. pylori* strains isolated from antrum and corpus of six patients were phylogenetically distinct each other.
Conclusion: *cagA* positive *H. pylori* strains correlate with inflammation of pits(active inflammation) in antrum and corpus and the presence of atrophy in corpus. The correlation could not be found between EPIYA motifs and histopathology markers.

Poster no.: P5.13
Association of *H. pylori* virulence factors with atrophic gastritis in dyspeptic patients from a population at high risk of gastric cancer in Costa Rica

J. Garita-Cambronero[1], C. Une[1], W. Malespín[1], R. Sierra[1], P. Golcher[1], F. Mégraud[2] and V. Ramírez[1]
[1]Institute for Health Research, University of Costa Rica, San Pedro de Montes de Oca, San José, Costa Rica; [2]University Victor Segalen, Bordeaux 2, Bordeaux, France

Background and aim: Costa Rica is one of the countries with the highest incidence and mortality rates from gastric cancer. Gastric cancer prevalence varies among different regions. However, the prevalence of *H. pylori* infection is high in the whole country. The aim of this study was to determine if *H. pylori* virulence factors are associated with atrophic gastritis in a dyspeptic population.
Methods: Seven biopsies and a blood sample were obtained from 264 patients referred to endoscopy for dyspeptic symptoms. In each case, a histopathological examination was performed. *H. pylori* infection was determined by PCR and serology. The presence of the *cag*A, *vac*A and *bab*A2 genes was analyzed by PCR in cultured strains. Odds ratio and 95% confidence intervals were calculated.
Results: Out of the 264 participants, 73% were infected with *H. pylori*, 6% presented normal mucosa, 68% non-atrophic gastritis and 26% atrophic gastritis. Infection with *H. pylori* was associated with atrophy (OR = 1,82; 95% CI = 0,88–3,88). A total of 56 strains were cultured:
Conclusion: Infection with *cag*A+ and *vac*A *s1m1* increases the risk on atrophic gastritis in this population; *bab*A+ is not associated with risk.

H. pylori babA2, cagA+, vacA s1m1 genes according to atrophic gastritis

Gene (n)	OR (AG vs NAG)	95% CI	(AG/NAG)
babA2 + (56)	0,57	0,18–1,79	18/38
cagA +(56)	4,16	0,82–20,9	18/38
vacA s1m1 (51)	2,8	0,53–14,62	16/35

OR: Odds ratio; CI: Confidence intervals; AG: Atrophic gastritis; NAG: Non atrophic gastritis.

Poster no.: P5.14
Pattern of *H. pylori cag* pathogenicity island genes among Egyptian dyspeptic patients

E. M. El Sherbini, M. S. Diab, S. Saad El Din, L. Abu El Fadl, M. Shemis, Z. Omran, A. Badawi, M. El Ghannam, A. El Raye, N. Fam, I. El Dafrawi and M. A. Saber

Theodor Bilharz Resarch Inst., Giza, Egypt

Infection with a strain of *H. pylori* possessing the virulent determinant Cag PAI increases the risk of progression from simple gastritis to severe pathology. The aim of this study was to determine *cag* PAI genes pattern among Egyptian dyspeptic patients and its correlation with varying degrees of associated gastritis,

Gastrointestinal antral biopsies were obtained from 106 dyspeptic patients undergoing upper endoscopy. Histopathological examination, urease test and PCR assay were performed to confirm *H. pylori* infection. DNA was extracted from positive *H. pylori* biopsies for *cag* PAI genotyping using PCR assay. There was a significant difference between dyspeptic patients with normal mucosa (30.2%) and those suffering from gastritis (69.8%). *H. pylori* was detected in 71.7% of the dyspeptic patients with a association between *H. pylori* infection and gastritis

Analysis of the entire *cag* PAI genes revealed that *cag A* and *cag E* were predominant genes while *cag T* and *tnpB* genes were not detected in all *H. pylori* positive biopsies. The entire *Cag* PAI was more substantiated in gastritis patients. The *cagA 1 / 2, cag 3 / 4, cag M* amd *cag E* were significantly ($p < 0.02$) associated with moderate gastritis while *tnpA gene* was detected in marked degree of gastritis ($p < 0.02$). In conclusion infection with a virulent strain carrying *cag* PAI is an indicator for the risk of gastric mucosal damage and its pathological consequences. In a developing country like Egypt where the prevalence of *H. pylori* infection is high *cag* PAI genotyping is important to speculate the clinical outcome and the management of the infection.

Poster no.: P5.15
Differentiation of relation of pathogenicity factors *H. pylori* with apoptosis in the gastric mucosa in genetically diverse populations

O. S. Amelchugova, V. V. Tsukanov and O. V. Tretyakova

State Scientific Medical Research Institute for Northern Problems of Siberian Division of Russian Ac, Krasnoyarsk, Russian Federation

Aim: To investigate the relationship of density dissemination (DD) and CagA strains of *H. pylori* with apoptosis in the gastric mucosa in chronic gastritis in Mongoloids and Europoids of Siberia.

Methods: The study included 22 adult Evenks and 24 Europoids. All subjects underwent upper digestive tract endoscopy and antrum mucosa biopsy specimens were taken. *H. pylori* and CagA *H. pylori* determined morphologically and by ELISA test systems in serum. Apoptotic indicators in gastric mucosa were determined using TUNEL-method.

Results: The apoptotic index (AI) in gastric antrum mucosa was 5,19% in Europoids, 4,04% in Evenks ($P = 0.001$) in gastric corpus - 4,99%, and 3.19%, respectively, $p < 0,001$. DD correlated with the AI in the antrum ($r = +0,80$; $p < 0,001$) and corpus ($r = +0,84$; $p < 0,001$) in Europoids greater than in Evenks ($r = +0,38$, $p = 0,03$; $r = +0,24$; $p = 0,18$). In antrum of patients with CagA AI was higher in comparison with persons without CagA in both populations. In greater curvature of stomach apoptosis was increased among patients with CagA in Evenks greater than aliens.

Conclusion: We found different levels of relationship CagA strains and DD *H. pylori* with apoptosis in antrum and gastric corpus in chronic gastritis in Mongoloids and Europoids in Siberia.

Poster no.: P5.16
Association of *H. pylori* with pepsinogen II level

O. V. Reshetnikov[1], S. Kurilovich[1] and A. Molchanova[2]

[1]Institute of Internal Medicine, Novosibirsk, Russian Federation; [2]Vector-Best, Novosibirsk, Russian Federation

The aim of this study was to investigate the influence of *H. pylori* to serum levels of pepsinogen II (PGII).

Methods: Two patient samples have been studied in Novosibirsk. The first one was drawn from The Population Cancer Registry with 25 gastric cancer cases vs 49 control persons (Sample 1). Another one used a population sample included 130 subjects aged 2–70 (Sample 2). Sera were tested with Gastropanel (Biohit, Finland).

Results: The presence of antibodies to *H. pylori* was associated with higher (> 3 ng/ml) levels of PGII (15.3 + 9.3 vs 9.8 + 6.8, $p = 0.026$) (Sample 1). In Sample 2 the presence of antibodies to *H. pylori* was associated alike with higher levels of PGII (21.1 + 13.6 vs 9.1 + 5.5, $p = 0.000$).

In multivariate analysis *H. pylori* was associated with the higher prevalence of PGII concentrations > 3 ng/ml (OR 5.51; 95% CI 1.16–26.23, $p = 0.032$).

Conclusions: Thus, some possible reasons may be proposed for the elevating levels of PGII in *H. pylori* infected persons. One not exclude that decrease of PGI synthesis and release into gastric lumen due to *H. pylori* infection may cause reciprocal and compensatory stimulation of PGII synthesis. *H. pylori* induce inflammation in antral mucosa and this may stimulate abundant leakage of PGII into local circulation out of destructed atrophic cells. Nevertheless, the PGII level is increased compared with the PGI level, proposing the use as a marker of *H. pylori* infection or eradication and of histological features of the gastric body (Miki et al., 2003).

Poster no.: P5.17
Genetic aspects of the pathogenesis corpus gastritis

O. S. Amelchugova, V. V. Tsukanov, O. V. Tretyakova and E. V. Kasparov
State Scientific Medical Research Institute for Northern Problems of Siberian Division of Russian Ac, Krasnoyarsk, Russian Federation

Aim: To study morphological structure of the corpus mucosa in correlation with CagA strains of *H. pylori* among Europoids and Mongoloids in Siberia.
Methods: Morphological study was carried out in 159 aliens and 136 Evenks and included light microscopy of biopsy specimens after staining by hematoxylin and eosin. Morphometry of structural elements of the gastric mucosa was carried out in 48 Europoids and 47 Evenks. All subjects were detected *H. pylori* and Cag A by urease, morphological and serological methods.
Results: The prevalence of corpus atrophic gastritis was 23. 9% among Europoids and 13.3% in Evenks (p = 0,02). Among the Evenks gastric corpus atrophy were found in 21.4% of persons with CagA strains and 8.3% of patients without CagA (p = 0,03). In Europoids with chronic gastritis, these figures were respectively 25.9% and 21.0% (P = 0.51). In aliens with CagA *H. pylori* compared with persons without CagA parietal cells decrease to 6,4% (p = 0.22), whereas among Evenks, the figures were 38,6% (p = 0,01). Among the CagA-positive Aboriginal share the proportion of glandular epithelium decreased by 28,1% (p = 0.03), compared with CagA - negative individuals, in Europoids - 6,9% (p = 0.12).
Conclusion: Registered varies of relationship CagA strains of *H. pylori* and morphometric structure of gastric corpus in two genetically diverse Siberian population.

P6 Epidemiology; Paediatric issues; Extradigestive and Hepatobiliary diseases; NSAIDs; Probiotics

Poster no.: P6.01
Risk factors for reinfection after successful eradication of *H. pylori* in three different populations in Alaska

M. Bruce[1], T. Hennessy[1], A. Reasonover[1], J. Morris[1], D. Bruden[1], F. Sacco[2], D. Hurlburt[1], J. Gove[2], A. Parkinson[1] and B. McMahon[2]
[1]CDC, Anchorage, AK, United States; [2]ANMC, Anchorage, AK, United States

Introduction: *H. pylori* infection is common among Alaska Native persons, is a major cause of peptic ulcer disease (PUD), and may be associated with gastric cancer. We performed a study to determine rates of *H. pylori* reinfection after cure in 3 groups followed for 2 years, and to determine risk factors for reinfection. Methods:
We enrolled adults diagnosed with *H. pylori* infection based on a positive UBT and/or one or more of the following: culture, CLO test or histology. After successful treatment was documented at 2 months, we tested each patient by UBT at 4, 6, 12 and 24 months.
Results: 229 persons were followed for 2 years. The median age of participants was 51 years; 55% were female. *H. pylori* reinfection occurred in 36 persons. The cumulative reinfection rate at 2 years was 16.1%. Participants who became reinfected were more likely to have a higher % of household members infected with *H. pylori* infection compared to participants who did not become reinfected (p = .04). On multivariate analysis, lower education, and a history of or PUD at enrollment were statistically significant risk factors associated with reinfection.
Conclusions: Among all 3 groups, reinfection after successful treatment for *H. pylori* occurred at rates higher than those reported for other US populations (< 5% at 2 years). For those patients successfully treated for infection (UBT-negative 2 months post treatment), consideration should be given in highly endemic areas, such as Alaska, to retesting these persons at a later date to rule out reinfection.

Poster no.: P6.02
Trends in prevalence of acid-related disorders in children in Lithuania

L. Kupcinskas[1], R. Kucinskiene[2] and J. Kupcinskas[1]
[1]Department of Gastroenterology, Kaunas University of Medicine, Kaunas, Lithuania; [2]Department of Children diseases, Kaunas University of Medicine, Kaunas, Lithuania

Introduction: The prevalence of *H. pylori* is significantly declining in Western Europe, with concomitant diminishing of prevalence of peptic ulcer disease. Prevalence of GERD is increasing in Western Society. There are only limited data on the changes in prevalence of acid-related disorders in children in East and Central European countries during the last decade.
Aims and Methods: The aims of current study were: 1) to analyse retrospectively the trends in prevalence of erosive esophagitis and peptic ulcer disease in Lithuania during period 1996–2008; 2) to compare changes of *H. pylori* prevalence in children during the same period. We retrospectively analyzed all endoscopy examination records (5348 children endoscopies) in

Kaunas medical university hospital during the period of 1996 - 2008. *H. pylori* prevalence were compared in 2 cohorts of consecutive children with dyspepsia, examined in 1997–1998 (n = 220) and in 2007–2008 (n = 136) in the same tertiary hospital. *H. pylori* infection was detected by rapid urease test, histology and serology.

Results: The prevalence of peptic ulcer among children who underwent endoscopy in 1996 was 8. 3%, in 1997 - 7.9%, and gradually decreased to 5.0% in 2007, and to 4.9% in 2008. The prevalence of erosive esophagitis gradually increased to from 0.6% (1996 and 1997) to 8.1% and 8.3% (2007 and 2008). *H. pylori* infection prevalence among dyspeptic children in Lithuania decreased from 76% (1997–1999) to 56% (2007–2008, $p < 0.001$).

Conclusion: During recent 12-years period in children the prevalence of peptic ulcer disease decreased 1. 7 times and the prevalence of erosive esophagitis increased 13 times in Lithuania.

Poster no.: P6.03
Differences in the prevalence of *H. pylori* between groups of HIV+ patients with various degrees of immunosuppression and one HIV- group

C. Aguiar[1], M. E. Igorra[1], C. Boccardo[1], M. Rondan[2], C. Mendez[1], S. Vazquez[1], G. Saona[3], S. Melle[3], C. Olano[1] and K. Mönkemüller[4]

[1]Hospital Pasteur, Montevideo, Uruguay; [2]Hospital Maciel, Montevideo, Uruguay; [3]Facultad de Medicina, Universidad de la Republica, Uruguay; [4]Marienhospital Bottrop, Bottrop, Germany

Background and study aims: To determine the prevalence of *H. pylori* infection in a population of HIV+ subjects with upper gastrointestinal tract symptoms, and to compare it with an HIV- population. Patients and Methods: This was an observational, cross-sectional and analytical study that enrolled a population of 107 patients referred for gastroscopy. Patients were divided into three groups: *Group A:* HIV+ patients with CD4 counts < 200 (n = 27), *Group B:* HIV+ patients with CD4 counts ≥ 200 CD4 (n = 19) and *Group C:* HIV- patients (n = 61). Five biopsies were taken from the antrum and 5 from the gastric body for urease test and histopathology (H&E, PAS and Giemsa). Gastritis was categorized according to the Sydney classification.

Results: The mean CD4-cell count for Group A was 67, for group B 467. Considering groups A and B, either jointly or separately, there were no significant differences in the prevalence of *H. pylori* when compared with Group C. A low percentage of ulcers were observed in the three groups. Chronic gastritis was divided into atrophic and non atrophic. The 3 groups showed similar percentages of *H. pylori*-negative active chronic gastritis.

Conclusions: Our study shows a tendency toward the development of chronic atrophic gastritis in correlation with the severity of immune depression. However, no significant differences were found in the prevalence of *H. pylori* infection among the groups studied. Our research does not support the hypothesis that immune depression would generate an environment hostile for the occurrence of *H. pylori* infection.

Poster no.: P6.04
Comparison of participants who consented to endoscopy and those who did not as part of community-driven research on *H. pylori* infection

L. Aplin, J. Huntington, A. Wynne, K. J. Goodman and CANHelp Working Group

University of Alberta, Edmonton, AB, Canada

In recent years, the health committee of the Aborignal hamlet of Aklavik, Northwest Territories identified *H. pylori* infection as a priority concern and advocated for research to find solutions. The resulting Aklavik *H. pylori* Project is the start of community-driven research on *H. pylori* infection in northern Canada. This report compares characteristics of participants who underwent endoscopy as part of this research with those who did not.

From November 2007- February 2008, participants were interviewed using a structured questionnaire to collect self-reported clinical history data, including family history of *H. pylori* infection or stomach cancer, and specific types of gastric discomfort. During this time, participants were offered testing for *H. pylori* by urea breath test (UBT) and/or evaluation by endoscopy. Endoscopy was offered in February 2008 and some Aklavik residents who did not participate by then had a UBT and interview later. This abstract presents a preliminary comparison of the selected characteristics among participants who did and did not undergo endoscopy. Multivariable logistic regression, to be added later, will include demographic and other factors to estimate their effects on an individual's decision to undergo endoscopy as part of community-driven research.

344 participants completed questionnaires, 321 had UBT, and 200 underwent endoscopy. Preliminary analysis does not show striking differences (see table), although those undergoing endoscopy were somewhat more likely to have relevant family history, prior testing and take heartburn medication.

Comparison of participants who consented to endoscopy and those who did not

	Had endoscopy n = 196, Proportion (95% CI)	No endoscopy n = 108, Proportion (95% CI)
GI Symptoms	–	–
None	0.38 (031–0.45)	0.40 (0.31–0.50)
1	0.19 (0.14–0.26)	0.18 (0.11–0.26)
2+	0.43 (0.36–0.50)	0.43 (0.33–0.52)
Family History	–	–
H. pylori infection	0.25 (0.19–0.32)	0.19 (0.12–0.28)
Stomach Cancer	0.31 (0.25–0.38)	0.24 (0.16–0.33)
Tested for *H. pylori* before this research?	0.20 (0.15–0.27)	0.12 (0.07–0.20)
	–	–
Currently on heartburn medication?	0.27 (0.21–0.34)	0.19 (0.12–0.28)

Poster no.: P6.05
Tracing *H. pylori* in the Oral and Gastric Yeasts by PCR and Live/Dead kit

F. Siavoshi[1], A. H. Salmanian[2], S. Pedramnia[1], A. Sarafnejad[3] and T. Shahrestani[3]
[1]Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran, Islamic Republic of; [2]Department of Plant Molecular Biology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran, Islamic Republic of; [3]School of Public Health, Medical Sciences, University of Tehran, Tehran, Iran, Islamic Republic of

Introduction: Oral yeast *Candida* has been proposed as the reservoir of *H. pylori* that could facilitate re-inoculation of the stomach. In this study yeasts from oral cavity and stomach of one patient were examined for the occurrence of *H. pylori*-specific genes. The amplified products were compared to those from *H. pylori* isolate from stomach of the same patient.
Methods: Two *Candida* yeasts which were isolated from the oral cavity and stomach of one dyspeptic patient with positive culture of gastric *H. pylori* were studied. Total DNAs from yeasts and *H. pylori* isolate were extracted and PCR was performed to amplify *H. pylori 16s rDNA, vacA (m)* and *jhp0947* genes. *H. pylori* and *E. coli* were used as controls. Yeast isolates were stained with Live/Dead BacLight Bacterial Viability Kit and examined by fluorescent microscope.
Results: *H. pylori*-specific *16s rDNA* (519 bp) gene was amplified from both oral and gastric yeasts. Sequencing results revealed 100% homology between the amplified products from oral and gastric yeasts and *H. pylori* isolate of the patient. Furthermore, the PCR products of *jhp0947* (611 bp) and *vacA* (*m1*:570 bp, *m2*:645 bp) genes amplified from yeasts and *H. pylori* showed similarity in size. Fluorescent microscopy demonstrated fast moving green fluorescent Bacterium-Like Bodies inside the yeasts vacuoles.
Discussion: *H. pylori* and yeast *Candida* are both the colonizers of gastrointestinal tract. However, interaction between these two microorganisms has not been studied. Results of this study demonstrate that both oral and gastric yeasts could harbor *H. pylori* in their vacuole. This indicates that yeast *Candida* could play an important role in accumulating *H. pylori* in the gastrointestinal tract.

Poster no.: P6.06
Risk factors for *H. pylori* (HP) infection: A prospective case-control study

A. Masgala[1], G. Nikolopoulos[1], Z. Tagalaki[1], E. Gova[1], N. Mathou[2], K. D. Paraskeva[2], E. Andrikopoulos[2] and J. A. Karagiannis[2]
[1]First Department of Internal Medicine, ''Konstantopoulio'' Hospital, Athens, Greece; [2]Gastroenterology Unit, ''Konstantopoulio'' Hospital, Athens, Greece

Background: HP infection consists a significant health-related issue worldwide but few data are available regarding predisposing risk factors for infection acquisition.
Aim: To calculate prospectively several predefined risk factors for acquisition of HP infection.
Patients and Methods: Cases (HP+) and controls (HP-) were matched only to the number. Predefined risk factors studied were: age, sex, nationality, education and socioeconomic status, diabetes mellitus, arterial hypertension, hyperlipidemia, thyroid disease, number of kids, smoke or alcohol abuse, and the use of NSAIDs. Statistical analysis was performed with logistic regression analysis.
Results: 50 consequetive cases and 50 controls were studied. 31 (62%) females - 19 (38%) males (cases) and 29 (58%) females - 21 (42%) males (controls). Mean age was 57.22 y and 52.7 y for cases and controls respectively. 86% of cases and 90% of controls were of Greek origin. Of the cases 44% had primary education and from the controls 48% had secondary education. Low income had 48% of the cases and 26% of the controls. Figures for diabetes mellitus were 16%, hypertension 38%, hyperlipidemia 19%, thyroid disease 18%, high alcohol consumption 3%, smoking 44% and NSAIDs use 16% of cases. Respective figures were 12%, 17%, 13%, 11%, 7%, 42% and 4% for the controls. Logistic regression analysis showed that low-income patients were most likely (58%) to be HP(+) compared with high-income controls (48%) (95% CI). High-income individuals had a 92% less probability for being HP+.
Conclusion: In the cohort studied, low income was the only statistically significant risk factor for HP infection acquisition.

Poster no.: P6.07
Epidemiological Characterization of Gastric Helicobacter Like Organisms (GHLO) infection in portuguese pets

I. F. Amorim[1], F. Faria[1], C. Lopes[1], J. Ribeiro[1], F. Augusto[1], C. A. Reis[2] and F. Gärtner[1,2]
[1]ICBAS, Porto, Portugal; [2]IPATIMUP, Porto, Portugal

Introduction: Relation between Helicobacter spp. and environment has been widely studied in humans and carnivorous pets. Promiscuity, non-controlled living conditions and poor sanitation are important predisposing factors. In Portugal, human prevalence of *H. pylori* is 83%. In pets this condition is underdiagnosed and number of animals infected, strains involved and its pathogenicity is not recognised.
Material and Methods: Group A: stomachs of 13 dogs and 1 cat collected in municipal kennel of Oporto (stray animals, apparently healthy). Group B - gastric endoscopic samples performed in ICBAS Veterinary Hospital from 14 dogs and 7 cats (all with GI signs). Sections of cardia, body and antrum were routinely processed and submitted to immunohistochemistry (antibody against *H. pylori* and several carbohydrates Le^a, Le^b, Le^x, Le^y, Sle^a, Sle^x). Group B animals were treated and follow-up was performed.
Results: All group A animals were infected. In group B, 6 dogs and 7 cats were infected. Histopathological findings are similar to those described in humans (mild to moderate chronic gastritis). Immunohistochemical results for glycan antigens revealed different expression when compared with humans.
Conclusions: Results in group A emphasize importance of stray living conditions in the infection pathogenesis. Incidence in pets is similar to that observed for *H. pylori* in humans in this same

region. This may result from environmental factors that both share. A full description and GHLOs clarification is needed to establish their role in human infection and to clarify whether these animals play a role as reservoir for the disease accounting in epidemiological studies.

Poster no.: P6.08
Prevalence of *H. pylori* infection in St.-Petersburg, Russia

N. V. Baryshnikova, Y. P. Uspenskiy, A. S. Smirnova and J. A. Fominykh
St-Petersburg State Medical Academy n.a. I.I. Mechnikov, St-Petersburg, Russian Federation

The aim: to define prevalence of *H. pylori* infection in St.-Petersburg, Russia
Materials and methods: It has been surveyed 200 healthy volunteers without any gastroenterological complaints and living in St. -Petersburg: 62 - men, 138 - women. Volunteers have been divided into 6 age groups: 15–19 years - 81 persons (40% from total number), 20–29 years - 47 persons (24%), 30–39 years - 15 persons (7%), 40–49 years - 23 persons (12%), 50–59 years - 16 persons (8%), also are more senior 60 years - 18 persons (9%).
For verificate of infection *H. pylori* has been used noninvasive breathing test - the helik-test with urea (Associate of medicine and Analytic, St-Petersburg), based on a kinetic estimation of concentration of ammonia in air of an oral cavity after reception by the patient of a portion of a carbamide (500 mg)/
Results: *H. pylori* has been revealed at 148 examinees (74%). *H. pylori* among men was above, than among women (accordingly, 77 and 72%), and also prevailed in age groups of 15–19 years (80%), 30–39 years (86%) and 40–49 years (82%), was lower in age group of 50–59 years (62%) and in age group of 60 years and is more senior (72%) and was the lowest in age group of 20–29 years (59%).
Conclusions: High prevalence of *H. pylori* in St-Petersburg dictates necessity of screening of people in age 30 years and elder for timely administrate of eradication therapy for *H. pylori*-positive persons. It helps to prevent clinically significant *H. pylori*-associated diseases and to prevent of a stomach cancer.

Poster no.: P6.09
H. pylori dissemination in Mongoloids and Europoids in Tuva Republic

V. V. Tsukanov[1], T. B. Bichurina[2], O. S. Amelchugova[1], E. Y. Kupershtein[1] and A. E. Ali Riza[2] and A. S. Maadi[3]
[1]State Scientific Medical Research Institute for Northern Problems of Siberian Division of Russian Ac, Krasnoyarsk, Russian Federation; [2]Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation; [3]Republican Hospital [1] 1 of Tyva, Kyzyl, Russian Federation

Aim: To study the frequency and rates of *H. pylori* dissemination in indigenous and aliens of Tuva Republic.
Methods: We carried out one-stage study of adults in Kyzyl. Clinical examination, upper digestive tract endoscopy and *H. pylori* definition (serological, morphological and urease methods) were performed in 274 Europoids and 316 Mongoloids. IgG Cag A was diagnosed by ELISA in serum of all patients. Morphological study included light microscopy, biopsy specimens of the three divisions of the stomach after hematoxylin and eosin and Giemsa staining with describing the results of visual analogue scale (Dixon MF et al, 1996) and was carried 131 Europoids and 154 tyvins. Dissemination density (DD) of *H. pylori* was determined.
Results: The detection rate of *H. pylori* morphological and urease method was among Europoids 92.6%, among tyvins - 96,4%. *H. pylori* DD in antrum was in Europoids 242,37 ± 16,23; in Mongoloids - 206,91 ± 14,16 (P = 0,1). Prevalence of Cag A *H. pylori* in tyvins was 60,0%, among Europoids - 59.8%.
Conclusion: No significant differences in *H. pylori* dissemination among Europoids and Mongoloids in Tuva Republic were detected.

Poster no.: P6.10
High prevalence of *H. pylori* dissemination in corpus gastric mucosa in patients with chronic gastritis

O. S. Amelchugova, V. V. Tsukanov and E. V. Kasparov
State Scientific Medical Research Institute for Northern Problems of Siberian Division of Russian Ac, Krasnoyarsk, Russian Federation

Aim: Examine indicators *H. pylori* dissemination in the antral mucosa and gastric corpus in patients with chronic gastritis.
Methods: The study included 128 Evenks and 142 Europoids with morphologically verified gastritis in age from 18 till 50 years living in Evenkia. All subjects underwent upper digestive tract endoscopy and antrum and corpus mucosa biopsy specimens were taken. *H. pylori* determined morphologically after Gimsa staining and urease method. Dissemination density (DD) of *H. pylori* was determined.
Results: The prevalence of *H. pylori* detection among patients with chronic gastritis was in Europoids in the antral mucosa 98,7%, in gastric corpus - 98,6%.
H. pylori dissemination rates were equal in Europoids: DD in antrum - 214,65 ± 8,75; in corpus - 179,78 ± 9,78 (p = 0,008). In Evenks with chronic gastritis prevalence determining *H. pylori* in antral mucosa was 98.5%, in gastric corpus - 98,4%. DD *H. pylori* in Mongoloids was in antrum 90,23 ± 4,02; in corpus - 95,15 ± 9,55.
Conclusion: In two different populations living in rural areas of Eastern Siberia, in patients with chronic gastritis in antrum and gastric corpus recorded similar *H. pylori* dissemination indicators.

Poster no.: P6.11
Iron deficiency anemia and nutritional status of children admitted in a digestive unit: a 3 year study

V. Hurduc, D. Plesca, M. Stefanescu, A. Zamfirescu and L. Bordei

Dr. Victor Gomoiu Clinical Children's Hospital, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Background: *H. pylori* infection has been reported to play a role in the development of iron deficiency anemia and poor nutritional status, both in adults and children.
Aim: To assess the relationship between *H. pylori* infection, iron and nutritional status in symptomatic *H. pylori* infected children.
Methods: We studied 232 consecutive symptomatic children (149 girls, age range 2–18 years) submitted for the first upper endoscopy (EGD) for various reasons, during the last 3 years (2007–2009). Socioeconomic status, medical, dietary and clinical data were analyzed. Weight, height, body mass index (BMI) for age and sex were recorded according to growth charts (CDC, 2000). *H. pylori* was confirmed by urease test and histological examination. Iron deficiency anemia was defined as a hemoglobin concentration < 11 g/dl, abnormal red cell indexed, serum iron < 60 mcg/dl and feritin < 12 ng/dl.
Results: Patients' nutritional status had the following repartition: 60 children (25,86%) were underweight, 130 (56,03%) had healthy weight, 27 (11,63%) were at risk of becoming overweight and 15 children (6,46%) were overweight. Of the 232 patients, 166 (71,55%) had documented *H. pylori* infection with a yearly decrease from 77,38% to 66,25% in 2009. Iron deficiency anemia was found most frequently in infected patients (31,32%) compared with uninfected ones (16,63%), with significant difference (p = 0,001) especially in adolescent girls, and was identified in both underweight and overweight infected children.
Conclusion: A statistically significant relationship was found between iron deficiency anemia, active *H. pylori* infection in children and poor nutritional status, coupled with socio-economic factors.

Poster no.: P6.12
Children with iron deficiency anemia: Celiac disease and *H. pylori*

T. Sabbi

Pediatric Unit - Belcolle Hospital, Rome, Italy

Background: *H. pylori* (Hp) causes one of the most widespread infections worldwide. This infection has been recognized as a cause of chronic gastritis, peptic ulcer, atrophic gastritis, gastric cancer, but it has a role in refractory iron deficiency anemia, delayed statural growth, dyspepsia and extradigestive diseases.
Aim: To analyzed the prevalence of celiac disease and Hp in patients with resistant iron deficiency anemia.
Patients and Methods: 20 patients (12 males; age range 6–18 years) with refractory iron deficiency anemia (not responding to iron therapy for 3 months). Blood sample was taken analysis of antibodies for celiac disease: antigliadin antibodies (AGA), antiendomysial antibodies (EMA) and antitissue Transglutaminase (tTg) antibodies. Fecal antigen and a (13) C-urea breath test (UBT) was done to all patients to diagnose Hp. All patients underwent to upper gastrointestinal endoscopy (to evaluate the presence of etiologies of resistant anemia) with antral (histological examination and culture for Hp) and duodenal biopsies.
Results: Hp infection was positive in 13 out of 20 patients (65%), while celiac disease in 5 out of 20 patients (25%), while in 2 patients endoscopy was positive for cardial erosions. In all positive Hp patients was made triple eradication therapy for 7 days (amoxicillin, clarithromicin and proton pump inhibitor) with iron supplementation with eradication of Hp infection and resolution of anemia. 2 patients with gastroesophageal reflux disease underwent to therapy with proton pump inhibitor while 5 patients with celiac disease are in gluten free diet rich in iron.
Conclusions: Celiac disease cause intestinal malabsorption in pediatric age with deficiency iron absorption and gluten free diet with iron supplementation can resolve the anemia. Eradication of Hp infection with concomitant iron therapy should correct the anemia.

Poster no.: P6.13
Pediatric helicobacter infections: differences between different ethnical groups

N. Dahhan[1,2], A. Kindermann[1], M. Ogtrop[3] and D. K. Bosman[1,3]

[1]Emma' s children hosptal/AMC., Amsterdam, Netherlands; [2]Saint lucas-andreas Hosptial, Amsterdam, Netherlands; [3]Onze Lieve Vrouwe Gasthuis, Amsterdam, Netherlands

In this study the differences between different ethnical paediatric groups infected with *H. pylori* (Hp) were studied. Outcome parameters used were among other antibiotic resistance, the presence of peptic ulcer diseases and the effect of therapy. Patients (n = 258) were indentified as Hp positive when both histology and culture were positive.
Peptic user disease was only found in both Surinam (4%) and Moroccan children (6%) whereas Dutch and Turkish children showed no peptic ulcer disease. Metronidazol resistance was remarkable higher in both children of Moroccan (21%) and Surinam origin (25%) then in dutch (5%) and turkisch (4%) children. Claritromycine resistance was only found in Dutch (16%) children. Triple therapy based on the antibiogram was in 73% effective in Moroccan children wheras it was effective in 48% of the dutch children.
These results demonstrate that with regard therapy and clinical presentation the ethnical background is an important factor in *H. pylori* infection in children. Both the presence of peptic ulcer disease and the antibiotic resistance pattern seems to be related to the ethnical background Although most children are of the so called 3th generation of migrants the pattern of antibiotic resistance of Hp in ethnical groups shows great similarities with the antibiotic resistance pattern of country of origin.

Poster no.: P6.14
Dynamics of *H. pylori* prevalence in asymptomatic children within 10 year period of time

D. Akmentina, S. Limeza, S. Remberga, I. Rumba-Rozenfelde, R. Seske, D. Senaratne and I. Daugule
University of Latvia, Riga, Latvia

Background and aims: The prevalence of *H. pylori (H. pylori)* infection is decreasing along with changes of socioeconomic situation. The aim of the study was to compare the prevalence of *H. pylori* in children in Latvia between 2000 and 2010 and to analyze the factors associated with the infection.
Methods: The patient sample included 130 children (aged 2 to 10 years) in kindergartens and primary care centres. The parents were asked to bring a stool sample of a child and to fill out a questionnaire (socioeconomic factors, demographic data, previous treatment with antibiotics, consumption of imported fruit). Presence of active *H. pylori* infection was detected by rapid stool antigen test (*Coris BioConcept, Belgium*). The data were compared to a study performed on 2000 *(H. pylori* detected by monoclonal stool antigen test). Statistical analysis: χ^2 test, *log* regression.
Results: The total prevalence of *H. pylori* infection was 8%(11/130) in 2010 compared to 18%(27/146) in 2000 ($p > 0.05$). In the univariate analysis *H. pylori* positivity was significantly associated with a lower parental educational level, lower socioeconomic status, less often consumption of imported fruit. In logistic regression analysis the investigated variables lost their strength.
Conclusions: Despite the changes of socioeconomic situation in Latvia the prevalence of *H. pylori* infection was only non-significantly lower in 2010 compared to 2000. However, the association of *H. pylori* positivity with lower socioeconomic status was more expressed in 2010 compared to 2000. Association of *H. pylori* positivity with consumption of imported fruit should be studied further.

Poster no.: P6.15
H. pylori Infection and Allergy in Children

S. Limeza, D. Akmentina, S. Remberga, I. Rumba-Rozenfelde, R. Seske and I. Daugule
University of Latvia, Riga, Latvia

Background and aims: Studies show a lower prevalence of *H. pylori (H. pylori)* infection among allergic children. However, the lower prevalence could be influenced by several other factors. The aim of the present study was to analyze the prevalence of *H. pylori* and associated risk factors in allergic children.
Methods: The study sample included 130 children (aged 2 to 10 years). Patient group (n = 42) included children with a diagnosed allergy. Control group (n = 88) consisted of children in kindergartens and primary care centres. The parents were asked to bring a stool sample of a child and to fill out a questionnaire (socioeconomic factors, demographic data, previous treatment with antibiotics). Presence of active *H. pylori* infection was detected by rapid stool antigen test (*Coris BioConcept, Belgium*). Statistical analysis: χ^2 test, *log* regression.
Results: In the univariate analysis *H. pylori* positivity was significantly lower in allergic children compared to children without allergy: 0% (0/42) vs. 12% (11/88); $p = 0.03$. *H. pylori* positivity was significantly associated with a lower parental education and markers of lower socioeconomic status. Previous antibacterial treatment did not show an association with *H. pylori* positivity. Allergy diagnosis did not show a significant association with parental education, previous antibacterial treatment and socioeconomic status. Logistic regression analysis could not be performed due to no *H. pylori* positive case in the allergy group.
Conclusions: Although lower *H. pylori* prevalence was observed in children with allergy in the univariate analysis, the interaction of other factors (previous treatment, higher living standards) should be studied further.

Poster no.: P6.16
Eradication of *H. pylori* modulates the progression of arteriosclerosis in healhty male smokers

T. Igarashi, T. Shimoyama, M. Matsuzaka, K. Danjo, S. Nakaji and S. Fukuda
Hirosaki university Graduate School of Medicine, Hirosaki, Japan

Background: *H. pylori* infection has been associated with the onset of ischemic heart disease and the promotion of arteriosclerosis. In Japanese elderly male smokers, infection of *H. pylori* increased brachial-ankle pulse-wave velocity (ba-PWV) score, which is an index for arteriosclerosis.
Aims: The aim of this study was to investigate whether eradication of *H. pylori* influences progression of arteriosclerosis.
Methods: Healthy subjects (>40 years old) who attended mass survey were examined *H. pylori* stool antigen and serum anti-*H. pylori* IgG antibodies. Subjects were defined as infected or uninfected when both examination were positive or negative. Subjects who have smoked until the examination were defined as smokers, and who don't have any smoking history as non-smokers. ba-PWV was measured non-invasively using an automatic device.
Results: Among *H. pylori*-infected subjects, 185 subjects were examined again in 2009. There were 51 *H. pylori*-negative subjects (eradicated group) and 131 *H. pylori*-positive subjects (non-eradicated group). Ba-PWV value was increased in both eradicated and non-eradicated groups after 4 years. In male subjects, ba-PWV value of non-eradicated group (n = 46) was increased significantly from 1686.5 ± 273.8 to 1732.2 ± 283.7 cm/s ($p < 0.05$). On the other side, in eradicated group males (n = 16), ba-PWV value was 1638.0 ± 405.9 in 2005 and 1663.3 ± 352.9 in 2009, respectively (NS). In male smokers, ba-PWV value of non-eradicated subjects also increased from 1484.3 ± 226.0 to 1602.1 ± 298.8 ($p < 0.05$). In contrast, ba-PWV of eradicated male smokers tended to decrease from 1545.8 ± 359.0 to 1477.0 ± 237.2.
Conclusion: Eradication of *H. pylori* would be beneficial to prevent the progression of arteriosclerosis in male smokers.

Poster no.: P6.17
H. pylori eradication changes course of reflux-esophagitis in the patients with gastroesophageal reflux disease (GERD)

A. A. Churikova and S. Khomeriki
Central Research Institute of Gastroenterology, Moscow, Russian Federation

Introduction: In recent years, much attention is paid to the study of GERD. Many issues of GERD, in particular, the communications of HP infection with reflux esophagitis, remain unsolved.
Objective: To study the influence of HP eradication on the course of reflux esophagitis (RE) in patients with GERD
Aims and methods: 73 (100%) HP-positive patients with GERD at the stage of reflux esophagitis, median age 39,1 ± 14,8. 33 (45%) of them male and 40 (55%) female, carried out eradication of HP. Before treatment and 6 months after the therapy were conducted: esophageal gastroduodenoscopy with biopsy of distal esophagus and antrum, 2-hour pH meter, the 5 methods detection of HP. A condition of mucosa was estimated by semi quantitative method with calculation of esophagopathic index (EPI) consisting of specific histological signs of esophagitis.
Results: Upon initial examination the value of a heartburn in points at GERD in the stage of RE was 3,4 ± 0,07. The pH of gastric corpus mucosa was 1.8 + 0.07, EPI - 2,33 ± 0,09. Eradication of HP was achieved in 45 (61.6%) patients (I group), HP eradication was not achieved in 28 (38,4%) patients (II group). After 6 months of observation heartburn was significantly more likely noted in patients with HP: In 89% of cases against 2% of I group, average values of heartburn in patients of group I was 1,1 ± 0,04, in group II - 2,5 ± 0.06 ($p < 0,05$). The pH of gastric corpus mucosa in patients of group I was 2,9 ± 0,012 vs 1,7 ± 0,012 in patients of group II ($p < 0,05$). The average value of EPI in group I decreased to 1,1 ± 0,06, while in II group has hardly changed - 2,24 ± 0,17 ($p < 0,05$). It is noted that the severity of heartburn in points had direct correlation with the EPI value ($rs=0,87; p < 0,01$).
Conclusion: The effective eradication of *H. pylori* in GERD at the stage of reflux esophagitis contributes to the normalization of acidogenic function of the stomach, which leads to the disappearance of inflammatory changes of the mucosa of the esophagus and the relief of clinical symptoms of RE.

Poster no.: P6.18
IgG and IgA-antibodies to Helicobacter pullorum, H bilis and *H. pylori* in sera and bile samples from patients with cholelithiasis

Å. Ljungh[1], I. Nilsson[1], A. Alvmark[2], F. Hoxha[1], V. Lönngren[1] and T. Wadström[1]
[1]Medical Microbiology, Lund University, Sweden; [2]Dept Surgery, Landskrona hospital, Landskrona, Sweden

Enterohepatic *Helicobacter* spp such as *H pullorum* and *H bilis* have been associated with hepatic infection in animals and might also be involved in the pathogenesis of chronic liver and intestinal diseases in humans. A total of 27 paired bile and serum samples collected from patients subjected to elective cholecystectomi were analyzed for IgG and IgA antibodies to cell surface proteins (CSP's) of *H pullorum, H bilis* and *H. pylori* by immunoblot (IB). Antibodies to *H pullorum* and *H bilis* were evaluated following pre-absorption of sera and bile samples with *H. pylori* sonicate (Vorobjova *et al* 2005).
Patients with IgG-antibodies to *H. pylori* were found in 11/27 (40%) sera, to *H pullorum* in 7/27 (25%) and to *H bilis* in 2/27 (7%) sera. With bile juice specimens *H. pylori* IgG-antibodies were found in 3/27 (11%) samples, to *H pullorum* in 2/27 (7%) and to *H bilis* in 2/27 (7%) bile samples. One bile sample showed IgG reactivity to all three species and IgA-antibodies to *H. pullorum*. Serum from this patient was negative for the enterohepatic strains but *H. pylori* positive. Three other patients with antibody reactivity in their bile samples for *H. pylori* (2 patients) and *H bilis* (one patient) were negative when corresponding sera were tested. Results of this study suggest the presence of a local immune response in the bile duct to *Helicobacter* spp in patients with biliary disease and possible co-infections.

Poster no.: P6.19
H. pylori infection and hepatic encephalopathy in cirrhotic patients: Facts or fictions

S. Naumovski - Mihalic[1], M. Katicic[1], V. Maricic[2], G. Cavric[1], B. Skurla[1], A. Mrzljak[1] and T. Filipec Kanizaj[1]
[1]Clinical Hospital Merkur, Zagreb, Croatia; [2]Pliva d.d., pharmaceutical industry, Zagreb, Croatia

Aim: of this study was to determine relationship between *H. pylori* (HP) infection and severity of hepatic encephalopathy (HE) in cirrhotic patients.
Patients and Methods: 80 patients (66M/14F) mean age 54. 3 yrs with cirrhosis of the liver were included in the study. The patients were divided into two groups. Group A: 40 patients with liver cirrhosis and HE and Group B:40 patients with liver cirrhosis without HE. All patients had upper gastroduodenal endoscopy and gastroduodenal pathology was identified. HP infection was confirmed by gastric histology. Patients were evaluated for biochemical tests, blood ammonia concentration, Child-Pugh class A, B and C, and active peptic ulcers.
Results: The incidence of HP infection was significantly higher in Group A:27 patients from 40 patients (67%) compared with group B:16 patients from 40 patients (40%). $P < 0.001$. In patients with encephalopathy HP infection was more among alcoholics (25 patients from 40 (62.5%) in comparison to non-alcoholics patients - 15 patients from 40 patients (37.5%). $P < 0.001$. The level of blood ammonia concentration was significantly higher in group A: 25 patients from 40 patients (62%) than in group B - 12 patients from 40 patients (30.0%). $P < 0.001$. In group A - 12 patients from 40 patients (30%) had peptic ulcer compared with group B- 5 patients from 40 patients (12.5%). $P < 0.001$.
Conclusion: Our data suggest that *H. pylori* infection is an important factor for inducing high blood ammonia concentration and hepatic encephalopathy in cirrhotic patients, especially in alcoholic patients. HP eradication may be useful for treatment

of HE. We found also that HP infection and liver cirrhosis are important factors associated with active peptic ulcer.

Poster no.: P6.20
H. pylori in gallbladder mucosa of patients with chronic calculous cholecystitis

G. Isaeva, L. Zaitseva, D. Volkov and R. Fakhrutdinov
Kazan State Medical University, Kazan, Russian Federation

Objective: In recent years the attention has been drawn to the possible association of *H. pylori* infection with biliary diseases.
Material and methods.
Using molecular method and immunohistochemistry we investigated gallbladder neck's mucosa specimens from 16 patients (12 - F, 4- M, mean age 56,2 years) with chronic calculous cholecystitis during cholecystomy. The detection of *H. pylori* DNA was performed by PCR using *H. pylori* -specific primers for *ureC* according to manufacturer's recommendation (Lytech, Russia). Gallbladder tissues were fixed in 10% formalin solution and then paraffin- deembedded sections were stained by haematoxylin-eosin. Immunohistochemical staining of same gallbladder sections was performed using avidin-biotin-peroxidase method according to manufacturer's recommendation (DakoCytomation, Denmark). As primary antibodies were used the polyclonal antibodies to *H. pylori* (BO471) with the system LSAB2 Kits. Sections were counterstained with Harris's haematoxylin.
Results: *H. pylori* DNA was detected in 1 (6,25%) sample. Histological examination showed pathomorphological signs of chronic cholecystitis: chronic inflammation, sclerolipomatosic transformation, local leukocytes infiltration, atrophy and cholesterosis. Microbial accumulation was observed inside the glands. No correlation was found between localization of bacteria and specific pathomorphological changes. Immunohistologically *H. pylori* was detected in five fields of vision in 1 sample as positive result and in 3 samples as doubtful result. Immunohistochemical observation demonstrated the presence curved s-shaped brawn bacteria mostly above the apical part of the gallbladder epithelial cells and in the mucous layer.
Conclusion: *H. pylori* may play the role in the pathogenesis of cholelithiasis, but these findings need to be confirmed in a larger study.

Poster no.: P6.21
Role of reactive oxygen metabolites and proinflammatory cytokines in *H. pylori* (*H. pylori*)-infected Mongolian gerbils exposed to aspirin (ASA)

T. Brzozowski, S. Kwiecien, R. Pajdo, D. Drozdowicz, A. Ptak-Belowska, S. Konturek and W. Pawlik
Department of Physiology, Jagiellonian University Medical College, Cracow, Poland

H. pylori and NSAID such as ASA are considered as two independent risk factors for the peptic ulcer disease but their interaction in the stomach remains controversial. We determined the effect of single and 5 day repetitive acidified aspirin (ASA; 5–50 mg/kg i.g.) treatment in the *H. pylori* infected Mongolian gerbils, which were inoculated with *H. pylori* strain (cagA+ vacA+, 5 x 106 CFU/ml i.g.) and 8 weeks later animals received acidified ASA (20 mg/kg i.g.) applied once (day 0) or for 5 days. At day 5 upon ASA treatment the histology, *H. pylori*-culture, gastric blood flow (GBF) assessed by H2-clearence technique, mucosal PGE2 generation, MPO activity, plasma levels and gene expression of IL-1β and TNF-α and MDA (lipid peroxidation) were evaluated. The gastric *H. pylori* infection was detected in all animals by histology and culture. ASA applied once produced gastric lesions which were exacerbated in *H. pylori*-infected gerbils followed by dramatic fall in the GBF, PGE2 generation, and the rise in MPO activity and MDA content. Five-day repetitive ASA treatment significantly reduced gastric lesions while decreasing MPO activity and MDA content. This adaptive response was reversed in *H. pylori*-infected gerbils and accompanied by the upregulation of mRNAs for IL-1β and TNF-α and their plasma levels, MDA content and MPO activity. We conclude that: 1) *H. pylori* acts in synergistic manner with ASA to aggravate acute gastric lesions induced by this NSAID, and 2) *H. pylori*-infection impairs ASA adaptation due to enhancement in lipid peroxidation, neutrophil-induced MPO activity and the overexpression and release of IL-1β and TNF-α.

Poster no.: P6.22
Upper Gastrointestinal Bleeding in Clopidogrel and Aspirin Users Receiving Concomitant Proton Pump Inhibitors

C. Kongchayanun[1], V. Mahachai[2] and R. Vilaichone[1]
[1]Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani, Thailand; [2]Division of Gastroenterology, Department of Medicine, Chulalongkon University Hospital, Bangkok, Thailand

Aims: Clopidogrel and ASA are wildly used in prevention of cardiovascular and embolic events. This case-control study was designed to evaluate the risk of UGIB in clopidogrel and aspirin users who continue receiving standard dose of PPI.
Methods: Data for clinical information and endoscopic findings were collected during January 2009 and November 2009 from patients who used clopidogrel and/or ASA and continue receiving standard dose of PPI. Clopidogrel or ASA user was defined as consumption of clopidogrel or ASA for at least 7 days preceding episode of bleeding. The UGIB was defined as overt bleeding or fall in baseline hematocrit ≥ 5 points within 24 hours of admission. Ulcer was defined at endoscope by breaking mucosa > 3 mm in diameter.
Results: Total of 175 patients (82 men and 93 women, mean age of 65. 3 years) were evaluated in this study including 54 patients (30.9%) with UGIB and 121 patients (69.1%) with dyspeptic symptoms. Male were significantly more common than female patients in bleeding group (61.1% vs 38.9%: P = 0.01). UGIB was significantly higher in current ASA (325 mg) plus clopidogrel user than non-user (16.7% vs 5.8%; P = 0.02). The multivariable model suggested that probability of UGIB event increased with current ASA (325 mg) plus clopi-

dogrel use (OR = 2.3, 95%CI = 1.2–3.9) even in patients receiving concomitant PPI.
Summary: Risk of UGIB events still occur in ASA and clopidogrel users in spite of receiving concomitant PPI. Lower dose of ASA and carefully monitoring of UGIB should be considered in combined ASA and clopidogrel users.

Poster no.: P6.23
Effect of Pretreatment with *Lactobacillus gasseri* OLL2716 on First Line Eradication

A. Takagi[1], H. Nakaminami[2], N. Noguchi[2], M. Sasatsu[2], R. Deguchi[1], T. Suzuki[1], M. Masashi[1] and T. Mine[1]

[1]Tokai University, Isehara, Japan; [2]Tokyo University of Pharmacy and Life Science, Hachioji, Japan

Background: *Lactobacillus gasseri* can suppress both clarithromycin-susceptible and -resistant strains of *H. pylori in vitro*.
Aim: To determine the effect of pretreatment with *L. gasseri* contained in yogurt on *H. pylori* eradication, we conducted a clinical trial in patients with *H. pylori* infection.
Methods: Two hundred thirty patients were randomized either to 1-week of triple therapy of rabeprazole (10 mg bid), amoxicillin (750 mg bid), clarithromycin (200 mg bid) or triple therapy with L. gasseri contained in yogurt. In the yogurt-plus-triple therapy groups, yogurt containing *L. gasseri* OLL2716 (90 g) was consumed twice daily for 4 weeks (3 weeks pretreatment followed by 1 week during eradication therapy). Clarithromycin resistance was determined by detection of a mutation in the 23S rRNA using nested PCR and direct sequencing of DNA from pretreatment feces. *H. pylori* eradication was diagnosed by UBT and stool antigen test after 8 weeks of eradication. The ethics committee of Tokai University Hospital approved the protocol, and written informed consent was obtained from all patients.
Results: Rate of infection with a CAM-resistant strain of *H. pylori* was 20.4%. Overall eradication (intention to treat/per protocol) was 69.7%/74.7% for triple therapy, and 83%/85.9% for triple therapy with yogurt ($p = 0.018$). Eradication in primary clarithromycin-resistant strains was higher in the group receiving yogurt-plus-triple therapy than that receiving triple therapy alone (38.5%vs. 20%: $p = 0.212$).
Conclusion: This study confirmed that the major cause of treatment failure is resistance to clarithromycin. Consuming yogurt containing *L. gasseri* for 4 weeks improved the efficacy of first-line triple therapy.

Poster no.: P6.24
Probiotic bacteria-induced gastroprotection. Evidence for the involvement of prostaglandins and nitric oxide (NO)

T. Brzozowski, A. Targosz, Z. Sliwowski, G. Krzysiek-Maczka, M. Pawlik, D. Drozdowicz, S. Konturek and W. Pawlik

Department of Physiology, Jagiellonian University Medical College, Cracow, Poland

Cultured food or milk derived products such as probiotics exhibit therapeutic properties and were recently shown to enhance the rate of *H. pylori* eradication. It is unknown whether probiotics could influence the formation acute gastric mucosal injury and exhibit gastroprotection. We studied the effect either of vehicle (saline), Escherichia coli Nissle 1917, (E.coliN), Lacidofil containing alive L. rhamnosus and L. acidophillus applied once or administered twice daily for 6 days on the formation of gastric lesions induced by i.g. administration of necrotizing substances such as 75% ethanol (ETH), 200 mM taurocholate (TC) and 25% NaCl. For comparison, heat-killed Lactobacilli strains were used. Exposure of gastric mucosa to ETH, TC and NaCl induced widespread hemorrhagic gastric erosions and significantly decreased the GBF accompanied by the rise in the plasma IL-1β and TNFα levels. Lacidofil or E.coliN (101–108/rat i.g.) dose-dependently inhibited gastric acid secretion and attenuated ETH-, TC- and NaCl-induced gastric lesions and raised the GBF, mucosal PGE2 concentration and plasma gastrin levels. Heat-killed bacteria failed to significantly affect gastric acid secretion, gastric lesions induced by ETH, TC and NaCl and accompanying fall in the GBF. Indomethacin, rofecoxib, a COX-2 selective inhibitor and L-NNA, the NO-synthase inhibitor, completely abolished E.coliN and Lacidofil-induced protection, that was restored by concurrent treatment with PGE2 analog (10 μ/kg i.p.) or L-arginine (200 mg/kg i.g.). We conclude that only alive probiotic bacteria affords gastroprotective action due to its antisecretory and antioxidizing activities and hyperemia mediated by COX-PG and NO-NOS systems and gastrin that exerts a trophic effect.

P7 Premalignant lesions & gastric cancer

Poster no.: P7.01
C677T polymorphism of 5,10-methylene-tetralydrofolate reductase MTHFR gene and the risk of gastric cancer among Iranian population

S. Jahangiri[1], Y. Talebkhan[1], N. Mohajerani[1], M. Bababeik[1], A. Oghalaie[1], A. Nahvijou[2], M. Mohagheghi[2], M. Eshagh Hosseini[3] and M. Mohammadi[1]

[1]Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of; [2]Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; [3]Endoscopy Unit, Amiralam Hospital, Tehran, Iran, Islamic Republic of

Introduction: The rate of gastric cancer in Iran has been rising in recent years as the leading cause of cancer related death in Iranian males. Recently analysis of host's genetic susceptibility to cancer development has come to the research forefront. Amongst these markers, the role of C677T single nucleotide polymorphism of the MTHFR gene as a risk factor for different gastrointestinal diseases and in particular gastric cancer has been evaluated in different populations.

Materials and methods: Two hundred and nine (119 cardia, 90 non-cardia) gastric cancer patients and 40 duodenal ulcer patients were evaluated against 248 non ulcer endoscopy patients as hospital based controls. Single nucleotide polymorphism (C677T) of MTHFR gene was assessed by polymerase chain reaction-restriction fragment length polymorphism.

Results: Performing multiple logistic regression analysis, assigning age, gender, ethnicity, smoking and Hp sero-status as potential confounders, identified MTHFR 677 TT genotype as a risk factor (OR = 3. 089, CI 95% = 1.085–8.793, P = 0.035) for duodenal ulcer development in an Hp dependent manner, when compared to hospital-based controls.

On the other hand, patients carrying the T allele were at additional risk for developing non-cardia gastric cancer (OR = 2.685, CI 95% = 1.439–5.008, P = 0.002) and diffuse type gastric cancer (OR = 1.930, CI 95% (1.005–3.708), P = 0.048). In addition a significant correlation was observed between TT genotype and any degree of gastric atrophy (5.269 (1.076–25.792), p = 0.040). In our analysis, TT genotype was also correlated with the presence of any PML in the stomach in an Hp dependent manner (OR: 3.529, CI 95% (0.989–12.591) p = 0.052), although the p-value includes a borderline amount.

Conclusion: Our study demonstrated that MTHFR 677 TT genotype and T carriage in general increase the risk for development of duodenal ulcers, atrophy and both non-cardia and diffuse type gastric cancer, respectively.

Poster no.: P7.02
Analysis of the influence of *H. pylori* infection on MLH1 promoter methylation status and microsatellite instability

M. Alvarez, M. Martini, J. Santos, N. Maniezzo, J. Pedrazzoli Jr and M. L. Ribeiro

Clinical Pharmacology and Gastroenterology Unit, Sao Francisco University Medical School, Bragança P, Braganca Paulista, Brazil

It is well known that many critical genes are silenced through DNA methylation during cancer development. Recent studies have shown that silencing of certain DNA repair genes through the process of DNA methylation might be related to the occurrence microsatellite instability during cancer development. It has been suggested that *H. pylori* may increase levels of DNA hyper-methylation in gastric tissue. The aim of this study is to analyse the effects of *H. pylori* infection on methylation of *MHL1* and the relationship of this modification with microsatellite instability. This study included 27 uninfected individuals, 152 individuals with chronic gastritis and positive for *H. pylori* infection, and 99 individuals with gastric cancer for a total of 252 individual patients. Microsatellite instability (MSI) was analysed using 4 markers (BAT-25, BAT-26, D2S123 and D17S250), and methylation of the promoter region of *MHL1* was analysed with MSP-PCR. MSI was detected in 53% of the chronic gastritis *H. pylori*-positive patients. Of these patients, 38% were MSI-Low and 15% MSI-High. Sixty-four percent of individuals with gastric cancer were positive for MSI (38% MSI-L and 25% MSI-H). We observed hyper-methylation of the promoter region of *MHL1* in 36% of gastric cancer patients and in 12% of the chronic gastritis *H. pylori*-positive patients. We observed hyper-methylation of the *MHL1* promoter, as well as an increase in the level of MSI, in gastric cancer patients.

Poster no.: P7.03
Is *H. pylori* infection a necessary cause of non-cardia gastric cancer? Evidence from the Eurgast-EPIC study

C. A. Gonzalez[1], F. Mégraud[2], L. Lujan[1], A. Buissonniere[2], A. Agudo[1] and E. J. Duell[1]

[1]Catalan Institute of Oncology, Barcelona, Spain; [2]Inserm, Bordeaux, France

H. pylori is a established cause of non cardia gastric cancer (NC GC), but whether it is a necessary cause remains controversial. *H. pylori* infection can clear from the mucosa during the progression of atrophy, there could be substantial under-detection of infection. Antibodies detected by Western blot are known to persist longer after the loss of the infection compared to those detected by enzyme-linked immunosorbent assay (ELISA). We report here *H. pylori* infection status in 76 NC GC cases (mean

age 62 ys; mean follow-up 6.6 ys), with and without severe chronic atrophic gastritis (SCAG) determined by plasma pepsinogen I, from the prospective Eurogast- EPIC study. *H. pylori* infection was assayed by ELISA (Pyloriset EIA-GIII®) and by immunoblot (HELICOBLOT 2.1); pepsinogen I by ELISA (Biohit). 62 cases (81.6%) were *H. pylori* positive for all assays, 8 cases (10.5%) were negative for *H. pylori* by ELISA but positive by Werstern blot, while 6 cases (7.9%) (4 with SCAG and 2 without SCAG) were negative by both ELISA and immunoblot. NC GC cases with SCAG were older than cases without SCAG. Negative cases according to ELISA is reduced to less than a half according to immunoblot. However, a small fraction of cases truly negative may exists, even in subjects without evidence of SCAG, but given that sensitivity of immunoblot is less than 100% for previous infection, is not yet possible to rule out the hypothesis that *H. pylori* infection is a necessary cause of GC, and further evidence is needed.

Poster no.: P7.04
Levels of serum antibodies (SA) to whole *H. pylori* (HP) antigens and prognosis of gastric cancer (GC) and patients' survival

D. Marrelli[1], N. Figura[2], M. Campagna[2], L. Garosi[1], V. Pascale[1], B. Franci[2], B. Lucani[2], C. Vindigni[1] and F. Roviello[1]

[1]Dipartimento di Patologia Umana e Oncologia, Sezione di Chirurgia Oncologica, University of Siena, Siena, Italy; [2]Department of Internal Medicine, University of Siena, Siena, Italy

Introduction: We have recently reported an increased survival of GC patients still infected by HP, compared to uninfected cases. Now, we aim at evaluating the potential correlations between the levels of anti-HP SA and GC prognosis and patients' survival according to GC histological type.
Methods: Serum samples from patients operated on between 1990 and 2005 were retrieved in the tissue and serum bank of our department; 173 patients with non-cardia GC, who still had HP DNA in gastric non-neoplastic mucosa (as determined by PCR), were included in this study. We determined anti-HP SA by ELISA using commercial products and expressed results in arbitrary units/ml (U/ml). The correlation between SA levels and clinical outcome was statistically analysed, considering death for cancer as the end-point. Levels of SA in subgroups were expressed as median and interquartile range (IQR).
Results: We observed a trend to increased anti-HP SA levels in patients with distant metastases at the time of surgery, respect to patients without metastases. After radical surgery, patients who survived to cancer had increased anti-HP SA levels, respect to patients who died (median 21 U/ml, IQR 8-82 vs. 12 U/ml, IQR 7–59). Differences in survival between serum HP-positive and HP-negative cases were significant in patients with Laurén diffuse histotype (5-yr survival rates: 65% in positive cases vs. 37% in negative cases, $p < 0.05$), while in patients with the intestinal histotype were not significant.
Conclusions: We suggest that immunological response to HP infection may affect prognosis of GC and survival in patients with diffuse GC.

Poster no.: P7.05
H. pylori-induced ERK1/2 activation depends on histological finding from patients with gastric cancer in AGS gastric epithelial cells

A. Ishiguro[1], T. Shimoyama[1], S. Fukuda[1], P. A. Robinson[2] and J. E. Crabtree[2]

[1]Hirosaki University, Hirosaki, Japan; [2]Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds, United Kingdom

Introduction: *H. pylori* activates extracellular-signal regulated kinases 1 and 2 (ERK1/2) in gastric epithelial cells, in part, following transactivation of EGFR or CagA. Identification of bacterial virulence factors contributing to ERK1/2 activation will provide insight into the bacterial mechanisms involved in the increased risk of carcinogenesis following *H. pylori* infection.
Methods: *H. pylori* strains were isolated from patients with intestinal cancer (N = 29), diffuse cancer (N = 11) and chronic gastritis (N = 29). Clinical strains were co-cultured with AGS gastric epithelial cells for 20–180 mins. ERK1/2 activation was quantified by 'In-Cell Western' (ICW) assay and phosphorylated ERK1/2 and total ERK levels were quantified by a Licor 'Odyssey' scanner and 'Odyssey' v1.2 software. Clinical strains were analyzed for detection of *cag*A by using PCR. Serum samples were also obtained to exam VacA seropositivity. Antibody to VacA was measured using Recombinant Immunoblot Assay and the cut-off for positivity was determined using sera of known *H. pylori* status.
Results: Analysis of ERK1/2 activation induced by 69 *H. pylori* strains showed marked variability. Strains isolated from diffuse cancer induced significantly reduced ($p < 0.05$) pERK compared to strains from intestinal cancer at 90 min. Moreover, in *cag*A positive strains, strains of diffuse cancer induced significantly lower ($p < 0.05$) ERK phosphorylation than intestinal cancer at 45, 90 and 180 min. Levels of pERK showed no difference between VacA seropositive and seronegative strains.
Conclusion: *H. pylori*-induced ERK1/2 activation is not associated with the recognition of VacA by host. In terms of *cag*A status, individual clinical strain related differences in ERK1/2 activation are evident.

Poster no.: P7.06
H. pylori (HP) or EBV infection and p53 protein expression in gastric carcinoma (GC)

A. Szkaradkiewicz, I. Chudzicka-Strugała, T. Karpiński, E. Andrzejewska and P. Majewski

University of Medical Sciences in Poznan, Poznan, Poland

Objectives: In etiopathogenesis of gastric carcinoma a significant role is played not only by HP, but also Epstein-Barr virus. It remains unclear whether in GC p53-abnormalities are dependent on presence of HP or EBV infection. Therefore present study aimed at analysis of incidence of HP manifestation or EBV infection and examination of p53 expression in GC.
Methods: The studies were performed on 48 unselected GC patients (12 females, 36 males, 41–79 years of age). In all

patients the material for studies involved routinely intraoperatively sampled fragments of tumour tissue. Cancer diagnosis (adenocarcinoma) was verified by analysis of hematoxylin and eosin stained preparations. In the obtained tissue material, following tests were performed: determination of HP-presence (by plating fragments of gastric mucosa on enriched Columbia-agar, followed by incubation in microaerophilic conditions for 4–6 days at 37°C, and examination using Giemsa staining) and EBV DNA product was detected in form of untranslated RNA (EBER1 and EBER2) particles using in situ hybridization (ISH Detection kit; DakoCytomation). For detection of *cagA* gene the diagnostic kit of PCR-HP (DNA Gdansk) was used. Presence of PCR reaction of product of 445 base pairs in size was accepted as positive test result. Expression of p53 protein was analysed using immunohistochemistry. Differences in frequencies of p53 positive results were compared with Fisher's exact test.
Results: Among the examined GC patients 9 (18. 75%) proved to be EBV-positive (group 1). Group 2 included 15 (31.25%) with HP infection (presence of *cagA* gene detected in 14 isolated strains of the bacteria). Group 3 included 24 (50%) (neither EBV infection nor HP infection was detected). In group 1 in 6 (66.6%) cases nuclear expression of p53 protein was noted in most tumour cells. In group 2 expression of p53 was demonstrated in 9 (60%) cases, while in group 3 included 14 cases (77.7%). Expression of p53 in patients of groups 1 and 2 was not significantly more frequent than group 3 ($p > 0.05$).
Conclusion: The results permit to conclude that HP infection or EBV infection does not affect the p53 pattern in GC cells.

Poster no.: P7.07
Indexes of surgical quality in gastric cancer surgery: experience of Latvia Oncology Center

A. Sivins[1,2], G. Ancans[1,3], C. Pedrazzani[4], G. Moisejevs[3], V. Krumins[1], V. Boka[1,3], F. Roviello[5] and M. Leja[1,2]

[1]Riga East University Hospital, Latvia Oncology Center, Riga, Latvia; [2]University of Latvia, Riga, Latvia; [3]Riga Stradins University, Riga, Latvia; [4]Department of Human Pathology and Oncology, Unit of Surgical Oncology, University of Siena, Siena, Italy; [5]Department of Human Pathology and Oncology, Unit of Surgical Oncology, University of Siena, Siena, Italy

Background: The proportion of postoperative complications (**PC**), splenectomies (**SP**) and blood transfusions (**BT**) have been described among the indexes of surgical quality in gastric cancer surgery. The aim of this study was to analyze the effect of **PC**, **SP** and **BT** on short- and long-term results of a large cohort of gastric cancer patients.
Methods: Retrospectively collected data from 479 patients who underwent R0 gastrectomy in Latvia Oncology Center from January 1999 to December 2005 were analyzed statistically.
Results: **PC** were more frequently observed in males (18% vs. 9%; P = 0.005), patients aged ≥68 years (20% vs. 8%; P < 0.001), ASA 3–4 (20% vs. 10%; P = 0.004), tumors requiring a proximal or total gastrectomy (24% vs. 17% vs. 11%; P = 0.089). **SP** was performed in 33 patients (7%). **SP** was carried out more frequently in tumors infiltrating the serosa (9% vs. 4%; P = 0.043) and treated by total or proximal gastrectomy (13% vs. 9.5% vs. 2%; P < 0.001). **BT** were required in 39 patients (8%). **BT** correlated with male gender (12% vs. 3%; P < 0.001), age ≥68 years (12% vs. 5%; P = 0.007), ASA 3–4 (13% vs. 5%; P = 0.002), proximal or total gastrectomy (19% vs. 13% vs. 4%; P = 0.001). Considering overall long-term results, patients **without PC** showed a better 5-year survival rate (53% vs. 34%; P = 0.001), as patients **without BT** (52.5% vs. 28%; P < 0.001) and **without SP** (52% vs. 36%; P = 0.025).
Conclusions: After excluding postoperative deaths from survival analysis, **SP**, **BT** and **PC** did not affect long-term prognosis.

Poster no.: P7.08
Risk factors and gastric cancer incidence in Russian population

V. V. Tsukanov[1], N. N. Butorin[2], E. V. Kasparov[1], A. S. Maadi[3] and O. S. Amelchugova[1]

[1]State Scientific Medical Research Institute for Northern Problems of Siberian Division of Russian Ac, Krasnoyarsk, Russian Federation; [2]Khakassian Republic hospital, Abakan, Russian Federation; [3]Republican Hospital [1] 1 of Tyva, Kyzyl, Russian Federation

Aim: To analyze the relationship of risk factors for gastric cancer incidence and atrophic gastritis in population of Russian Federation.
Methods: We analyzed the reported data on the prevalence of risk factors and incidence of gastric cancer among the population of various regions of Russia. Performed large epidemiological study of atrophic gastritis in adults in Tyva, Khakassia and Evenkia. Upper digestive tract endoscopy and identification of *H. pylori* were detected in 3494 patients. Morphological study was carried in 759 patients.
Results: The gastric cancer incidence among the Russian population varies in different regions of about 30 per 100,000, reaching a maximum (50–60 per 100000) in Mongoloids in the north of European part of Russia, in Tuva and Buryatia (Siberia). The prevalence of *H. pylori* in an urban and rural Russia varies around 80% -90%. Smoking tobacco is about 80% of men of the country. Systematic use salted fish and pickled vegetables, strong alcohol 50% of the population of Russia. The prevalence of atrophic gastritis in different ethnic groups of Siberia correlated with gastric cancer.
Conclusion: Relationship of high prevalence of risk factors and high incidence of gastric cancer in the population of the Russian Federation was registered.

Poster no.: P7.09
The special features of gastric mucosal inflammation in patient with gastric cancer infected by *H. pylori*

M. Senchukova, A. Stadnicov, N. Shevlyuk and D. Bokov

Orenburg State Medical Academy, Orenburg, Russian Federation

The gastric mucosal inflammation in patient with gastric cancer (GC) can be connected with *H. pylori* (HP) infection. We have

studied special features of the gastric inflammatory infiltration depending on a degree of its HP colonization at 32 patients with a GC. HP infection was defined by histological and by rapid urease test. All patients were divided into 2 groups. The first group (n = 18) included patients with a high and middle degree of HP colonization of a gastric mucosa, the second group (n = 14) - the patients with a low degree of HP colonization and not infected patients. The level of mononuclear cell infiltration of the gastric mucosa was 16,04 ± 1,57 cells on a standard unit of the area (SUA) in the first group and 15,86 ± 2,45 cells on SUA in the second group (p = 0,8). The quantity of lymphoid follicles were 0,58 ± 0,14 and 0,50 ± 0,20 on SUA in the first and second groups respectively. It is necessary to note, that 8 from 32 patients before operation have received HP eradication therapy in connection with the preliminary diagnosis of a gastritis or a stomach ulcer. The decrease gastric mucosal infiltration (14,93 ± 0,8 and 17,18 ± 0,92 cells on SUA respectively, p = 0,05) and quantities of the lymphoid follicles (0,44 ± 0,18 and 0,65 ± 0,16 on SUA respectively, p = 0,1) is noted in this group of patients. Thus, it is not revealed dependences a expressiveness inflammatory infiltration of the gastric mucosa from a degree of HP contamination mucosa. However, carrying out before operation antibacterial therapy authentically reduced a level of gastric mucosal infiltration.

Poster no.: P7.10
The phenotype lymphocytes of patients with *H. pylori*-associated the gastric cancer

O. V. Shtygasheva[1], E. S. Ageeva[1], M. O. Maksimov[2] and G. N. Strizhakov[1]

[1]Khakas State University, Abakan, Russian Federation;
[2]Oncological clinic, Abakan, Russian Federation

Infection Helicobacter (HP) is one of the possible reasons of the Gastric Cancer (GC).

Aim of investigation: to study the level of CD3, CD4 and CD8 cells of peripheral blood in patients with HP-associated GC.

Objects of investigation: patients with established diagnosis HP-associated GC, at the age of 49–74 years old.

Methods: a gastric biopsy sampling was assessed according to Sydney classification. By the means of cytofluorimetric method CD3, CD4 and CD8 cells were estimated.

Results: among patients with HP-associated GC absolute quantity of peripheral blood lymphocytes was observed (2,52 ± 1,8) x 10^9/l, in control group - (2,28 ± 1,4) x 10^9/l. Relative quantity lymphocytes - (38,4 ± 3,8)% and (34,3 ± 4,1)% accordingly.

In patients with GC there was a CD3 cells reduction (1,39 ± 0,87) x $10^9/\mu$l in comparison with the control group (1,55 ± 0,21) x $10^9/\mu$l. The similar tendency of reduction was observed in case with CD4 cells too.

CD4 cells in patients with GC were (0,82 ± 0,56) x $10^9/\mu$l and (0,88 ± 0,09) x $10^9/\mu$l in control group. Rather CD8 cells had original reduction in comparison with the control group which has shown (0,46 ± 0,28) x $10^9/\mu$l in patients with GC and (0,61 ± 0,14) x $10^9/\mu$l in control group. At the same time in patients with GC there was an increase of helper-suppressor cell ratio (CD4/CD8) in comparison with the control group (1,78 ± 0,47 and 1,44 ± 0,46). Thus, patients with GC had an immune deficiency which was characterized by the lack of CD3, CD4 and CD8 cells.

Poster no.: P7.11
Comparison of the long-term treatment results of the gastric cancer patients received and not received antibacterial agents before the radical operation

M. Senchukova and A. Stadnicov

Orenburg State Medical Academy, Orenburg, Russian Federation

Considering, that the some patients with a gastric cancer (GC) receive before operation Helicobacter eradication therapy in connection with the preliminary diagnosis of a gastritis or a stomach ulcer we decided to analyses the long-term treatment results of the patients with GC depending on presence in the anamnesis of the antibacterial therapy. 81 patients with GC after ragical surgery were divided into two groups. Patients of the first group (n = 37) received before operation antibacterial treatment (not less two antibiotics during not less seven days). Patients of the second group (n = 44) did not receive it. We established, that management of the eradication therapy at a pre-hospital period considerably improved the long-term treatment results of the patients with GC which had metastasises in regional lymph nodes (RLN). Relapse-free survival was 43.1 months and overall survival was 43,6 months at the patients of the first group (n = 14), compared with 23,2 months and 16,4 months at the patients of the second group (n = 22, p = 0,025 and p = 0,017 respectively). 1-year overall and relapse-free survival rate were 100% and 85,7% in first group, compared 81,8% and 54,5% in second group (p = 0,25 and p = 0,11 respectively); 2-year - 85,7% and 64,2%, compared 36,4 and 18,2% (p = 0,01 and p = 0,11) and 3-year - 64,2% and 57,1%, compared 18,2% and 13,6% respectively (p = 0,014 and p = 0,017). At absence of metastasises in lymph nodes the differences were not observed between the 2 groups. The further researches are necessary for specification of a role of antibacterial agents in treatment invasive GC.

Poster no.: P7.12
H. pylori infection in gastric cancer patient in Orenburg region of Russian Federation

M. Senchukova

Orenburg State Medical Academy, Orenburg, Russian Federation

We studied specific features of *H. pylori* infection by rapid urease test and histological method in 74 gastric cancer patients who had undergone surgery in Orenburg Regional Cancer Clinic. Urease activity was studied in gastric mucosa, omentum and lymph nodes by HELPIL test (AMA, Russia). Rate of *H. pylori* positivity among gastric cancer patients in the Orenburg region

was very high (96%). The high degree urease activity in gastric mucosa has been revealed at 13 patients (17,6%), the average degree - at 32 (43,2%), the low degree - at 17 (22,9%). The HELPIL test (HT) was negative at 12 patients (16,3%). Alongside with gastric mucosa, urease activity was detected in the omentum and lymph nodes without metastasis. From 12 patients with negative result of the HT in mucous stomach at 9 patients HT was positive in an omentum and 8 from them received before operation eradication therapy. Urease activity in a tumour was usually below, than in located near to it gastric mucosa. 16 from 17 lymph nodes without metastasises had positive result of the test on the ureqse activity whereas all 17 lymph nodes with metastasises had negative result of it. It was established, that the high degree *H. pylori* colonization of the serous tunic of a stomach considerably increases risk of development of complications in the early postoperative period. Further study is needed to elucidate reasons for positive rapid urease tests in the omentum and lymph nodes.

Poster no.: P7.13
Usefulness of GastroPanel for screening of atrophic gastritis in patients with autoimmune thyroid disease

F. Di Mario

University of Parma, Clinical Science, Chair Of Gastroenterology, Parma, Italy

A serological panel (GastropanelTM) gives information of gastric atrophic damage. Atrophic gastritis could be related to autoimmune thyroid diseases.

Aims: To evaluate the presence of atrophic gastritis by using GastropanelTM in patients affected by autoimmune thyroid disease.

159 patients (141 F, range 44–67), 115 with Hashimoto and 44 with Graves disease were submitted to clinical examination, validated questionnaire for upper gastrointestinal symptoms. Blood sample was performed to evaluate: gastrin-17, pepsinogens I, II, *H. pylori* antibodies, FT3, FT4, TSH, APCA, Anti-TPO, Anti-TG, anti-TSH-receptor antibodies. Patients with levels ≥100 U/L of one antithyroid antibody at least were considered to have a high thyroid autoimmunity. In patients with serologically diagnosed gastric atrophy an EGDS was performed (Sydney System). Association between AITD and other autoimmune diseases was assessed. Using Gastropanel, the gastric mucosa status was classified as follows: normal gastric mucosa (57%), non atrophic gastritis (18%) and atrophic gastritis (16%). Biopsies (18/26 patients with serologically diagnosed gastric atrophy) confirmed such diagnosis in all patients. Upper gastrointestinal symptoms weren't so different among groups. Patients with gastric atrophy were significantly more associated with APCAb than the others (68% vs 32%, $p < 0,0001$). Patients with high thyroid autoimmunity presented gastric atrophy in 23% of cases, while only 2% with low thyroid autoimmunity showed gastric alterations ($p < 0,001$).Gastric atrophy was more frequent in patients with other autoimmune diseases. The majority of patients with AITD doesn't complain upper GI symptoms. Gastropanel could be a useful, non invasive tool for management of patients with autoimmune thyroid diseases to look for the best candidate for EGDS.

Poster no.: P7.14
Premalignant gastric lesions in patients with gastric MALT lymphoma and metachronous gastric carcinoma: a case-control study

C. M. den Hoed[1], L. G. Capelle[1], K. Biermann[2], M. K. Casparie[3] and E. J. Kuipers[1]

[1]Erasmus MC University Medical Center, department of Gastroenterology and Hepatology, Rotterdam, Netherlands; [2]Erasmus MC University Medical Center, department of Pathology, Rotterdam, Netherlands; [3]Foundation PALGA, Utrecht, Netherlands

Patients with low grade gastric MALT lymphoma (gMALT) and diffuse large B-cell lymphoma (DLBCL), have an increased risk of developing gastric carcinoma (GC). Identifying gMALT/DLBCL patients at high GC risk may allow early endoscopic intervention; lead to improved survival and prognosis.

Can we identify premalignant gastric lesions (PM): atrophic gastritis (AG), intestinal metaplasia (IM) and dysplasia (DYS), in gMALT/DLBCL patients and demonstrate whether these lesions are more severe in gMALT/DLBCL patients with subsequent GC?

Patients with a first diagnosis of gMALT/DLBCL 1991–2006 in a nationwide histopathology registry (PALGA); Cases: patients with a diagnosis of gMALT/DLBCL and subsequent GC. Controls: no GC during follow-up (FU), matched by age and FU. Histopathology evaluated by a pathologist, blinded for case/control status.

8 cases (M/F 3/5) and 31 controls (M/F 19/12), mean age 60 yrs (18–86 yrs).Cases with gMALT developed GC within 6.2 yrs, controls had a mean FU of 5.2 yrs. DLBCL patients GC after a mean of 3.6 yrs, controls had a mean FU of 5.7 yrs. Cases gMALT/DLBCL 75% PM; 1 AG, 3 IM, and 2 DYS as most severe diagnosis. 67% of controls PM; 7 AG, 11 IM and 3 DYS ($p = 0.638$). 10 controls (32%) demonstrated progression of PM after a mean of 5.3 years.

The majority of patients with gMALT have premalignant gastric lesions. No specific parameters which enable early identification of those likely to progress to cancer. The prevalence of severe PM (DYS) is high in both groups of patients, warranting careful surveillance of PM in gMALT/DLBCL patients.

Poster no.: P7.15
Gastric cancer and *H. pylori* infection in Thailand

V. Mahachai[1], T. Khuhaprema[2], N. Phadet[3], L. Comson[4] and R. Vilaichone[5]

[1]Division of Gastroenterology, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand; [2]Director, National Cancer Institute,, Bangkok,, Thailand; [3]Division of Gastroenterology, Department of Medicine, Surin Hospital, Surin, Thailand; [4]Division of Gastroenterology, Department of Medicine, Chonburi Hospital, Chonburi, Thailand; [5]Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani, Thailand

Background and aims: Gastric adenocarcinoma is the second leading cause of cancer death worldwide. This study was

designed to evaluate the clinical, endoscopic findings, pathological features and molecular study of *H. pylori* infection in Thai gastric adenocarcinoma patients.
Methods: Clinical information, endoscopic findings, histological features and *H. pylori* status were collected from all gastric adenocarcinoma patients during June 2000-May 2010. *H. pylori* isolates were genotyped by PCR based on cagA, cag right end junction and vacA genotypes.
Results: Total of 117 gastric adenocarcinoma patients were enrolled in this study. Common presenting symptoms were dyspepsia (70%) and weight loss (64%). Mean duration of symptoms was 107 days. All gastric adenocarcinoma patients were in advanced stage. Overall prevalence of *H. pylori* infection was 83.6%. There was no difference of *H. pylori* infection in diffuse type and intestinal type (80% vs. 84%; $P > 0.05$). East Asian genotype (cagA 1a, vacA s1c and/or vacA m1b) was highly prevalent in Thai gastric adenocarcinoma patients (65%). East Asian genotype was more common in gastric adenocarcinoma age ≤ 50 years than patients age > 50 (74% vs 43%: $P < 0.01$) and more common in patients from city than those from rural area (79% vs 50%; $P < 0.05$).
Conclusion: Gastric adenocarcinoma in Thailand usually presents in the advanced stage with a 5-year survival less than 15%. *H. pylori* infection was highly prevalent and associated with both intestinal type and diffuse type. East Asian genotype might be regarded as an important risk factor of gastric adenocarcinoma in Thailand.

Poster no.: P7.16
H. pylori does not affect the expression of microsatellite instability and P53 in early gastric cancer treated by endoscopic submucosal dissection

S. Choi[1], J. Cho[2] and W. Cho[2]
[1]Dong-A University College of Medicine, Busan, Korea, Republic of; [2]Soonchunhyang University, Seoul, Korea, Republic of

Backgrounds and Objectives: To investigate the relationship between *H. pylori* (*H. pylori*) infection, microsatellite instability(MSI) and the expressions of the p53 in gastric adenocarcinoma and to elucidate the mechanism of gastric carcinogenesis relating to *H. pylori* infection in early gastric cancers (EGCs) treated by endoscopic submucosal dissection (ESD).
Patients and Methods: Since May 2005 through August 2009, patients with EGC who were candidate of endoscopic submucosal dissection were enrolled. MSI loci were studied by PCR-SSCP-CE using the markers BAT-26, D17S261, D3S1283, D2S123, and D3S1611. MSI was defined as the peak shift in the DNA of the gastric tissue compared with that of the peripheral blood samples. Based on the number of mutated MSI markers, specimens were characterized as MSI if they manifested instability at least one marker and microsatellite stable (MSS) if they showed no instability at any marker. *H. pylori* infection was detected by urea breath test and rapid urease test. p53 expression was detected by tissue immunohistochemical staining.
Results: 140 patients were enrolled. Male were 89 and female were 51. Average age was 62.9. Presence of *H. pylori* was noted in 71 patients (50.7%). Presence between MSS and MSI was expressed in 127(90.7%) and 13(9.3%). Presence of P53 was noted in 52(37.1%) of all patients. Of 52 patients, 28 patients (53.8%) had positive findings of *H. pylori*. There were no statistical differences in presence of *H. pylori* related with MSI and P53.
Conclusions: *H. pylori* does not affect the expression of P53 and MSI in EGC that are included in the indication of endoscopic resection.

Poster no.: P7.17
Establishment of a new human gastric epithelial cell line by over-expression of human telomerase catalytic subunit in NCI-N87 cells

K. D. Saraiva-Pava[1], A. I. Lopes[2], M. Oleastro[3] and M. Roxo-Rosa[1]
[1]Faculty of Engineering, Catholic University of Portugal, Rio de Mouro, Portugal; [2]Hospital de Santa Maria, Lisboa, Portugal; [3]National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal

One major limitation on *H. pylori* research is the inexistence of an *in vitro* cellular model that truly mimics its natural niche. The widely used heterogenic NCI-N87 (ATCC CRL-5822) gastric cell line presents typical epithelial markers and a progenitor-like phenotype (simultaneous synthesis of mucus and zymogens) but only in some cell subpopulations, exhibiting therefore a colony-forming growth pattern[1]. Aiming to isolate clones of this subpopulation of cells and to improve their epithelial properties, we stably-transduced NCI-N87 cells with human telomerase reverse-transcriptase (hTERT) catalytic subunit (pGRN145 plasmid, ATCC MBA-141), using the FuGENE-HD reagent (Roche). In fact, hTERT expression was shown to improve the classical CHO-K1 (Chinese Hamster Ovary k1) cell line, reducing its levels of apoptosis and enhancing both attachment and cellular viability under serum-deprived conditions[2].
Cellular characterization of 8 NCI-N87-derived clones by Periodic-Acid-Schiff and Hematoxilin staining, for mucus and zymogens detection, respectively, indicated the presence of homotypic cells exhibiting the progenitor-like phenotype. Improved epithelial properties were found for two of these clones with their more coherent growth pattern and with their ability of efficient mucus-secretion. *Per si*, this is a huge advantage for their use in the study of the molecular mechanisms of *H. pylori* infection. The expression and subcellular localization (immunocytochemistry) of *E*-cadherin and ZO-1, *i.e.*, adherens and tight-junctions' proteins, as well as, the polarization status (transepithelial electrical resistance generation) are now under evaluation.
Supported by PPCDT/SAL-IMI/57297/2004 research-grant.
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P8 Drug resistance; Other Helicobacters

Poster no.: P8.01
Evaluation of GenoType HelicoDR for molecular detection of *H. pylori* and its resistance against clarithromycin and fluoroquinolones

N. Wüppenhorst, B. Hobmaier and M. Kist

Institut für Med. Mikrobiologie und Hygiene, Freiburg, Germany.

Background: In Germany, primary resistance rates of *H. pylori* are 32% for metronidazole (MZ), 11% for clarithromycin (CLA), and 18% for fluoroquinolones (FQ) (data from the 3rd European antimicrobial resistance survey). To provide an optimized therapy for patients and to prevent further spread of antibiotic resistance, susceptibility testing appears mandatory. GenoType HelicoDR is a PCR-based test system and enables susceptibility testing of *H. pylori* for CLA and FQ from culture or biopsy. To evaluate GenoType HelicoDR we investigated this test system using pre-tested samples (isolates and biopsies) from our reference stock.

Methods: In total 99 samples were cultivated and susceptibility against clarithromycin and fluoroquinolones was determined by E-test. DNA was prepared from 48 culture samples and 51 biopsy samples. GenoType HelicoDR and an in-house CLA-RealTime PCR were performed.

Results: For CLA the concordance between the results of GenoType HelicoDR, E-test and in-house PCR was 100%. For FQ the results of GenoType HelicoDR were discordant in 4 samples when compared to E-test. Two samples harboured rare silent mutations in the probe region of GenoType HelicoDR, leading to misidentification. Two samples showed a wild-type pattern in GenoType HelicoDR but were resistant according to the E-test. Sequencing analysis revealed wild-type *gyrA* sequence, so other unknown mechanisms may be causative for resistance.

Conclusion: GenoType HelicoDR allows a reliable detection of *H. pylori* and its resistance against CLA and FQ either from biopsy or culture. The test system can be used for susceptibility testing, especially from biopsies if culture is not possible.

Poster no.: P8.02
Evaluation of metronidazole susceptibility testing of *H. pylori* using the E-test method

N. Wüppenhorst, B. Hobmaier and M. Kist

Institut für Med. Mikrobiologie und Hygiene, Freiburg, Germany

Background: Susceptibility testing of metronidazole is actually not approved by the FDA due to ''variance in test results, lack of reproducibility and clinical relevance''. Maastricht III does not recommend metronidazole testing in clinical practice because in vitro resistance to metronidazole may not accurately reflect in vivo resistance. In Germany, 32% of *H. pylori* isolates tested from non pre-treated patients are resistant to metronidazole, which is a problem because metronidazole is a recommended first line drug. Furthermore, phenotypic susceptibility testing is the only option for detection of resistance because molecular mechanisms of resistance are not conclusively explained. The aim of our study is to evaluate the precision and reproducibility of metronidazole susceptibility testing using the E-test method.

Methods: 50 randomly selected *H. pylori* isolates cultured from routine gastric biopsy specimens (no selection concerning resistance patterns, pre-treatments, patient data etc.) are actually under study. E-tests of each isolate are performed repeatedly at the same time point (precision) and at different time points (reproducibility). Further variables included are the culture media (Mueller-Hinton + 10% horse blood vs. Isosensitest + 10% horse blood), McFarland turbidity standard (2 vs. 3) and incubation period (48 h vs. 72 h).

Results: Preliminary results show that Minimal Inhibitory Concentration (MIC) seems not to be significantly influenced by the culture media, the McFarland turbidity standard and the incubation period used. Measurement uncertainty at one time point ranges within 2 MIC levels.

Conclusion: Metronidazole susceptibility testing by E-test appears more robust than expected. Further data are needed to confirm our preliminary observations.

Poster no.: P8.03
Primary levofloxacin resistance among *H. pylori* in Colombia

A. Trespalacios[1], A. Arévalo[1], W. Otero[2], M. Mercado[1], E. Caminos[2], R. Poutou[1], D. Graham[3] and E. Rimbara[3]

[1]Pontificia Universidad Javeriana, Bogotá, D.C, Colombia; [2]Universidad Nacional de Colombia, Bogotá, D.C, Colombia; [3]Department of Medicine, Michael E. DeBakey Veterans Affairs Medical Center, and Baylor College of Medicine, Houston, TX, United States

Aim: Resistance to antibiotics, especially clarithromycin, is the major cause of the failure to eradicate *H. pylori*. Alternative regimens including fluoroquinolones have been proposed. However, fluoroquinolone resistance has been reported to be increasing rapidly worldwide such that it has been recommended that a low prevalence of resistance is confirmed before using fluoroquinolones empirically. In this study, we aimed to investigate the susceptibility to levofloxacin in clinical isolates originating from Colombian patients.

Methods: A total of 107 clinical isolates of *H. pylori* were collected from April 2008 to January 2010 from patients in Bogotá, Colombia. Minimal inhibitory concentration (MIC) values of levofloxacin were determined by the agar dilution method. *H. pylori* isolates were considered resistant when the MIC values were ≥1 μg/mL. The mutations in quinolone resistance-

determining regions (QRDR) of the gyrA were investigated by amplification and nucleotide sequence.
Results: 6/107 (5.6%) of the strains were resistant to levofloxacin. The prevalence increased from 3% in 2008, 5% in 2009, to 11% in 2010. QRDR sequencing revealed mutations of the codons corresponding to Asn-87 and Asp-91, in 5 of the 6 resistant strains. The sequence analysis showed 2 isolates with D91G mutation, 1 with D91N mutation and 2 with N87I mutation. One resistant strain no had mutation. The MIC distribution in resistant strains ranged from 1 to 16 μg/mL.
Conclusions: The results suggest levofloxacin as a possible option for *H. pylori* eradication therapy in Colombia although careful monitoring of resistance will be required it the trend for increase in prevalence is real.

Poster no.: P8.04
Resistance to Levofloxacin, Rifabutin and Tetracycline Amongst *H. pylori* Strains in a Reference Centre

A. O'Connor[1,2], I. Taneike[1,2], D. McNamara[1,2], B. Ryan[1,2], N. Breslin[1,2], H. O'Connor[1,2] and C. O'Morain[1,2]
[1]Adelaide and Meath Hospital, Tallaght, Ireland; [2]Trinity College Dublin, Dublin, Ireland

Introduction: *H. pylori* eradication rates using standard triple therapy have fallen over recent years. Alternative antibiotic combinations are therefore being used more frequently. It is therefore important to understand resistance patterns to these medications.
Aims: Our study examined levels of resistance to levofloxacin, rifabutin and tetracycline in *H. pylori* isolates in a reference centre in Ireland from 2008.
Method: Antimicrobial susceptibilities were tested by E-test. Frequencies of spontaneous resistance were measured on agar plate containing the antibiotics at concentrations of 2x and 4x MIC value. Resistance was defined as MIC greater than 0.5 μg/mL for levofloxacin 1 μg/mL for rifabutin and 1 μg/mL for tetracycline. Clinical data was obtained from charts, laboratory and endoscopy reports.
Results: 85 patients were analysed, 50.6% were females. Mean age was 47 years. 10 had prior attempts at eradication therapy with amoxicillin-clarithromycin-PPI, 2 had levofloxacin based second line therapy. 11.7% [95%CI(6.5% to 20.3%)] had strains resistant to levofloxacin. There was no resistance to rifabutin or tetracycline. Neither of patients who had had levofloxacin based eradication treatment previously had resistance to it. Therefore levofloxacin resistance in the treatment naïve population was 12.0%. There was no significant difference between genders. Average age in years of the levofloxacin sensitive cohort was 45 [95%CI(40.9% to 49.1%)] and 61[95%CI(49.4% to 72.6%)] in the resistant group.
Conclusion: Levofloxacin resistance is frequent and is more common in older adults. It is commoner than clarithromycin resistance amongst treatment-naïve isolates found in our population. Rifabutin and Tetracycline resistance is exceptional our population.

Poster no.: P8.05
High rate of *H. pylori* resistance to clarithromycin in children in comparison to adults in Lithuania

L. Kupcinskas[1], R. Kucinskiene[1], N. Urbanaviciene[1], G. Pranculiene[1], L. Rasmussen[2], L. Jonaitis[1] and L. Andersen[2]
[1]Department of Gastroenterology, Kaunas University of Medicine, Kaunas, Lithuania; [2]Department of Clinical Microbiology, Copenhagen University Hospital, Copenhagen, Denmark

Introduction: An increase of *H. pylori* resistance to clarithromycin in many countries is worrying.
Aims & Methods: The aim of the study was to compare the prevalence of *H. pylori* strains with primary resistance to clarithromycin and other antibiotics used for *H. pylori* eradication in children and adults in Lithuania. *H. pylori* susceptibilities to antibiotics were tested in 2007–2008 yrs for 90 adults and 48 children from a single tertiary care university centre. Susceptibility to metronidazole (Mtz), clarithromycin (Cla), amoxicillin (Amx) tetracycline (Tet), and ciprofloxacin (Cip) were tested by E test (BioDisk, Sweden). Resistance breakpoints for Mtz >8 mg/l; for Cla >1 mg/l; for Amx and Tet >2 mg/l; and for Cip >1 mg/l were used.
Results: Primary *H. pylori* resistance rates in adults were for Mtz 35.6%, for Cla 3.3%, and for Cip 6%, respectively. The resistance rates in children were for Mtz 20.7%, for Cla 16.8%, and for Cip 0%, respectively. No cases of Amx and Tet resistance have been detected. The primary *H. pylori* resistance rate to Cla in children was 5 times higher than in adults ($p = 0.008$); Mtz resistance was more prevalent in adults ($p = 0.03$). Multi-drug resistance both to Cla and Mtz were observed in 4.2% of children and in 2.2% of adults ($p > 0.05$).
Conclusion: We have observed high and alerting prevalence of *H. pylori* resistance to clarithromycin in children in comparison to adults in Lithuania. This finding might be related to more frequent macrolide use in children due to different antibiotics compensation policy for children and adults in Lithuania.

Poster no.: P8.06
CagA status and drug resistance of clinical *H. pylori* strains isolated in the recent 5 years

E. Andrzejewska, A. Szkaradkiewicz, T. Karpiński and B. Zwoździak
University of Medical Sciences in Poznan, Poznan, Poland

Objectives: Colonization of the human gastric mucosa by *H. pylori* may lead to acute and chronic infection resulting in gastritis, ulcer and gastric cancer. The study aimed at analysis of *cagA* gene presence (a marker for the PAI) and evaluation of drug resistance of *H. pylori* strains associated with various clinical forms of the infection: *chronic gastritis, gastric ulcer and gastric cancer.*
Methods: Evaluation included 123 strains of *H. pylori* isolated in years of 2005–2010, including 59 strains originating from

patients with gastritis (group 1), 44 strains from patients with gastric ulcerative disease (group 2) and 20 strains originating from patients with gastric tumour (group 3). Manifestation of *cagA* gene was analyzed using PCR technique (DNA Gdansk). Evaluation of drug resistance in the strains was conducted using E-tests (bio-Disc Sweden). The culture was conducted using Columbia agar supplemented with 7% of sheep blood in microaerophilic conditions (Genbag microaer-bioMerieux) for 4–5 days. Strain sensitivity was evaluated to amoxicillin, tetracycline, clarithromycin and metronidazole.

Results: In group 1 (*gastritis*) 25 strains (42. 2%) were found to contain *cagA* gene. All the examined strains proved to be sensitive to amoxicillin and tetracycline. Resistance to metronidazole was detected in 25 strains (42.4%), and to clarithromycin in 6 strains (10.1%). In group 2 (*ulcer*) 39 strains were found to be *cagA* positive (88.6%). All the examined strains were sensitive to amoxicillin and tetracycline but 14 of them (31.8%) demonstrated resistance to metronidazole and 5 strains (11.4%) to clarithromycin. In group 3 (*cancer)* presence of 18 strains with *cagA* gene (90%) was disclosed. Also in this group all strains were sensitive to amoxicillin and tetracycline while 12 strains (60%) were resistant to metronidazole and 4 strains (20%) to clarithromycin. Analysis of *cagA* gene manifestation demonstrated that *H. pylori* strains with *cagA* gene were identified with a significantly higher frequency in patients with gastric ulcerative disease or with gastric cancer. Despite inter-group differences in proportions of strains resistant to metronidazole and clarithromycin the differences proved to be insignificant ($p > 0.05$).

Conclusion: 1. The risk of development of gastric ulcerative disease and/or gastric cancer is markedly higher in infections with *H. pylori* carrying *cagA* gene than in infections with *H. pylori* without *cagA* gene.

2. No significant relationship could be detected between presence of *cagA* gene and resistance to metronidazole among studied *H. pylori* strains.

Poster no.: P8.07
Correlation between mutations in penicillin-binding protein 1, 2 and 3 and amoxicillin resistance in *H. pylori*

J. G. Kim[1,2], W. Cheon[2], B. Kim[2], J. Kim[3] and J. Kim[2]

[1]Korean College of Helicobacter and Upper Gastrointestinal Research, Seoul, Korea, Republic of; [2]Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea, Republic of; [3]Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of

Objective: The correlation between the mutations of penicillin-binding protein (PBP) 2 and PBP3 and amoxicillin resistance has not been elucidated, although it has been reported that the mutations of PBP1 confer amoxicillin resistance in *H. pylori*. We evaluated the relationship between the mutations of PBP1, PBP2 and PBP3 and amoxicillin resistance in *H. pylori*.

Methods: One hundred fifty *H. pylori* strains were isolated in 2005 and 2006 from Korean patients with chronic gastritis, peptic ulcer or stomach cancer. The minimum inhibitory concentrations (MIC) of the strains were determined with serial 2-fold agar dilution method. The resistance breakpoint for amoxicillin was defined as >0.5 μg/ml. The PBP1, PBP2 and PBP3 amino acid sequence of resistant strains were analyzed. The *pbp1, ftsI* and *pbp2* genes from clinical amoxicillin-resistant strains were naturally transformed to four amoxicillin-susceptible strains including ATCC 700392.

Results: Nine of one hundred fifty *H. pylori* strains showed amoxicillin resistance (6.0%). The MIC values of resistant strains were ranged from 1 μg/ml to 4 μg/ml. The amino acid sequence analysis of resistant strains revealed multiple amino acid substitutions: seven of PBP1, one of PBP2 and two of PBP3, respectively. Each natural transformant of these three mutated genes from amoxicillin-sensitive strains showed moderate increase of amoxicillin MIC. However, none of them reached the values of original strains.

Conclusion: Multiple mutations in PBP2 and PBP3, as well as mutations in PBP1, affect amoxicillin resistance in *H. pylori*. This suggests interaction among PBP1, PBP2, and PBP3 mutations in the mechanism of amoxicillin resistance of *H. pylori.*

Poster no.: P8.08
H. pylori resistance to various antibiotics in Croatia; results of Croatian Reference Centre for *H. pylori* infection

M. Katicic[1], V. Plecko[2], T. Filipec-Kanizaj[1], S. Naumovski-Mihalic[1], V. Maricic[3], B. Skurla[1], M. Prskalo[1], A. Mrzljak[1] and T. Borcic[1]

[1]University Hospital Merkur, Zagreb, Croatia; [2]University Centre Rebro, Zagreb, Croatia; [3]Pharmaceutical Indust. Pliva d.o.o., Zagreb, Croatia

Introduction: The progresive increasing of antimicrobial resistance is now recognised as the main reason for decline success rate of *H. pylori* infection eradication.

The aim of this study was to evaluate the prevalence of primary resistance to different antibiotics in *H. pylori.* Isolates were taken in Reference Centre for *H. pylori* in Republic Croatia during 15 years (1995–2010).

Methods: In last 15 years, 2915 patients (F/M 1356/1559; age range 15–94) were prospectively included in scientific program for *H. pylori* detection and treatment. Endoscopy was performed before treatment, with 4 biopsies for histology and 2 for culture. Susceptibility testing was performed with agar dilution method for metronidazole (MIC>8), clarithromycin (MIC≥1),amoxicillin (MIC≥1), and tetracycline (MIC≥2). Primary resistance was calculated for every period of three years (1995–1997; 1998–2000; 2001–2003; 2004–2006; and 2007–2010).

Results: *H. pylori* was isolated from 1240 of 2915 (42,5%) persons undergoing upper endoscopy The primary resistance in 5 time periods for clarithromycin/azithromycin was, respectively: 7,2%, 10,5%, 11,0%, 10,9% and 19,4%; for metronidazole: 26,2%, 28,4%, 26,3%, 29,7%, and 34,3%, for amoxicillin 0%, and for tetracycline 1,6% in first period, and 0% after that. We also calculated the prevalence of primary multidrug antibacterial resistance during all five periods.

Conclusion: This result shows that solitary resistance to clarithromycin/azithromycin, and metronidazole, and multidrug resistance to both drugs, among HP positive Croatian patients

has progressively increased, particularly in last three years. We are now in Croatia reached Maastricht's limit for necessary use of sensitivity testing before decision for the best treatment option.

Poster no.: P8.09
Murine Gastritis Model to study antibacterial effect of *Camellia sinensis* (Green Tea) against *H. pylori* infection

S. K. Sherwani[1,2], F. Aziz[1], T. R. Siddiqui[3], S. S. Akhter[4], W. Ahmed[5] and S. U. Kazmi[1]

[1]University of Karachi, Karachi, Pakistan; [2]Federal Urdu University, Karachi, Pakistan; [3]PMRC, JPMC, Karachi, Pakistan; [4]Dow Medical College, Civil Hospital, Karachi, Pakistan; [5]PMRC, JPMC, Karachi, Pakistan

Background: This study is meant to evaluate the antimicrobial potential of aqueous crude extract of dried leaves of Green Tea against *H. pylori*.
Methods: In this study, 250 biopsies were collected from the patients of gastro-duodenal pathology. All biopsies processed for detection of *H. pylori* by two rapid Helicourease and PCR for the 16S ribosomal gene of *H. pylori*. The isolates screened for their susceptibility against antibiotics and aqueous extract of *Green Tea* by agar well diffusion and micro-broth dilution. In-vivo studies carried out by developing Gastritis Models in BALB/c mice by infecting orally with sublethal doses of 100 μl of *H. pylori* culture. The gastritis models allowed them to cause gastritis for about 7 days, 14 days, 21 days, 28 days and 34 days with doses of 5% aqueous extract of geen tea whose MIC was 28 mg/ml.
Results: In this study, 88 clinical *H. pylori* isolates successfully cultured and identified by rapid and molecular methods. Most of our isolates showed resistance towards Tetracyclin (60%), Metronidazole (80%), Amoxicillin (60%), Erythromycin (40%), Clarithromycin (30%), Ofloxicin (8%).A significantly large zone of an average 21 mm was found on most of the isolates as result Significant reduction of Cfu/g was noted in 7 days, 14 days and 21 days, but moderate CFU/g was observed in the rest of the models as compared with those who were left untreated case of organ culture of gastro intestinal tract organs.
Conclusion: The results of the current study provide useful insights to the developments of new antimicrobial agent like green tea as a therapeutic intervention tool.

Poster no.: P8.10
Clinical evaluation of GenoType HelicoDR kit for the detection and antimicrobial susceptibility testing of *H. pylori*

S. Jeverica, M. Skvarč and P. Čamernik

Institute of microbiology and immunology, Ljubljana, Slovenia

Background and Objective: Culture based phenotypic methods were for a long time considered the only means of antimicrobial susceptibility detection for *H. pylori* (HP). With regard to fastidious nature of HP, such methods have some inherent difficulties. GenoType HelicoDR test is a promising alternative to culture based tests. Our objective was to evaluate success rate of HP culture during past 5 years and to perform a pilot study of the impact the introduction of GenoType HelicoDR test would have for the routine HP diagnostics.
Patients and Methods: The evaluation of the GenoType HelicoDR was done one 36 selected strains. It is amplification and hybridisation based method, that uses biotinilated primers and hybridisation strips. Concordance of the results for clarithromycin and levofloxacin with Etests was assessed.
Results: HP culture positivity in a clinical practice is at our best 80%. Results for GenoType HelicoDR are in good overall agreement with Etests. Essential agreement for clarithromycin and fluoroquinolon was 92% and 95%. 2 strains repeatedly produced major error for clarithromicin, 1 strain produced very major error for clarithromycin and 1 for fluoroquinolon. When testing 22 culture negative biopsies GenoType HelicoDR detected 4 more positives. Algorithm that would include testing all culture negative biopsies with HelicoDR test would increase detection yield of laboratory by 20%.
Discussion: HP culture based methods are not ideal. GenoType HelicoDR produce reliable results of the resistance to clarithromycin and fluoroquinolon. Testing all negative biopsies with GenoType HelicoDR would increase diagnostic yield of laboratory and would prevent some additional gastroscopy.

Poster no.: P8.11
Susceptibility pattern of *H. pylori* after treatment failure

L. Rasmussen[1,2], J. Alimoradi[3], A. Friis-Møller[4], S. Skovgaard[4], F. Jafari[1], A. Boschian[1,2] and L. P. Andersen[2]

[1]Dept of Clinical Microbiology, Copenhagen, Denmark; [2]Dept of Infection Control, Copenhagen, Denmark; [3]Glostrup Hospital, Glostrup, Denmark; [4]Clinical Microbiology, Hvidovre, Denmark

Objectives: According to the Maastricht III Consensus, *H. pylori* test and treat is the strategy of choice in most *H. pylori* infected patients and PPI combined with amoxicillin and clarithromycin/metronidazole is recommendated as first line treatment. There is a growing concern regarding antibiotic resistance in *H. pylori*, which can result in treatment failure. The aim of this study was to evaluate the susceptibility pattern of *H. pylori* strains after treatment failure in Danish patients.
Methods: 50 clinically isolated *H. pylori* strains were susceptibility tested by E-test for amoxicillin, metronidazole, ciprofloxacin, levofloxacin, clindamycin, erythromycin, clarithromycin, rifampicin, tetracycline and meropenem.
The bacteria were grown and susceptibility tested according to the manufacturer.
Results: 74% of the strains were resistant to metronidazole, 54% were resistant to clindamycin and clarithromycin and 52% were resistant to erythromycin while 36% of the strains were resistant to all those 4 antibiotics at the same time. All strains

were susceptible to amoxicillin, ciprofloxacin, levafloxacin, tetracycline and rifampicin.
Conclusion: This study shows a high rate of resistance to the most commonly used antibiotics and a high rate of multiresistant strains in patients with treatment failure. This suggests that antimicrobial susceptibility testing should be done after the first treatment failure.

Poster no.: P8.12
The primary resistance of *H. pylori* strains in pediatric and adult patients in South-West Poland

G. Gościniak[1], M. Biernat[1], J. Grabińska[1], F. Mégraud[2] and B. Iwańczak[3]
[1]Department of Microbiology, Medical University of Wrocław, Poland; [2]Laboratoire de Becteriologie, Universite de Victor Segalen, Bordeaux 2, France; [3]2nd Department and Clinic of Paediatrics, Gastroenterology and Nutrition, Medical University of Wrocław, Poland

The increasing resistance rates in *H. pylori* strains leads to the necessity for creating new treatment strategies.
The aim of the study: to assess the prevalence of antimicrobial resistance of *H. pylori* strains isolated from children and adults with primary infection.
Materials and Methods: Sixty eight clinical isolates were obtained from children, aged 2–18 years with chronic gastritis and duodenal/gastric ulcer, whereas 46 strains were isolated from adults with gastritis and ulcers. All strains were collecetd in years 2008–2010. Antimicrobial susceptibility to 6 drugs (metronidazole MZ, clarihtromycin CH, levofloxacin LE, rifabutin RB, tetracycline TC and amoxicillin AC) was tested by the E-test method.
Results: From 68 *H. pylori* strains isolated from children, 15% (n = 10) showed resistance to CH, 23,5% (n = 16) to both MZ and CH, 23,5% (n = 16) to MZ alone. In adults the resistance to both MZ and CH was detected in 28% (n = 13) of tested strains whereas resistance to MZ alone in 43%(n = 20), respectively. The prevalence of LE-resistant *H. pylori* isolates was 2,9% in children and 13% in adults, respectively. Among these strains 75% were resistant also to CH and/or MZ. Resistance to RB, TC as well as to AC was not observed.
Conclusions: The *H. pylori* strains isolated from adults showed higher resistance than those from children. The prevalence of multi-drug resistant isolates in both children and adults with primary infection is high in our region, that is why pretreatment antimicrobial susceptibility testing is strongly recommended in this patients.

Poster no.: P8.13
Evolution of antimicrobial resistance in *H. pylori* Spanish clinical isolates in a 10-year period

M. J. Moreno[1], T. Alarcon[1], S. Agudo[1], M. J. Martinez[2], P. Urruzuno[3] and M. Lopez-Brea[1]
[1]HOSP. DE LA PRINCESA, MADRID, Spain; [2]HOSP. DEL NIÑO JESUS, MADRID, Spain; [3]HOSP. DOCE DE OCTUBRE, MADRID, Spain

The aim of this study was to determine the resistance to several antimicrobial agents in *H. pylori* clinical isolates in a 10-year period.
Material and Methods: A total of 532 clinical isolates of *H. pylori* were studied for metronidazole, clarithromycin, amoxicillin, and tetracycline and 359 strains for rifampicin and levofloxacin The *in vitro* activity of clarithromycin and metronidazol was determined by E-test using an inoculum of 3 McFarland. Plates were incubated at 37°C during 2 or 5 days in a CO_2 increased atmosphere. MIC was determined as the point where ellipse cross the E-test strip. Strains were considered resistant to clarithromycin when MIC>0.5 mg/L, intermediate when MIC = 0.5 mg/l, resistant to metronidazole when MIC≥8 mg/l, to amoxicillin when ≥2 mg/l, to tetracicline and levofloxacin when ≥4 mg/l and to rifampicin when ≥32 mg/L.
Results: Considering the total number of strains studied, metronidazole resistance was found in 48.1%, clarithromycin resistance in 45%, levofloxacin resistance in 5,5%, rifampicin resistance in 2,9%, amoxicillin resistance in 2,2% and 1 strain was tetracicline resistant (0,1%).
Conclusions: Resistance to claritromycin and metronidazole was very high in the *H. pylori* strains studied herein. Amoxicillin resistance was higher in the last period studied. Tetracycline resistance was still very unfrequent.

Percentage resistance in 2000–2005 period	
AMX	0%
TET	0%
CLA	57.8%
MET	59.3%
Percentage resistance in 2006–2009 period	
AMX	3.3%
TET	0.2%
CLA	47.9%
MET	45.2%

Poster no.: P8.14
Mutations in rdxA & 23s rRNA genes are associated with metronidazole & clarithromycin resistance in *H. pylori* isolates in Kerman, Iran

H. Abdollahi, M. Savari, M. Zahedi, S. Darvish-Moghadam and M. Hayatbakhsh-Abasi
kerman university of medical sciences, kerman, Iran, Islamic Republic of

Introduction: Clarithromycin and metronidazole are among the most commonly used antibiotics for eradication of *H. pylori*, leading to increased resistance towards them. Mutation in rdxA

gene leads to functional changes of oxygen-insensitive nitroreductase, which in turn is associated with metronidazole resistance in *H. pylori*. Resistance to clarithromycin in this bacterium is associated with one point mutation in the 23s rRNA gene.
Methods: 63 *H. pylori* isolates from 191 patients referred to endoscopy unit of Afzalipour hospital in Kerman, Iran during 2009 were evaluated for their antibiotic susceptibility using a modified disk diffusion test. To detect deletion in rdxA gene a simple PCR method was used, but point mutations in 23s rRNA gene was detected by PCR-RFLP method.
Results: From 35 metronidazole resistance isolates only 22. 9% (8 isolates) had deletion in rdxA gene. No rdxA deletion was detected in sensitive isolates. All of the 20 clarithromycin resistant isolates had at least one of the three common point mutations in 23s rRNA. the clarithromycin sensitive isolates had no point mutations in 23s rRNA.
Conclusion: Antibiotics resistance of *H. pylori* is a serious concern but outburst of MDR isolates is even more important as the whole treatments can go waste. According to rdxA deletion rates in this study it seems that some other nitroreductases are involved in metronidazole activation or there are other metronidazole resistance mechanisms involved. The clarithromycin resistance of *H. pylori* was associated with point mutations in 23s rRNA in all of our local isolates, and probably is true for other parts of the world.

Poster no.: P8.15
Antimicrobial susceptibility pattern of *H. pylori* strains isolated from adult dyspeptic patients in Latvia

D. Rudzite[1,2], A. Sudraba[1,2], I. Daugule[2], K. Funka[2], I. Tolmanis[3], A. Vanags[3], L. Engstrand[4], D. Janciauskas[5], L. Jonaitis[5], L. Kupcinskas[5], A. Ivanauskas[5] and M. Leja[2,3,1]

[1]Riga East Clinical University Hospital, Riga, Latvia; [2]University of Latvia, Riga, Latvia; [3]Digestive Diseases Centre GASTRO, Riga, Latvia; [4]Swedish Institute for Infectious Diseases Control, Stockholm, Sweden; [5]Kaunas University of Medicine, Kaunas, Lithuania

Introduction: To make a choice for the best therapeutic strategy for *H. pylori (Hp)* eradication the local knowledge of resistance pattern is necessary. However, there is lack of information on the antimicrobial susceptibility pattern of *Hp* in Latvia.
Aim: To determine the current state of metronidazole, clarithromycin and tetracycline primary resistance in *Hp* clinical isolates.
Patients and Methods: In total 173 *Hp* strains (142 - from antrum, 31 - from corpus) from 146 patients referred for upper endoscopy due to dyspeptic problems were analysed. Patients with gastric cancer, peptic ulcer, previous gastric surgery or eradication therapy were excluded. Antibacterial susceptibility to metronidazole (MTR), clarithromycin (CLR) and tetracycline (TCY) was determined by disk diffusion and E-test methods.
Results: The resistance rates were 47,6% for metronidazole, 2,7% for clarithromycin and 1,4% for tetracycline. Single antimicrobial resistance was found in 61(42%) and double - in 5(3%) patients. In 12 patients (8,2%) co-infection by two strains with different resistance pattern was detected. 5 antibiotypes were revealed (table).
Conclusions: The primary resistance to clarithromycin was low compared to other European countries. Double resistance and coinfection was observed in 3% and 8% of previously untreated patients, respectively.

Table 1 Revealed antibiotypes in dyspeptic patients.

Resistance profile	Number of patients	Patients (%)
No resistance	79	54
CLR	1	0
MTR	60	41
MTR and TCY	2	1
MTR and CLR	3	2

Poster no.: P8.16
Identification and Characterization of Antibiotic Resistant *H. pylori* Isolates in Mongolia

T. Daria[1], J. Sarantuya[2], T. Khosbayar[2] and N. Bira[3]

[1]Central Scientific Research Laboratory, National Institute of Medicine of Mongolia, Ulanbator, Mongolia; [2]Department of Molecular biology and Genetics, Ulanbator, Mongolia; [3]Department of Gastroenterology, Health Sciences University of Mongolia, Ulanbator, Mongolia

Introduction: *H. pylori* infection prevalence dramatically increased among Mongolian population. Antibiotic resistance varies widely between geographical regions and among subgroups within a study population. Numerous regimens of several antimicrobial agents are suggested to eradicate the bacterium. Among them, triple therapy containing a proton pump inhibitor or bismuth and two antibiotics, which are usually among metronidazole, amoxicillin, tetracycline or clarithromycin, for 2 weeks are applied widely. However, lack of pure culture cells, the antibiotic resistance of *H. pylori* has not been studied in Mongolia.
Aims & Methods: 83 biopsy samples were obtained during upper gastrointestinal endoscopy and the specimens were inoculated onto plates Columbia with 10% blood and a selective *H. pylori* agar. *H. pylori* were identified by the appearance of milky colonies, gram negative bacillus in gram stained smear and positive urease test. Evaluation of antibiotic susceptibility testing and resistance breakpoints (MIC) of antibiotics furazolidone, clarithromycin, amoxicillin, erythromycin and tetracycline were performed by E-tests and disc diffusion tests.
Results: In our study, from 27 pure cultured samples of *H. pylori* resistance rate to metronidazole (>256 mg/l) was 62.9% (17 isolates), and to clarithrimycin (8->256 mg/l) was 25.9% (7) and intermediate sensitivity 7.4% (2), and resistance rates to tetracycline, erythromycin amoxicillin and nitroimidazole were 18.5% (5), 14.8% (4), 11.1% (3) and 14.8% (4) respectively.
Conclusion: It is essential to be aware of the local antibiotic resistance rate to be able to prescribe the proper regimen to eradicate *H. pylori*. Most prevalent resistance rate are to metronidazole and clarithrimycin in our patients.

Poster no.: P8.17
Comparison of microarray statistical algorithms to analyse the response of human liver cells to *Helicobacter pullorum*

Q. V. Tu[1] and G. L. Mendz[2]
[1]The University of New South Wales, Sydney, Australia; [2]The University of Notre Dame Australia, Sydney, Australia

Several statistical algorithms have been developed to analyse microarray data of modulation of gene expression under various conditions. Their different modeling assumptions potentially can yield significantly different results. The aim of this work was to compare the results of applying the software packages Partek™ Genomics Suite (PGS), Genomatrix™ ChipInspector (GCI) and Extraction and Analysis of Differential Gene Expression (EDGE) to the microarray data of the 48 h response of Huh-7 cells to the bacterium *Helicobacter pullorum.*

PGS and EDGE employ GC-adjusted robust multiarray analysis and quantile normalisation taking into account batch factor as source of variance, whereas GCI applies single probe-transcript annotation to normalize the total intensity of the probe set. The number of genes with a modulated expression that resulted from applying each of the packages to the raw microarray data were 805, 1586 and 780 ($p < 0.05$) for the PGS, GCI and EDGE algorithms, respectively. The three methods yielded only 7 common genes differentially expressed, and the overlap of modulated genes between PGS and GCI, GCI and EDGE and PGS and EDGE was 53, 43 and 36, respectively. The results of each algorithm were compared to: (1) the number of genes which appeared simultaneously differentially expressed in specific networks of functional pathways, and to (2) qRT-PCR data of selected genes.

The comparisons of the results yielded by the three methods indicated that the analysis of the PGS algorithm provided the most complete sets of genes in networks of functional pathways and correlated best with the qRT-PCR data.

Poster no.: P8.18
Infection with *H. pylori* and hepatitis C virus are the risks of Helicobacter hepaticus seropositivity in a Japanese population

C. Iino[1], T. Shimoyama[1], R. Takahashi[2], D. Abe[2] and S. Fukuda[1]
[1]Hirosaki University Graduate School of Medicine, Hirosaki, Japan; [2]Wakamoto Pharmaceutical Co., Ltd., Kanagawa, Japan

Backgrounds: Presence of serum antibodies to Helicobacter hepaticus was higher among elderly subjects and patients infected with hepatitis C virus (HCV) or *H. pylori* in Japan.
Aims: In this study, we performed a logistic regression multivariable analysis to identify the factors, which would increase the risk of H. hepaticus seropositivity.
Methods: Serum samples were obtained from 182 patients infected with HCV, 51 patients infected with chronic hepatitis B virus (HBV) and 142 control subjects who were not infected with hepatitis virus. Infection of viral hepatitis was considered positive when HCV-RNA or HBs antigen were detected in serum. Seropositivity of *H. pylori* was tested by E-plate. Antigen-capture ELISA using monoclonal antibody to a H. hepaticus specific antigen (HH15) were performed. Serum samples were also tested after absorption with H. hepaticus cell lysate to define cut-off value of the ELISA. A logistic regression multivariable model was conducted to determine the risk for H. hepaticus seropositivity.
Results: Infection of HCV was an increased risk for H. hepaticus seropositivity (OR 1.91; 95% CI 1.05–3.47, $p < 0.03$) while infection with HBV was not (OR 0.82; 95% CI 0.32–2.06). Infection of *H. pylori* was also an increased risk (OR 1.91; 95% CI 1.09–3.36, $p < 0.03$) comparing with subjects without *H. pylori* infection. OR for men was 1.33 (95% CI 0.81–2.19) and OR for higher age (>60 years old) was 0.68 (0.35–1.32), respectively.
Conclusion: Both infection of *H. pylori* and HCV were the risks of serum antibody to H. hepaticus in a Japanese population.

Author Index

Keyword Index

