Helicobacter

European Helicobacter Study Group

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Contents

Workshop Presentations

WS1 Molecular Genetics & Virulence Factors

Abstract no.: W1.1

CHROMOSOME PAINTING IN SILICO IN A BACTERIAL SPECIES REVEALS FINE POPULATION STRUCTURE

K. Yahara,*,†,‡ Y. Furuta,*,† K. Oshima,§ M. Yoshida,¶ T. Azuma,¶ M. Hattori,§ I. Uchiyama** and I. Kobayashi*,†

*Department of Medical Genome Sciences, Graduate School of Frontier Sciences, University of Tokyo, Tokyo, Japan; †Institute of Medical Science, University of Tokyo, Tokyo, Japan; ‡Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany; §Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Japan; ¶Department of Gastroenterology, Graduate School of Medicine, Kobe University, Kobe, Japan; **Laboratory of Genome Informatics, National Institute for Basic Biology, Okazaki, Aichi, Japan

Identifying population structure forms an important basis for genetic and evolutionary studies. Most current methods to identify population structure have limitations in analyzing haplotypes and recombination across the genome. Recently, a method of chromosome painting in silico has been developed to overcome these shortcomings and has been applied to multiple human genome sequences. This method detects the genome-wide transfer of DNA sequence chunks through homologous recombination. Here, we apply it to the fre quently recombining bacterial species *Helicobacter pylori* that has infected Homo sapiens since their birth in Africa and shows wide phylogeographic divergence. Multiple complete genome sequences were analyzed including sequences from Okinawa, Japan, that we recently sequenced. The newer method revealed a finer population structure than revealed by a previous method that examines only MLST housekeeping genes or a phylogenetic network analysis of the core genome. Novel subgroups were found in Europe, Amerind, and East Asia groups. Examination of genetic flux showed some singleton strains to be hybrids of subgroups and revealed evident signs of population admixture in Africa, Europe, and parts of Asia. We expect this approach to further our understanding of intraspecific bacterial evolution by revealing population structure at a finer scale (Molecular Biology and Evolution (2013) 30 (6): 1454–1464.).

Abstract no.: W1.2

GENOMES OF *HELICOBACTER PYLORI* FROM INDIA AND MALAYSIA AND THE SYSTEMS EPIDEMIOLOGY AT THE ANVIL

N. Kumar,* J. Vadivelu† and N. Ahmed*

*Department of Biotechnology and Bioinformatics, University of Hyderabad, Hyderabad, India; †Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

High rates of prevalence of *H. pylori* infection culminating in minuscule number of serious outcomes such as gastric cancer in India and some other countries of Asia are seen as an enigma. Some of the ethnic groups are probably more susceptible to gastric cancer than the others living in the same region and practicing similar or different cultural and culinary traditions. Not enough genomic information from the pathogen side has been integrated in to epidemiology of *H. pylori*. Here we report high-throughput genome sequencing, annotation and comparative analysis of 20 *H. pylori* strains isolated from India and Malaysia, representing patients of different clinical presentations and ethno-geographical history. The genomes were sequenced using Solexa/Illumina platform; reads were assembled into contigs and scaffolds using paired-end information and the scaffolds were reordered according to a chosen reference and manually curated using Tablet. The whole genome based phylogeny portrayed existence of dissimilar genotypes. The strains from Chinese patients in Malaysia clustered together with other East-Asian genomes while the Indian and Malay strains shared genomic lineage(s) representative of an Indo-European gene pool. This provides an interesting account of bacterial gene flow influenced by historical and or contemporary human migration. Further, we attempted to refurbish core and accessory gene pools of *H. pylori* and defined extended plasticity zone (s) on the basis of ours and the federated genome data. Functional analysis of some of the novel genes within the refurbished regions would allow better understanding of adaptive mechanisms. We believe that improved knowledge of the complex interactions between genetically different hosts and their co-evolved pathogens amidst the contrasting geographical and cultural scenarios, would help consolidation of genome inspired systems biology with anthropology resulting in what might be described as "systems epidemiology" of *H. pylori*.

Abstract no.: W1.3

BACTERIOPHAGE GENES WITHIN GENOMES OF *HELICOBACTER PYLORI* CLINICAL ISOLATES

A. Timóteo,*,† P. Lehours,‡ M. Oleastro,§ J. M. B. Vítor,¶ T. T. Perets,** Y. Niv,** F. Mégraud‡ and F. F. Vale*,††

*Faculdade de Engenharia – Universidade Católica Portuguesa, Sintra, Portugal; †Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal; ‡Université Bordeaux Segalen, INSERM U853, Bordeaux, France; §Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa, Portugal; ¶IMed.UL. Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; **Rabin Medical Center – Beilinson Hospital, Petah Tikva, Israel; ††Centro de Estudos do Ambiente e do Mar (CESAM/FCUL) – Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

The description of *Helicobater pylori* prophages is a new topic in the literature, with the first complete prophage described in 2011 (phiHP33) and a remnant prophage described in 2010 (strain B38). To date, four strains were described with complete prophage sequences: French B45; Peruvian Cuz20; Indian India 7 and Taiwan 1961P; plus two *H. pylori* phages, KHP30 and KHP40. Remnant prophages sequences have been described for three *H. pylori* strains: French B38, Japanese F16 and Gambia 94/24. Previously, we have showed that the prophage integrase gene was present in approximately 20% of the *H. pylori* genomes. Now, we aim to extend this finding to a larger number of isolates, and to determine if other prophage genes are also present. We have analyzed a set of 849 isolates (670 European, 60 African and 119 Asian, corresponding to 218 gastritis, 229 peptic ulcer, 76 adenocarcinoma, 63 MALT lymphoma, and 263 without data), by PCR using degenerated primers for the integrase, primase and transcriptional regulator genes. Results confirm that the integrase gene is present in about 20%, but the majority of the prophages appear to be remnant, i.e. absence of amplification of the other two tested genes. Phages typically present a lack of DNA homology, therefore false negative PCR results are possible. Thus the complete sequence of the prophage is necessary to confirm this result. Moreover, the primase gene appear to be related to the origin of the strain, showing higher prevalence among Asian strains; while the transcriptional regulator sequence may be associated with the disease, with higher prevalence in peptic ulcer.

Abstract no.: W1.4

HELICOBACTER PYLORI CAGA DIRECTS GASTRIC INTESTINAL METAPLASIA BY INDUCING STEMNESS-RELATED REPROGRAMMING FACTORS

M. Hatakeyama and Y. Fujii

Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Chronic infection with CagA-producing *Helicobacter pylori* is the strongest risk factor for the development of gastric carcinoma. Intestinal metaplasia of the stomach, a pathological mucosal change characterized by the conversion of gastric epithelium into an intestinal phenotype, is thought to be a precancerous lesion from which gastric carcinoma arises. We previously reported that *H. pylori* CagA, which is delivered into gastric epithelial cells via type IV secretion, elicits aberrant activation of Wnt signaling and thereby induces ectopic expression of the Wnt target CDX1, an intestinal-specific transcription factor that mediates development and maintenance of intestinal epithelia. Here we show that, ectopically expressed CDX1 in the stomach transactivates stemness-related reprogramming factors, SALL4 and KLF5, both of which are related to the induction of iPS cells. Consequently, CagA-expressing gastric epithelial cells acquire the ability to defifferentiate into those expressing intestinal stemness markers such as Lgr5, DCLK1 and Bmi1, which in turn transdifferentiate into variable lineages of intestinal cells. Given that tissue stem cells share multiple traits in common with cancer stem cells, *H. pylori* CagA may enforce generation of such stem/precursor-like cells that predispose oncogenic transformation. Our study thus reveals a novel CagA-Wnt-CDX1-SALL4/KLF5 signaling axis that underpins the development of precancerous intestinal metaplasia in the stomach infected with *H. pylori* CagA-producing strains.

Abstract no.: W1.5

LRP-1 MEDIATES AUTOPHAGY AND APOPTOSIS CAUSED BY *HELICOBACTER PYLORI* VACUOLATING CYTOTOXIN, VACA

T. Hirayama,* M. Nakano,* M. Noda† and K. Yahiro†
*Nagasaki University, Nagasaki, Japan; †Chiba University, Chiba, Japan

In *Helicobacter pylori* infection, vacuolating cytotoxin (VacA) induces mitochondrial damage leading to eventually apoptotic cell death. Recent studies of Dr. Jones's group have shown that VacA induces autophagy (Autophagy 2009, Gastroenterology 2012), suggesting that VacA-induced autophagy serves as a host mechanism to limit toxin-induced cellular damage. Apoptosis and autophagy are two dynamic and opposing processes that must be balanced to regulate cell death and survival. The VacA receptor(s) responsible for apoptotic cell death and autophagy has not been identified during intoxication.

Here we identify the low-density lipoprotein receptor-related protein-1 (LRP-1) as the VacA receptor for toxin-induced autophagy in the human gastric epithelial cell line AZ-521, and show that VacA internalization through binding to LRP1 specifically regulates the autophagic process including generation of LC3-II from LC3-I, which is involved in formation of autophagosomes and autolysosomes. Knockdown of LRP1 and Agt5 inhibited generation of LC3-II as well as cleavage of PARP, a marker of apoptosis, in response to VacA, whereas caspase inhibitor, Z-VAD-FMK, and necroptosis inhibitor, Necrostatin-1, did not inhibit VacA-induced autophagy, suggesting that VacA-induced autophagy via LRP1 binding precedes apoptosis. In addition, Both NPPB and DIDS inhibited VacA-induced LC3-II generation in AZ-521 cells suggesting that channel activity may be required for LRP1-dependent autophagy. Other VacA receptors such as RPTP alpha, RPTP beta, and fibronectin did not affect VacA-induced autophagy or apoptosis. Therefore, we propose that a single cell surface receptor, LRP1, mediates VacA-induced autophagy as well as apoptosis in gastric epithelial cell line AZ-521 cells.

Abstract no.: W1.6

PHOSPHORYLATION OF BACTERIAL EFFECTOR CAGA MAY BE REQUIRED FOR THE INDUCTION OF MOLECULES INVOLVED IN EXTRACELLULAR MATRIX REMODELING IN *HELICOBACTER PYLORI* EXPERIMENTAL IN VITRO INFECTION OF GASTRIC EPITHELIAL CELLS

I. Sougleri, K. Papadakos, A. F. Mentis and D. N. Sgouras
Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece

Following its translocation inside gastric epithelial cells, CagA is hierarchically tyrosine phosphorylated by Src and Abl kinases, on repetitive EPIYA sequences, inducing the appearance of a scattering phenotype resembling the epithelial to mesenchymal transition. In western clinical isolates the type of EPIYA motifs varies depending on the surrounding sequence namely, EPIYA-*A*:EPIYAKVNK, EPIYA-*B*:EPIYAQVAKK and EPIYA-*C*:EPIYATIDDLG. The number of EPIYA-C repeats has been positively correlated to scattering. We investigated the potential involvement of CagA protein in the activation of matrix metalloproteinase-9 (MMP-9) and its activator MMP-3 in *H. pylori*-infected gastric epithelial cells (AGS). We utilized isogenic P12 *H. pylori* mutants, expressing CagA protein with variable numbers of functional EPIYA-C and phosphorylation-deficient EPIFA-C motifs, as well as the corresponding P12 *cagA*- and *cagE*- knock out strains. AGS cells were infected in vitro and MMP-specific transcriptional activation was measured by quantitative reverse transcriptase Real Time PCR, at several time points. MMP expression in total cell lysates and cell culture supernatants was also determined by western blot analysis at 24 hours post-infection. Nearly 100-fold increase in MMP-3 and 80-fold increase in MMP-9 was observed in the presence of CagA protein and proportional to the number of EPIYA-C terminal motifs. On the contrary, infection with CagA phosphorylation-deficient or the *cagA*-and *cagE*-knock out mutants, induced only background levels of MMP transcription. CagA-dependent increase in gelatinase and caseinolytic activity was detected in infected supernatants, utilizing zymography. Phosphorylation of the CagA effector may be required for induction and secretion of MMP-3 and MMP-9 in experimental *H. pylori* infection.

WS2 Epidemiology and Pediatrics

Abstract no.: W2.1

HELICOBACTER PYLORI COLONIZATION RATE IN CHILDREN IS HIGHLY VARIABLE AMONG DIFFERENT ETHNIC GROUPS IN WESTERN POPULATIONS: THE GENERATION R STUDY

W. J. den Hollander,* I. L. Holster,* B. van Gilst,* A. J. van Vuuren,* V. W. Jaddoe,* G. I. Perez-Perez,† M. J. Blaser,† H. A. Moll* and E. J. Kuipers*
*Erasmus MC University Medical Center, Rotterdam, The Netherlands; †New York Langone Medicine Center, New York, NY, USA

Introduction: *Helicobacter pylori* (*H. pylori*) is usually acquired during childhood. Although colonization rates have been declining in Western countries, less is known about *H. pylori* prevalence among children living in a multi-ethnic Western city.
Aims and Methods: Our aim was to identify *H. pylori* and CagA status and *H. pylori*-related risk factors in children living in Rotterdam, a large European city. We measured IgG anti-*H. pylori* and CagA-antibodies in children participating in the Generation R study, a population-based prospective cohort study. Information on demographics and maternal characteristics was collected by questionnaires. Odds ratios (OR) for *H. pylori* colonization were adjusted for potential confounders.
Results: Serum of 4467 children was analysed (mean age 6.2 years ± 0.48 SD), of which 2164 were female (48%). Overall, 438 children were *H. pylori* positive (10%), and 142 of them were CagA-positive (32%). Highest colonization rate was found in Moroccan children (27%), followed by children originating from Cape Verde (23%), Dutch Antilles (15%), Turkey (13%), other non-western countries (12%), Surinam (10%), other western countries (10%), and the Netherlands (6%) respectively ($p < 0.001$). Colonization rate if all non-Dutch children were pooled was 15% compared with 6% of the Dutch children ($p < 0.001$). Multivariate regression analyses revealed following associations with *H. pylori* colonization: non-Dutch ethnicity (OR 2.30; 95% confidence interval 1.82–2.90), male gender (0.69; 0.56–0.84), and day care attendance (0.60; 0.41–0.88). *H. pylori*-positive Dutch children were CagA-positive in 15% of the cases compared with 39% of the non-Dutch subjects ($p < 0.001$).
Conclusion: We identified large differences in colonization rates among children living in a multi-ethnic population. Highest *H. pylori* and CagA prevalence was found in children from non-Western countries, implying that in coming decades *H. pylori* and related diseases will be prevalent in this multi-ethnic population.

Abstract no.: W2.2

THE CHANGING PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN DYSPEPTIC PATIENTS FROM BUDAPEST, HUNGARY: A RETROSPECTIVE ENDOSCOPIC STUDY, 1997–2012

G. M. Buzás,* G. Lotz,† F. Schneider‡ and J. Józan§
*Ferencváros Health Center, Gastroenterology, Budapest, Hungary; †Second Institute of Pathology, Semmelweis University, Budapest, Hungary; ‡Cell Diagnostics Ltd, Budapest, Hungary; §Institute of Physiology, Semmelweis University, Budapest, Hungary

Background: The prevalence of *H. pylori* is decreasing in developed countries. The time-frame of this process is largely unknown.
Material and Methods: This retrospective study included 4647 patients who underwent upper digestive endoscopy for dyspeptic symptoms between 1997 and 2012. *H. pylori* was determined from antral and corpus biopsies by the modified Giemsa stain and rapid urease tests. The patients were previously untreated for the infection. The prevalence of *H. pylori* was calculated for age decades from 18 to 85 years, birth cohorts from 1920 to 1994, and years of examination.
Results: In the population with a mean age of 48.1 ± 24.5 years, the prevalence of *H. pylori* was 55%. Male to female ratio was 40.6/59.4%. According to diagnosis, 37.9% of the cases had functional dyspepsia, 25.3% duodenal ulcer, 3.8% gastric ulcer, 1.9% gastric + duodenal ulcer, 24.2% reflux disease and 6.7% chronic erosive gastritis.
According to the year of examination, the prevalence of infection peaked in 1997 at 71.3%, and gradually reached its lowest level of 32.6% in 2011. The decrease became significant in the cohort of patients born in the mid-1960's and 1970's as compared to cohorts from 1920 to 1950's and was continuous until the recent years.

Table 1 The prevalence of *H. pylori* according to age decades and birth cohorts

Age (years)	<19	20-29	30-39	40-49	50-59	60-69	70-79	>80
Prevalence %	**21.8**	**34.9**	**46.5**	**63.7**	**63.2**	**59.2**	**61.8**	**57.6**
Birth cohorts	1920-29	1930-39	1940-49	1950-59	1960-69	1970-79	1980-89	1990-94
Prevalence %	**63.6**	**62.8**	**63.0**	**62.1**	**57.4.**	**39.0**	**26.7**	**12.2**

Conclusions: The age-and birth-related prevalence of *H. pylori* infection in dyspeptic patients from Budapest is decreasing: the phenomenon occurred in the birth cohorts of 1960–1970's, two decades before the discovery of bacterium.

Abstract no.: W2.3

REINFECTION AFTER SUCCESSFUL ERADICATION OF *HELICOBACTER PYLORI* IN THREE DIFFERENT POPULATIONS IN ALASKA

M. G. Bruce,* D. Bruden,* J. Morris,* A. Reasonover,* F. Sacco,† D. Hurlburt,* T. Hennessy,* J. Gove,‡ A. Parkinson,* G. Sahagun,§ P. Davis,† J. Klejka¶ and B. McMahon**
*Centers for Disease Contol and Prevention, Anchorage, AK, USA; †The Alaska Native Medical Center, Anchorage, AK, USA; ‡Liver Disease and Hepatitis Program Alaska Native Tribal Health Consortium, Anchorage, AK, USA; §Internal Medicine Associate INC, Anchorage, AK, USA; ¶Yukon Kuskokwim Health Corporation, Bethel, AK, USA; **Liver Disease and Hepatitis Program, Division of Community Health Services, Anchorage, AK, USA

Introduction: *H. pylori* infection is common among American Indian/Alaska Native persons (AI/AN), is a major cause of peptic ulcer disease, and is associated with gastric cancer. We performed a study to determine rates of reinfection in three groups followed for 2 years after successful treatment: AI/AN living in urban (Group1) and rural (Group2) communities, and urban Alaska non-Native persons (Group3).
Methods: We enrolled adults diagnosed with *H. pylori* infection based on a positive urea breath test (UBT). After successful treatment was documented at 2 months, we tested each patient by ^{13}C-UBT at 4, 6, 12 and 24 months. At each visit, participants were asked about medication use, illnesses and risk factors for reinfection.
Results: We followed 229 persons for 2 years or until they became reinfected. *H. pylori* reinfection occurred in 36 persons total; proportional cumulative reinfection was 14.5%, 22.1%, and 12.0% for groups 1, 2 and 3, respectively. Study participants who became reinfected were more likely to have peptic ulcer disease (past or present, $p = 0.02$), low education level ($p = 0.04$), or have a higher proportion of household members infected with *H. pylori* compared to participants who did not become reinfected ($p = 0.03$). Among all three groups, the proportion reinfected after successful treatment for *H. pylori* was higher than those reported for other US populations (<5% at 2 years).
Conclusions: Rural AI/AN were at highest risk for reinfection. Persons with peptic ulcer disease (past or present) should be followed, after successful treatment for *H. pylori,* with periodic UBT to detect reinfection.

Abstract no.: W2.4

LOW EFFICACY OF RESCUE TREATMENTS FOR *H. PYLORI* ERADICATION IN CHILDREN AFTER FAILURE OF A FIRST SEQUENTIAL REGIMEN

N. Genis, A. Salame, E. Lenga, T. Mahler, M. Scaillon, S. Cadranel and P. Bontems
Queen Fabiola Children's University Hospital, Université Libre de Bruxelles, Brussels, Belgium

Objective: No data have been published so far concerning the efficacy of second line eradication treatments for *Helicobacter pylori* infection.
Methods: Open, mono-center, non-randomized, retrospective study. Through July 2007–August 2011, naïve children with *Helicobacter pylori* infection were systematically treated with a sequential regimen (SR). Eradication was assessed at least 8 weeks after treatment by 13C-urea breath test (UBT). A primary antimicrobial susceptibility testing was performed by *E*-test. Those who remained

infected received a second line treatment by either a repeated SR or a tailored triple therapy (TT) for 10–14 days according to the results of the primary antimicrobial susceptibility test. Eradication was then assessed by UBT again.
Results: Two hundred and thirty-two naïve children were enrolled during the study period, aged 1.5–18 years (median 10.5 years) and treated by a SR. Among them, 13 (5.6%) were lost of follow-up and 38 (16.3%) remained infected (intention to treat eradication rate, ITT 78%, per protocol eradication rate, PP 83%). Among these 38 children, 30 received a second line treatment: 24/30 a tailored TT and 6/30 a SR. Eradication rates were low, achieved only in 13/24 children in the TT arm (ITT 54%, PP 13/20 – 65%) and in 3/6 children in the SR arm (ITT 50%, PP 2/5 – 60%). Compliance and tolerance were good.
Conclusion: The efficacy of rescue treatments is low after failure of a first SR. This may be due to a high rate of acquired secondary resistance since the compliance was satisfactory. Our results should be assessed in a large prospective multicenter study.

Abstract no.: W2.5

STRING TEST UTILITY TO OBTAIN *HELICOBACTER PYLORI* AND PERFORM ANTIMICROBIAL SUSCEPTIBILITY. A 13 YEAR EXPERIENCE

M. Montes,* M. Fernández-Reyes,* Y. Salicio,* A. Cosme,† L. Bujanda†,‡ and E. Pérez-Trallero*,‡
*Microbiology Department, Hospital U. Donostia, Donostia-San Sebastián, Spain; †Gastroenterology Department, Hospital U. Donostia, Donostia-San Sebastián, Spain; ‡Faculty of Medicine, University of the Basque Country, UPV/EHU, Donostia-San Sebastián, Spain

Introduction: Eradication of *H. pylori* is greatest when treatment is cultured-guided. To know the susceptibility pattern it is necessary to obtain the microorganism. When endoscopy is not essential (e.g. relapses after treatment) the string test is the most comfortable alternative for the patient.
Aim: To demonstrate the utility of the string test to perform antimicrobial susceptibility of *H. pylori* compared with biopsy culture for 13 years of hospital practice.
Methods: The pediatric string test (ENTERO-TEST^{R}) was used for both children and adults and it was performed with the patient fasting the night before. The capsule was swallowed and the string was held in the stomach for 1.5 hours being culture after extraction.
Results: During 2000–2012, 2813 string test and 9838 gastric biopsies were studied. The results are referred in Table 1.

Table 1 Results of string tests and gastric biopsies 2000–2012

	Number investigated	Positive cultures (%)	With antibiogram (%)
Biopsies	9838	4774 (48.5)	4661 (97.6)
String test	2813	1402 (49.8)	1337 (95.4)
Total	12 651	6176 (48.8)	5998 (97.1)

Cultures were "not evaluable" in 126 (4.5%) strings because of pharyngeal flora overgrowth and in 18 biopsies (0.2%) with accidental contamination.
Conclusions: In areas with a high prevalence of resistant strains, the antibiogram to guide the treatment is necessary. The string test greatly facilitates the susceptibility study because, unlike biopsy, it does not require an expert endoscopist, being only necessary a microbiology laboratory moderately equipped. The string test is a cheap-noninvasive technique, well tolerated by patients and very useful to recover *Helicobacter* strains and to know their antimicrobial susceptibility.

Abstract no.: W2.6

A NEW APPROACH AS A NON-INVASIVE MOLECULAR METHOD TO DETECT *HELICOBACTER PYLORI* AND DETERMINE CLARITHROMYCIN AND LEVOFLOXACIN SUSCEPTIBILITY: STOOL GENOTYPE HELICODR

T. Becerikli,* Ö. Yılmaz,* E. Demiray-Gürbüz,* S. Sarıoğlu,† M. Soytürk,‡ H. Ellidokuz§ and I. Şimşek‡
*Department of Medical Microbiology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; †Department of Pathology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; ‡Department of Gastroenterology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; §Department of Medical Informatics and Biostatistics, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey

Aim: To evaluate applicability of GenotypeHelicoDR for stool specimens.
Methods: Fifty-five patients with dyspepsia (38F, 17M; 41.1 ± 12.7 years) were included in this study. Antrum and corpus biopsies were taken and *H. pylori* positivity was defined according to at least two positivity of culture, histopathology and RUT. Stool specimens were collected simultaneously with gastric biopsy specimens. Culture, E-test and GenotypeHelicoDR were all performed to antrum and corpus biopsy specimens for confirmation. DNA was extracted from stool specimens with QIAamp DNA Stool Mini Kit (QIAGEN). Stool GenotypeHelicoDR was applied to assess *H. pylori* and its resistance to clarithromycin and levofloxacin simultaneously with gastric biopsy specimens, was used with some modifications of the recommended colony and biopsy kit procedures to adapt to obtain best results with stool specimens.
Results: Fourty-eight (87.3%) patients were *H. pylori* positive according to our criteria. Hybridization bands of stool specimens were as strongly visible as gastric biopsy specimens for both *H. pylori,* wild-type and resistant probes of clarithromycin and levofloxacin. Fifty-four (98.2%) patients were positive and 1 (1.8%) patient was negative for *H. pylori* by stool GenotypeHelicoDR. Thirty-four (58.6%) patients and 8 (13.8%) patients hybridized with the sequences of both wild-type and mutated alleles for clarithromycin and levofloxacin, respectively. Thirty-five (63.6%) and 40 (72.7%) patients were correlated with the biopsy results of GenotypeHelicoDR for clarithromycin and levofloxacin, respectively. The sensitivity of stool GenotypeHelicoDR was 100% ($\kappa = 0.225$).
Conclusion: As a non-invasive method, ability of using stool GenotypeHelicoDR is very useful in clinical setting. To obtain different results from biopsy and stool specimens may be associated with genotype diversity among biopsy and stool specimens. Genotype diversity showed that more than one *H. pylori* genotype may exist in the same patient with dyspepsia.

WS3 Treatment and Drug Resistance

Abstract no.: W3.1

EFFECT OF DOSING SCHEDULES OF AMOXICILLIN ON ERADICATION RATES OF *H. PYLORI* BY TRIPLE THERAPY WITH PPI, AMOXICILLIN AND CLARITHROMYCIN OR METRONIDAZOLE

T. Furuta, M. Sugimoto, T. Uotani, S. Sahara and H. Ichikawa
Hamamatsu University School of Medicine, Hamamatsu, Japan

Objectives: Usually, drugs used in standard regimens for *H. pylori* infection are dosed twice daily. However, the bactericidal effect of amoxicillin depends on the time-above-MIC, not Cmax or AUC. Then, we aimed to examine the influence of different dosing schedules of amoxicillin on eradication rates of *H. pylori* by triple therapies.
Methods: Patients infected with clarithromycin-sensitive strains of *H. pylori* were treated with a PPI, clarithromycin 200 mg bid and amoxicillin 750 mg bid, 500 mg tid or 500 mg qid for 1 week and those infected with clarithromycin-resistant strains were treated with a PPI, metronidazole 250 mg bid and amoxicillin 750 mg bid, 500 mg tid or 500 mg qid for 1 week. In the rapid metabolizers of CYP2C19, a PPI was dosed four times daily, whereas intermediate and poor metabolizers, a PPI was dosed twice daily.
Results: Ten patients were excluded from the analysis. The eradication rates (PP) of the triple PPI/amoxicillin/clarithromycin therapy with bid, tid and qid dosings of amoxicillin were 80.3% (49/61), 96.7% (58/60) and 95.0% (57/60), respectively. Those of the triple PPI/amoxicillin/metronidazole therapy were 82.5% (33/40), 95.0% (38/40) and 97.6% (40/41), respectively. The eradication rates in the regimens with tid and qid dosings of amoxicillin were higher than that of the regimen with the bid dosing of amoxicillin.
Conclusions: The dosing schedule of amoxicillin significantly influenced the eradication rates of the standard triple therapies. Although amoxicillin is empirically dosed twice daily, three or four times daily dosing is appropriate for amoxicillin in *H. pylori* eradication therapy.

Abstract no.: W3.2

THE MOLECULAR BASIS OF INACTIVATION OF METRONIDAZOLE RESISTANT *HELICOBACTER PYLORI* BY POLYETHYLENEIMINE (PEI) FUNCTIONALIZED ZINC OXIDE (ZNO) NANO-PARTICLES

S. Bhattacharya,* S. Chakraborti,† P. Chakrabarti† and R. Chowdhury*
*Indian Institute of Chemical Biology, Kolkata, India; †Bose Institute, Kolkata, India

In view of the world wide prevalence of *Helicobacter pylori* infection, its potentially serious consequences, and the increasing emergence of antibiotic resistant *H. pylori* strains there is an urgent need for the development of alternative strategies to combat the infection. In this study polyethyleneimine (PEI) functionalized zinc oxide (ZnO) nanoparticles (NPs) have been engineered which when compared to conventional ZnO NPs, have been demonstrated to be more water soluble, relatively acid stable, more potent in generating reactive oxygen species (ROS) and more specifically attracted towards gram negative bacteria by virtue of its ability to intercalate into the outer membrane lipopolysaccharide. It has been demonstrated that ZnO-PEI NPs efficiently inhibit the growth of a metronidazole-resistant strain of *H. pylori* and the molecular basis of its anti-bacterial activity has been elucidated. Internalization and uniform distribution of ZnO-PEI NP without agglomeration was observed in *H. pylori* cytosol by electron microscopy. Several lines of evidence including scanning electron microscopy, propidium iodide uptake and ATP assay indicate severe membrane damage in ZnO-PEI NP treated *H. pylori*. Intracellular ROS generation increased rapidly following the treatment of *H. pylori* with ZnO-PEI NP and extensive degradation of 16S and 23S rRNA was observed by quantitative reverse-transcriptase PCR. Finally, considerable synergy between ZnO-PEI NP and antibiotics was observed and it has been demonstrated that the concentration of ZnO-PEI NP (20 μg/mL) that is non-toxic to human cells could be safely used in combination with sub-inhibitory concentrations of antibiotics for the inhibition of *H. pylori* growth.

Abstract no.: W3.3

SEQUENTIAL AND CONCOMITANT TREATMENTS IN *H. PYLORI* ERADICATION: A NETWORK META-ANALYSIS

A. G. McNicholl, O. P. Nyssen and J. P. Gisbert
La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain

Background: Conventional meta-analyses comparing non-bismuth quadruple sequential (SEQ) and concomitant (CON) regimens in *H. pylori* eradication have been unable to demonstrate differences on treatment efficacy. Network meta-analyses combining pooled data from direct comparisons and comparisons with a common control treatment (standard triple therapy, STT) may provide more complete and consistent information for the selection of the most effective treatment.
Aim: To perform a network meta-analysis of randomized trials comparing SEQ versus CON treatment, or with STT as common comparator.
Methods: *Selection of studies*: randomized clinical trials comparing CON versus SEQ, or comparing them with STT. Studies with different treatment arm lengths were excluded. *Search strategy:* bibliographical searches in electronic databases, and manual search of abstracts from Congresses, were conducted up to May-2013. *Data synthesis:* intention-to-treat eradication rate. *Outcome:* Odds Ratio (OR) pooled using random-effects-model.
Results: Twenty-six trials were included: 13 SEQvsSTT (3648 patients), eight CONvsSTT (1230 patients) and five CONvsSEQ (966 patients). Only the SEQvsSTT comparison was heterogeneous (I^2 = 62%). Direct comparisons showed significantly lower eradication efficacy of STT than SEQ (OR = 1.74) and CON (OR = 2.57) treatments. Direct CONvsSEQ meta-analysis showed significantly better results for CON than for SEQ treatment (OR = 1.47; 95% CI = 1.02–2.12). Indirect comparison obtained similar results OR = 1.48; 95% CI = 0.98–2.36. Network meta-analysis (combining the results from direct and indirect comparisons) demonstrated that CON regimen was significantly more effective than SEQ (OR = 1.47; 95% CI = 1.06–2.05) and that results were consistent (detailed data of all comparisons are shown in the table). Number needed to treat was 11.
Conclusion: The results from this network meta-analysis demonstrate that non-bismuth quadruple concomitant treatment offers consistent and significantly better cure rates than sequential treatment in the eradication of *H. pylori*.

Table 1 Direct and Indirect comparison of SEQ and CON regimens

Comparison	Odds ratio	95% CI
Direct SEQvsSTT	1.74	1.27–2.38
Direct CONvsSTT	2.57	1.85–3.58
Direct CONvsSEQ	1.47	1.02–2.12
Indirect CONvsSEQ	1.48	0.92–2.36
Network CONvsSEQ	1.47	1.06–2.04

Abstract no.: W3.4

SEQUENTIAL VERSUS TRIPLE THERAPY FOR THE TREATMENT OF *HELICOBACTER PYLORI*: A NATIONWIDE STUDY

J. S. Kim, B. Kim, J. Ji, B. Lee and H. Choi
Incheon St. Mary's Hospital, Incheon, Korea

Background/Aims: Eradication of *Helicobacter pylori* infection with triple therapy has declined in part to increased antibiotic resistance. Sequential therapy has shown promise in several studies published from Korea. The aim of this study was to compare the efficacy of sequential therapy with that of triple therapy in Korea by performing a nationwide study.
Methods: For this multicentre, nationwide, randomized trial, patients (≥20 years of age) with *H. pylori* infection from 13 centers in Korea were recruited. Patients were randomly allocated to either sequential therapy (PPI and amoxicillin for the first 5 days, followed by PPI, clarithromycin and metronidazole for the next 5 days) or triple therapy (PPI, amoxicillin and clarithromycin for 10 days). The primary outcome was the eradication rate of each treatment by intention-to-treat (ITT) and per-protocol (PP) analysis.
Results: Between March, 2012 and January, 2013, 380 patients were enrolled. In the ITT analysis, the eradication rates of sequential therapy and triple therapy were 81.9% (158/193) and 69.0% (129/187), respectively (p = 0.004). In the PP analysis, the eradication rates were 87.1% (155/178) and 73.8% (127/172), respectively (p = 0.002). There was no statistically significant difference

between the two groups regarding the occurrence of adverse events (52.3% vs 47.1%, $p = 0.304$).

Conclusions: The sequential therapy regimen achieved higher eradication rates than the standard triple therapy regimen in Korea. Additional study results from more patients are expected.

Abstract no.: W3.5

CLARITHROMYCIN FOR FIRST-LINE TREATMENT OF *HELICOBACTER PYLORI* INFECTION IN HIGH ANTIBIOTIC RESISTANCE REGIONS: BETTER TESTING ANTIMICROBIAL SUSCEPTIBILITY BEFORE TREATING

M. Martos, A. Cosme, M. Montes, B. Ibarra, U. Mendarte, M. Fernandez, C. Sarasqueta and L. Bujanda

Hospital Universitario Donostia, San Sebastisn, Spain

Background: Resistance to antibiotics is the major cause of treatment failure of *Helicobacter pylori* infection. The culture-guided triple therapy (chosen based on a preliminary in vitro susceptibility test) might help to increase treatment success in high antibiotic resistance regions.

Methods: This was a prospective trial of 100 patients naïve *H. pylori* positive recruited consecutively from February 2011 to May 2012, in Hospital Universitario Donostia, San Sebastian (Basque Country). The patients were randomized in a 1:1 ratio to receive standard triple therapy (OAC), omeprazole (20 mg b.i.d.), amoxicillin (1 g b.i.d.) and clarithromycin (500 mg b.i.d.) for 10-days, after antimicrobial susceptibility testing if there is no resistance to clarithromycin, or empirical 10 days OAC for first-line therapy of *H. pylori*. Eradication was confirmed using 13C-labelled urea breath test (UBT) 6–8 weeks after therapy. Incidence of adverse effects was evaluated by means of a questionnaire.

Results: The succes rate of 10-days OAC therapy in the 50 patients diagnosed by culture with clarithromycin-susceptibility was 94% (95% CI: 0.83–0.98) whereas in the gruop of patients treated with empirical 10-days OAC therapy was 72% (95% CI: 0.58–0.85) ($p < 0.006$). During recruitment period, considering 937 *H. pylori* isolates, the goblal resistance was 20.8% for clarithromycin and 45.8% for metronidazole. Adverse effects were registered in 13% of the patients, the most common being metallic taste (5%) and diarrhea (3%), having no differences among the groups.

Conclusions: Knowledge of antimicrobial susceptibility of *H. pylori* is necesary before first-line treatment with standard triple therapy (OAC) for 10 days, particulary in high antibiotic resistance regions (Clinical Trials.gov number NCT01486082).

Abstract no.: W3.6

DOES THE MAASTRICHT IV CONSENSUS REPORT ON *HELICOBACTER PYLORI* ERADICATION HAVE ANY IMPLICATIONS IN A SOUTH-EUROPEAN COUNTRY?

N. M. Almeida,* J. M. Romãozinho,* M. M. Donato,† C. Luxo,‡ O. Cardoso,‡ M. A. Cipriano,* C. Marinho* and C. M. R. Sofia*

*Coimbra University Hospital, Coimbra, Portugal; †Faculty of Medicine, Coimbra, Portugal; ‡Faculty of Pharmacy, Coimbra, Portugal

Introduction: The Maastricht IV consensus report recommends that the most commonly used empirical triple-therapy protocol for *Helicobacter pylori* (Hp) eradication should not be prescribed if clarithromycin resistance rate in a specific population exceeds 15–20%.

Aims and Methods: This prospective unicentric study, involving adult patients with positive 13C Urea Breath Test (UBT), was performed to evaluate the success rate of standard triple therapy for Hp eradication in a south-european country, the corresponding clarithromycin resistance rate and possible factors associated with treatment failure (age, sex, urban/rural residence, personal and family history of gastric pathology, consumption of olive oil/alcohol/tobacco, Body Mass Index, frequent infections requiring antibiotherapy, genetic profiles of Hp, therapy compliance and adverse events). All patients were submitted to upper digestive endoscopy with gastric biopsies for histological and microbiological characterization (genotyping for cagA, babA, vacA and IceA status, and minimum inhibitory concentration determination against metronidazole, clarithromycin, levofloxacin, amoxicillin and tetracycline). They were then treated with triple-therapy protocol (Pantoprazol + Amoxicillin + Clarithromycin, 14 days). Hp eradication rate was assessed with UBT after 8–12 weeks. Statistical analysis with SPSS v20.0.

Results: Eighty patients were enrolled in the protocol (males-23; mean age-41 ± 13 years; completed therapy-75%; adverse effects-46.3%). Hp eradication was sucessful in 68.8%. Clarithromycin resistance rate was 20% and mutation A2143G was the most commonly identified (18.8%). Factors associated with treatment failure (Hp+ vs Hp−) were: frequent infections (32% vs 10.9%), active smoking (20% vs 3.6%); vacAs2 gene (72% vs 47.3%); vacAm2 gene (96% vs 74.5%).

Conclusions: In this country Hp eradication rate with empirical triple-therapy is very low. Clarithromycin resistance rates are high and, following the Maastricht IV recommendations, the traditional triple treatment should not be used as first choice, especially in patients with a history of smoking or frequent infections.

WS4 Other Helicobacters and Animal Models

Abstract no.: W4.1

UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (UPAR) IS INDUCED IN GASTRIC EPITHELIAL CELLS IN RESPONSE TO *HELICOBACTER PYLORI* INFECTION IN A C57BL/6 MOUSE MODEL

W. Alpizar-Alpizar,*,† M. E. Skindersø,‡ L. Rasmussen,§,¶ I. J. Christensen,† I. K. Lund,† O. D. Laerum,† K. A. Krogfelt,‡ L. P. Andersen§,¶ and M. Ploug†
*Centre for Research on Microscopic Structures (CIEMic), University of Costa Rica, San Jose, Costa Rica; †The Finsen Laboratory, Copenhagen University Hospital, Copenhagen, Denmark; ‡Department of Microbiology and Infection Control, Statens Serum Institute, Copenhagen, Denmark; §Department of Infection Control, Copenhagen University Hospital, Copenhagen, Denmark; ¶Department of Clinical Microbiology, Copenhagen University Hospital, Copenhagen, Denmark

H. pylori infection induces chronic inflammation in gastric mucosa, which in susceptible individuals may ultimately result in gastric cancer. uPAR plays a central role in extracellular matrix remodeling by mediating plasminogen activation and is highly upregulated during cancer invasion and inflammation. Circumstantial evidence keeps accumulating, which correlates *H. pylori* colonization with increased uPAR expression in the human gastric epithelium. In this study, we investigated the time-course of uPAR induction in the gastric mucosa in response to *H. pylori* infection in a mouse model. Histopathological evaluation of the *H. pylori*-colonized mouse stomachs revealed a positive correlation between increased inflammation and the duration of infection. In *H. pylori*-infected mice, intense uPAR immunoreactivity was observed in foveolar epithelial cells of the gastric corpus and this was absent in non-infected animals. *H. pylori*-dependent uPAR induction was a very early event, but it increased progressively during all 30 weeks of the experiment ($p = 0.006$; $r_s = 0.57$). This increased uPAR expression was correlated to the number of infiltrating CD3-positive and F4/80-positive cells ($p < 0.0001$; $r_s = 0.65$ and $p < 0.0001$; $r_s = 0.74$, respectively) and to epithelial cell proliferation ($p < 0.0001$; $r_s = 0.70$). Eradication of *H. pylori* infection by antimicrobial therapy caused a complete regression of the uPAR expression to base-line levels observed in the non-infected mouse gastric epithelium. In contrast, suppression of the inflammatory response by prostaglandin E_2 did attenuate uPAR expression, but not to base-line levels. In conclusion, uPAR is induced in foveolar epithelial cells in response persistent *H. pylori* colonization of the gastric mucosa and this occurs early in the time-course of infection.

Abstract no.: W4.2

PATHOGENIC PROPERTIES OF ENTEROHEPATIC *HELICOBACTER* SPP. ASSOCIATED WITH INTESTINAL ADENOCARCINOMA IN RHESUS MACAQUES

K. Lertpiriyapong,* Y. Feng,* L. Handt,† T. W. Mitchell,† K. Lodge,† Z. Shen,* F. Dewhirst† and J. G. Fox*
*Massachusetts Institute of Technology, Cambridge, MA, USA; †The Forsyth Institute, Cambridge, MA, USA

We determined the prevalence of Enterohepatic *Helicobacter* species (EHS) infection in a cohort of geriatric rhesus monkeys in which intestinal adenocarcinoma (IAC) is common and investigated its association with EHS. The cohort consisted of 36 animals, 14 of which (age 26–35 years) had IAC. Of the 36 rhesus, 35 (97%) were positive for EHS using PCR or bacterial isolation from feces, colonic or tumor tissues. Only one rhesus, which had IAC, was negative for EHS by all detection methods. 16S rRNA gene sequencing revealed that EHS identified in these rhesus are related to a human clinical isolate of EHS, *H. fennelliae* CCUG 18820, and EHS previously reported by our laboratory, *H. macacae*, and *Helicobacter* sp. MIT 99-5507 Rhesus monkey 2. Thirteen of 14 monkeys with IAC were positive for either H. macacae (7/13 [54%]), EHS similar to *H. fennelliae* CCUG 18820 (4/13 [31%]) or a mixture of the two EHS (2/13 [15%]). These results indicate that EHS is prevalent among aged rhesus macaques with IAC and reveal an association between EHS and IAC. Using *Helicobacter*-family specific fluorescence in situ hybridization, EHS could be detected on the surface of colonic epithelia of infected monkeys. All Helicobacter isolates, including *H. macacae*, effectively adhered to, invaded, and significantly induced proinflammatory genes, including *IL8, IL6, TNF-α,* and *iNOS*, while down regulating genes involved in the function of inflammasomes, particularly *IL-1β, NRLP3,* and *NLRP6* in the human colonic T84 cell line ($p < 0.0001$). Human EHS may represent an etiological agent mediating diarrhea, chronic inflammation, and possibly intestinal cancer in nonhuman primates, and may play a role in similar human diseases. As EHS often persists within hosts, down regulation of inflammasome function may represent a strategy for EHS to establish chronic colonization in the host.

Abstract no.: W4.3

ORAL GLUTATHIONE SUPPLEMENTATION REDUCES *HELICOBACTER SUIS* – RELATED GASTRIC PATHOLOGIES IN A MONGOLIAN GERBIL MODEL

E. De Bruyne, R. Ducatelle, A. Smet, F. Pasmans, F. Haesebrouck and B. Flahou
Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Helicobacter (*H.*) *suis* causes gastric pathologies in both pigs and humans. An important virulence factor of *H. suis,* -glutamyl-transpeptidase (GGT), is responsible for the degradation of extracellular components like glutathione (GSH). Both for *H. pylori* and *H. suis,* it has been hypothesized that the degradation of extracellular GSH can lead to a deficiency for the host, possibly initiating or promoting several pathologies. In this study, we aimed at reducing *H. suis*-linked gastric pathologies by supplementing GSH to the diet of Mongolian gerbils. Both uninfected and *H. suis*-infected gerbils were divided into two groups, each receiving a specific standard rodent diet or the same diet supplemented with 0.8% GSH. Twelve weeks after inoculation, the animals were euthanized. Histopathological analysis showed that GSH supplementation significantly reduced gastric inflammation and epithelial cell proliferation in *H. suis* infected animals compared to infected animals receiving the standard diet. Compared to uninfected control animals, expression levels of IL-1β, IL-10, IL-17 and INF- mRNA were markedly upregulated in infected animals receiving the standard diet. In contrast, infected animals receiving the GSH diet showed a normalization of cytokine expression levels, comparable to the expression levels found in the control animals. These results clearly show that GSH supplementation can drastically reduce *H. suis* infection-related gastric pathologies, possibly opening doors for new treatment regimens.

Abstract no.: W4.4

THE GYRA GENE: A PERTINENT TARGET FOR *HELICOBACTERACEAE* SPECIES TAXONOMY

A. Ménard,*,† A. Buissonnière,*,† V. Prouzet-Mauléon*,† and F. Mégraud*,†
*Université de Bordeaux, Centre National de Référence des Helicobacters et Campylobacters, Bordeaux, France; †INSERM U853, Bordeaux, France

Taxonomy of *Epsilonproteobacteria* is based on sequencing of the 16S rRNA gene. However, 16S rRNA gene taxonomy is not sufficiently discriminant in *Helicobacter* species and discordant results were reported for 16S and 23S rRNA gene sequences, suggesting that an alternative scheme to ribosomal gene phylogeny would be useful. The *gyrA* gene encoding the subunit A of DNA gyrase was reported to be an important tool for bacterial phylogeny and was used to identify several organisms including enteric bacteria. In this study, the *gyrA* gene of 55 Helicobacter strains belonging to 25 species was sequenced using chromosome walking and PCR sequencing. Phylogenetic trees were generated by the neighbor-joining method using the entire *gyrA* gene. Preliminary results of the phylogenetic analyses of the *gyrA* gene indicated a good separation of these species, especially between gastric and enterohepatic *Helicobacter* species. Moreover, the phylogenetic analysis of the *gyrA* gene revealed some differences in clustering when compared to the 16S and 23S rRNA gene trees previously reported while a similar clustering to that of the partial *gyrB* gene was observed indicating that the gyrase genes are pertinent target for *Helicobacteraceae* species taxonomy.

Abstract no.: W4.5

PARKINSON'S DISEASE AND *HELICOBACTER SUIS*: PIGS, PORK OR TRANSMISSION OF HUMAN-ADAPTED *H. SUIS*-LIKE BACTERIA?

F. Haesebrouck,* A. Smet,* B. Flahou,* C. Blaecher,* F. Pasmans,* R. Ducatelle,* I. Bjarnason,† A. J. Lawson,‡ A. Charlett,§,¶ D. Taylor,¶,** C. Weller,¶ R. J. Dobbs†,¶,** and S. M. Dobbs†,¶,**

*Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; †Department of Gastroenterology, King's College Hospital, London, UK; ‡Laboratory of Gastrointestinal Pathogens, Public Health England, London, UK; §Statistics Unit Centre for Infections, Public Health England, London, UK; ¶Pharmaceutical Sciences, King's College, London, UK; **Maudsley Hospital, London, UK

Background: *Helicobacter suis* is a very fastidious bacterium often present in pigs' stomachs. Using multilocus sequence typing (MLST), we demonstrated that a *H. suis* strain from the stomach of a pig-veterinarian with gastric complaints was closely related to porcine strains, confirming the zoonotic potential of this *Helicobacter* species. We also demonstrated that *H. suis* can be present and survive in minced pork, indicating that raw or undercooked pork might constitute a source of *H. suis* infection.

Methods: Archived DNA-extracts from gastric biopsies of 60 patients with idiopathic parkinsonism (IP) and 256 "controls" from gastroenterology-services were examined for *H. suis* using an *ureA*-based species-specific qPCR.

Results: Overall, *H. suis* DNA (confirmed by sequencing) was present in 27% (binomial exact 95% confidence interval (C.I.) 15, 38) of the IP-patients and in 2% (CI 0, 3) of controls. Relative frequency of *H. suis* compared with *H. pylori* (culture or PCR) was 10 times greater in IP than controls. In IP-patients, *H. suis* DNA was detected in DNA-extracts from 11 of 19 (58%) with biopsy-proven *H. pylori* eradication and 5/41 (12%) never exposed to anti-*H. pylori* therapy. In controls, it was detected in 3/100 (3%) patients with recorded exposure to anti-*H. pylori* therapy and in 1/156 (0.6%) without.

Conclusions: Greater frequency of *H. suis* DNA detection in IP-patients, exaggerated where *H. pylori* had been eradicated (usual therapy amoxicillin/clarithromycin/proton pump inhibitor), warrants further studies. MLST comparison with porcine *H. suis* strains may clarify whether these infections originate from pigs/porcine products or by transmission of *H. suis*-like (human-adapted) bacteria.

Abstract no.: W4.6

ANALYSIS OF *HELICOBACTER* SPP. SPECIFIC ANTIBODIES WITH MULTIPLEX SEROLOGY

J. Butt,* A. Michel,* M. Willhauck-Fleckenstein,* W. Nicklas,† J. G. Fox‡ and M. Pawlita*

*Infection and Cancer Program, German Cancer Research Center (DKFZ), Heidelberg, Germany; †Microbiological Diagnostics, German Cancer Research Center (DKFZ), Heidelberg, Germany; ‡Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA, USA

Helicobacter pylori is recognized as causal agent for gastric cancer in humans. *H. hepaticus* and *H. bilis* are discussed to be associated with diseases of the enterohepatic and biliary tract in mice and humans. To allow for specific analysis of antibodies to *Helicobacter* spp. we developed and validated an assay suited to further assess prevalence and disease association of *Helicobacter* spp. infections in large sero-epidemiological studies. The assay is based on fluorescent polystyrene bead technology (Luminex). Antigens are membrane proteins of *H. pylori, H. hepaticus* and *H. bilis* extracted by a mild detergent (N-octyl-B-D-glucopyranoside) and cross-linked to the bead surfaces. Analysis of 50 gerbil and mouse sera with defined status of *Helicobacter* spp. infection showed that *Helicobacter* spp. membrane extracts directly coupled to polystyrene beads are technically feasible with a multiplex serology approach. Agreement of infection status and detection of antibodies was considered to be very good for *H. pylori* ($\kappa = 0.8$, 95% CI: 0.44–1.16) and *H. hepaticus* ($\kappa = 0.8$, 95% CI: 0.54–1.06) but poor for *H. bilis* ($\kappa = 0.2$, 95% CI: −0.06 to 0.48) due to a four times lower sensitivity compared to *H. pylori* and *H. hepaticus*. Signals of crossreacting antibodies between species can be eliminated with an additional pre-blocking of murine sera with *Helicobacter* spp. membrane extracts. The assay is suited to discriminate between *Helicobacter* spp. specific antibodies that may be associated with enterohepatic diseases.

WS5 Gastric Cancer and Pre-malignant Lesions

Abstract no.: W5.1

CHANGES IN GASTRIC CANCER PREVALENCE AND TREATMENT STRATEGY

S. Lee and S. Park
Yeouido St. Mary's hospital, medical college, the Catholic University of Korea, Seoul, Korea

Background: The recommended interval of endoscopic screening for gastric cancer (GC) in general population is 2-year after National Cancer Screening (NCS) program started since 2007 in Korea. After NCS program was started, total number of endoscopy had been increased. The aim of this study is to evaluate the early detection rate of early gastric cancer (EGC) and the rate of endoscopic submucosal dissection (ESD) or endoscopic mucosal resection (EMR) in patients diagnosed with EGC.
Patients and Methods: We retrospectively reviewed all consecutive patients diagnosed and treated with GC from 2005 to 2012 at Yeouido St. Mary's hospital in Seoul, Korea. All patients were categorized according to whether they had been diagnosed with advanced gastric cancer (AGC) or not undergone endoscopic exam. Clinical and pathologic characteristics of GC between the two groups were analyzed. Next, the number and ratio of patients undergone ESD or EMR were identified.
Results: In total, 1058 patients were included in this study. Among them, 653 patients were diagnosed with EGC, the others were diagnosed with AGC. And among 1058 patients, 127 patients were undergone ESD or EMR in this hospital. According to annual analysis, EGC was more frequently diagnosed since 2007 – maybe after carrying out NCS (annual odd ratio 0.987, 95% CI, $p = 0.576$ vs 1.275, 95% CI, $p < 0.001$). And the cases of ESD or EMR also increased annually (annual odd ratio 1.203, 95% CI, $p < 0.001$).
Conclusion: With these results, the ratio of patients diagnosed with EGC has increased after NCS program started. And this early detection of gastric cancer may make ESD or EMR increase as the treatment of EGC.

Abstract no.: W5.2

RISK FACTORS AND CLINICAL OUTCOMES FOR 327 PATIENTS WITH GASTRIC CANCER IDENTIFIED BY SCREENING ENDOSCOPY: RETROSPECTIVE CASE–CONTROL STUDY

J. Ahn, H. Jung, E. Gong, K. Choi, J. Lee, D. Kim, K. Choi, H. Song, G. Lee and J. Kim
University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea

Introduction: A customized screening program for gastric cancer would optimize the benefits and cost-effectiveness of screening endoscopy. This study investigated the risk factors for gastric cancer detected during screening, and those factors affecting clinical outcomes.
Methods: Of 109 530 subjects who underwent screening endoscopy at Asan medical Center between April 2000 and December 2010, 327 were diagnosed with gastric cancer (screening group). These patients were compared with 327 age- and sex-matched gastric cancer negative individuals who underwent screening endoscopy (control group). The clinical outcomes of the screening group were compared with those of 663 individuals, matched by age, sex, and date of diagnosis, who were diagnosed with gastric cancer in the outpatient clinic (outpatient group).
Results: The median age of the screening group was 63.6 years, and the male to female ratio was 2.4:1 during 70.7 ± 41.5 months of follow-up periods. When comparing with the control group, *H. pylori* seropositivity (odds ratio [OR] 2.933, 95% confidence interval [CI] 1.876–4.587, $p < 0.001$), carcinoembryonic antigen (OR 8.633, 1.974–37.754, $p = 0.004$), family history of gastric cancer (OR 2.254, 1.256–4.046, $p = 0.007$), and alcohol consumption (OR 3.312, 2.103–5.216, $p < 0.001$) were independent positive risk factors, and the use of aspirin a negative risk factor for gastric cancer (OR 0.445, 0.236–0.840, $p = 0.012$) in multivariate analysis. Low density lipoprotein cholesterol (hazard ratio [HR], 95% CI 0.987, 0.979–0.995, $p = 0.005$), cancer antigen 19-9 (HR 21.713, 6.124–26.391, $p < 0.001$), resectability (HR 59.833, 25.888–138.285, $p < 0.001$), and family history (HR 0.308, 0.127–0.746, $p = 0.009$) were independent risk factors for death. The 5-year survival rate was significantly higher in the screening group than in the outpatient group (82.4% vs 56.3%, $p < 0.001$).
Conclusion: Early detection of gastric cancer by screening endoscopy while asymptomatic enhances patient outcomes, especially in high risk groups.

Abstract no.: W5.3

INCISURA ANGULARIS REPRESENTS THE MORE SEVERE ATROPHIC AND CHRONIC INFLAMMATORY CHANGES COMPARED TO ANTRUM MUCOSA AND CHARACTERIZED BY HIGHER INTEROBSERVER AGREEMENT

S. Isajevs,*[,†,‡] I. Liepniece-Karele,*[,†,‡] D. Janciauskas,[§] G. Moisejevs,*[,†,¶] K. Funka,* I. Kikuste,** A. Vanags,** I. Tolmanis** and M. Leja*[,†,**]
*Faculty of Medicine, University of Latvia, Riga, Latvia; [†]Riga East University Hospital, Riga, Latvia; [‡]Academic Histology Laboratory, Riga, Latvia; [§]Lithuanian University of Health Sciences, Faculty of Medicine, Kaunas, Lithuania; [¶]Riga Stradins University, Riga, Latvia; **Digestive Diseases centre GASTRO, Riga, Latvia

Background: The value of the diagnostic yield of biopsy of incisura angularis has been addressed in several studies. Despite being considered the earliest location of the onset of metaplastic transformation, some studies concluded that few histological findings were detectable only in biopsies of the incisura angularis.
Objective: The aim of our study was to compare the atrophic, metaplastic, acute and chronic inflammatory changes as well the colonization of *H. pylori* in incisura angularis and antrum mucosa.
Methods: Seven hundred and thirty-one patients undergoing upper endoscopy were enrolled in the study. Three expert pathologists graded biopsy specimens according to the Sydney classification and evaluated atrophy, intestinal metaplasia and *H. pylori* colonization in antrum and incisura angularis.
Results: Overall, atrophic mucosa changes in antrum was observed in 20.6% of cases, whereas atrophic changes in incisura angularis was found in 17.4%. However, the moderate and severe atrophy stage (II–III) was more frequently found in incisura angularis compared to antrum mucosa. In addition, the incisura angularis was characterized by frequent incidence of moderate and severe intestinal metaplasia compared to antrum. Furthermore, the frequency of acute inflammatory responses (mucosal leukocytes' infiltration) was higher in antrum mucosa compared to incisura angularis, whereas the frequency of chronic inflammatory responses (mucosal lymphocytes infiltration) was higher in incisura angularis compared to antrum mucosa. *H. pylori* by Giemsa staining was detected slightly often in incisura angularis compared to antrum (respectively, 46.36% vs 47.79% of cases). In addition, the interobserver agreement between pathologists was higher for incisura angularis compared to antrum mucosal changes.
Conclusion: Incisura angularis represents the more severe atrophic and chronic inflammatory changes compared to antrum mucosa and characterized by higher interobserver agreement between the pathologists. The study was supported by ERDF project Nr.2010/0302/2DP/2.1.1.1.0/10/APIA/VIAA/158.

Abstract no.: W5.5

ANCESTRAL ORIGIN AND VIRULENCE MARKERS OF *H. PYLORI* (HP) STRAINS AND HOST GENETIC STRUCTURE AS PREDICTORS OF GASTRIC CANCER (GC)

D. M. M. Queiroz,* G. A. Rocha,* A. M. C. Rocha,* F. F. Melo,* C. A. Oliveira,* M. M. D. A. Cabral,* R. P. A. Cunha,* A. D. Gomes,* C. Anacleto,* K. N. Teixeira,* C. G. M. C. Trant,* R. Calixto,* E. M. Tarazona-Santos,* F. S. G. Kehdy,* K. S. Brito,* R. S. Coimbra* and A. A. M. Lima[†]
*Universidade Federal de Minas Gerais/Faculade de Medicina, Belo Horizonte, Brazil; [†]Universidade Federal do Ceará, Belo Horizonte, Brazil

We aimed to investigate the phylogeographic origin of HP strains from GC patients as well as the genetic structure of the patients to determine whether they are predictors of GC in an admixed population from South-East Brazil. Phylogeographic origin was evaluated in 103 HP strains from patients with GC (n = 27), DU (n = 28) and gastritis, control group (n = 48), by sequencing of both strands of 397–690 bp per gene of the atpA, efp, mutY, trpC, ureI, and yph house-keeping genes. The sequences were aligned (MUSCLE program) and deposited in the multi-locus sequence typing-MLST database. Neighbor joining tree of HP strains (1201 classified in ancestral haplogroups and our 103 strains) was created by software MEGA 5.1 using the Kimura model with 10 000 bootstraps. To determine the ethnicity of each patient, 106 validated SNPs were evaluated by Sequenon iPLEX Plataform. We estimated individuals' ancestry using parental groups: African, European and Brazilian Amerindians employing

the software Structure 2.3.3. Data were analyzed by Fisher, chi-square, Student and correlation tests. HP strains were classified as hpAfrica1 (73–70.9%) and hpEurope (30–29.1%). hpAfrica1 strains were observed in 88.9% (GC), 85.7% (DU) and 47.9% (gastritis) patients (p0.25). s1i1m1 vacA associated with GC and s1m1/m2 with DU ($p < 0.001$). The percentage of each ancestry was similar in patients with gastritis and GC ($p > 0.46$). European ancestry correlated with corpus gastritis ($r = 0.2$, $p = 0.05$) and intestinal metaplasia ($r = 0.2$, $p = 0.04$) in the s1 vacA gastritis group. European ancestry also correlated with European origin of HP strains ($r = 0.5$, $p = 0.01$). HP virulence markers, more than HP ancestry "per se" and genetic structure of the population, are the most important predictors of GC in the studied admixed population.

Abstract no.: W5.6

CHARACTERIZATION OF THE GASTRIC MICROBIOTA IN PATIENTS WITH CHRONIC GASTRITIS AND GASTRIC CARCINOMA

R. M. Ferreira,* J. L. Costa,* C. Figueiredo*,† and J. C. Machado*,†

*IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; †Department of Pathology and Oncology, Faculty of Medicine of the University of Porto, Porto, Portugal

Background and Aim: *Helicobacter pylori* is the major trigger for gastric carcinogenesis. However, during the process of carcinogenesis other bacteria may grow in the stomach as a result of the changes induced by *H. pylori*. The new bacterial growth that competes for the gastric niche may potentially enhance inflammation, thus contributing to gastric carcinogenesis. Our aim is to characterize the gastric microbiota in patients with chronic gastritis and gastric carcinoma.

Methods: So far, data from 10 gastritis and seven carcinoma patients was analyzed. Next generation sequencing platform Ion PGM was used to sequence part of the 16SrRNA gene and the resulting data were analyzed in Qiime pipeline. Sample clustering was evaluated by Principal Coordinate Analysis (PCoA).

Results: A complex and diverse gastric microbiota was identified with considerable variation between individuals, and which was distributed in five main phyla: *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes,* and *Fusobacteria*. In patients with gastritis and carcinoma the most representative phylum was the *Proteobacteria*, with 69.5% and 84.2% reads, respectively. The remaining phyla were less represented in carcinoma than in gastritis patients. The abundance of *Helicobacter* sp. was significantly lower in carcinoma (15.8%) than in gastritis (63.9%) patients. Analysis of the diversity between groups of patients by PCoA revealed clustering based on disease type.

Conclusion: Apart from the complex and diverse gastric microbiota found within individuals, we could identify gastric microbiota profiles that distinguish chronic gastritis from gastric carcinoma patients. The microbiota profiles identified may have an important role in *H. pylori*-associated gastric carcinogenesis. SFRH/BPD/84084/2012.

WS6 Modulation of Inflammation and Immunity

Abstract no.: W6.1

HELICOBACTER PYLORI AND THE RISK OF UPPER GI BLEEDING IN NSAID AND OR LOW-DOSE ASA USERS

C. Sostres,* R. Benito,* P. Carrera,† M. Arroyo,* M. Roncales,* S. Hernandez-Díaz,‡ L. García-Rodriguez§ and A. Lanas*,†,¶

*HCU Lozano Blesa, Zaragoza, Spain; †CiberEHD, Madrid, Spain; ‡Oxford University, Oxford, UK; §CEIFE, Madrid, Spain; ¶Health Research Institute Aragón, Zaragoza, Spain

Helicobacter pylori (Hp) infection and NSAID/ASA use are independent risk factors for peptic ulcer disease. Meta-analysis of observational studies, have shown apparent synergism in the development of uncomplicated peptic ulcer between Hp infection and NSAID use, however the interaction between these two factors or between low-dose ASA and Hp infection for the development of upper gastro-intestinal bleeding (UGIB) remain uncertain.

Aim: To determine the risk of UGIB associated with the interaction between Hp infection and current use of NSAIDs or low dose ASA.

Multicentric hospital based case-control study carried out between 2006 and 2012. The study included consecutive patients hospitalised because of UGIB. Controls matched by age, sex and month of admission. Cases and controls were interviewed with a structured questionnaire including NSAID and low-dose ASA current use. Hp infection status was determined by Western Blot (Bioblot Helicobacter, Biokit SA), which was locally validated. Relative risk (RR) associated with different factors and the interaction between NSAID/ASA and Hp infection was estimated by logistic regression analysis.

Results: We collected 666 cases and 666 controls with mean age 61.6 ± 16.1 and 60.4 ± 15.6 years respectively; 28.8% in both groups were females. 71.3% of cases and 50.3% of controls were Hp positive (RR: 2.5 [1.9–3.1]); 34.5% of cases and 13.4% of controls were current NSAID users (RR: 4 [3.0–5.4]), whereas 15.8% and 12% respectively were ASA users (RR: 1.9 [1.3–2.7]). RRs of the interaction between these factors overall and depending on location are summarized in the table.

Conclusions: Hp infection, NSAID and/or ASA use are independent risk factors for UGIB.

Hp infection potentiates significantly the risk of UGIB in NSAID but not among low-dose ASA users.

Table 1 Interaction of Hp infection, NSAID and/or ASA depending on location

OR (95% CI)*	Overall	Gastric	Duodenal
No NSAID/ASA – No HP	1	1	1
NSAID – No HP	4.8 (3.0–7.6)	5.8 (3.1–10.7)	3.8 (1.9–7.7)
No NSAID – HP	2.6 (2.0–3.5)	1.9 (1.3–2.8)	3.7 (2.5–5.6)
NSAID – HP	9.4 (6.3–14.1)	9.1 (5.2–15.9)	10.0 (5.7–17.8)
ASA – No HP	3.0 (1.7–5.1)	2.9 (1.5–5.9)	3.1 (1.4–7.0)
No ASA – HP	2.8 (2.1–3.6)	2.1 (1.5–3.1)	3.8 (2.6–5.7)
ASA – HP	3.7 (2.3–5.9)	2.6 (1.4–4.9)	5.8 (2.9–11.8)

*Adjusted for sex, age, ulcer history, smoking status, PPI use, ANTICOAGULANT use and NSAID or ASA use.

Abstract no.: W6.2

NON STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) DO NOT ALTER MUCIN PROTEIN EXPRESSION BUT MAY AFFECT GLYCOSYLATION IN THE STOMACH OF GASTRIC ULCER PATIENTS

Y. Niv,* M. Cohen,† M. Halpern,* S. Morgenstern,* D. Boltin,* Z. Levi,* S. Batra‡ and S. B. Ho†

*Rabin Medical Center, Tel Aviv University, Petach Tikva, Israel; †UCSD, San Diego, CA, USA; ‡UNMC, Nebraska, NE, USA

Introduction: *NSAID* therapy is a significant cause for peptic ulcer disease in addition to *Helicobacter pylori* infection. NSAID may alter mucin synthesis and glycosylation by inhibiting cyclo-oxygenase activity and formation of prostaglandin E2 in the stomach.

Aim: To describe gastric mucin expression in *NSAID* associated peptic ulcer in a *post hoc* calculation.

Method: In a previous paper we randomly selected 92 gastric ulcer patients and performed immunohistochemistry for secreted and membrane-bound gastric mucins, lectins and T-cell CD4/CD8. Staining was performed on sections of the mucosa from the ulcer margins. Inflammation score was assessed according to the Sidney system. The patients were grouped according to *NSAID treatment, treated* (group 1, n = 42), or not-treated (group 2, n = 50).

Results: In *NSAID not-treated* patients gland ECA *(Erythrina Cristagalli* agglutinin is a probe to detect Galβ1-4GlcNAc) staining intensity was higher than in NSAID-treated group ($p = 0.037$). A trend was found also for higher MUC17 expression in the glands in NSAID not-treated group ($p = 0.065$). Inflammation score and CD4/CD8 ratio were not different between the groups, except a trend for higher CD4 staining score for NSAID not-treated patients ($p = 0.055$). No statistically significant difference was demonstrated for other mucins or sugar side chains.

Conclusion: Decreased expression of Galβ1-4GlcNAc, type 2 backbone sugar structure, and of MUC17 membrane-bound mucin in NSAID-treated patients may reflect the effect of NSAID on PGE2 synthesis from archaidonic acid in the stomach. Our observation may explain the disruption of the mucin protective layer and NSAID – ulcer pathogenesis.

Abstract no.: W6.3

THERAPEUTIC VACCINATION AGAINST *HELICOBACTER PYLORI*: NEUTRALIZING ANTIBODIES AND T-CELLS

F. Anderl, C. Bolz, B. Kalali, D. H. Busch and M. Gerhard

Institut für medizinische Mikrobiologie, Technische Universität München, Munich, Germany

Helicobacter pylori (*H.p.*) is one of the most prevalent bacterial infections worldwide affecting half of the world's population, thereby causing peptic ulcers and gastric cancer. Although big efforts have been initiated to develop a vaccine against this pathogen no human vaccination study was successful until now. Thus, an approved vaccine for humans is still missing. If this is due to the vaccine formulation – antigen and adjuvant composition, the type of immunity induced, systemic or mucosal delivery – has to be figured out. Our group described a virulence factor of *H. pylori*, the *H.p.* gamma-glutmyltranspeptidase (HPgGT) that inhibits the proliferation of T-cells and thus prevents the generation of an effective immune response. We used HPgGT in an experimental mouse infection model for a novel vaccination approach. As HPgGT is a secreted protein, HPgGT specific T-cells can hardly target the pathogen. Therefore HPgGT was combined with an outer membrane protein to induce protective T-cell responses. Notably, immunization with HPgGT induced a strong antibody response, which blocked its enzymatic activity and thereby counteracting the immunosuppressive effect of HPgGT. In therapeutic infection experiments this vaccination led to a substantial decrease of bacterial colonization in the stomach (>80% of the mice cleared the infection). Thus this immunization strategy could lead to a novel vaccine candidate against *H. pylori*. Currently we are testing this vaccination approach in in additional animal models, the Mongolian gerbil and the Rhesus macaque.

Abstract no.: W6.4

THE INTERACTION BETWEEN *HELICOBACTER PYLORI* AND INFLUENZA A – FRIENDS OR FOES?

J. Pang, M. A. Estrin, W. B. Puryear, N. J. Hill, N. M. Parry, I. T. Hussein, S. Ihezie, M. K. Ramachandran, J. G. Fox and J. A. Runstadler

Massachusetts Institute of Technology, Cambridge, MA, USA

Throughout the course of life, the body is exposed to a multitude of microorganisms. Organisms often synergise to cause more harm to the host, while other microbes confer protection against other pathogens. To study the effects of chronic infections and their role in the host's response to acute onslaughts, 6-week-old C57BL/6 male mice were inoculated with *Helicobacer pylori* SS1 (Hp SS1) or sham dosed, then challenged with Influenza A virus (strain A/Puerto Rico/8/1934 H1N1) 6 weeks later. Weights were monitored daily and serial blood samples for cytokine analysis were collected after Influenza challenge. Stomach tissues were analysed for Hp SS1 colonisation, lung tissues for Influenza virus titres, and both submitted for histopathology. Pilot results demonstrated a significant difference in weight loss between the Hp SS1 and sham dosed groups when challenged with influenza ($p < 0.05$). One hundred percent

of the mice inoculated with Hp SS1 were colonised (mean = 5.36 × 104SS1/μg 18S DNA), and Hp SS1 mice had a lower lung viral titre compared to sham dosed mice at days 3 and 7 (16- and 2-fold respectively). Interferon-γ, detectable in both groups at the day 7 timepoint only, was comparable (145.9 pg/mL in sham dosed vs 124.2 pg/mL in Hp SS1). *H. pylori* infection has been shown to have an inverse relationship with asthma, a chronic respiratory disease (Taube & Müller, 2012). Our experiments suggest that there may be a similar interaction in *H. pylori* infected hosts with acute respiratory viral infections. Further study is warranted to elucidate the interaction and pathways by which this occurs.

Abstract no.: W6.5

COMPARISON OF CLINICAL OUTCOME OF PEPTIC ULCER BLEEDING ACCORDING TO THE DIFFERENT ETIOLOGY: *HELICOBACTER PYLORI* INFECTION OR DRUG USE

H. Sung, W. Chung, K. Lee, Y. Kim, H. Lee, E. Jeon, B. Kim and S. Park
St. Vincent Hospital, the Catholic University of Korea, Suwon, Korea

Background: The peptic ulcer has two major etiologies, *Helicobacter pylori* (*H. pylori*) and drug. Its bleeding can be severe, but the severity of bleeding according to the etiology has rarely been reported. We aim to evaluate the clinical outcomes and severities of peptic ulcer bleeding (PUB) according to the etiology.
Method: A consecutive series of patients who had PUB and admitted to the hospital between 2006 and 2011 were retrospectively analyzed. A total of 232 patients were enrolled in this study, and we compared the clinical characteristics, outcomes according to the different etiologies (*H. pylori* only/Drug only/*H. pylori* and drug/Idiopathic group). We also evaluated severity using Blatchford score and Rockall score between four groups.
Results: In drug only group, patients were older (68.49 ± 14.76 vs 47.84 ± 15.14 years), the duration of admission was longer, (8.52 ± 8.97 vs 5.60 ± 2.41 days), the ulcer size were larger (1.24 ± 0.92 vs 0.86 ± 0.51 cm) and transfusion need is more frequent (3.58 ± 4.95 vs 2.21 ± 1.98) than *H. pylori* only group. Blachford score and Rockall score of drug only group are higher than *H. pylori* only group. Blachford and Rockall score of *H. pylori* & drug group are higher than *H. pylori* only group. In idiopathic group, the ulcer size were larger (1.21 ± 0.65 vs 0.86 ± 0.51 cm) and re-bleeding rate after initial hemostasis were higher (25% vs 7.8%) than *H. pylori* only group.
Conclusions: Clinically, drug induced PUB seems to be more severe than *H. pylori* associated PUB. Idiopathic ulcer has bigger size and more frequent rate of re-bleeding than peptic ulcers with *H. pylori* infection.

Abstract no.: W6.6

MITOCHONDRIA BIOGENESIS IS INDUCED IN RESPONSE TO *H. PYLORI* INFECTION IN A PARTIALLY VACA-DEPENDENT MANNER

L. Chatre,* J. Fernandes,† V. Michel,† M. Ricchetti* and E. Touati†
*Unit of Molecular Genetic of Yeast, Institut Pasteur, Paris, France; †Unit of Helicobacter Pathogenesis, Institut Pasteur, Paris, France

Mitochondria alterations including mitochondrial DNA (mtDNA) instabilities are associated with human diseases and cancer. *Helicobacter pylori* is a major risk factor for gastric cancer. Up to now, only its pro-apoptotic cytotoxin VacA is known to target and to damage mitochondria forming anion-channels in mitochondrial membrane. Previously, we reported that *H. pylori* induces mtDNA mutations in vitro and in mice. In the present study we investigated the mitochondrial response to *H. pylori* infection and the influence of VacA on the processing and stability of mtDNA. Using a high-resolution imaging approach and immunofluorescence analysis, mitochondria and mtDNA replication/transcription were analysed in vitro in gastric epithelial cells (AGS) infected with *H. pylori* 26695 and 26695ΔvacA or treated with the VacA protein from 2 to 48 hours. MtDNA was quantified by real time PCR. This study was also performed in INS-GAS transgenic mice that develop gastric intraepithelial neoplasia after 6/12 months infection with *H. pylori* SS1. We demonstrate that *H. pylori* induces mitochondria biogenesis, promotes mtDNA replication/transcription with the concomitant increase of mtDNA level in the first hours of infection. VacA has an early and strong inducer effect on mitochondria and mtDNA levels. At later time points, this induction is VacA-independent, suggesting the involvement of other undetermined *H. pylori* factors. These data were also validated in INS-GAS mice. In conclusions, we report first evidences that *H. pylori* infection up-regulates mtDNA replication and transcription, thereby promoting mitochondrial biogenesis. Our study also points out mitochondria as an important player in the *H. pylori*-induced gastric lesions and possibly in the related-carcinogenesis.

Poster Presentations

P01 Microbiology

Abstract no.: P01.01

THE ROLE OF MLTD AND RPON IN *H. PYLORI* BIOFILM FORMATION IN VITRO

B. T. Alhaj-Hussein,* E. I. Kazakos,† B. W. Wren‡ and N. Dorrell‡

*Department of Pathology and Laboratory Medicine, King Abdullah International Medical Research Centre, Jeddah, Saudi Arabia; †Department of Medicine, Second Medical Clinic, Aristotle University of Thessaloniki, Ippokration Hospital, Thessaloniki, Greece; ‡Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Background: Several proteins have been investigated for a potential role in in-vitro biofilm formation by the human gastric pathogen *Helicobacter pylori* including those implicated in motility, quorum sensing, adhesion, secretion, potential matrix production and morphology transformation. In addition to luxS which encodes the enzyme responsible for the synthesis of autoinducer-II; a quorum sensing molecule involved in biofilm formation, two previously uninvestigated genes were investigated in this study including rpoN (essential for flagellar biosynthesis and secretion via the flagella), mltD which encodes a lytic transglycosylase essential for the peptidoglycan assembly and is a homologue of Cj0645 which was investigated for potential resuscitation promotion activity in *C. jejuni*

Methods: Isogenic mutants were constructed in 26695 and J99. In addition to the wild-type strains, rpoN and mltD mutants were investigated compared to luxS mutant which is known to exhibit enhanced biofilm formation. Twenty-four-well plates incubated microaerobically with shaking were tested for crystal violet biofilm assays every 3–4 days over a 14-day period. Electron microscopy was performed on formalin-fixed aggregates.

Results: All mutants showed varied pattern of biofilm formation over time where mltD mutant biofilms deteriorated after day 10, luxS mutants stabilised after day 10 while rpoN mutant continued increasing steadily. Although rpoN and luxS mutants demonstrated loss of motility associated with absent or deformed flagella, autoaggregation increased only in luxS mutant and dropped significantly in the other two mutants.

Conclusions: Several additional biological parameters such as intact flagellar morphology, correct peptidoglycan biogenesis and adaptation to environmental stress may influence biofilm formation in *H. pylori* hence affecting its virulence capacity.

Abstract no.: P01.02

ISOLATION OF *H. PYLORI* FROM GASTRIC TISSUES BY MICROCULTURE METHOD: THE EVER FIRST EXPERIENCE WORLDWIDE

B. S. Kocazeybek,* M. Aslan,* A. M. Allahverdiyev,† H. Bahar Tokman,* M. Bagirova,† R. Caliskan Algingil,* G. Unal,† P. Yuksel,* F. Kalayci,* O. Nehir Oztel,† Y. Z. Erzin‡ and K. Bal‡

*Department of Medical Microbiology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; †Department of Bioengineering, Cell Culture and Tissue Engineering Laboratory, Faculty of Chemistry-Metallurgical, Yildiz Technical University, Istanbul, Turkey; ‡Department of Gastroenterology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey

The main purpose of clinical and laboratory diagnosis of *H. pylori* is to cure the patient by an effective treatment. The culture of this bacterium is both costly and requires specific atmospheric conditions and specific culture media. The aim of this cross-sectional case-control study is to isolate *H. pylori* for the first time in the world, by microculture method and to compare this method with classical culture, histopathology and PCR used for the laboratory diagnosis of *H. pylori*.

This study was performed between October 2012- December 2012, with 26 patients whose histopathological examination of biopsy samples and/or culture revealed the presence of *H. pylori* and with 26 control whose *H. pylori* was not found. The biopsy samples were homogenized and 60 μL was tranfered to four capillary tubes. They were closed with silicone and were incubated 48 hours at 37°C. Any atmospheric conditions like CO_2 was not provided. The bacteria that grew in capillary tubes was confirmed as *H. pylori* with PCR.

From 25 of 26 biopsy, *H. pylori* was isolated with microculture and from 14 with classical culture. *H. pylori* was detected by histopathology in only 17 samples. The sensitivity of the micro culture method was found as 96% the specificity as 80% the positive predictive value (PPV) as 83%, the negative predictive value (NPV) as 95% and Kappa coefficient of concordance was found as 76%.

In this study, for the first time in the world, *H. pylori* was isolated from gastric biopsies by micro-culture method and the culture was confirmed by PCR. Furthermore, this new method was compared with histopathology and classical culture and appeared to be more sensitive. We are beliving that the micro-culture method will be useful for the isolation of *H. pylori* from symptomatic patients, as well as asymptomatic patients.

Abstract no.: P01.03

THE PHYTOTHERAPEUTIC POTENTIAL OF *ORIGANUM MINUTIFLORUM* FOR ERADICATION OF *HELICOBACTER PYLORI*

F. Özen, F. Y. Ekinci and M. Korachi

Yeditepe University, Biotechnology Department, Istanbul, Turkey

Phytotherapy is a growing market with the increasing awareness of microbial resistance a demand for alternative antimicrobial treatments that are more effective, non-invasive, less side effects and economical than conventional medical treatments. Origanum minutiflorum is an endemic plant of Isparta, Turkey, locally called "Toka kekiği" which has been shown to possess strong antimicrobial properties against a wide range of medical, oral and food pathogens.

The aim of this study was to investigate the antimicrobial activity of different O. minutiflorum extracts against *H. pylori* J99 (ATCC 700824). Methanol, ethanol, aqueous and essential oil extracts of O. minutiflorum were collected via soxhlet extractor and clevenger apparatus. Following extraction, the anti-bacterial activity (agar well diffusion method) of all extracts and the minimum bactericidal concentration (MBC) were determined according to CLSI protocols. The chemical composition of the extract showing the strongest antimicrobial activity was determined via gas chromatography-mass spectrometry (GC-MS). Furthermore, the cell viability effects of the chosen extract were determined on AGS (human gastric adenocarcinoma) (ATCC CRL 1739) cell line by WST-1 assay for 24, 48, and 72 hours.

Results showed the EO of O. minutiflorum to have the highest zone of inhibition (≥90 mm) at 100% concentration (v/v) with an MBC of 1/20 000 (v/v) dilution. WST-1 results displayed no cytotoxic effect on cells at the MBC concentration. Compositional analysis identified 40 different components including carvacrol (29.22%), a known antimicrobial agent, as the major component. These results show the potential future use of O. minutiflorum EO as an alternative antimicrobial therapy against *H. pylori*.

Abstract no.: P01.04

CLINICAL SIGNIFICANCE OF COMBINATIONS OF *HELICOBACTER PYLORI* VIRULENCE GENES IN SLOVENIAN PAEDIATRIC POPULATION

A. Šterbenc,* B. J. Kocjan,* B. Luzar,† N. Zidar,† M. Homan‡ and M. Poljak*

*Faculty of Medicine, Institute of Microbiology and Immunology, University of Ljubljana, Ljubljana, Slovenia; †Faculty of Medicine, Institute of Pathology, University of Ljubljana, Ljubljana, Slovenia; ‡Department of Gastroenterology, Hepatology and Nutrition, University Children's Hospital, Ljubljana, Slovenia

Background: It has been shown that the combination of certain *Helicobacter pylori* (*H. pylori*) genes can result in more virulent strains, thus increasing the risk of developing severe gastroduodenal disease.

Objective: To determine whether different *H. pylori* virulence genes act synergistically in causing gastritis in Slovenian paediatric population.

Material and Methods: A total of 168 *H. pylori* positive gastric biopsies, obtained from children, were examined for the presence of five virulence genes: *cagA, vacAs1, vacAm1, babA2* and *homB*. All virulence genes were detected by polymerase chain reaction. The combinations of genes were compared with density score, activity and chronic inflammation of *H. pylori* infection using a Mann Whitney *U* test.

Results: *H. pylori* strains were divided into five groups on the basis of positive or negative PCR findings for the virulence genes: group A: *cagA+, vacAs1 m1, babA2+, homB+*; group B: *cagA+, vacAs1 m1, babA2 + , homB−*; group C: *cagA+, vacAs1 m1, babA2−, homB+*; group D: *cagA+, vacAs1 m1, babA2−, homB−*; group E: *cagA−, vacAs2 m2, babA2−, homB−*. When histological parameters of biopsy samples of children infected with group E and group D strains were compared, a statistically significant association regarding a higher degree of bacterial density and chronic inflammation was found in patients infected with *cagA+,*

vacAs1 m1, *babA2−*, *homB−* strains. There were no statistically significant associations between any of the histological parameters and strains in other groups.
Conclusion: When co-expressed by the same *H. pylori* strain, *cagA* and *vacAs1 m1* work synergistically in the development of gastric disease, causing more pronounced chronic gastritis in paediatric patients.

Abstract no.: P01.05

CYTOKINE RELATIONS IN DIFFERENT GENOTYPES OF *H. PYLORI* STRAINS ISOLATED IN TURKEY: A PROSPECTIVE STUDY IN MONOCYTE CELL LINE (THP-1) MODEL

R. Caliskan Algingil,* H. Bahar Tokman,* A. Sayi Yazgan,† P. Yuksel,* E. Sofyali,† O. Akgul,* F. Kalayci,* A. Kurt,* Y. Z. Erzin,‡ K. Bal‡ and B. S. Kocazeybek*
*Medical Microbiology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey; †Molecular Biology and Genetics Department, ITU Faculty of Science and Letters, Istanbul, Turkey; ‡Gastroenterology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey

More than 50% of the world's population are infected. with *H. pylori*. Different *H. pylori* genotypes, isolated from a patient population of Istanbul and its suburbs from Turkey which has an Eurasian geographic characteristic, have been studied to determine their cytokine responses in THP-1 (monocyte) cell line and their roles in pathogenesis were evaluated. Twenty-one randomly selected *H. pylori* genotypes were divided into five genotypes: cagA+ vacAs1m2+, cagA+ vacAs1m1+, cagA+ vacAs1m2+ babA2+, cagA− vacAs2m2+, cagA+ vacAs2m2+ as genotype 1, 2, 3, 4, 5, respectively. After performing their co-culture on THP-1 (monocyte) cell lines, their IL1β, IL-6, IL-8, IL-12, TNF-α and IL-10 levels were determined quantitatively by flow cytometry. *H. pylori* inoculated in a cell line without THP-1 was used as a negative control. In THP-1 cell lines, all genotypes secreted high level of IL-1β, IL-6, IL-8, IL-12, TNF-α and IL-10 ($p < 0.05$). Genotype 5 secreted the highest level of IL-1β, IL-6, TNF-α and IL-10, and inflammation in the antrum with severe chronic gastritis was determined in the patient harboring this genotype. Genotype 1 secreted the maximum level of IL-8, and inflammation in the corpus and mild chronic gastritis were defined in the patient harboring this genotype. In this study, cagA, vacAs2m2 genotypes were found to secrete the highest level of IL-1β, IL-6, TNF-α and IL-10 except IL-8, However, IL-8 secretion, which is the most important chemotactic cytokine for *H. pylori*-associated gastric pathogenesis, was found to be high in the patient who was endoscopically diagnosed with normal GIS harboring cagA, vacAs1m2 genotype. As a result, the cytokine levels and the histopathologic pattern may be affected by genotype differences. Howewer, we believe that prospective studies with higher number of different genotypes and different gastroduodenal pathologies should be done to evaluate a clear correlation with histopathology, different genotypes and cytokine levels

Abstract no.: P01.06

THE CYTOKINE RESPONSES OF DIFFERENT GENOTYPE OF *H. PYLORI* STRAINS IN NEUTROPHIL DIFFERENTIATED HL-60 CELLS LINES

M. Aslan,* R. Caliskan Algingil,* A. Sayi Yazgan,† P. Yuksel,* E. Sofyali,† H. Bahar Tokman,* O. Yilmazli,* O. akgul,* Y. Z. Erzin,‡ K. Bal‡ and B. S. Kocazeybek*
*Medical Microbiology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey; †Molecular Biology and Genetics Department, ITU Faculty of Science and Letters, Istanbul, Turkey; ‡Gastroenterology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey

The main virulence factors of *H. pylori,* cagA and vacA and their cytokines are very important for gastroduodenal pathology. We determined the cytokine response of different *H. pylori* genotypes, isolated from a patient population of Istanbul and its suburbs from Turkey having an Eurasian geographic characteristic, in neutrophil differentiated (ND) HL-60 cell lines to evaluate their roles in the pathogenesis. Five different genotypes: cagA+ vacAs1m2+, cagA+ vacAs1m1+, cagA+ vacAs1m2+ babA2+, cagA− vacAs2m2+, cagA+ vacAs2m2+ were selected as genotype 1, 2, 3, 4, 5, respectively. Their effects on the cytokine response was determined in ND HL-60 cell lines by evaluating quantitatively by flow cytometry the IL1β, IL-6, IL-8, IL-12, TNF-α and IL-10 levels. Comparing with the negative control, genotype 4 influenced a significantly high level secretion of IL-6 ($p < 0.001$) and genotype 3 and 4 influenced a high level secretion of IL-8 ($p < 0.001$). The maximum level of IL-6 and IL-8 secretion was found influenced by genotype 4. As a result, genotype 4 was determined as an important genotype influencing the secretion of IL-6 and IL-8 from ND HL-60 cell lines. Mild chronic gastritis with inflammation in the antrum was defined in the patient harboring this genotype. Additionnaly, genotype 3 was found to influence a high level secretion of IL-8 and the patient harboring this genotype had also intestinal metaplasia and chronic gastritis with inflammation in the corpus and antrum. Comparing with controls, a significan difference was not found in IL-1β, IL-12, TNF-α and IL-10 levels secreted from ND HL-60 cell lines in all genotypes ($p > 0.05$). These results indicated that cytokine levels and histopathologic patterns might be affected by genotype diversity. We believe that new prospective studies with higher number of different genotypes and gastroduodenal pathologies should be done to evaluate a clear correlation between histopathology- different genotype- cytokine levels.

Abstract no.: P01.07

COMPARING YIELDS OF EXTRACTION FOR CELL SURFACE PROTEINS AND 26 KDA PROTEIN FROM *HELICOBACTER PYLORI* USING VARIOUS METHODS

H. AminiNajafabadi,* T. Mohammadian,† M. Paknejad,* S. Farshad,‡ H. Ghahramani,* B. Amini* and A. AminiNajafabadi*
*Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; †Department of Microbiology, Faculty of Beasic Sciences, Islamic Azad University, shahr-e Qods-Branch, Qods, Iran; ‡Professor Alborzi Clinical Microbiology Research Center, Namazi Hospital, Shiraz, Iran

Background: Characterization of 26 kDa protein of *H. pylori* and its application to raise monoclonal antibody to detect *H. pylori* infection using stool samples requires its extraction. This study was performed with the objective of comparing yields of extraction for cell surface proteins (CSPs) using octyl-glucoside and acid-glycine methods and 26 kDa protein extraction of the CSPs with various detection methods in SDS- PAGE gel and different elution methods from the gel.
Methods: The method consisted of bacterial isolation and cultivation, extraction of cell surface proteins (CSPs) using octyl-glucoside and acid-glycine methods, protein quantification of CSPs, preparative SDS- PAGE, detection of 26 kDa protein with Zinc sulfate and Coomassie brilliant blue staining in the gel, 26 kDa protein elution from the gel by electroelution and passive elution, SDS removal through dialysis and 26 kDa protein quantification.
Results: The yields of cell surface proteins from *H. pylori* using octyl glucoside and acid-glycine methods were 1.034% and 1.05% respectively. The yields of 26 kDa protein extraction from CSPs using the combinations of Zinc sulfate-electroelution, Zinc sulfate- passive elution, Coomassie brilliant blue- electroelution and Coomassie brilliant blue- passive elution were 7%, 5.33%, 5.22% and 3.5% respectively.
Conclusion: Octyl glucoside and acid-glycine methods have approximately equal yields of cell surface protein extraction from *H. pylori*. The combination of Zinc sulfate- electroelution has higher yield of 26 kDa protein extraction from CSPs than other combinations.

P02 Molecular genetics and genomics

Abstract no.: P02.01

EVIDENCE OF RECOMBINATION FROM ENVIRONMENTAL SOURCES IN THE HOUSE-KEEPING GENES OF *H. PYLORI*

T. Perkins, A. Tay, F. Thirriot and B. Marshall
University of Western Australia, Perth, WA, Australia

The motile, extremophile gram-negative bacterium *Helicobacter pylori* (*H. pylori*) colonises the human stomach in half of the world's population and resides there for the life of the host unless treated with antibiotics. It is panmictic and analyses of its housekeeping genes (MLST genes) suggest it is weakly clonal. Previously, MLST genes have been assigned to geographic regions, and have provided clues to pre-historic migrational patterns throughout the world. We analysed the MLST genes of 113 strains isolated in clinics in Perth, Western Australia in the context of the significant global diversity of its population. Our analysis suggests that resident *H. pylori* from diverse ethnic groups, recombines with the Indigenous Australian clade (coined hpSahul) in a significant proportion of patients presenting to our clinic. We hypothesise that this is most likely due to environmental sources of DNA or live bacteria recombining with the colonising bacteria. We believe this challenges the fidelity of the accepted phylogeographic classification of *H. pylori*.

Abstract no.: P02.02

GEOGRAPHIC ORIGIN OF *HELICOBACTER PYLORI* ISOLATED FROM COSTA RICAN PATIENTS

S. Molina-Castro,* D. Herrera-Ríos,* J. Garita,* W. Malespín-Bendaña,* F. Mégraud,† V. Ramírez Mayorga* and C. Une*
*Institute for Health Research, San José, Costa Rica; †University Bordeaux Segalen, Bordeaux, France

Costa Rica is one of the countries with the highest incidence and mortality rates from gastric cancer. The prevalence varies among regions but the frequency of *Helicobacter pylori* infection is high in the whole country. *H. pylori* is genetically highly diverse and geographic origin may be associated with virulence of the strain. Using a MLST scheme, the global population structure for *H. pylori* has been established, grouping the bacterial isolates in populations with specific geographical origins. The aim of this study was to determine the geographical origin of the *H. pylori* isolates circulating in Costa Rica.

Seven biopsies were obtained from 501 dyspeptic patients of the Calderón Guardia Hospital. In each case, a histopathological examination was performed and the bacterium was cultured. The isolates were identified as *H. pylori* by different techniques. Seven housekeeping genes (ureI, mutY, efp, ppa, yphC, atpA and trpC) from 24 isolates were amplified by PCR and sequenced. The obtained sequences were compared with those from 82 isolates of *Helicobacter pylori* from other parts of the world obtained from the *Helicobacter pylori* Public MLST Database. The comparison was made with Bayesian inference and a tree was constructed using the Mr. Bayes 3.2 software.

The majority of Costa Rican isolates grouped with the hpEurope group, 18 in a branch predominantly formed by strains isolated from Spanish and Latin American patients, three grouped with strains from northern European and Australian patients and three grouped with hspWAfrica isolates. The geographical origin of cagA+ and cagA− isolates did not differ significantly in their distribution.

The studied isolates from Costa Rica clustered with strains from hpEurope and hspWAfrica. The Asian contribution (hpEastAsia and hpAsia2) is of little importance. This distribution is consistent with the genetic admixture of the Costa Rican population.

Abstract no.: P02.03

EPIGENETIC SILENCING OF RASSF1A GENE IS INDUCED BY HYPERMETHYLATION OF CPG ISLANDS AND RESTORED BY DEMETHYLATING AGENT IN GASTRIC CANCER CELL LINES

J. Park,* S. Lee,† M. Joo,* H. Yoo,* B. Lee,* S. Kim,* S. Kim,* J. Choe,* W. Lee,* J. Kim* and Y. Bak*
*Korea University College of Medicine Guro Hospital, Seoul, Korea; †Korea University College of Medicine Ansan Hospital, Seoul, Korea

Among critical tumor suppressor genes located within 3p21.3 locus, *RASSF1A* has been extensively investigated recently because it has broad spectrum of effects involving tumorigenesis, cell cycle progression, cell adhesion/migration/apoptosis, transcription and angiogenesis. The gene expression level of *RASSF1A* is down-regulated in various neoplastic tissues, including lung, breast, kidney, ovary, neuroblastoma or colon, and silencing of its transcription is regulated by epigenetic mechanism such as aberrant hypermethylation of CpG islands. Recent studies revealed epigenetic silencing of *RASSF1A* in gastric adenocarcinoma tissues using methylation-specific PCR (MSP) method, however this has not been supported by bisulfite sequencing or demethylating agent. In this study, we found that mRNA expression of *RASSF1A* was nearly eliminated in gastric cancer cell lines including AGS, SNU5, SNU719, MKN28 by RT-PCR, while it was observed in SNU16, SNU638, MKN45 and KATO-III, as well as normal gastric tissue. We also designed methylation- or unmethylation-specific primers to detect the methylation status of CpG islands anchoring from −66 to +570 bp (based on the transcription initiation site) and performed MSP. We identified positive band by methylation-specific primers in AGS, SNU5, SNU16, SNU719, MKN28, however, it was not observed in SNU638, MKN45, KATO-III and normal gastric tissue. By treating with demathylating agent 5-Aza-2′-deoxycytidine for 48 hour, gene expression of *RASSF1A* was restored in AGS. Furthermore, bisulfite genomic sequencing of AGS revealed that 87.5% (21/24) of CpG were methylated. Taken together, hypermethylation of *RASSF1A* promoter occurred in various gastric cancer cell lines, and this could be confirmed by demathylation using 5-Aza compound, and bisulfite sequencing.

Abstract no.: P02.04

PSCA, MUC1, PLCE1 GENE POLYMORPHISMS IN GASTRIC CANCER AND HIGH RISK ATROPHIC GASTRITIS

J. Kupcinskas,* T. Wex,† A. Link,† R. Noreikiene,* M. Leja,‡ L. Jonaitis,* G. Kiudelis,* J. Skieceviciene,* L. Kupcinskas* and P. Malfertheiner†
*Lithuanian University of Health Sciences, Kaunas, Lithuania; †Otto Von Guericke University, Magdeburg, Germany; ‡University of Riga, Riga, Latvia

Background/Introduction: Recent genome-wide association studies (GWAS) revieled a link between gastric cancer (GC) and single nucleotide polymorphisms (SNPs) of the genes encoding PSCA, PLCE1 and MUC1. Replication studies of these GWAS data in Caucasian subjects are higly lacking.

Aims: To evaluate the relationship between SNPs of the genes encoding PSCA (rs2976392, rs2294008), MUC1 (rs4072037) and PLCE1 (rs2274223) and the risk of developing GC or high risk atrophic gastritis (HRAG) in individuals of Caucasian ethnicity.

Methods: Gene polymorphisms were analyzed in 634 subjects (GC: n = 252; HRAG: n = 136, controls: n = 246) of Caucasian origin. PSCA A>G (rs2976392), PSCA C>T (rs2294008), MUC1 G>A (rs4072037) and PLCE1 A>G (rs2274223) SNPs were genotyped by real-time PCR (RT-PCR).

Results: MUC1 A allele (rs4072037) was associated with higher incidence of GC (53.2%, OR – 1.57; $p = 0.00064$) and HRAG (51.5%, OR-1.47, $p = 0.0136$) when compared to controls (42.0%). PSCA T/T genotype of rs2294008 was linked with higher risk of GC (26.3%, OR – 2.41, $p = 0.00021$) and HRAG (17.2%, OR – 1.72, $p = 0.04303$) when comparing to the control group (12.9%). There was a higher frequency of PSCA rs2976392 A/A genotype in GC group (41.2%, OR-2.35, $p = 0.00032$) and HRAG group (34.8%, OR – 1.82, $p = 0.0285$) than in control group (23.3%). No significant differences were determined between PLCE1 rs2274223 genotypes and the presence of GC or HRAG.

Conclusions: SNPs of PSCA (rs2976392, rs2294008) and MUC1 (rs4072037) genes are linked with the presence of GC and HRAG. No significant association was determined between PLCE1 rs2274223 and the risk of GC or HRAG.

Abstract no.: P02.05

ALTERED EXPRESSION OF PYK-REG-90 NON CODING RNA IN GASTRIC CANCER

S. Lee, J. Lee, C. Park, J. Park, H. Lee, S. Shin and Y. Lee
Yonsei University College of Medicine Severance Hospital, Seoul, Korea

Non-coding RNAs (ncRNAs) exceeds by large the number of protein-coding genes. However, for the vast majority of ncRNA, the function remains unknown. The long ncRNA gene pyk-reg-90 which map to the chromosomal 3 (p21.1) and located in the intergenic region and expression of this transcript was found to altered in colon cancer recently. We therefore explored their significance in gastric cancer.

Pyk-reg-90 and U6 expression level were determined by qRT-PCR in various gastric cancer cell lines and normal tissues, respectively. Fresh 50 gastric cancer tissues and adjacent normal tissues obtained by surgery and clinical data were collected prospectively. For qRT-PCR, total RNA was purified using TRIzol reagent. qRT-PCR analysis was carried out with iQ SYBR Green Supermix. Down regulation of pyk-reg-90 was obtained by three different siRNAs transfection and cell number and viability was checked after treatment of siRNAs. For apoptotic analysis, PARP was determined by western blot and caspase 3/7 were analyzed by luminescent assay.

Compared to normal tissue, expression of pyk-reg-90 was up-regulated in various gastric cancer cell lines. In gastric cancer tissue expression of pyk-reg-90 was significantly high in cancer than adjacent normal tissue ($p < 0.05$). Three different siRNAs for pyk-reg-90 down regulated expression of pyk-reg-90 in SNU-719 cells, and pool of siRNAs repressed expression of pyk-reg-90 by 50%. The number of SNU-719 cells was significantly reduced after 48 hours after siRNA treatment. Treatment with siRNAs increased expression of cleaved PARP-1 and caspase 3/7 compared to scramble. Our results suggested that pyk-reg-90 was upregulated in gastric cancer and down-regulation of a pyk-reg-90 can induce cell death.

Abstract no.: P02.06

GENE EXPRESSION PROFILING IN CHRONIC GASTRITIS ASSOCIATED WITH *HELICOBACTER PYLORI* INFECTION

S. E. Morales-Guerrero,* G. Castillo-Rojas,* C. Rangel-Escareño,† I. Imaz-Rosshandler,† S. Ponce de León‡ and Y. LópezVidal*
*Universidad Nacional Autónoma de Mexico, Mexico City, Mexico; †Instituto Nacional de Medicina Genomica, Mexico City, Mexico; ‡Instituto Nacional de Ciencias Médicas y de la Nutrición SZ, Mexico City, Mexico

Helicobacter pylori is a gram negative bacterium that colonizes the stomach of almost half of the world's population and the infection usually persists for several decades. The infection with *H. pylori* produces a chronic gastritis, which is asymptomatic in the majority of the infected subjects; however, this chronic inflammation can progress to the development of gastric cancer, for which is considered as a type I carcinogen in humans.

Analysis of host gene expression in subjects with chronic gastritis could help to understand the first molecular mechanism altered in the host that could lead to de development of more serious conditions.

Aim: Determine the gene expression profile in subjects infected with *H. pylori* and with chronic gastritis and functional dyspepsia.

Methodology: Samples from patients with chronic gastritis and samples from patients with functional dyspepsia were evaluated through microarray assays. Gene expression values for each gene across all samples were analyzed using the Gene Set Enrichment Analysis tool (GSEA), to determine the gene ontology terms enriched in each of the phenotypes evaluated.

Results and Conclusions: The GSEA analysis in functional dyspepsia and chronic gastritis shows that ontology terms related with mitochondria are enriched in functional dyspepsia, whereas ontology terms related with immune response are enriched in chronic gastritis samples. These results suggest that in chronic gastritis exists a down-regulation of genes associated with mitochondria, which can be related with *H. pylori* infection and could be the first alterations involved in the development of more serious diseases.

Abstract no.: P02.07

GENOME SEQUENCING OF *HELICOBACTER PYLORI* STRAINS ISOLATED IN MEXICO FROM GASTRIC CANCER AND CHRONIC GASTRITIS LESSIONS

E. J. Mucito-Varela,* V. Flores-López,† G. Castillo-Rojas,* M. A. Cevallos-Gaos* and Y. López-Vidal*
*Universidad Nacional Autónoma de Mexico, Mexico City, Mexico; †CINVESTAV, IPN, Mexico City, Mexico

H. pylori infection is associated to gastritis, peptic ulcer and gastric cancer development. It has been proposed that virulence factors like CagA and VacA containing specific polymorphisms increase the risk of disease development but distribution of these polymorphisms worldwide doesn't always correlate to disease acquisition suggesting that other bacterial factors play an important role in gastric cell damage and transformation. Whole genome sequencing provides information of the total gene content allowing the search for new genetic markers associated to virulence. Representative sequences from different parts of the world must be included to gain insights in common processes of *H. pylori* disease-associated strains. Until now it had not been sequenced any genome of *H. pylori* isolated in Mexico so we obtained the whole genome sequence of two *H. pylori* strains by pyrosequencing. Assembly was done de novo and we finally constructed 17 and 39 contigs that cover more than 92% of the genome and 1.6 Mb combined length. One thousand four hundred genes have been annotated for each genome. The genome sequence of these strains allowed us to do comparative genomics to better understand gastric cancer pathogenesis.

Abstract no.: P02.08

SODIUM BUTYRATE INDUCES FOXO3 DOWNSTREAM SIGNAL PATHWAYS BY PTEN MODULATION IN GASTRIC CANCER CELL

Y. Lee, D. Yu, S. Kim, Y. Lee and D. Lee
Yonsei University College of Medicine, Seoul, Korea

Background/Aims: Sodium Butyrate (NaB) has various effects including the regulation of cell growth and differentiation. PTEN (phosphatase and tensin homologue deleted on chromosome 10) is known to promote gastrointestinal cell differentiation. Forkhead transcription factors of class O (FoxO) are implicated in the regulation of apoptosis, cell survival, and pathogenesis. In this study, we aimed to determine whether NaB regulated the expression of the PTEN through the PI3K/Akt pathway and examine the relationship between PTEN and FoxO3a in gastric cancer.

Method: Gastric cancer cell line AGS was used. NaB 2 μmol/L treated cells for respective hours were used. The cells were used for proliferation assay or were lysed directly for protein analysis. The effects of NaB on gastric cancer cells in PI3k/PTEN/AKT signal pathways were analyzed by using MTS assay, western blotting, and qRT-PCR. siPTEN or inhibitors for PI3K/AKT signal pathways were used respectively. FHRE luciferase assay were employed to measure FOXO signal activity.

Results: AGS cells treated with NaB for 24–72 hours showed the inhibition of cell growth in a time dependent manner. Cell growth was significantly inhibited by 50% at 72 hours. NaB treatment markedly increased the expression of PTEN, while down-regulating the PI3K/Akt pathway. NaB treatment resulted in the enhancement the translocation of FoxO3a to the nucleus. The induction of FoxO3a's downstream target genes, GADD45a and p27 was confirmed by the western blotting and qRT-PCR.

Conclusion: The increase of PTEN activity by NaB induce the induction of FoxO3a through the down regulation of PI3K/Akt signaling pathway.

Abstract no.: P02.09

THE ROLE OF POLYMORPHISMS IN GENES −330 T/G IL-2 AND −174 G/C IL-6 IN PATHOGENESIS AT THE *HELICOBACTER PYLORI*-ASSOCIATED DISEASES

E. S. Ageeva, O. V. Shtygasheva and Y. V. Saranchina
Khakas State University, Abakan, Russian Federation

Aim of Investigation: The purpose of the research is to estimate polymorphism genes (IL-2, IL-6) pathogenesis at the *Helicobacter pylori*-associated gastritis (CG) and ulcerative disease (UD).

The objects of the research were peripheral blood from 71 patients with diagnosis UD – 35 Caucasians and 36 Mongoloids; 130 patients with *Helicobacter pylori*-associated CG – 69 Caucasians and 61 Mongoloids.

Methods: Endoscopy, gastric biopsy samples were investigated according to Sydney classification. Genomic DNA was typed for polymorphisms at position −330 T/G in the IL-2 gene using RFLP analysis (FauND I), −174 G/C IL-6 (SfaNI). Analysis was performed by PCR and 4% agarose gel electrophoresis.

Results: Genotype GT-330 IL-2 at Caucasians is associated with risk of CG (OR = 2.35). TT −330 IL-2 (OR = 0.28) is protective genotype of development of CG at Caucasians. At Khakas the risk of development of UD is associated with GG −330 IL-2 (OR = 1.91), the risk of development of CG is reduced at the Khakas carriers of TT −330 IL-2 (OR = 0.42). Polymorphism −174 G/C IL-6 at the Khakas of sick UD and CG didn't differ in control comparison. At Caucasians the risk of development of UD is associated with genotype of CC −174 IL-6 (OR = 2.25).

P03 Virulence factors and pathogenesis

Abstract no.: P03.01

ROLE OF A CAG PATHOGENICITY ISLAND ENCODED SMALL NON-CODING RNA IN THE SURVIVAL AND PATHOGENESIS OF *HELICOBACTER PYLORI*

S. Bhattacharya and R. Chowdhury
Indian Institute of Chemical Biology, Kolkata, India

Helicobacter pylori, a major human pathogen, is associated with the development of a range of gastric disorders. The most severe disease states have been attributed to strains harboring the cag pathogenicity island (PAI). Many small non-coding RNAs (sRNAs), encoded in the cag PAI of *H. pylori,* appear to be involved in gene regulation at the post transcriptional level. Most of these are encoded antisense to their target genes, however one sRNA (HPnc2630) is located at the 5′ UTR of the *cag15* gene. Under normal growth conditions, an abundant short transcript and a long transcript corresponding to HPnc2630 and HPnc2630-*cag15* fusion respectively are synthesized from the same promoter upstream of HPnc2630. However, on contact with host cells (AGS cells), an alternative downstream promoter is specifically activated resulting in significant upregulation of *cag15* expression. In view of the fact that Cag15 shares homology with Pilin and has putative trans-membrane domains, these results suggest that it might have a role in adherence and infection. On the other hand, acid stress specifically induced expression of HPnc2630 as a discrete sRNA moiety which appears to be involved in the regulation of trans encoded genes. Studies on isogenic HPnc2630 deletion mutant suggest that the mRNAs encoding CytC 551 peroxidase and NADH dehydrogenase κ subunit are negatively regulated by HPnc2630 under acid stress, which might contribute to the critical process of acid acclimation of this bacterium. Affinity capture studies suggest that this sRNA can also interact with the proteins CytC 551 peroxidase and Gamma glutamyl transpeptidase.

Abstract no.: P03.02

EFFECT OF THE EXTRACELLULAR VESICLES DERIVED FROM *HELICOBACTER PYLORI* ON STOMACH DISEASES

H. Choi,* Y. Kim* and J. G. Kim†
*Department of Life Science and Division of Molecular and Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang, Korea; †Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea

Background/Aims: Extracellular Vesicles derived from *H. pylori* contains representative virulent factors such as CagA and VacA. The interaction between extracellular vesicles derived from *H. pylori* and stomach diseases has been investigated.
Method: The *H. pylori* vesicle has been isolated on optimal growth phages conditions. Transmission electron microscope images and dynamic light scattering sizes have been used to analyze the characterization. In vitro, vesicles were treated into mouse, human peritoneal macrophage and human stomach epithelial cell line. Consequently, it has been confirmed that the secretion of important Th1& Th17 inducers such as IL-8, IL-6 and TNF-α can be activated. In vivo, 6-week-old C_{57}BL/6 mice were fed with vesicles and the bacteria for 4 weeks. The harvested T cells from spleen and lymph node were restimulated and total IgG1 and IgA levels in serum were evaluated.
Results: The *H. pylori* produced extracellular vesicles and the size of vesicles was measured to be 10–100 nm. In vitro, *H. pylori* vesicles also induced proinflammatory cytokines. In vivo, mice fed with vesicles increase production of cytokine more than mice group fed with bacteria. Total IgG1 and IgA levels were also higher than mice group fed with bacteria.
Conclusion: *H. pylori* vesicles penetrate into mucosal layer on the stomach epithelial cell barrier. This suggests that vesicles induce more severe inflammation in the stomach than the bacteria at the early stage of a stomach disease.

Abstract no.: P03.03

THE ROLE OF THE TFS4 TYPE IV SECRETION SYSTEM IN CHROMOSOMAL DNA TRANSFER IN *HELICOBACTER PYLORI*

A. S. D. Stephens and R. M. Delahay
Centre for Biomolecular Sciences and Nottingham Digestive Diseases Centre Biomedical Research Unit, University of Nottingham, Nottingham, UK

Helicobacter pylori are a genetically diverse species due to polymorphism and intra- and intergenomic recombination promoting the loss and gain of chromosomal genes within the population. However, the mechanisms underlying gene acquisition have not been clearly defined. The Com system is known to function in DNA uptake and a further role in plasmid release has recently been established. Additionally, the mobilisation of plasticity zone (PZ) *tfs4* gene clusters has also been reported as a function of the *tfs4*-encoded XerD recombinase, defining a potential mechanism for transfer of large segments of the chromosome.
In this study, we aim to define the role of the Tfs4 T4SS and key accessory proteins in the transfer of PZ clusters and non-PZ genes.
Initial DNase I resistant mating assays were performed using mutant *H. pylori* strains inactivated in key components of each T4SS. Genes were disrupted by insertion of either/both kanamycin (Kan^R) or chloramphenicol (Cm^R) resistance cassettes and included *cagE, comH, tfs4 virB4/virD2 and tfs3 virB4/virD2*. Donor strains were incubated in the presence of DNaseI and mated with isogenic spectinomycin (Spc^R) resistant recipients. DNA transfer was demonstrated by selection for Kan^R/Spc^R or Cm^R/Spc^R transconjugants on antibiotic plates as appropriate.
Preliminary studies show chromosomal DNA transfer to be reduced in *tfs4* mutants. Transfer efficiencies are strain dependent, perhaps due to non-reciprocal activity of T4SSs in different recipient strain backgrounds. Ongoing work will better distinguish between general and specific Tfs4 DNA transfer mechanisms and the range of substrates transferred.

Abstract no.: P03.04

EFFECTS OF *HELICOBACTER PYLORI* VACUOLATING CYTOTOXIN ON APOPTOSIS IN DENDRITIC CELLS

J. M. Kim* and H. Y. Jung†
*Hanyang University College of Medicine, Seoul, Korea; †Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Vacuolating cytotoxin (VacA) is one of the major virulence factors in the pathogenesis of *Helicobacter pylori*-related diseases. In the *H. pylori*-infected mucosa, an infiltration of dendritic cells (DCs) is observed. Considering that DCs play an important role in the regulation of inflammation, DC response to *H. pylori* infection may contribute to the pathogenesis. Although stimulation of gastric epithelial cells and eosinophils with *H. pylori* VacA has been reported to induce the apoptotic cell death, the effects of VacA on DC apoptotic responses have not been well elucidated. The goal of this study was to investigate the role of VacA in modulating the apoptotic process of DCs. Treatment of DCs with *H. pylori* VacA resulted in the induction of apoptosis. Stimulation with VacA led to the cytoplasmic Bax translocation to the mitochondria and the cytochrome *c* release from mitochondria in DCs. In addition, suppression of the activated Bax signals significantly decreased the release of cytochrome *c* and DNA fragmentation, suggesting that Bax activation is directly related to the apoptotic process in VacA-stimulated DCs. In addition, stimulation of DCs with *H. pylori* VacA induced ER stress-related molecules and the suppression of ER stress resulted in a significant inhibition of VacA-induced apoptotic responses. These results suggest that the exposure of DCs to *H. pylori* VacA can induce a sequential pathway including induction of ER stress, Bax translocation, cytochrome *c* release, and apoptotic cell death.

Abstract no.: P03.05

STRAIN-SPECIFIC SEQUENCES AT THE *HELICOBACTER PYLORI CAGA* PROMOTER INFLUENCE CAGA EXPRESSION AND INTERLEUKIN-8 SECRETION

R. M. Ferreira,* I. Pinto-Ribeiro,* J. C. Machado*,† and C. Figueiredo*,†
*IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; †Department of Pathology and Oncology, Faculty of Medicine of the University of Porto, Porto, Portugal

Background and Aim: CagA is a major *Helicobacter pylori* virulence factor associated with increased gastric inflammation. Strain-specific sequences at the

promoter region of *cagA* influence the level of CagA expression and relate to the histopathological scores in the gastric mucosa of patients.
The aim of this study was to characterize the relationships between strain-specific sequences on the *cagA* promoter and CagA expression, as well as the influence of variation in this region and of CagA expression on IL-8 secretion from gastric epithelial cells.
Methods: The *cagA* promoter region was sequenced in 46 *H. pylori* clinical isolates and two reference strains. CagA expression was evaluated by western blot. IL-8 secretion was evaluated by ELISA in culture supernatants of AGS cells infected with *H. pylori*.
Results: The *cagA* promoter region showed high sequence heterogeneity, which included variation in copy number and sequence of motifs located at −344, −53, −10, and +59 bp from the TTS. CagA expression was associated with the −10 TATAATGA sequence ($p = 0.012$) and with the presence of the +59 motif ($p = 0.003$). IL-8 secretion by AGS cells was correlated with CagA expression ($r_p = 0.391$; $p = 0.009$), and with the presence of the +59 motif in the *cagA* promoter ($p < 0.001$).
Conclusion: Variation in *H. pylori cagA* promoter region is associated with CagA expression and influences IL-8 secretion by gastric cells. Further studies are needed to confirm the usefulness of specific regions on the *cagA* promoter as markers to predict disease risk.
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Abstract no.: P03.06

JHP0940 EXPRESSED FROM *HELICOBACTER PYLORI* INDUCES A PROINFLAMMATORY RESPONSE FROM GASTRIC EPITHELIAL CELLS IN VITRO

M. N. Alandiyjany, J. Grove and R. M. Delahay
Centre for Biomolecular Sciences and Nottingham Digestive Diseases Centre, Nottingham, UK

Many *H. pylori* strain-specific genes are encoded within highly variable chromosomal plasticity zones (PZs). Most PZ genes are of unknown function but the encoded products of some are recognised to contribute to the virulence potential of infecting strains. The JHP0940 protein (or cell translocating kinase, CtkA) encoded within the PZ of strain J99 is one such example. Studies have shown it to be highly expressed in response to the interaction of *H. pylori* with the gerbil gastric mucosa and addition of purified recombinant JHP0940 to cultured macrophages has been demonstrated to activate NF-κB via phosphorylation of the p65 subunit leading to upregulation of proinflammatory cytokines such as TNFα and IL-8.
In this study, we aimed to investigate the ability of native JHP0940 expressed from *H. pylori* to interact with cultured epithelial cells in vitro. A module comprising a kanamycin resistance cassette and 5′-GSK-tagged jhp0940 under transcriptional control of the constitutive flaA promotor was inserted into the coding sequence of cagE in several clinical strain backgrounds in order to abrogate proinflammatory effects of CagA and the Cag type IV secretion system. Then strains were assessed for JHP0940 expression, secretion and host cell interaction. Our data indicates that natively expressed JHP0940 is secreted from certain strains and induces expression of IL-8 from gastric epithelial cells in vitro. The C-terminus of the protein is determined to be important in these respects. These observations reinforce the potential of PZ genes to influence the outcome of *H. pylori* infection.

Abstract no.: P03.07

GASTRIC PRIMARY CELL MODELS TO STUDY IN VITRO TRANSFORMATION EVENTS

P. Schlaermann,* M. Glanemann,† S. Schmidt,† J. Ordemann‡ and T. Meyer*
*Max Planck Institute for Infection Biology, Dept. Molecular Biology, Berlin, Germany; †Charité – University Medicine, Clinics for General and Visceral Surgery, Berlin, Germany; ‡Charité – University Medicine, Center for Adipositas and Metabolic Surgery, Berlin, Germany

Helicobacter pylori infections play an important role in gastric cancerogenesis and significantly increase the risk of developing gastric adenocarcinoma. So far, tumour cell lines, exclusively generated from gastric adenocarcinomas, are used as an in vitro infection model. However, this approach is in part artificial and particularly inappropriate for investigations on early steps in gastric cancerogenesis. Therefore, a primary cell system was developed to better approximate the in vivo situation. Under specific long-term culture conditions, single gastric glands, isolated from healthy tissue samples, undergo multiple fission events resulting in 3-dimensional organoids with epithelial domains. Once transferred into 2-dimensional primary cell layers, these cells constitute ideal conditions to study infections in vitro. Until now, we employed this technique successfully for human, simian and murine stomach tissue.
Future applications of this primary cell system will facilitate obtaining insight into processes, such as molecular signalling and (epi-)genetic alteration elicited by *H. pylori* in normal human gastric cells. This is of particular relevance for studies of tumorigenic pathogens when it comes to an analysis of the mechanisms underlying the transition from a normal to a transformed cell stage.

Abstract no.: P03.08

HELICOBACTER PYLORI INTERACTION WITH MUCOADHESIVE FILMS UNDER GASTRIC CONDITIONS

F. Nogueira,*,† I. C. Gonçalves*,†,‡ and C. L. Martins*,§
*INEB – Intituto de Engenharia Biomédica, Porto, Portugal; †Faculdade de Engenharia, Universidade do Porto, Porto, Portugal; ‡IPATIMUP–Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; §Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Introduction: The mucoadhesive polymer, chitosan, has been investigated as gastric antibiotic delivery system to improve *H. pylori* treatment. However, since soluble chitosan has antimicrobial properties, this work aims to evaluate *H. pylori* interactions with chitosan films under simulated gastric environments with pepsin and urea.
Methods: Thin chitosan-films crosslinked with genipin, were characterized in different pH (2.6; 4.0; 6.0) in presence/absence of pepsin and/or urea, using ellipsometry, Infrared Spectroscopy and electrokinetic analyzer. The amount, morphology and viability of adherent bacteria (J99 strain), performed under different pH with/without pepsin and/or urea, were determined by scanning electron and fluorescence microscopy. Bacteria Zeta potential was calculated in different pH at 25°C.
Results/Discussion: Chitosan films (11.7 ± 0.6 nm) adsorbed pepsin in all pH, but not urea (only small adsorption at pH 6.0). In suspension, the decrease in pH changed *H. pylori* Z-potential from negative to positive. *H. pylori* adhered to chitosan in all pH used, but binding was higher at pH2.6 although most adherent bacteria were dead and in a rod morphology. The presence of pepsin decreased bacterial adhesion and increased its viability. The presence of urea did not affect the amount, morphology or viability of chitosan-adherent bacteria.
Conclusion: Chitosan was able to bind and kill *H. pylori* in a pH range that simulates gastric conditions, revealing the potential use of chitosan-based biomaterials as adjuvants in the elimination of *H. pylori* gastric infection. However, the presence of pepsin might impair this strategy.
Acknowledgements: COMPETE/FCT (PTDC/CTM/65330/2006; PTDC/CTM-BPC/121149/2010; SFRH/BPD/63722/2009). Prof. Borén for *H. pylori* strains.

Abstract no.: P03.09

DIFFERENTIAL EXPRESSION OF THE *HELICOBACTER PYLORI* VACUOLATING CYTOTOXIN GENE, *VACA*, IN THE HUMAN STOMACH

K. R. Amilon,* D. P. Letley,* R. J. M. Ingram,* A. Zaitoun† and J. C. Atherton*
*Nottingham Digestive Diseases Centre Biomedical Research Unit, University of Nottingham, Nottingham, UK; †Department of Pathology, Nottingham University Hospitals NHS Trust, Nottingham, UK

Background and Aims: The vacuolating cytotoxin (VacA) is one of the main virulence factors produced by *H. pylori*. Certain *vacA* genotypes are strongly associated with increased disease risk, but this association is not absolute. We hypothesised that the amount of VacA produced during infection is also likely to be important and aimed to characterise *vacA* expression in vivo and its association with promoter region polymorphisms and disease.
Methods: We obtained antrum and corpus biopsies from 15 *H. pylori*-infected patients attending endoscopy for dyspeptic symptoms with informed consent. We measured *vacA* mRNA levels in biopsies by RT-qPCR, and performed modified Sydney scoring on histological sections. The *vacA* promoter region was sequenced from the isolated strains.
Results: *vacA* transcript levels varied over a 19 fold range. Extensive variation existed within the *vacA* promoter region (10% mean pair-wise nucleotide diversity), and several common polymorphisms within regulatory regions were iden-

tified. Interestingly, for most strains *vacA* expression was higher in the corpus than the antrum (median 1.78 fold difference; $p = 0.04$, Wilcoxon matched-paired signed rank test). No significant correlation between promoter region diversity, in vivo *vacA* transcription level, inflammation score or disease status was found.
Conclusions: Increased *vacA* mRNA levels in the stomach corpus suggest a role for environmental factors regulating *vacA* transcription. We aim to investigate this further by assessing the effect of pH on *vacA* transcription in vitro.

Abstract no.: P03.10

THE CAGL GLU59 POLYMORPHISM OF *H. PYLORI* IS ASSOCIATED WITH AN INCREASED RISK OF GASTRIC CANCER. PRELIMINARY RESULTS FROM AN ITALIAN MULTICENTER STUDY

C. F. Zambon,*,† M. Pelloso,† A. Tessari,† D. Basso,† D. Bozzato,† A. Marchet,‡ G. de Manzoni,§ L. Cristadoro,¶ A. Brandimarte,¶ L. Gerard,** D. Nitti‡ and M. Plebani*,†
*Department of Medicine – DIMED, University of Padova, Padova, Italy; †Department of Laboratory Medicine, University of Padova, Padova, Italy; ‡Surgery Branch, Department of Surgery Oncology and Gastroenterology, University of Padova, Padova, Italy; §First Division of General Surgery, University of Verona, Verona, Italy; ¶General Surgery, Pieve di Coriano Hospital, Mantova, Italy; **General Surgery, Carlo Poma Hospital, Mantova, Italy

Objectives: The risk of *H. pylori*-associated gastric cancer is increased when the infecting strain bears the cag-pathogenicity island or the s1/m1 *vacA* gene polymorphisms. A recent report showed that coding polymorphisms of *cagL* gene, belonging to the cag-pathogenicity island, are associated with gastric cancer risk in Taiwanese patients (Yeh YC et al. *Mol Carcinog* 2011;50:751–9). Our study was aimed to evaluate this association in Italian subjects infected by Western *H. pylori* strains.
Methods: We retrospectively selected 98 patients infected by *H. pylori* strains bearing the cag-pathogenicity island, 39 with gastric cancer (21 male, 18 female; mean age 68 years, range 39–88 years) and 59 with benign *H. pylori* associated pathologies (control group) (26 male, 33 female; mean age 52 years, range 20–78 years). Histology was used to diagnose gastric cancer and, in control group, to evaluate inflammation, activity, intestinal metaplasia and bacterial load. *H. pylori* was cultured from gastric specimens and genomic DNA extracted from isolates. CagL aminoacidic polymorphisms at residues 58 and 59 and *vacA* s1 and m1 polymorphisms were determined by direct sequencing and PCR respectively.
Results: Glutammic acid at residue 59 of CagL protein (Glu59) was significantly more frequent among gastric cancer patients than controls (61.5% and 40.7% respectively, $\chi^2 = 4.089$, $p < 0.05$, OR = 2.333, 95% CI 1.019–5.342). CagL polymorphisms were not significantly associated with any histological finding in control group. All infecting strains bore the s1/m1 *vacA* genotype.
Conclusions: CagL Glu59 polymorphism appears to be a risk factor for gastric cancer onset in Italian patients independently from *H. pylori vacA* gene polymorphisms.

Abstract no.: P03.11

ASSOCIATION OF *HELICOBACTER PYLORI* OUTER MEMBRANE PROTEIN A/B AND GASTRIC CANCER

T. Samadi,* S. Barani,* Y. Talebkhan,* S. Saberi,* A. Oghalaie,* M. Esmaili,* N. Karami,* M. Eshagh Hosseini,† M. Mohagheghi‡ and M. Mohammadi*
*Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran; †Endoscopy Clinic, Amiralam Hospital, Tehran University of Medical Sciences, Tehran, Iran; ‡Cancer Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran

Introduction: *Helicobacter pylori* hom gene encodes an outer membrane protein with allelic divergence; homB and its nearly 90% similar allele, homA. In this study, we have evaluated the association of gastric cancer with hom gene status and other virulence factors including cagA and vacA in an Iranian case-control study.
Methods: A total of 241 *H. pylori* strains were isolated from 73 Gastric cancer (GC) cases and 168 non-ulcer dyspeptic (NUD) subjects as controls. Genotyping (homB/A), (cagA+/−) and (vacA s and m regions) was done by gene-specific PCR. Chi square test, Pearson's correlation and linear regression analysis was used for data analyses (SPSS v.16.0).
Results: homB was more frequent (64.3%) among GC cases than NUD (47.4%) controls, inducing a 2.0 fold increased risk (OR = 2.0; 95% CI = 1.0–4.2), while adjusting for age and gender. Furthermore, the majority (89.4%) of *H. pylori* strains isolated from the GC group were of the vacA s1m1 genotype which produced a 7.0 fold increased risk (OR = 7.8; 95% CI = 2.7–22.9). Accordingly, a significant positive correlation was observed between homB and vacA s1m1 genotype amongst GC cases ($R = 0.558$; $p < 0.001$), as well as between cagA positivity and vacA s1m1 amongst NUD subjects ($R = 0.368$; $p = 0.002$). Due to the high prevalence (>90%) of cagA positivity amongst Iranian Hp strains, there was no statistically significant association between cagA gene and gastric cancer.
Conclusion: Our findings support the role of homB as a GC-related virulence factor which in association with other well-studied virulence factors such as cagA+ and vacA s1m1 may help create grounds for gastric cancer development.

Abstract no.: P03.12

PRO-INFLAMMATORY PROPERTIES AND NEUTROPHIL ACTIVATION BY *HELICOBACTER PYLORI* UREASE

A. F. Uberti,* D. Olivera-Severo,* G. E. Wassermann,* A. Scopel-Guerra,* J. A. Moraes,† P. Barcellos-de-Souza,† C. Barja-Fidalgo† and C. R. Carlini*,‡
*Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; †Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; ‡Universidade Luterana do Brasil, Canoas, Brazil

The gastric pathogen *Helicobacter pylori* produces large amounts of urease, whose enzyme activity enables the bacterium to survive in the stomach. We have previously shown that ureases display enzyme-independent effects in mammalian models, most through lipoxygenases-mediated pathways. Here, we evaluated potential pro-inflammatory properties of *H. pylori* urease (HPU). Mouse paw edema and activation of human neutrophils were tested using a purified, cell-free, recombinant HPU. rHPU induced paw edema with intense neutrophil infiltration. In vitro 100 nmol/L rHPU was chemotactic to human neutrophils, inducing production of reactive oxygen species. rHPU-activated neutrophils showed increased lifespan, with inhibition of apoptosis accompanied by alterations of Bcl-XL and Bad contents. These effects of rHPU persisted in the absence of enzyme activity. rHPU-induced paw edema, neutrophil chemotaxis and apoptosis inhibition reverted in the presence of the lipoxygenase inhibitors esculetin or AA861. Neutrophils exposed to rHPU showed increased content of lipoxygenase(s) and no alteration of cyclooxygenase(s). Altogether, our data indicate that HPU, besides allowing the bacterial survival in the stomach, could play an important role in the pathogenesis of the gastrointestinal inflammatory disease caused by *H. pylori*.

Abstract no.: P03.13

ASSOCIATION OF *HELICOBACTER PYLORI* DUPA GENE WITH CAGA AND VACA GENES IN BRAZILIAN PATIENTS

L. T. Rasmussen,* W. N. Pereira,* W. A. Orcini,* J. B. Ximenez,* A. C. Neto,† R. W. Delabio† and S. L. M. Payão*
*Universidade do Sagrado Coração – USC, Bauru, Brazil; †Faculdade de Medicina de Marília, Marilia, Brazil

Virulence factors of *Helicobacter pylori* have been demonstrated to be predictors of gastric diseases. Duodenal ulcer-promoting gene A (*dupA*), is a novel virulence factor, in *Helicobacter pylori* and was associated with duodenal ulcer development and reduced risk for gastric carcinoma in some populations. The present study aimed to determine the presence of *dupA* gene and evaluated the association among *dupA* and other virulence factors such as *cagA, vacA* in Brazilian patients. Gastric biopsies, was obtained from 205 dyspeptic patient, 100 pediatric and 105 adult. DNA was extracted and analyzed for the presence of *H. pylori* and virulence factors using polymerase chain reaction method. Patients with gastritis had a higher frequency of *H. pylori*-positive. The *dupA* gene was detected in 41.5% (85/205); *cagA* gene was obtained with 98 isolates (47.8%) and *vacA* genotypes s1/m1 (50.2%), s1/m2 (8.3%), s2/m2 (36.6%), s2/m1 (0.5%) and s1/s2/m1/m2 (4.4%) were found respectively. We also verified a significant association between *cagA* and *dupA* gene ($p = 0.0003$, relative risk (RR) 1.73 and confidence interval [CI] = 1.3–2.3). The genotypes s1/m1 also was associated with *dupA* gene ($p = 0.0001$, RR: 1.72 and CI: 1.3–2.2). The same associations were verified when analyzing pediatric and adults groups of patients individually. In conclusion, *dupA* is highly frequent in Brazilian patients and was associated with *cagA* gene and *vacA* s1/m1 genotype, and may

considered important virulence factor in development of gastric diseases in adults or pediatric patients.

Keywords: *Helicobacter pylori, dupA, cagA.*

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Abstract no.: P03.14

ASSOCIATION OF CAGA AND VACA WITH GASTRIC MUCOSA ATROPHY

G. Moisejevs,*,†,‡ I. Daugule,* D. Rudzite,*,†,‡ M. Gerhard,§ G. Gottner,¶ T. Zacher,¶ D. Janciauskas,*,** I. Liepniece-Karele,*,†,†† S. Isajevs,*,† I. Lasina,* I. Kikuste,*,† K. Funka,*,‡‡ A. Vanags,‡‡ I. Tolmanis‡,‡‡ and M. Leja*,†,‡‡

*University of Latvia, Riga, Latvia; †Riga East University Hospital, Riga, Latvia; ‡Riga Stradins University, Riga, Latvia; §The Entrepreneurial University, Munich, Germany; ¶Mikrogen Diagnostik, GmbH, Munich, Germany; **Lithuanian University of Health Sciences, Kaunas, Lithuania; ††Academic Histology laboratory, Riga, Latvia; ‡‡Center of Digestive Diseases "GASTRO", Riga, Latvia

Introduction: Persistent *Helicobacter pylori* (HP) infection may lead to development of chronic atrophic gastritis – important precancerous condition. Still it is not clear why mucosal damage occurs only in some patients infected with HP. Aim of the study was to find out if such HP virulence factors as cytotoxin associated gene A (CagA) and vacuolating cytotoxin A (VacA) are associated with the development of gastric mucosa atrophy.

Material and Methods: Patients (n = 304, median of age 61 years, males/females 113/191) due to current gastrointestinal symptoms underwent upper gastrointestinal tract endoscopy with standard biopsy sampling and further histopathological examination. Gastric mucosa atrophy was elevated according to Operative Link of Gastritis Assessment (OLGA). Patients having OLGA 0-I score were considered as non-atrophy patients and those with OLGA II-IV score – atrophy.

Blood sample from each patient was collected; IgG for HP (Biohit, Finland) and anti-CagA and anti-VacA IgG (Mikrogen Diagnostik, Germany) were detected. Statistical analysis: chi-square test.

Results: HP infection rate among atrophy group was significantly higher compared to non-atrophy group (76.4% vs 63.8%, $p = 0.025$). Levels of anti-CagA and anti-VacA antibodies were significantly higher in atrophy group compared to non-atrophy group (correspondingly, 83.6% vs 62.4%, $p < 0.001$ and 22.7% vs 9.8%, $p = 0.002$).

Conclusions: Presence of anti-CagA and anti-VacA antibodies is associated with the development of gastric mucosa atrophy. Although other candidate HP virulence factors, predicting development of gastric mucosa damage, should be identified and their role have to be clarified.

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Abstract no.: P03.15

EVALUATION OF IGG IMMUNE RESPONSES TO 15 *H. PYLORI PROTEINS* BY LINE ASSAY

L. Formichella,* L. Romberg,* M. Vieth,† M. Geppert,‡ G. Göttner,§ C. Nölting,§ W. Schepp,¶ A. Schneide,¶ K. Ulm,** P. Wolf,** D. Busch,*,†† E. Soutschek§ and M. Gerhard*,††

*Institut for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany; †Department of Pathology, Klinikum Bayreuth, Bayreuth, Germany; ‡Private Practice for Gastroenterology, Bayreuth, Germany; §Mikrogen GmbH, Neuried, Germany; ¶Department of Gastroenterology, Hepatology and Gastrointestinal, Oncology, Bogenhausen Academic Teaching Hospital, Technical University of Munich, Munich, Germany; **Institute for Medical Statistics and Epidemiology, Technische Universität München, Munich, Germany; ††DZIF German Centre for Infection Research, Munich, Germany

H. pylori colonizes half of the world's population, but only a minority of infected individuals develop associated diseases. Nevertheless, it is a major health problem because of its high prevalence worldwide and the link to the development of ulcers, gastric cancer and the MALT-lymphoma. To date, it is not possible to identify patients at increased risk for disease. *H. pylori* virulence factors have been associated with disease development, but direct assessment of virulence factors requires invasive methods to obtain gastric biopsies.

Our study aimed at the evaluation of serological immune responses against important *H. pylori* virulence factors. For this evaluation highly immunogenic proteins where selected, some of which are associated with chronic atrophic gastritis, ulcers, and also with the development of gastric cancer. Fifteen *H. pylori* proteins were overexpressed, purified and immobilised to nitrocellulose membranes to detect serological immune responses. For the evaluation of these antigens a German cohort of 980 patients was screened and immune responses were compared to histological findings. In this population fife antigens showed significant correlations to high degree inflammation and preneoplastic changes by logistic regression. Furthermore, in combination, these antibodies indicate a higher risk for premalignant changes among *H. pylori*-seropositive subjects. For the evaluation of these antigens also different populations (Europe, China, South America) were screened to analyse their diagnostic value in different countries.

Abstract no.: P03.16

HELICOBACTER PYLORI DUPA IS HETEROGENEOUS AND DOES NOT INFLUENCE INTERLEUKIN-8 SECRETION

R. M. Ferreira,* I. Pinto-Ribeiro,* M. S. Marques,* L. Pinto*,† and C. Figueiredo*,†

*IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; †Department of Pathology and Oncology, Faculty of Medicine of the University of Porto, Porto, Portugal

Background and Aim: The duodenal ulcer promoting gene *dupA* is a putative *Helicobacter pylori* virulence marker. While initial studies were based on PCR detection of *jhp0917* and *jhp0918* and on the presence of the C/T insertion after position 1385 of *jhp0917*, more recent nucleotide sequence data have shown that a significant number of strains harbor mutations that lead to premature stop codons.

The aim of this study was to characterize the full *dupA* locus in *H. pylori* clinical isolates and to evaluate the influence of the dupA status on IL-8 secretion by gastric epithelial cells.

Methods: The *dupA* of 65 clinical isolates was sequenced using primers covering the *jhp0917* and *jhp0918 loci*. Strains were considered *dupA*-positive if there was a C/T insertion after nt1385 and if the size was ≥1839 bp. IL-8 secretion was measured by ELISA in supernatants of AGS cells infected with *H. pylori*.

Results: Most strains were classified as *dupA*-positive (n = 52; 80%), the majority of which had an ORF of 1884 bp (n = 50; 96.2%). The *dupA*-negative strains (n = 13; 20%), showed sequence variation leading to premature stop codons and giving rise to ORFs that ranged from 501 to 1635 bp; the most common form spanned 1635 bp (n = 5; 38.5%). No significant differences were observed in IL-8 secretion by AGS cells between infections with *dupA*-positive and *dupA*-negative strains.

Conclusions: *dupA* is more heterogeneous than initially thought and sequencing of the full *dupA* locus should be considered when evaluating the clinical relevance of this virulence factor. The *dupA* status does not influence IL-8 secretion by epithelial cells.

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Abstract no.: P03.17

SYSTEMATIC CHARACTERIZATION OF CAGA IN *H. PYLORI* ISOLATES FROM PATIENTS WITH GASTRIC DISORDERS: PROSPECTIVE STUDY

C. Langner,* W. Habendorf,* A. Link,* M. Varbanova,* I. Tammer,† D. Jechorek,‡ T. Wex*,§ and P. Malfertheiner*

*Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Germany; †Institute of Medical Microbiology, Otto-von-Guericke University, Magdeburg, Germany; ‡Institute of Pathology, Otto-von-Guericke University, Magdeburg, Germany; §Department Molecular Genetics, Medical Laboratory for Clinical Chemistry, Microbiology and Infectious Diseases, Magdeburg, Germany

Background: *H. pylori* infection has a key trigger function in gastric cancer and cagA is a relevant factor in this context. In this prospective study, we aimed to characterize *H. pylori* isolates in regard to cagA status systematically and correlate the expression with host immunological response and gastric pathology.

Methods: Three hundred and ten patients underwent upper gastrointestinal endoscopy and based on histological findings subgroups were formed as followed: patients with normal mucosa, chronic gastritis (CG) without atrophy/intestinal metaplasia, intestinal metaplasia with atrophic gastritis (AG) and gastric cancer. Isolates of *H. pylori* have been obtained from gastric biopsies and cagA gene as well as its expression (mRNA) was determined by PCR. ELISA was performed to evaluate anti-CagA IgG status.

Results: *H. pylori* culture was successful in 70 (61.9%) patients with positive *H. pylori* serology. From 50 (71.4%) patients *H. pylori* isolates were obtained from both gastric sites. Ninety percent of the isolates showed concordant cagA status both in antrum and corpus. The cagA gene was present in 105 of total 120 (86.7%) isolates and expression was confirmed in 75%. However, from 70 patients with isolated *H. pylori* strains only 29.2% were positive for anti-CagA IgG. Furthermore, cagA gene and mRNA were more frequent in patients with AG compared with CG 85% versus 51%, respectively ($p < 0.05$).
Conclusions: Most patients with *H. pylori* infection harbored cagA positive strains. However, the negative CagA IgG serology does not reflect the bacterial cagA status. This difference may be related to bacterial as well as host factors.

Table 1 Clinical data of study group

Diagnosis	Number of patients (number of isolated strains)	cagA gene (%)	cagA mRNA (%)	Anti-CagA IgG antibody (>6.25 U/mL)
Chronic Gastritis (CG)	41 (70)	58 (82.8)	46 (65.7)	14 (20%)
Atrophic gastrits ± intestinal metaplasia (AG)	26 (45)	42 (93.3)	39 (86.7)	16 (35.5%)
Gastric cancer (GC)	3 (5)	5 (100)	5 (100)	5 (100%)
Total number	70 (120)	105 (87.5)	90 (75)	35 (29.2%)

Abstract no.: P03.18

THE IL-8 LEVELS OF DIFFERENT EPIYA MOTIFS DETECTED FROM DIFFERENT GENOTYPE OF *H. PYLORI* ISOLATED IN TURKEY: A PROSPECTIVE STUDY IN MONOCYTE AND NEUTROPHIL CELL LINE MODELS

H. Cakan,* B. S. Kocazeybek,† R. Caliskan Algingil,† P. Yuksel,† H. Bahar Tokman,† A. S. Saribas,† O. Akgul,† Y. Z. Erzin‡ and K. Bal‡

*Department of Microbiology, Faculty of Forensic Science, Istanbul University, Istanbul, Turkey; †Medical Microbiology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey; ‡Gastroenterology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey

Major virulence factors of *H. pylori,* cagA and vacA, associated with gastroduodenal pathologies are also factors used for genotyping *H. pylori* and recently they become important in terms of the relation of different CagA EPIYA motifs and gastroduodenal pathologies. We aimed to determine the IL-8 level on the association of *H. pylori* cagA genotypes and EPIYA motifs in in-vitro cell lines. Twenty-one randomly selected *H. pylori* genotypes were divided in cagA+ vacAs1 m2+, cagA+ vacAs1m1+, cagA+ vacAs1m2+ babA2+, cagA+ vacAs2m2+ as genotype 1, 2, 3 and 4 respectively. CagA-P1C, cagA-P2CG, cagA-P2TA and cagA-P3E primers were used to determine EPIYA A, B, C and D motifs respectively. EPIYA motifs were determined by comparing our results with the RIGLD-HC139 cagA genome using the BLAST program at NCBF. IL-8 levels were determined quantitatively by flow cytometry in four strains performing their co-culture in THP-1 (monocyte) and neutrophil differentiated (ND) HL-60 cell lines. In our study, EPIYA-ABC and EPIYA-AB were found in genotype 1, 2, 4, and in genotype 3 respectively. In THP-1 cell line, four strains with different genotypes/EPIYA motifs influenced a high level of IL-8 secretion ($p < 0.05$) and the highest IL-8 secretion was determined with the influence of the genotip1/EPIYA-ABC strain. In ND HL-60 cell line, the genotype 3/EPIYA-AB strain influenced the highest IL-8 secretion ($p < 0.001$). As a result we determined that IL-8 plays an important role in the pathogenesis of *H. pylori* and could be secreted by the influence of both single repeated EPIYA-C genotypes and no repeated genotypes. However, non EPIYA-C with BapA2 genotype was found to influence the release of the highest IL-8 secretion in ND HLA 60 cell lines. We belive that, further studies with larger groups of strain are needed to elucidate clearely the effect of different pattern of genotype/EPIYA association and cytokine secretion on the gastroduodenal pathology.

Abstract no.: P03.19

EPIYA MOTIF PATTERNS AMONG CAGA- POSITIVE *H. PYLORI* STRAINS: A PROSPECTIVE STUDY FROM TURKEY, A COUNTRY WITH A POPULATION HAVING EUROPEAN AND ASIAN GEOGRAPHICAL FEATURES

B. S. Kocazeybek,* P. Yuksel,* R. Caliskan Algingil,* A. S. Saribas,* F. Kalayci,* O. Akgul,* H. Bahar Tokman,* E. Caglar,† A. Karakullukcu,* T. Ziver,* Y. Z. Erzin† and K. Bal†

*Medical Microbiology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey; †Gastroenterology Department, Cerrahpasa Faculty of Medicine, The University of Istanbul, Istanbul, Turkey

Helicobacter pylori has many virulence factors such as cagA and vacA playing an important role in the development of gastroduodenal pathologies. Geographical differences in the incidence of gastroduodenal pathology have been reported to be in close relation with different geographic distribution of cagA EPIYA patterns. The aims of our study were to determine the pattern of EPIYA motifs from a patient population of Istanbul and its suburbs which have Eurasian geographical features of Turkey and to evaluate its relation with endoscopic findings.

Ninety-eight cagA-positive *H. pylori* strains isolated from 318 cases with dyspeptic complaints were studied. Primers such as cagA-P1C, cagA-P2CG, cagA-P2TA and cagA-P3E were used for detecting EPIYA segments. Sequence analysis was performed in EPIYA positive bands. EPIYA motifs were determined by comparing the results with RIGLD-HC139 cagA genome.

EPIYA-A, B and C were found in 84, 80 and 70 strains respectively, EPIYA-D was not detected. and 14 strains were EPIYA negative. EPIYA segment's combinations were as EPIYA ABC, ABCC, AB and AC in 62, 4, 14, and 4 strains respectively. EPIYT motif was detected in 10 strains with EPIYA-B segment. EPIYA-ABCC, AB and AC was found in 4, 14 and 4 patients with gastritis(G) respectively. Additionnaly EPIYA-ABC was detected in 50 patients with G, two patients with G + DU (duodenal ulcer) and in 10 patients with normal GIS. EPIYA motif was negative in four patients.

In our study, EPIYA-C was found to be the predominant type with two repeats (EPIYA-CC) in four cases. EPIYA-D was not found in our cagA positive cases. Furthermore, in this region of Turkey, the presence of the relation with mild histopathological pattern west type EPIYA-C, and the importance of the predic-

tive diagnostic value of this pathological relation are highlighted with our results as parallel in the litterature.

Abstract no.: P03.20

PREVALENCE OF GENES FROM *H. PYLORI*-PLASTICITY REGION ASSOCIATED TO DISEASE IN ISOLATES FROM MEXICAN CHILDREN

C. Romo-González,* A. Consuelo-Sánchez,† N. Velázquez- Guadarrama,† M. Camorlinga-Ponce,‡ J. Burgueño-Ferreira,§ M. García-Zúñiga* and R. Coria-Jímenez*

*Instituto Nacional de Pediatría, Mexico, Mexico; †Hospital Infantil de México "Federico Gómez", Mexico, Mexico; ‡Hospital de Pediatría, CMN Siglo XXI. IMSS, Mexico, Mexico; §Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico, Mexico

Background: The genes jhp0940, jhp0945, jhp0947 and jhp0949 belong to *H. pylori*-plasticity region. These genes have been proposed as markers for gastroduodenal diseases and to associate with an increase in inflammatory cytokines and enhancement of NF-kB signaling pathway.

Aim: To assess the prevalence of jhp0940-jhp0945-jhp0947-jhp0949 genes in *H. pylori* isolates from Mexican children.

Methods: In 45 *H. pylori* isolates, we identified jhp0940, jhp0945, jhp0947 and jhp0949 genes by PCR and evaluated the relationship between cagA and the presence of these genes.

Results: Of the 45 *H. pylori* isolates, 28 (62.2%) were positive for jhp0940, 17 (37.7%) for jhp0945, 33 (73.3%) for jhp0947, 36 (80%) for jhp0949 and 28 (62.2%) for cagA. The combinations of these genes generate a genotype pattern between the isolates, represented as 1 = present; 0 = absent. jhp0940-jhp0945-jhp0947-jhp0949 (1/1/1/1 genotype) = 10 (22.2%); (1/0/1/1 genotype) = 10 (22.2%); (0/1/1/1 genotype) = 6 (13%); (0/0/1/1 genotype) = 6 (13%); (1/0/0/1 genotype) = 4 (8.8%); (0/0/0/0 genotype) = 3 (6.6%); (1/0/0/0 genotype) = 2 (4.4%); (0/0/0/1 genotype) = 1 (2%) and (0/0/1/0 genotype) = 1 (2%). There was no association between these four genes and cagA. However, we found an association between jhp0949 and jhp0947, $p = 0.0005$ and $\phi = 0.5779$ (analysis with Fisher's exact test).

Conclusions: The prevalence of jhp0940 gene in this isolates is high in comparison with what was published for adults. The rest of the genes showed similar prevalence to what was found in adults with some kind of gastroduodenal disease. We suggest that these genes could play a role in inflammation process at the beginning of the infection with *H. pylori*.

P04 Epidemiology and transmission

Abstract no.: P04.01

ERADICATION RATES OF *HELICOBACTER PYLORI* IN KOREA OVER THE LAST 11 YEARS: NATION-WIDE SURVEY

S. Lee,* W. Shin,† S. Lee,‡ G. Baik,§ K. Huh,¶ S. Lee,** J. Chung,†† W. Jung,‡‡ M. Park,§§ H. Jung,¶¶ H. Kim,*** J. Kim,††† S. Seol,‡‡‡ S. Yoon,§§§ S. Jeon,¶¶¶ S. Hong,**** G. Kim,†††† D. Lee,‡‡‡‡ H. Kim,§§§§ S. Choi,¶¶¶¶ H. Kang,***** J. G. Kim,* J. J. Kim††††† and Korean College of Helicobacter and Upper Gastrointestinal Research

*Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea; †Department of Internal Medicine, Hallym University College of Medicine, Seoul, Korea; ‡Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea; §Department of Internal Medicine, Hallym University College of Medicine, Chun Cheon, Korea; ¶Department of Internal Medicine, Konyang University College of Medicine, Daejeon, Korea; **Department of Internal Medicine, Institute of Gastroenterology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; ††Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; ‡‡Department of Internal Medicine, Kyungsang National University School of Medicine, Daegu, Korea; §§Department of Internal Medicine, Kosin University College of Medicine, Busan, Korea; ¶¶Department of Internal Medicine, Ewha Womans University School of Medicine, Seoul, Korea; ***Department of Internal Medicine, School of Medicine, Jeju National University, Jeju, Korea; †††Department of Internal Medicine, Konkuk University School of Medicine, Digestive Disease Center, Konkuk University Medical Center, Seoul, Korea; ‡‡‡Department of Internal Medicine, Busan Paik Hospital, Inje University College of Medicine, Busan, Korea; §§§Department of Internal Medicine, Chungbuk National University College of Medicine, Cheongju, Korea; ¶¶¶Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu, Korea; ****Department of Internal Medicine, Soonchunhyang University College of Medicine, Bucheon, Korea; ††††Department of Internal Medicine, School of Medicine, Pusan National University, Busan, Korea; ‡‡‡‡Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul, Korea; §§§§Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Korea; ¶¶¶¶Department of Internal Medicine, School of Medicine, Wonkwang University, Chonbuk, Korea; *****Department of Finance and Information Statistics, Hallym University College of Natural Science, Chuncheon, Korea; †††††Department of Medicine, Sungkyunkwan University College of Medicine, Samsung Medical Center, Seoul, Korea

Objectives: We assessed the time trend of *H. pylori* eradication rates over the last 11 years in a country with a high prevalence of *H. pylori* infection.
Methods: This nationwide multi-center study was conducted nationwide for adult subjects aged ≥20 years who were treated *H. pylori* infection in the 20 secondary or tertiary medical centers from January 2000 to December 2010. A total of 34 846 patients (32 682, 2017, and 147 patients in the first-, second-, and third-line therapy group, respectively) were included in this analyses.
Results: The eradication rates of clarithromycin-based triple therapy as a first-line therapy were 95.9%, 86.1%, 87.8%, 86.8%, 84.6%, 84.2%, 85.1%, 83.4%, 81.2%, 80.5%, and 80.7% from 2000 to 2010 with a decreasing trend ($p < 0.0001$). The decreasing trend of eradication rates of overall first-line therapy was observed in the three of nine geographic areas including Chungcheong province, Seoul, and Busan ($p < 0.0001$). The eradication rates of bismuth-based quadruple therapy as a second-line therapy were 100%, 81.3%, 80.6%, 88.9%, 86.0%, 88.6%, 92.6%, 90.8%, 91.1%, 89.9%, and 88.7% from 2000 to 2010 without significant change ($p = 0.352$). The increasing trend of eradication rates of overall second-line therapy was observed in the two of nine geographic areas including Seoul ($p < 0.0001$) and Busan ($p = 0.009$).
Conclusions: The eradication rates of clarithromycin-based triple therapy had been decreased, where as those of bismuth-based quadruple therapy unchanged over the past 11 years in Korea, though these trends were different among the geographic areas.

Abstract no.: P04.02

COMMUNITY-BASED RESEARCH ON *H. PYLORI* INFECTION IN THE CANADIAN ARCTIC: HISTOPATHOLOGICAL FINDINGS SHOW A HIGH PREVALENCE OF SEVERE GASTRITIS

E. V. Hastings,* S. J. O. Veldhuyzen Van Zanten,* S. Girgis† and K. J. Goodman*

*Division of Gastroenterology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada; †Pathology and Laboratory Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

Prevalence of *H. pylori* infection appears to be declining in southern Canada, yet residents of northern Aboriginal communities continue to experience a disproportionately high prevalence and frequent treatment failure. In response to concerns raised by community leaders, the Canadian North *Helicobacter pylori* (CANHelp) Working Group established community projects that aim to investigate the disease burden and improve treatment strategies in the region. We present histopathological findings and treatment trial results from Aklavik, Northwest Territories and Old Crow, Yukon, where community-wide *H. pylori* prevalence (by 13C-UBT) was 58% (n = 332) and 70% (n = 186), respectively.
Consenting participants underwent upper endoscopy with gastric biopsy in 2008 in Aklavik and 2011 in Old Crow. For each participant, five biopsies were assessed by a single pathologist using the Sydney classification; two biopsies were used for culture for susceptibility testing. Consenting participants aged ≥15 years received a randomly selected *H. pylori* treatment regimen. Follow-up 13C-UBT was used to determine treatment success.
The prevalence of severe gastritis and atrophy were high, especially in Old Crow (Table 1). Treatment was effective in 59% (29/49) assigned triple therapy, 69% (40/58) assigned sequential therapy, and 85% (17/20) assigned quadruple therapy.
Our results confirm that these populations have high frequencies of severe gastritis and precancerous lesions with variation across communities. We will present data from other Canadian Arctic communities, and show treatment success by gastritis severity and antibiotic susceptibility status.

Table 1 Histopathological findings of gastric biopsies from CANHelp projects

Histopathological finding	Aklavik *H. pylori* project % of total [% of HP+]	Old crow *H. pylori* project % of total [% of HP+]	All participants % of total [% of HP+]
Inflammation			
Mild (%)	7 [8]	5 [3]	7 [7]
Moderate (%)	31 [47]	29 [32]	31 [42]
Severe (%)	29 [43]	59 [65]	36 [50]
Atrophy (%)	14 [21]	67 [74]	27 [37]
Intestinal metaplasia (%)	8 [11]	33 [35]	14 [18]

Abstract no.: P04.03

HELICOBACTER PYLORI IN THE WATER. JUST CONTAMINATION OR SOURCE OF TRANSMISSION?

M. Plonka, A. Targosz, U. Szczyrk, M. Strzalka, A. Ptak-Belowska and T. Brzozowski

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

The major transmission pathway of *Helicobacter pylori* (*H. pylori*) has not been so far identified but recent evidence suggests the waterborne aspect of *H. pylori* transmission. We conducted the innovative project in south region of Poland to detect the presence of Hp in drinking water samples in Cracow and surroundings. The water contamination and consideration of *H. pylori* in water as source of transmission in polish population have not been extensively studied. More than 150 water samples from different municipal water distribution systems, rivers, drinking water tanks and wells were collected and analyzed between June and December of year 2012. Samples of 1000 mL of water were concentrated by centrifugation. Obtained pellet was resuspended in 1 mL of PBS used for the *H. pylori* culture. The remaining portion was stored at −20°C for DNA extraction and subsequent gastric colonization of Mongolian gerbils. Water samples were subjected to PCR for the presence of Hp using primer pair: Cluster2: GGCGTTATCAACAGAATGGC and B1J99: CTCAGTTCGGATTGTAGGCTGC targeting the hypervariable region flanking the 16S rRNA gene in *H. pylori*. All samples were negative for *H. pylori* culture but 12 out of 150 samples collected from the Cra-

cow municipal water distribution system were *H. pylori* DNA positive. These *H. pylori* positive samples unsuccessfully colonized the stomach of Mongolian gerbils. Among 20 samples which were tested twice (June and October), eight were positive only in June, but not in October. We conclude that (1) *H. pylori* DNA could be detectable in municipal drinking water samples; (2) bacteria detected in water was non-culturable and failed to colonize animal stomach possibly due to bug transition from spiral to its coccoid form, and (3) water *H. pylori* detection could be influenced by the variation of temperature at different seasons (supported by grant No 2011/01/B/NZ/01539 to M.P. and A.T.).

Abstract no.: P04.04

INCREASED TREND IN *HELICOBACTER PYLORI* ANTIMICROBIAL RESISTANCE DURING A 13 YEAR PERIOD (2000–2012, GIPUZKOA, BASQUE COUNTRY, SPAIN)

M. Fernández-Reyes,* M. Montes,* J. Lizasoain,† L. Piñeiro,* L. Bujanda†,‡ and E. Pérez-Trallero*,‡

*Microbiology Department, Hospital U. Donostia, Donostia-San Sebastián, Spain; †Gastroenterology Department, Hospital U. Donostia, Donostia-San Sebastián, Spain; ‡Faculty of Medicine, University of the Basque Country, UPV/EHU, Donostia-San Sebastián, Spain

Introduction: Treatment of infectious pathogens usually is based in the knowledge of their antimicrobial susceptibility. Until recently, *H. pylori* infection has been treated empirically although many relapses occurred due to the high resistance rates of the antimicrobials used.
Aim of the Study: To know the antimicrobial susceptibility of *H. pylori* isolates obtained in Gipuzkoa, northern Spain.
Material and Methods: Between 2000 and 2012, 5998 isolates of *H. pylori* were tested for antimicrobial susceptibility: 4661 and 1337 strains were isolated from gastric biopsies (obtained by endoscopy) and the string test, respectively. Susceptibility against amoxicillin, clarithromycin, metronidazole, levofloxacin and tetracycline was performed by E-test strips using Brucella agar plates plus 5% hemolyzed horse blood, and 1% Vitox, at 37°C incubation for 48–72 hours under microaerophilic conditions with 80% humidity.
Results: Although this series included naïve infections and relapses, the increased resistance rates of clarithromycin, levofloxacin and metronidazole during the last years is highlighted. Simultaneous resistance to two antimicrobials (metronidazole, levofloxacin, or clarithromycin) was observed in 724 isolates (12.4%), and multiresistance including the three antimicrobials in 140 isolates (2.4%).

Table 2 Percentage of resistant *H. pylory* isolates in Gipuzkoa

	2000-2005	2006	2007	2008	2009	2010	2011	2012
No. isolates	n=1928	n=350	n=383	n=437	n=677	n=770	n=749	n=704
Metronidazole	29.9%	32.0%	38.8%	37.2%	37.2%	41.1%	44.7 %	44.3%
Clarithromycin	16.9%	15.7%	17.8%	21.1%	17.6%	18.1%	20.2%	21.6%
Levofloxacin	10.2%	11.7%	19.3%	15.1%	16.1%	16.6%	18.2%	19.5%
Tetracycline	0.2%	0%	0%	0%	0.6%	0.4%	0.5%	0%
Amoxicillin	0%	0%	0%	0%	0%	0%	0%	0%

Conclusions: 1Resistance to clarithromycin, levofloxacin, and metronidazole was very high in Gipuzkoa, northern Spain.2Simultaneous resistance to two of the three antimicrobials most used in empirical treatments was higher than 10%.3Routine susceptibility testing is compulsory before treatment for *H. pylori* infection.

Abstract no.: P04.05

FOLLOW-UP OF *HELICOBACTER PYLORI* INFECTION IN CHILDREN OVER TWO DECADES (1988–2007): PERSISTENCE, RELAPSE AND ACQUISITION RATES

J. Vanderpas,* P. Bontems,† V. Y. Miendje Deyi‡ and S. Cadranel†

*Medical Microbiology Laboratory, Scientific Institute of Public Health, Brussels, Belgium; †Queen Fabiola Children's University Hospital, Université Libre de Bruxelles, Brussels, Belgium; ‡Microbiology Department, Brugmann University Hospital, Université Libre de Bruxelles, Brussels, Belgium

Helicobacter pylori culture on gastric biopsy was performed in 4964 subjects <18-year-old from 1988 to 2007 in a Central laboratory of Brussels. The total number of biopsies increased markedly from 941 in 1988–1993 to 1608 in 2004–2007. Biopsies were repeated at least once in 922 subjects (603 initially negative and 319 initially positive for *H. pylori*). Persistence rate of *H. pylori* at 1 year after initial positive biopsy was greater in the 1998–2007 cohort than in the 1988–1997 cohort (72.7% vs 45.8%, $p = 1.96*10^{-3}$), suggesting a tailored selection of candidates for biopsy and *H. pylori* culture with the non-invasive tests for *H. pylori* (13C urea breath test test). Within 68 subjects initially positive and re-examined subsequently with a documented cure, reinfection/relapse rate was 48.6% within 10 years post-elimination of *H. pylori*. Acquisition rate over the same time span in the initial cohort of 603 negative patients was 38.7% (Relapse vs acquisition: $p = 3.81*10^{-6}$). Multivariate analysis showed a fourfold greater risk of acquisition of *H. pylori* in non-European origin versus European origin ($p < 0.001$). Clarithromycin and metronidazole susceptibility were determined in 226 and in 223 paired positive cultures in case of relapse or persistence. Initial non-susceptibility profile was highly predictive of subsequent non-susceptibility profile, and the proportion of non-susceptibility increased markedly present from 13.3% to 21.2% for Clarithromycine ($p < 0.001$) and from 27.3% to 35.0% for Metronidazole ($p = 0.014$), with no difference according to European or non-European origin.

Abstract no.: P04.06

HELICOBACTER PYLORI INFECTION IN PEDIATRIC DIGESTIVE ENDOSCOPY: PREVALENCE TREND AND CLINICAL PROFILE

M. Almeida,* T. Rodrigues,† A. Palha,‡ M. Oleatro§ and A. I. Lopes*

*Pediatric Gastroenterology Unit, Department of Pediatrics, Medical Faculty of Lisbon, University Hospital of Santa Maria, Lisboa, Portugal; †Biomathematics Department, Medical Faculty of Lisbon, Lisboa, Portugal; ‡Anatomic Pathology Service, University Hospital of Santa Maria, Lisboa, Portugal; §Department of Infectious Diseases, National Health Institute Dr. Ricardo Jorge, Lisboa, Portugal

Background: Decreasing prevalence of *H. pylori* (*Hp*) infection has been reported worldwide, in parallel with the improvement in sociodemographic conditions. So far there are no prevalence studies concerning symptomatic Portuguese children submitted to digestive endoscopy.
Aims: To evaluate the prevalence of *Hp* infection in a 10-year period in a sample of symptomatic children/adolescents submitted to upper digestive endoscopy; To describe clinical features associated with *Hp* infection in the same population sample.
Methods: Descriptive, analytical and retrospective study; review of 359 diagnostic endoscopy records performed in 2002, 2006 and 2011 in a Lisbon tertiary Gastroenterology care center, age ≤18-year-old; *Hp* status was considered (+) if histology and/or culture were positive; *Hp*(−) if both histology and culture were negative. Statistics: Chi-square test, Fishers exact test; significance $p < 0.05$.
Results: One hundred and seventy-five (48.7%) children/adolescents presented with *Hp* infection and distributed as follows: 11.4% ≤5 years, 48% 5 ≤ 11 years, 40.6% 11 ≤ 18 years. Mean annual prevalence was 57.1% in 2002, 55.5% in 2006 and 41.3% in 2011 ($p = 0.02$ in the interval 2006–2011). Endoscopic features associated with *Hp*(+) were normal esophagus ($p = 0.032$), nodularity in the antrum and corpus ($p < 0.001$) and duodenal ulcer ($p = 0.013$). Histological features associated with *Hp*(+) included moderate inflammation, moderate activity ($p < 0.001$) and the presence of lymphoid aggregates/follicles ($p < 0.005$).
Conclusions: Differently from data concerning other populations at similar settings, our study has shown a yet high *Hp* prevalence, although suggesting a recent decrease trend. These results emphasize the clinical relevance of *Hp* infection in symptomatic pediatric population and the need of cost-effective management strategies.

Abstract no.: P04.07

RETROSPECTIVE ANALYSIS OF THE *H. PYLORI* PREVALENCE AND ERADICATION RATES IN CRIMEAN POPULATION WITH GASTRO-INTESTINAL DISORDERS

V. Kryvy, I. Klyaryts'ka, I. Rabotiagova and S. Matraou

S.I.Georgievskyi Crimean State Medical University, Simferopol, Ukraine

Background: Maastricht IV Consensus recommends that, as a first-line therapy clarithromycin or bismuth containing regimes. The different prevalence levels and eradication rates of *Helicobacter pylori* (*H. pylori*) in reports from different regions requires permanent local monitoring of these indices.

Aim: Assessed the levels of infection *H. pylori* and eradication rates with proton pump inhibitor (PPI)- clarithromycin-based triple and PPI-bismuth-based quadruple therapy in the Crimean population.
Methods: Within the time period from September 2011 to April 2013 were examined 977 patients by 13C-Urea breath test (UBT).
Results: Four hundred and forty-eight patients were examined for the first time (261 *H. pylori*-positive – 58.3%). Five hundred and twenty-nine patients were examined after first-line eradication therapy. The eradication rates for PPI-clarithromycin-based triple therapy (337 treated patients [63.7%]) dependent on the duration of treatment: 7 days – (ITT, 61.67%; PP, 65.0%), 10 days – (ITT, 78.7%; PP, 84.4%), 14 days (ITT, 79.4%; PP, 88.2%).
The 102 (19.3%) *H. pylori*-positive patients received second-line treatment (PPI, bismuth, metronidazole, and tetracycline). *H. pylori* eradication rates were for 10 days: ITT, 70.4%; PP; 81.2%, for 14 days: ITT, 74.9%; PP, 87.6%.
Conclusions: Found a moderate prevalence of *H. pylori* infection in our region. Longer eradication first-line regimes (triple and quadruple therapy) showed not only improvement of eradication, but increase in side effects. This requires in further optimization of therapy in Crimean Population.

Abstract no.: P04.08

PREVALENCE OF *HELICOBACTER PYLORI* AMONG OUTPATIENT MIDDLE-AGED PATIENTS IN LITHUANIA AND ITS RELATION TO DYSPEPTIC SYMPTOMS

L. Jonaitis, G. Kiudelis, L. Kupcinskas and J. Kupcinskas
Lithuanian University of Health Sciences, Kaunas, Lithuania

Introduction: The prevalence of *Helicobacter pylori* infection is decreasing in Western countries, but remains comparably high in developing regions. There are very limited data about the prevalence of *Helicobacter pylori* in Eastern and Central European regions.
Aim: To establish the prevalence of *Helicobacter pylori* among 40–50 years old ambulatory patients routinely consulted by general practitioner (GP) in Kaunas (350 000 inhabitants) and to evaluate the relation of different dyspeptic symptoms with the *Helicobacter pylori*.
Methods: The middle-aged patients from 40 to 50 years old persons who visited their GP were invited to participate in the study. Exclusion criteria were: former *Helicobacter pylori* eradication treatment, patients on dialysis, use of immunosuppressive medications patients.
SureScreen HPSC *Helicobacter pylori* test from finger blood was used to detect antibodies against *Helicobacter pylori*. Patients anonymously filled-in the gastrointestinal symptoms rating scale questionnaire. The intensity of different upper dyspeptic symptoms (epigastric pain or discomfort, heartburn, regurgitation, hunger-like-pain, nausea, epigastric fullness, belching) was assessed during last week rating symptoms in 7 grade Likert scale.
Results: One hundred and three patients included: 71 (69%) female and 32 (31%). Mean age – 46.8 ± 4.8 year. Mean age of female – 46.3 ± 4.5, mean age of male 47.8 ± 5.3, $p > 0.05$. *Helicobacter pylori* infection was established in 71 (69%) patients: among females in 46 (65%), among males in 25 (79%), $p > 0.05$.
The overall prevalence and intensity of investigated symptoms was not different between *Helicobacter pylori*-positive and *Helicobacter pylori*-negative patients.
Conclusion: The prevalence of *Helicobacter pylori* among Lithuanian middle-aged (40–50 years old) patients is quite high – 69%. HP is more prevalent in males though it did not reach the statistical difference due to little sample size.
We did not find any correlations between the status of *Helicobacter pylori* and the prevalence and intensity of different dyspeptic symptoms.

Abstract no.: P04.09

IDENTIFICATION OF VIABLE *HELICOBACTER PYLORI* IN WATER SUPPLIES BY DVC-FISH

P. Santiago, M. Ferrús, L. Moreno and Y. Moreno
Universidad Politécnica de Valencia, Valencia, Spain

Numerous epidemiological studies confirm the relationship between water use and infection with *H. pylori*. However, this pathogen has not been isolated from drinking water yet. The Direct viable count method (DVC) is based on the incubation in the presence of an inhibitor agent of the DNA-gyrase, allowing for discriminating between viable and non viable cells. The combination of DVC with Fluorescence in situ hybridization (FISH) for identification of viable *H. pylori* cells has been successfully reported by our group (Piqueres et al.2006).
The aim of this work was to investigate the presence of viable *H. pylori* cells in drinking water samples by DVC-FISH.
Methods: Samples collected from public tap drinking water were filtered through 0.45 μm nitrocellulose filters. Membranes were introduced into Brucella broth with 5% fetal bovine serum and subcultured in DVC broth (Brucella broth, 5% fetal bovine serum, 0.5 μg/μL novobiocin). Afterwards, samples were fixed with 4% paraformaldehyde and subsequently hybridized with a specific probe (HPY) designed as LNA/DNA probe (Moreno et al., 2001).
Results: Viable elongated *H. pylori* cells were identified in four out of 11 drinking water samples with a specific LNA/DNA probe. Viable cells could be discriminated from nonviable cells because the viable cells were significantly elongated.
Conclusions: DVC-FISH combination is a rapid and specific method to detect viable *H. pylori* cells in drinking water. Our results demonstrate the presence of metabolically active *H. pylori* cells in drinking water public supplies, showing that water distribution systems could be a potential route of *H. pylori* transmission.

Abstract no.: P04.10

CLARITHROMYCIN RESISTANT *HELICOBACTER PYLORI* DETECTION IN TREATED WASTEWATER

Y. Moreno, P. Santiago and M. Ferrús
Universidad Politécnica de Valencia, Valencia, Spain

Helicobacter pylori is an important cause of gastritis in humans, and plays a significant role in duodenal ulcers and gastric adenocarciroma. Clarithromycin resistance occurring in *H. pylori* strains is the most important cause of treatment failure, with an increase of reports worldwide. This resistance is caused by single base mutations within the peptidyl transeferase centre of 23S rRNA. Fluorescence in situ hibridization (FISH) allows for direct identification of microorganisms without previous selective isolation, and has been proposed for direct determination of *H. pylori* clarithromycin resistant strains.
Due to the risk of fecal transmission of these antibiotic resistant strains, the aim of this work has been to determine the prevalence of *H. pylori* clarythromicin resistant cells after disinfection treatment of wastewater samples by FISH.
Methods: Fourty-two wastewater samples from influent, secondary treatment and after disinfection were evaluated for the presence of *H. pylori* clarithromycin resistant cells. All samples were fixed with three volumes of 4% paraformaldehyde.
Samples were hybridized with a mix of probes CLAR1, CLAR2, and CLAR3, specific for detecting 23S rRNA point mutations in clarithromycin resistant *H. pylori*.
Results: FISH detection showed 25 *H. pylori* positive samples among 42, of which 54% were clarithromycin resistant. Furthermore, the two *H. pylori* positive water samples after disinfection were positive for CLAR hybridization.
Conclusions: Presence of macrolide resistant *H. pylori* in samples from wastewater treatment plant even after disinfection process represents a health risk because this treated water is reused for irrigation purposes and can act as a transmission vehicle.

Abstract no.: P04.11

LOW INCIDENCE OF GASTRIC CANCER IN THAI POPULATION IS ASSOCIATED WITH ANTRUM LIMITED *H. PYLORI* GASTRITIS

T. Uchida,* N. Wisedopas,† T. Ratanachu-ek,‡ R. Vilaichone§ and V. Mahachai†
*Oita University, Oita, Japan; †Chulalongkorn University, Bangkok, Thailand; ‡Rajavithi Hospital, Bangkok, Thailand; §Thammasat University, Bangkok, Japan

Backgrounds: Infection with *Helicobacter pylori* (*H. pylori*) almost always causes chronic gastritis and a small proportion of infected patients develop gastric cancer based on a long-term infection of *H. pylori*. Gastric cancer incidence is quite low in Thailand (4.2/100 000 Globocan 2008) in spite of normal rate of *H. pylori* infection. In this study, we conducted nation-wide survey of histopathological analysis of gastric mucosa in Thailand.
Methods: A total of 1389 Thai dyspeptic patients (909 females, 480 males) aged between 13 and 85 years (mean age: 50.5 years) from four regions (469 North, 542 Northeast, 287 Central and 195 South). Patients who were taking PPIs or NSAIDs, or had a history of eradication were excluded. All patients underwent upper gastrointestinal endoscopy in which two samples (one from antrum and one from body) were taken for histopathological analysis. Biopsy specimens were evaluated based on updated Sydney system. *H. pylori* infection

was diagnosed by Giemsa staining and immunohistochemistry with anti-*H. pylori* antibody.
Results: Overall, 676 (45.3%) patients were judged to be positive for *H. pylori*. The prevalence of *H. pylori* infection was significantly low in South region. All histology scores (neutrophil, mononuclear cell infiltration, atrophy and intestinal metaplasia) were high in *H. pylori* infected patients than in non-infected patients. Among the *H. pylori* infected patients, histology scores at the antrum were significantly higher than that at the body and C/A ratio was below 1.0 in all age groups. Intestinal metaplasia was found in 7.7% of the patients.
Conclusion: Low incidence of gastric cancer in Thai population is associated with antrum limited *H. pylori* gastritis.

Abstract no.: P04.12

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION AND PRECANCEROUS GASTRIC LESIONS IN MOROCCO

A. Chakkor, L. Amrani, I. Serraj, Z. Chaoui, M. Salihoun, N. Kabbaj, M. Acharki and N. Amrani
Digestive Functional Exploration Unit-Hepato-Gastro-Enterology Department, Rabat, Morocco

This study aims to evaluate the prevalence of *H. pylori* infection and premalignant gastric lesions and to determine their relationship in Morocco where published studies related to this infection are very scarce.
Materials and Methods: We retrospectively reviewed medical records of 2195 patients with a history of upper digestive disorders who underwent upper gastro-intestinal endoscopy with biopsies screening in our unit. The biopsy specimens were taken from antrum, incisura angularis and corpus for histopathological study according to the Modified Sydney system.
Results: *H. pylori* seroprevalence is 71%. Mean age is 51 years (17–84 years), with sex ratio F/M: 1, 5. Clinical data are dominated by stomach pain 43.6% followed by ulcer syndrome 25.5%, dyspepsia 11.4% and finally hematemesis.
The difference in prevalence between the age group 40–60 years and other age groups are statistically significant; and gender has no significant association.
Normal looking mucosa is the most common endoscopic finding, accounting for 41.6%, followed by gastritis 36% and duodenal ulcer 21.2%.
H. pylori infection is found in 92% of chronic gastritis cases. Its prevalence is significantly higher in the antrum (73%) than in the corpus (21%) and the incisura angularis (6%).
The frequency of gastric atrophy is 15%; it is higher in antrum than in fundus (77% vs 23%). Intestinal metaplasia is noted in 13.4% of patients localised in antrum in 70.6% and fundus in 29.4%. Dysplasia is noted in 3.1% of cases (low grade (2, 8%) and high grade (0, 3%).
Conclusion: Our study shows high prevalence of *H. pylori* infection and dissociation between this latter and low rate of precancerous lesions in Morocco. This confirms previous observations in African developing countries suggesting that other risk factors should be involved in the carcinogenesis process.

Abstract no.: P04.13

LOCALIZATION OF *H. PYLORI* WITHIN THE VACUOLE OF CANDIDA YEAST BY DIRECT IMMUNOFLUORESCENCE TECHNIQUE

P. Saniee,* F. Siavoshi,* G. Nikbakht Broujeni,† M. Khormali,† A. Sarrafnejad‡ and R. Malekzadeh§
*Department of Microbiology, School of Biology, University College of Sciences, University of Tehran, Tehran, Iran; †Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ‡Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; §Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Background: Reports indicate that *H. pylori* in capable of invading the eukaryotic cells and establishing inside their vacuole. FITC-conjugated IgY-Hp was recruited to localize *H. pylori* inside the vacuole of Candida yeast. Existence of the intracellular *H. pylori* inside the new generations of yeast cells was also examined by light microscopy and Live/Dead BacLight staining method.
Methods: G1 yeast was cultivated in a 100-µL medium containing yeast extract and fetal bovine Serum. After 12 hours incubation at 37°C, FITC-conjugated IgY-Hp was added. After 3 hours, 10 µL of yeast suspension was smeared on a glass slide, air-dried and examined by the fluorescent microscopy. Wet mounts of yeast culture and Live/Dead BacLight stained preparations were examined by light and fluorescent microscopy, respectively. Photographs were taken from the fast-moving *H. pylori* inside the yeasts vacuole.
Results: Fluorescent microscopy observations showed that FITC-conjugated IgY-Hp could enter the yeast cells and specifically react with *H. pylori*, revealing the bacterial localization inside the yeast vacuole. Photographs taken from light and fluorescent microscopy showed fast-moving *H. pylori* cells in the vacuole of mother as well as daughter yeast cells. The intravacuolar *H. pylori* cells stained green, showing their viability.
Conclusion: Intracellular life of prokaryotes inside eukaryotes has been described as an evolutionary phenomenon with a great impact on the bacterial persistence against environmental stresses. In this study *H. pylori* was localized inside the Candida yeast vacuole by FITC-conjugated IgY-Hp. The intracellular bacteria were viable and existed in the vacuole of next generations of yeast cells. It appears that *H. pylori* is well-equipped to establish within the vacuole of eukaryotic cells where it is protected against the stressful conditions, including antibacterial therapy. The intracellular association of *H. pylori* with yeast appears to be so intimate that bacteria are transmitted to the next generations of yeast cells.

Abstract no.: P04.14

LEARNING FROM ONE ANOTHER: THE DISSEMINATION OF MICROBIOLOGY RESEARCH RESULTS IN INDIGENOUS ARCTIC COMMUNITIES THROUGH A JOINT COMMUNITY-UNIVERSITY KNOWLEDGE EXCHANGE PROJECT

A. Colquhoun,* S. Carraher,† M. Keelan,* B. L. Koe,‡ P. D. Edwards,‡ K. J. Goodman* and CANHelp Working Group
*University of Alberta, Edmonton, AB, Canada; †McMaster University, Hamilton, ON, Canada; ‡Aklavik resident, Aklavik, NT, Canada

To address community concerns about a high frequency of *Helicobacter pylori* in northern Canadian Indigenous communities, a community-driven project brings together community members, researchers, and healthcare providers. An important element of this work is the exchange of knowledge between community members and researchers to support the meaningful movement of knowledge generated through research into implementation by users such as community members and healthcare providers. In one participating community (Aklavik, Northwest Territories, Canada), a knowledge exchange project was developed by community representatives and researchers. As part of this initiative, researchers traveled to Aklavik to meet with community members, share research results, and learn about life in the community. While there, through the guidance of community members, they recruited two youth to travel to Edmonton, Alberta to learn about laboratory and other research components conducted at the University of Alberta. In Edmonton, the two recruited community members applied microbiology methods used to study the antimicrobial susceptibility and genetic characteristics of the *H. pylori* bacteria, and learned how to interpret the data. They also observed and participated in work conducted by public health researchers and gastroenterologists. Upon returning to Aklavik, they presented what they had learned to other members of the community, including high school science students. They also attended a national scientific conference where they shared their experiences with various researchers. This knowledge exchange initiative permitted the dissemination of research results in a meaningful, culturally appropriate way to community members, and informed future collaborative research methodologies and knowledge dissemination strategies.

Abstract no.: P04.15

COMMUNITY-DRIVEN RESEARCH IN NORTHERN CANADA: LOCAL VISUAL REPRESENTATIONS OF A HEALTH RESEARCH PROJECT ON *HELICOBACTER PYLORI*

A. Colquhoun,* S. Carraher,† R. Papik,‡ M. Moses,§ J. Edwards,¶ R. Munday,** M. Keelan,* S. Veldhuyzen van Zanten,* K. J. Goodman* and CANHelp Working Group
*University of Alberta, Edmonton, AB, Canada; †McMaster University, Hamilton, ON, Canada; ‡Aklavik resident, Aklavik, NT, Canada; §Old Crow resident, Old Crow, YT, Canada; ¶Fort McPherson resident, Fort McPherson, NT, Canada; **Susie Husky Health Centre, Aklavik, NT, Canada

Northern Canadian Aboriginal populations have a disproportionately high frequency of *Helicobacter pylori* infection and associated diseases. To address concerns, a multidisciplinary community-driven project has been established. The

overall aims of the program are to describe disease burden and risk factors associated with *H. pylori* infection, and to identify effective public health and knowledge translation strategies to reduce associated health risks.
Research implementation occurs through guidance from community-specific planning committees comprising various local representatives and research staff. These teams work together to achieve community-specific goals and to uphold the integrity of the research. In three participating communities, community members developed a visual symbol, or logo, representing the community project. In each case, through community guidance, contests were held for "the best" *H. pylori* project logo.
This initiative opened discussions about the project, the bacterium and its health effects, and resulted in dozens of submissions from various members of each community. The "winning" design was selected through a process chosen by community representatives; a graphically designed version of this drawing was created. Each of the three graphically designed images is now used to represent the respective community project. We will describe, in the artist's own words, each of the official community project logos and share dozens of drawn submissions.
These images capture the imagination and innovation of community members, visually conceptualizing the community-based research and the bacterium from their perspectives. These figures also promote and represent a strong community-researcher partnership where questions are posed and solutions to health problems are discovered together.

Abstract no.: P04.16

ERADICATION THERAPY FOR *HELICOBACTER PYLORI* IN GASTRODUODENAL ULCER

E. Montes de Oca Megías,* G. Noa Pedroso,† B. Gutiérrez Carrillo,‡ O. Seijas Cabrera,* E. García Jordá* and F. Pérez Triana*
*Centro Nacional de Cirugía de Mínimo Acceso, La Habana, Cuba; †Hermanos Ameijeiras Hospital, La Habana, Cuba; ‡Instituto Nacional de Oncología y Radiología, La Habana, Cuba

Introduction: *Helicobacter pylori* Infection affect half of the World population. The discovery of this bacterium revolutionized the etiology and treatment in ulcer disease and it is considered their principal etiological agent. A successful eradication therapy may contribute to the prevention of gastric cancer development.
Objective: To describe the results of eradication therapy to *Helicobacter pylori* in gastroduodenal ulcer.
Methods: This observational study was realized in two medicals Center of Yaracuy, Venezuela during June 2007–December 2009. The sample was constituted by 166 patients with ulcer (65 gastric and 101 duodenal) with *Helicobacter pylori,* diagnosed by upper digestive endoscopy and biopsy. Standard triple therapy (omeprazole, amoxicillin and metronidazole) were indicated for 14 days and 8 weeks later patients were evaluated by upper digestive endoscopy and biopsy. The SPSS program was performed to analyze the information.
Results: Male (63.1%) and 50–59 years old patients (36.9%) were predominated in gastric ulcer while female (57.4%) and 40–49 years old patients (29.7%) were most frequent in duodenal ulcer. 79.2% of the patients completed the treatment for 14 days and 20.8% among 9–13 days. After the eradication therapy, 90.8% of gastric ulcers and 89.1% of duodenal ulcer eliminated the infection; this persisted in 17 patients (10.2%) and was eradicated with rescue therapy (omeprazole, bismuth, tetracycline and metronidazole) for 14 days. The metallic taste (7.2%), epigastric pain (5.4%) and diarrhea (3.01%) were the most reported adverse effects.
Conclusions: High eradication rates of *Helicobacter pylori* were found after therapy with good adherence and few adverse effects.

Abstract no.: P04.17

THE PREVALENCE OF *HELICOBACTER PYLORI* – INFECTION IN KAZAN (REPUBLIC OF TATARSTAN)

G. Isaeva
Center of Hygiene and Epidemiology in the Republic of Tatarstan, Kazan, Russian Federation

Aim: Analysis of the prevalence of *H. pylori* – infection in Kazan.
Materials and Methods: Two hundred and thirty-nine adults and 68 children with gastropeptyc complaints were examined. The breath test ("AMA", St. Petersburg) for the detection of *H. pylori* by means of the determination of the exhaled air's composition were carried out among children before the fibrogastroduodenoscopie. Detection of *H. pylori* in the biopsy of gastric mucosa was carried out by means of the cytological method using cationic blue (basic) stain. A serological study of 97 inhabitants without gastropeptyc complaints at the age from 18 to 60 years was carried out for the detection of specific antibodies to *H. pylori* (IgG, IgM, IgA) using immunochromatographic rapid method by means of the test-system Hexagon *H. pylori* (Human GmbH).
Results: Among adults, the frequency of infection was varied from 79.6 ± 5.68 up to 100% (results are presented in the table). Minimal rate of infection was detected among children of younger age group (from 7 to 10 years old), it was 78.94 ± 9.4%. The rate of infection among the teenagers (11–14, 15–17) was 88.23 ± 7.8% and 93.75 ± 4.3% appropriately, what is more it was nearly as high as among adults. *H. pylori*- infection among adults and children was detected in 92.5 ± 1.5% cases. Seroprevalence study among the inhabitants of Kazan without gastropeptyc complaints was revealed antibodies against *H. pylori* in 62% cases.
Conclusion: Revealed high rate of *H. pylori* infection among children and adult population of Kazan city shows a high risk of gastroduodenal oncological diseases's development for this population.

Table 1 Prevalence of *H. pylori* infection among adults

Age: 18–29	30–39	40–49	>50
57 patients	58 patients	49 patients	75 patients
H. pylori + 55 patients (96 ± 2.6%)	*H. pylori* + 55 patients (94.8 ± 2.9%)	*H. pylori* + 39 patients (79.6 ± 5.6%)	*H. pylori* + 75 patients (100%)

Abstract no.: P04.18

SEROPREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN LARGE SERIES OF PATIENTS IN AN URBAN AREA OF SAUDI ARABIA

I. Alshunaibir
King Saud University, Riyadh, Saudi Arabia

Background/Aims: Despite that *Helicobacter pylori* is a serious pathogenic bacteria few studies focusing on the epidemiology of the infection caused by this nasty bacteria in Saudi Arabia. This is an urban based study which was done to determine and specified the prevalence of *H. pylori* infection among outpatients suffering from gastrointestinal symptoms in the capital of Saudi Arabia.
Methods: Enzyme-linked immunosorbent assay (ELISA) was used for this study with more than 5700 samples collected from the outpatients suffering from gastrointestinal symptoms with age ranging between 2 and 82 years.
Results: *Helicobacter pylori* seroprevalence was 67% increasing with age. High prevalence of *Helicobacter pylori* infection in female than male. The infection was more common in patients suffering from epigastric pain.
Conclusions: The seroprevalence percentage was higher in female than male in the capital of Saudi Arabia which is Riyadh.

P05 Inflammation and host response

Abstract no.: P05.01

POLYMORPHISM STUDY OF TOLL-LIKE RECEPTORS GENES 2, 4, 5 AND 9 AND ITS ASSOCIATION WITH DISEASES CAUSED BY *HELICOBACTER PYLORI* IN TWO LATINAMERICAN POPULATIONS

P. Hernández,* M. Pérez,* L. Flores,† E. Lazcano,† J. Torres* and M. Maldonado‡
*Instituto Mexicano del Seguro Social, México, Mexico; †Instituto Nacional de Salud Pública, México, Mexico; ‡Hospital Infantil de México "Federico Gómez", México, Mexico

Helicobacter pylori (Hp) is a Gram negative bacillus that infects 80% of the world population and is associated with gastroduodenal diseases. However, only 30% of the infected population develops pathology. Toll-like receptors (TLRs) are receptors of the innate immune system that recognize *Hp* and activate signals that activate inflammatory genes. Polymorphisms D299G and T399I in TLR4, R753Q in TLR2 and Arg392stop in TLR5 have been associated with an increased risk of developing infections. The polymorphism in the promoter region of TLR9 gene −1237T/C has been associated with autoimmune diseases and gastroduodenal diseases. Because these studies have been conducted in Caucasians, the aim was to evaluate the presence of these polymorphisms in two Latin American populations and their association with pathologies caused by *Hp*. Real-time PCR was used to determine allelic discrimination between the polymorphisms above mentioned in 495 samples from a Paraguayan and Colombian population. Diagnosis of patients were: non-atrophic gastritis, atrophic gastritis, metaplasia, gastric cancer and duodenal ulcer; the control group was non-atrophic gastritis. We found that neither of the two populations studied had statistically significant differences in the frequency of polymorphisms in TLRs 2, 4 and 5 and nor had association with the pathologies studied. However, we observed an increase in the frequency of the TLR9 −1237T/C polymorphism in the Paraguayan population with a statistically significant difference ($p = 0.04$) in the group of duodenal ulcer with an OR of 3.7. These results suggest that the −1237T/C polymorphism in TLR9 is a risk factor for developing duodenal ulcer in the Paraguayan population and would require a larger number of samples to conclude whether such polymorphism is similarly important in Colombians.

Abstract no.: P05.02

IL-1B-511 ALLELE T AND IL-1RN-L/L PLAY A PATHOLOGICAL ROLE IN *HELICOBACTER PYLORI* (*H. PYLORI*) DISEASE OUTCOME IN THE AFRICAN POPULATION

A. N. Kimang'a
Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Background: Many of the pathogenic effects of *Helicobacter pylori* infection are related to chronic active inflammation, which is controlled and maintained by the complex interplay of pro-inflammatory and anti-inflammatory mediators. Pro-inflammatory genetic polymorphisms tend to increase the risk of development of gastric cancer. In Africa, the data are scarce regarding the effects of these polymorphisms on gastric pathology. The objective of this study is therefore to investigate the pro-inflammatory genetic polymorphisms and their role in *H. pylori*-related gastric disorders in a select African population.
Methods: This cross-sectional prospective study recruited 696 adult subjects with a history of uninvestigated dyspepsia. The *H. pylori* status was determined by tissue Giemsa staining, Rapid Urease Test (RUT), *H. pylori* stool antigen test (HpSAT), and PCR using the 16s-rRNA gene. The polymorphisms in IL-1B (511 C/T), TNF-A (_308 G/A) and IL-1RN were assessed by the PCR-restricted fragment length polymorphism (RFLP).
Results: *H. pylori* was significantly associated with gastric pathologies investigated ($p = 0.0000$). Heterozygous allele TC of IL-1 β −511 was significantly associated with *H. pylori* infection ($p = 0.003815$). Similarly, allele IL-1 RN*2/2 and allele IL-1 RN-L/L were associated with *H. pylori* infection ($p = 0.0025$ and $p = 0.0203$). Allele T of IL-1 β −511 and IL-1 RN-L/L are more frequent in *H. pylori* associated gastric pathologies in this series.
Conclusion: Allele T of IL-1 β −511 and long allele IL-1 RN-L/L play a role in *H. pylori* disease in this population.

Abstract no.: P05.03

ISOPROPRANOL EXTRACTS OF *ARTEMISIA* IMPOSED ANTI-OXIDATIVE AND ANTI-INFLAMMATORY ACTIVITIES AS WELL AS CYTOPROTECTIVE ACTIONS AGAINST *HELICOBACTER PYLORI* INFECTION

K. Hahm, J. Park, Y. Han, S. Hong, W. Kim and E. Kim
CHA University, Seongnam, Korea

Artemisia asiatica has been used to treat inflammation, gynecological disorder, and microbial infection as traditional folk medicine and its ethanol extracts had been prescribed for the treatment of gastritis and peptic ulcer disease during last 10 years in Korea and some Asian countries. The aim of this study was to compare the antioxidant and anti-inflammatory effects as well as cytoprotective action between current ethanol extracts and novel isopropanol extracts of Artemisia. The isopropanol extracts of Artemisia showed the higher DPPH radical scavenging activity and lesser LPS-induced ROS productions in RGM1 cells comparing with ethanol extracts. Isopropanol extracts significantly increased HO-1 proteins expression through increased nuclear translocation of Nrf2 transcription factor than ethanol extracts and the expression level of an HSP70 protein was only increased with isopropanol extracts. Isopropanol extracts showed a concentration-dependent inhibition of LPS-induced inflammatory mediators including COX-2 and iNOS even at a lower concentration. Cytokine protein array revealed that anti-inflammatory mechanisms of isopropanol extracts were based on attenuated cytokines and chemokines. Conclusively, isopropanol extracts of Artemisia had the more potent antioxidant and anti-inflammatory activity than ethanol extracts, supporting the anticipation that isopropranol extracts might impose higher clinical efficacy than current ethanol extracts, leading to anticipation of higher efficacy against gastritis treatment in clinic.

Abstract no.: P05.04

GHRELIN AS A SEROLOGIC MARKER FOR ATROPHIC GASTRITIS: COMPARING WITH PEPSINOGEN PROFILE

J. Lee, H. Song, H. Lim, J. Ahn, K. Choi, D. Kim, Y. Park, K. Choi, G. Lee, S. Jang, H. Jung and J. Kim
Asan Medical Center, Seoul, Korea

Background: Chronic atrophy gastritis (CAG) is considered as precancerous in the stomach. This study was aimed to evaluate serum ghrelin as a serologic marker as well as serum pepsinogen profile in *Helicobacter pylori* CAG patients.
Material and Methods: Data were analyzed from 86 endoscopic mucosal resection patients who were *H. pylori* infected. Three paired biopsies for histology were taken at antrum, corpus lesser, and greater curve (CGC). Total serum ghrelin, pepsinogen, gastrin-17, leptin levels were analyzed.
Results: Total ghrelin level correlated with pepsinogen I level ($R^2 = 0.15$, $p < 0.001$). Total ghrelin level was lower in the patients with CGC atrophy than those without CGC atrophy (189.8 ± 57.3 vs 219.5 ± 52.0, $p = 0.026$). Serologic atrophy determined by pepsinogen I < 60 μg/L and pepsinogen I/II < 3.5 was estimated in 40.7%. Total ghrelin level of serologic atrophy patients was lower than that of non-serologic atrophy patients (193.8 vs 222.7 pg/mL, $p = 0.012$). Areas under the receiver-operating characteristic curve of ghrelin for discriminating presence of corpus greater curve atrophy was 0.75 and did not differ from that of pepsinogen I and pepsinogen I/II ratio ($p = 0.383$ and $p = 0.877$ respectively).
Conclusions: Total ghrelin is useful biomarker for the prediction of CGC atrophy and comparable to serum pepsinogen profile.

Abstract no.: P05.05

DIFFERENT IL-8 EXPRESSION IN *HELICOBACTER PYLORI* INFECTED GASTRIC MUCOSA BETWEEN BHUTAN AND THE DOMINICAN REPUBLIC

H. Nagashima,*,† S. Iwatani,*,† M. Cruz,‡ J. A. Jimenez Abreu,§ V. Mahachai,¶ R. Vilaichone,** T. Uchida,* S. Shiota,* L. Tshering,†† T. Fujioka,‡‡ D. Y. Graham† and Y. Yamaoka*,†
*Oita University Faculty of Medicine, Yufu, Japan; †Baylor College of Medicine, Houston, TX, USA; ‡Universidad Autonoma de Santo Domingo, Santo Domingo, Dominican Republic; §Dr. Luís E. Aybar Health and Hygiene City, Santo Domingo, Dominican Republic; ¶Bangkok Medical Center, Bangkok, Thailand; **Thammasat University Hospital, Pathumthani, Thailand; ††Jigme Dorji Wangchuk National Referral Hospital, Thimphu, Bhutan; ‡‡Oita University, Oita, Japan

Background: Relations between several cytokines and *Helicobacter pylori* infection have been reported; however most of these were limited to one region and

no previous data are available from Bhutan (Asian) and the Dominican Republic (DR) (Western) where even little data are available for the prevalence of *H. pylori* infection.

Methods: Mucosal cytokines were measured using real-time PCR. *H. pylori* status was assessed by the combination of rapid urease test, culture and histology. Gastritis was scored using the updated Sydney System.

Results: One hundred and forty patients from Bhutan and 155 from DR were included. *H. pylori*-positive ratio was 65% in Bhutan and 58% in DR. The severity of gastritis was significantly higher in infected person from Bhutan compared to those from DR. IL-8 expression by *H. pylori* infection adjusted by beta-actin ($\times 10^4$) was 5.51 in Bhutan versus 3.03 in DR ($p < 0.001$); whereas IL-10 expression was similar. In DR, expression level of IL-8 was significantly higher in CagA-positive than CagA-negative cases (3.27 vs 2.00) ($p = 0.04$); whereas all cases were CagA-positive in Bhutan. We compared the expression of IL-8 between countries for the same histologic grades and IL-8 was significant higher in Bhutan than in DR for low grade.

Conclusions: This is the first study, to our knowledge, to measure gastric mucosal cytokines levels in Bhutan and DR. The significant difference of IL-8 expression between Bhutan and DR might be due to the differences in *H. pylori* (e.g., CagA type) or differences in host- *H. pylori*-environmental interactions.

Abstract no.: P05.06

VALUTATION OF HUMAN β-DEFENSIN 2 AND 3 IN SERA OF *HELICOBACTER PYLORI* INFECTED OR NON-INFECTED PATIENTS

G. Donnarumma,* T. Fasciana,† A. Fusco,* C. Calà,† C. Bonura,† G. Scarpulla‡ and A. Giammanco†

*Section of Microbiology and Clinical Microbiology, Department of Experimental Medicine, Second University of Naples, Naples, Italy; †Section of Microbiology "A. Chiarini", Department of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro" University of Palermo, Palermo, Italy; ‡Gastroenterology Division, M. Raimondi Hospital, San Cataldo (CL), Italy

In recent years, anti-microbial peptides have emerged as a critical component of host innate defense. These peptides are gene-encoded natural antibiotics expressed by immune and non-immune cell types including epithelia. Human beta defensins are disulphide linked, low molecular weight cationic peptides that are known to be a major components of innate immune defense mechanisms at mucosal surfaces. Their expression level differs between those that are constitutively expressed and those that are induced upon challenge with inflammatory or pathogen-derived stimuli. To date six members of the human-β-defensins (hBD1-6) family have been identified. *Helicobacter pylori* (*H. pylori*), a pathogenic but noninvasive Gram-negative bacterium, appears to be unique in its adaptation to long-term survival in the acidic environment of human stomach. hBD3 as well hBD2 is known to be induced in gastric epithelial cells in relation to *H. pylori* infection and may be involved in the pathogenesis of *H. pylori*-associated gastritis, possibly through its function as immune and inflammatory mediator. In our study we detected by ELISA the HBD-2 and 3 in a collection of 45 sera from patients selected as follows: 12 with an active infection, 11 uninfected, 11 with chronic infection and 11 with eradicated infection.

Abstract no.: P05.07

THE CONTRIBUTION OF NEUTROPHILS AT DEVELOPMENT OF THE *HELICOBACTER PYLORI*-INFECTION

Y. V. Saranchina, E. S. Ageeva and O. V. Shtygasheva

Khakas State University, Abakan, Russian Federation

Aim of investigation was phagocytosis activity of neutrophils at patients with infection *Helicobacter pylori* (HP). The objects of the research were neutrophils in peripheral blood from 28 patients with diagnosis atrophic chronic gastritis; 30 patients with diagnosis HP-associated chronic gastritis without atrophic and 10 HP-associated gastric cancer.

Methods: Endoscopy, gastric biopsy samples were investigated according to Sydney classification. Estimated by definition of a index of phagocytosis (PI) and number of phagocytosis (PN) of neutrophils.

Results: PI at the patients with chronic gastritis with and without atrophic consist 72.4% and 74.0%, respective. PI of neutrophils at patients with gastric cancer was 77.4%. In the group of patients with chronic gastritis without atrophic the PN was 13.6 absolute units (a.u.), with atrophic – 9.3 a.u. and at gastric cancer the PN consist 9.0 a.u.

Conclusions: The chronic infection of HP leads to decrease at the absorbing ability but preservation of functional activity of neutrophils – cell type involved in the innate immune response.

P06 Pathology and Pathophysiology

Abstract no.: P06.01

THE ASSOCIATION BETWEEN *H. PYLORI* INFECTION AND GALL STONE DISEASE IN BASRA

S. M. Imran,* N. Ajeel† and Q. Z. Mohammed*
*Alsadder Teaching Hospital, Basra, Iraq; †Basra Medical College, Basra, Iraq

Background: The old axiom, that a typical gall stone sufferer is a fat, fertile, female of 50, is only partially true, as the disease has been found in women soon after their first delivery and also in underweight and thin people. Recent studies showed that bacterial population includes *Helicobacter pylori (H. pylori*) could have a possible role in gall stone formation. *H. pylori* infection is most widely spread among the developing region due to poor standard of public health.
Aim: To determine the association of *H. pylori* infection and gallstone in Basra.
Material and Method: The study was conducted from November 2007 to May 2008. The study sample involved 325 patients referred to the endoscopy unit in AL-Sadder Teaching hospital for dyspeptic symptoms. Only patients with positive endoscopic findings like (gastritis, gastric ulcer) were included in the study. During the procedure antral biopsy was immediately immersed in a urease solution for detection of *H. pylori*. Upper abdominal ultrasonography examination was performed on each patient underwent endoscopy for detection of hidden gall stone.
Statistical analysis used SPSS version 15 and chi square test. The p-value < 0.05 was considered significant.
Result: The prevalence of gall stones in the *H. pylori* positive patients was 50.8%; this is compared to 37.5% for *H. pylori* negative patients. The association between *H. pylori* infection and gall stone disease was statistically significant ($p < 0.05$).
Conclusions: The prevalence of gall stones among *H. pylori* positive patients was much higher than that among *H. pylori* negative patients. These findings may suggest the presence of an association between *H. pylori* infection and gall stone disease.

Abstract no.: P06.02

DEVELOPMENT OF A MURINE MODEL OF CLINICAL ISOLATES *HELICOBACTER PYLORI* INFECTION

A. J. Flores-Palacios, Y. LópezVidal, G. Chavéz-Gris, A. Verdugo-Rodríguez and G. Castillo-Rojas
Universidad Nacional Autónoma de Mexico, Mexico City, Mexico

Helicobacter pylori infects more than 50% of the population causing chronic gastritis, peptic ulcer and gastric adenocarcinoma. The latter is considered the second leading cause of death worldwide. Several host and bacterial factors have been implicated in gastric cancer development. Such as virulence proteins like CagA and VacA have been related to gastric disease but they are not always necessary to be present in gastric cancer development. A better way to identify biomarkers involved in gastric tissue damage is the use of animal models to determine the association between the expression of mRNA of *H. pylori* and histopathological damage. So we are developing a murine model of *H. pylori* infection with clinical isolates to asses this goal. We inoculated 1 × 107 CFU of three *H. pylori* strains isolated from gastric biopsies of patients with chronic gastritis, bleeding peptic ulcer and gastric cancer in groups of Balb/C mice orogastrically. Colonization of gastric mucosa by *H. pylori* was assed by immunohistochemistry and histological damage by HE staining of mice gastric biopsies at different times up to 6 months post infection. The presence of *H. pylori* by immunohistochemistry was determined at 1 day and 6 months postinfection in the mice infected with the *H. pylori* gastric cancer strain. Histopathological damage reveals a constant inflammation of the mucosa during the follow up of the study. To our knowledge this is the first study to evaluate a gastric cancer isolate in a murine model. Currently we are working to improove and characterize our murine model.

Abstract no.: P06.03

RECOVERY OF GASTRIC FUNCTION AFTER *HELICOBACTER PYLORI* ERADICATION AND ACETIUM ADMINISTRATION: A 6 YEARS STUDY IN ATROPHIC GASTRITIS SUBJECTS

F. Di Mario,* M. Rugge,† F. Ferrara,* T. Slongo,* N. Dal Bo',* G. Guarnieri,* R. Marcello,* H. Heras Salvat,* R. Rigoli,‡ A. Dei Tos§ and C. Scarpignato¶
*Gastroenterology Unit, Department of Specialist Medicine, Treviso General Hospital, Treviso, Italy; †Surgical Pathology and Cytopathology Unit, Department of Diagnostic Medical Sciences and Special Therapies, University of Padova, Padova, Italy; ‡Department of Clinical Pathology, Treviso General Hospital, Treviso, Italy; §Pathology Unit, Department of Clinical Pathology, Treviso General Hospital, Treviso, Italy; ¶Department of Clinical Sciences, Laboratory of Clinical Pharmacology, University of Parma, Parma, Italy

Introduction: The relationship between *H. pylori* eradication and atrophy in the gastric mucosa has not yet been fully defined. Athough studies report a partial restoration of serum Pepsinogen I levels after eradication. Recently a new compound (L-cystein Acetium, Biohit, Finland) has been proposed for prevention of gastric carcinogenesis in patients with atrophic gastritis, by reducing acetaldehyde production.
Aims and Methods: To assess alteration in gastric function after H.p. eradication on moderate-severe body atrophic gastritis. Seventy-four dyspeptic patients, selected from 738 consecutive H.p. positive patients, with histological features of atrophic gastritis and sPGI < 25 μg/L, underwent an upper gastrointestinal endoscopy with gastric biopsies and sPGI and sG17 determination. Patients underwent eradication therapy. Serum sPGI and sG17 were measured after 6 months, 1, 2, 3, 5 and 6 years after eradication.
Results: Mean sPGI levels at baseline were 13.4 μg/L (range 1.5–24 μg/L). Six months after eradication, mean sPGI levels significantly increase to 16.6 μg/L ($p = 0.05$). At the completion of the study, 6 years after eradication, sPGI levels increased to 27.3 μg/L ($p = 0.01$). Conversely, the sG17 dropped out from 84.8 at baseline to 67.6 pmol/L after the 6 years follow up period ($p < 0.01$). Twenty-one patients (16 female, mean age 44) out of the 74 pts experienced a 3 month period of treatment with Acetium 100 mg at dosage of three capsules daily before eating. The mean levels of sPGI increases from 7.9 μg/L at baseline to 11.4 after 90 days of Acetium intake as well as sG17 dropped out from 30.3 to 25.5 pmol/L.
Conclusion: After H.p. eradication subjects with body atrophic gastritis showed improvement of physiological gastric function. L-cysteine seem to be usefull in restoring gastric secretion in subjects with chronic atrophic gastritis, but other studies are required to confirm and clarify this finding.

Abstract no.: P06.04

THE PRESENCE OF *HELICOBACTER PYLORI* DOES NOT CHANGE THE HISTOLOGICAL INFLAMMATORY PROFILE IN ADVANCED ATROPHIC BODY GASTRITIS

A. J. A. Barbosa, R. D. Pereira and C. S. C. Silva
Faculdade de Medicina, Belo Horizonte, Brazil

The etiopathogenesis of inflammatory reaction in atrophic body gastritis (ABG) is still a matter of debate. The present work aims to analyze the inflammatory changes of the gastric mucosa in advanced cases of ABG associated and not associated to Hp infection. Between 2007 and 2009 we surveyed 2564 consecutive cases of chronic gastritis as the main pathological condition of the gastric mucosa at a general hospital (Belo Horizonte, Brazil). We confirmed 196 (7.6%) cases presenting severe ABG and Hp infection was found in 9 (4.6%) of them. The following parameters were analyzed: intestinal metaplasia (IM); lymphoid follicles (LF); slight (+), moderate (++) or intense (+++) infiltration of polymorph nuclear neutrophils (PMN), eosinophils (EOS), and mononuclear (MON) cells. The results found in the nine Hp-positive patients were compared with the results obtained from 18 randomly selected Hp-negative subjects. Gastric corpus in Hp-negative: IM present in 94% and LF in 29%; MON+ (70.6%), MON++ (29.4%); PMN+ (70.6%), PMN++ (29.4%); EOS+ (23.5%), EOS++ (76.5%). Gastric corpus in Hp-positive: IM present in 100% and LF in 67%; MON+ (55.6%), MON++ (44.4%); PMN+ (77.8%), PMN++ (22.2%); EOS + (22.2%), EOS++ (77.8%). Gastric antropyloric mucosa was preserved in all patients, frequently showing just few MON cells infiltration, LF present in 22.2% of Hp-positive patients, and absence of IM. In conclusion, it seems that Hp do not elicit PMN response in severe ABG. In addition, the antropyloric mucosa in ABG appears to be resistant to the pathogenic role of this microorganism.

Abstract no.: P06.05

HELICOBACTER PYLORI ASSOCIATED WITH *GIARDIA LAMBLIA*

G. Isaeva
Center of hygiene and epidemiology in Republique of Tatarstan, Kazan, Russian Federation

Aim: The study of association between *H. pylori* infection and *G. lamblia*.
Materials and Methods: The study involved 160 patients (M – 50, F – 110) with chronic cholecystitis associated with chronic gastroduodenitis. The mean age was 39.9 years. Obtaining biopsy specimens of gastric mucosa and bile samples allowed to compare the microbial picture and the morphological structure of gastric mucosa in the same patient, to identify patterns of colonization of the stomach, duodenum and gall bladder by *H. pylori* and *G. lamblia*. The smears prepared from gastric mucosa and the bile (portions A, B, C) were stained by cationic blue basic for cytological examination. The control group which included 14 patients without the gastroduodenal pathology was observed using immonochromatographic tests for detection of antigens *H. pylori* and G. lamblia in feces ("HelicoStick", "Giardia Test", Novamed Ltd., Israel).
Results: By cytological examination *G. lamblia* was detected in the gall bladder in 47.5% cases, in the stomach – in 29% cases. The frequency of *H. pylori* detection in gastric mucosa amounted to 98% cases, in duodenum – 93%, in the gall bladder – 54%. Morphological changes of gastric mucosa in the form of lymphoid infiltration detected mainly in the mixed infection *H. pylori* and *G. lamblia*. In control group all specimens were negative for *H. pylori* and *G. lamblia*.
Conclusion: The strong association between the detection of *H. pylori* and *G. lamblia* in the stomach was found.

Abstract no.: P06.06

GASTRIC CANCER RISK ESTIMATE IN PATIENTS WITH CHRONIC GASTRITIS ASSOCIATED WITH *HELICOBACTER PYLORI* INFECTION IN A CLINICAL SETTING

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

Background: The severity of chronic gastritis associated with *Helicobacter pylori* infection (CGA*Hp*I) in the antrum and/or corpus could play a role in evaluating the potential risk for patients with CGA*Hp*I to develop gastric cancer.
Aims: To estimate the risk for gastric cancer in a clinical setting, according to histopathologic criteria, in patients with CGA*Hp*I, by applying the gastric cancer risk index (GCRI) proposed by Meining et al.
Methods: Histopathologic study of the gastric biopsies (corpus-antrum) from 111 adult patients that underwent gastroesophageal duodenoscopy was carried out, and the GCRI was applied in patients presenting with CGA*Hp*I.
Results: Forty-five percent of the patients with CGA*Hp*I had *pangastritis* (23%) and *corpus-predominant gastritis* (22%). GCRI was high in 20.7% (16 patients) and four of them (25%) presented with metaplastic atrophy (all of them with *pangastritis* and *corpus-predominant gastritis*).
Conclusions: The estimated gastric cancer risk in patients with CGA*Hp*I in the clinical setting studied was relatively low. Five percent of the patients had a histopathologic phenotype associated with an elevated risk for developing gastric cancer (metaplastic atrophic pangastritis and metaplastic atrophic chronic corpus-predominant gastritis). It could be advantageous to combine the histopathologic phenotype of CGA*Hp*I, the GCRI, and the demographic and geographic characteristics and presence of *Helicobacter pylori*, in order to obtain a more precise individual assessment of the patient risk for gastric cancer. Finally, a clinical and pathologic gastric cancer risk index could be designed for each country or geographic sub-region.

Abstract no.: P06.07

THE ASSOCIATION BETWEEN SYMPTOMS OF FUNCTIONAL DYSPEPSISA AND GASTRIC HORMONE, LEPTIN AND GHRELIN ACCORDING TO *HELICOBACTER PYLORI* INFECTION

K. Lee, O. Lee, B. Yoon, H. Lee, H. Tae, J. Kim, D. Jun, H. Choi, E. Doo, K. Yoo and Y. Ahn
Hanyang University College of Medicine, Seoul, Korea

Background and Aims: Leptin and ghrelin are known as gastric hormones to control feeding behavior by modulating gastrointestinal (GI) motility. *Helicobacter pylori* infection may affect gastric synthesis and secretion of leptin and ghrelin. The present study aimed to investigate whether leptin or ghrelin is associated with symptoms in functional dyspepsia or is affected by *Helicobacter pylori* infection.
Methods: Forty seven patients with FD (ulcer-like dyspepsia in 30 and dysmotility-like dyspepsia in 17) according to the Rome II criteria (29 females, mean age 45.2 ± 1.8 year) and 18 controls (10 females, 45.7 ± 3.4 year) were enrolled for one consecutive year. All the patients filled out a questionnaire composed of upper GI symptoms such as nausea, vomiting, abdominal discomfort, epigastric pain, epigastric sorness, and reflux. Serum levels and gastric mucosal mRNA expressions of leptin and ghrelin were measured and compared between each group by multivariate analyses.
Results: *Helicobacter pylori* infection assessed by CLO test was not different between patients with FD (27/47 [57.4%]) and controls (9/18 [50%]). Both serum and gastric mucosal levels of leptin and ghrelin were not different between patients with FD and controls, between CLO (+) and (−) patients with FD, and between CLO (+) and (−) controls. In subgroup analysis, gastric mucosal expressions of leptin were higher in patients with dysmotility-like dyspepsia than controls, regardless of *Helicobacter pylori* infection ($p = 0.029$). In patients with FD, gastric mucosal expressions of leptin also tended to be higher in those with vomiting than without ($p = 0.070$).
Conclusions: We conclude that gastric leptin is associated with certain type or symptom, but not with *Helicobacter pylori* infection in FD. Our results suggest that leptin rather than ghrelin might be a potential target to control symptoms in FD, particularly in dysmotility like dyspepsia and those with vomiting.

P07 Preneoplastic and neoplastic diseases

Abstract no.: P07.01

SPANISH FOLLOW-UP MULTICENTRIC STUDY ON PHENOTYPIC, EPIGENETIC, GENETIC AND *H. PYLORI* VIRULENCE FACTORS ASSOCIATED WITH THE PROGRESSION OF GASTRIC CANCER PRECURSOR LESIONS

C. A. González,* J. Sanz-Anquela,† O. Companioni,‡ C. Bonet,* M. Berdasco,§ C. López,¶ J. Mendoza,** E. Collantes,†† G. Ruiz,‡‡ E. Rey,§§ F. Sánchez-Ceballos,§§ E. Poves,¶¶ L. Espinosa,¶¶ M. Torres,*** B. Madrigal,*** M. Cuatrecases,††† I. Elizalde,‡‡‡ L. Bujanda,§§§ A. Cosme,§§§ Á. Ferrández,¶¶¶ G. Muñoz,**** V. Andreu,†††† M. Paules,‡‡‡‡ S. Lario,§§§§ M. Ramírez§§§§ and J. P. Gisbert**

*Catalan Institute of Oncology, Hospitalet del Llobregat, Barcelona, Spain; †Department of Pathology, Hospital "Principe de Asturias", University of Alcala de Henares, Alcalá de Henares, Madrid, Spain; ‡Unit of Nutrition, Environment and Cancer, Catalan Institute of Oncology (IDIBELL-ICO), Hospitalet del Llobregat, Barcelona, Spain; §Cancer Epigenetics Group, Cancer Epigenetics and Biology Program, Bellvitge Biomedical Research Institute, Barcelona, Spain; ¶Department of Pathology, Hospital Universitario de la Princesa, Madrid, Spain; **Department of Gastroenterology, Hospital Universitario de la Princesa, IP and CIBEREHD, Madrid, Spain; ††Department of Gastroenterology, Hospital Universitario La Paz, Madrid, Spain; ‡‡Department of Pathology, Hospital Universitario La Paz, Madrid, Spain; §§Department of Gastroenterology, Hospital Clínico San Carlos, Madrid, Spain; ¶¶Department of Gastroenterology, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain; ***Department of Pathology, Hospital Universitario Río Hortega, Valladolid, Spain; †††Department of Pathology, Hospital Universitari Clínic de Barcelona, Barcelona, Spain; ‡‡‡Department of Gastroenterology, Hospital Universitari Clínic de Barcelona, Barcelona, Spain; §§§Department of Gastroenterology, Hospital Universitario Donostia, Donostia, Spain; ¶¶¶Department of Gastroenterology, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain; ****Department of Pathology, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain; ††††Department of Gastroenterology, Hospital de Viladecans, Viladecans, Barcelona, Spain; ‡‡‡‡Department of Pathology, Hospital Universitari de Bellvitge – IDIBELL, Hospitalet del Llobregat, Barcelona, Spain; §§§§Departament de Medicina, Digestive Diseases Service, Hospital de Sabadell, Institut Universitari Parc Taulí, Universitat Autònoma de Barcelona, Sabadell, Spain

Background: Surveillance of patients at risk of progression from precursor lesions (PL) to gastric cancer (GC) is recommended but more research is needed to identify markers of progression.
Aims: (1) To evaluate the risk of progression to GC in patients with PL (atrophic gastritis, complete/incomplete intestinal metaplasia); (2) To assess the effect of virulence factors of *H. pylori* infection (cagA and s1/m1 vagA alleles), the effect of polimorphisms of candidate genes, the effect of epigenetic variants, and (3) To elaborate an score of different markers of risk to allow identification of patients with high risk of progression to GC.
Design: A follow up study is ongoing including about 900 patients of 25–69 years, diagnosed with PL between 1995 and 2004, in nine participating hospitals. Endoscopy and biopsy is being repeated during 2011–2013, and fresh gastric mucosa is being collected. A sample of saliva and a questionnaire with medical information and habits is also being collected. SNPs of candidate genes will be evaluated in DNA from saliva. Genotyping of virulent factors by PCR is being analysed from DNA of paraffin blocks. Patterns of methylation is being analysed by the Infinium 450 K methylation arrays which allows to interrogate more than 485 000 CpGs of the whole genome and validation of candidates genes is tested by pyrosequencing. Occurrence of all GC cases is being identified during follow-up. Incidence of GC and progression/regression of PL according to different factors will be analysed by multiple regression models.
Results: According to a preliminary analysis, 40.3% of patients had chronic atrophic gastritis, 37.1% complete intestinal metaplasia, 13.2% incomplete intestinal metaplasia and 9.4% dysplasia. The mean time of follow-up was 12.8 years (SD 1.8). More results will be available in September and will be included in the poster.

Abstract no.: P07.02

PROTON PUMP INHIBITORS AND RISK OF GASTRIC CANCER: A RETROSPECTIVE COHORT STUDY

J. Choi,* K. S. Hong,† S. J. Kang‡ and J. S. Kim‡

*Department of Internal medicine, National Medical Center, Seoul, Korea; †Seoul National University College of Medicine, Seoul, Korea; ‡Department of Internal Medicine, Seoul Nationl University Hospital, Seoul, Korea

Background: The effect of long-term PPI administration on gastric cancer is still unclear. We evaluated the association between PPI use and risk of gastric cancer in a high incidence area of *Helicobacter pylori* and gastric cancer.
Methods: All adult patients who had received a prescription for PPIs among those who visited Seoul National University Hospital from January 1, 2005 to December 31, 2009 were identified. The patients who were diagnosed with atrophic gastritis or gastric cancer before PPI administration were excluded. The patients were divided into three groups according to treatment duration (group 1: <3 month; group 2: 3 month–1 year; group 3: ≥1 year) and were followed up from the time they took the first prescription of PPIs until their last visit. Logistic regression analysis was used to calculate the relative risks (RR) and 95% CI, adjusting for covariates.
Results: Among the 23 385 patients exposed to PPIs (17 837 in group 1; 5080 in group 2; 1304 in group 3), 610 patients were diagnosed with gastric cancer during 690 800 person-years of follow-up. Of 610 patients, the 510 were excluded because they were diagnosed with gastric cancer within 1 year of the first prescription for PPIs. The incidence of active gastric cancer was 0.13 per 1000 person-years in group 1, 0.16 per 1000 person- years in group 2 and 0.19 per 1000 person-years in group 3. After adjusting for age, gender and helicobacter infection, longer PPI treatment period was not associated with increased risk of gastric cancer (RR: 1 [reference] in group 1; 1.25 [95% CI: 0.80–1.96] in group 2; 1.40 [95% CI: 0.74–2.62], $p = 0.45$).
Conclusion: Long-term PPI therapy does not seem to be associated with increased risk of gastric cancer.

Abstract no.: P07.03

GASTRIC CANCER IN THAILAND: A 15 YEARS REVIEW

R. Vilaichone*,† and V. Mahachai†,‡

*Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani, Thailand; †National Gastric Cancer and Helicobacter pylori Research Center, Bangkok, Thailand; ‡Division of Gastroenterology, Department of Medicine, Chulalongkorn University, Bangkok, Thailand

Background and Aims: Gastric cancer is the second leading course of cancer death worldwide. This study was design to evaluate the clinical, pathological features, prevalence of *H. pylori* infection in gastric cancer of Thai patients.
Patients and Methods: Clinical information, histological features and *H. pylori* status were collected from gastric cancer patients from Thammasat university hospital and Chulalongkorn university hospital during June 1996–December 2011. *H. pylori* infection was assessed by histological evaluation, serological test, culture and/or rapid urease test. Clinical information, endoscopic findings and histopathology of all patients were recorded and compared between patients with active or non-active *H. pylori* infection.
Results: Total of 100 gastric cancer patients were enrolled in this study. Common presenting symptoms were dyspepsia (70%), weight loss (69%) and anorexia (42%). Mean duration of symptoms prior to diagnosis was 98 days. Overall prevalence of *H. pylori* infection was 83% and active *H. pylori* infection was 28%. One-year and 5-year survival rates were 47% and 0%. There was no difference of mean age, sex, duration of presenting symptom, endoscopic findings and staging between active and non-active *H. pylori* infection group. Furthermore, there were no significant difference of location (proximal vs non-proximal) and histology (diffuse type vs intestinal type) of gastric cancer between active and non-active *H. pylori* infection group in this study (25% vs 30%, p-value = 0.1 and 29% vs 29%, p-value = 0.1 respectively)
Conclusion: Prevalence of *H. pylori* infection in Thai gastric cancer patients was high but active infection was low. Most of gastric cancer patients presented in advance stage and had grave prognosis. There was no significant difference of clinical information, endoscopic findings and histological type of gastric cancer between active and non-active *H. pylori* infection in Thailand.

Abstract no.: P07.04

DIFFERENTIAL PROTEOMICS OF *HELICOBACTER PYLORI* ASSOCIATED TO AUTOIMMUNE ATROPHIC GASTRITIS

O. Repetto, S. Zanussi, M. Casarotto, G. Basaglia, S. Giacomini, V. Canzonieri, R. Cannizzaro, P. De Paoli and V. De Re
National Cancer Institute, IRCCS, Aviano, Italy

Atrophic autoimmune gastritis (AAG) is a condition of chronic inflammation and atrophy of the stomach mucosa, whose development can be partially triggered by the bacterial pathogen *Helicobacter pylori* (HP). In this work, a comparative proteomic approach was used by two dimensional difference gel electrophoresis (2D DIGE) to identify differentially expressed proteins of HP strains isolated from patients with AAG, in order to identify candidate markers for strains associated with clinical outcomes of AAG pathology and HP infection. Proteome profiles of HP isolated from GC or DU were used as reference to compare proteomic levels. Proteomics analyses revealed 27 differentially expressed spots in AAG-associated HP in comparison with GC, while only nine differential spots were found for AAG-associated HP profiles compared with DU. Proteins were identified after MALDI-TOF and peptide mass fingerprinting. Some AAG-HP differential proteins were common between DU- and GC-HP (probable peroxiredoxin, ATP synthase subunit alpha, flagellin A). Our results may suggest that comparative proteomes of HP isolated from AAG and DU share more common protein expression than in regards to GC, and provide subsets of putative AAG-specific up- or down-regulated proteins, that could be proposed as putative markers of AAG-associated HP. Other comparative studies by 2D maps integrated with functional genomics of candidate proteins will undoubtedly contribute to better decipher the biology of AAG-associated HP strains.

Abstract no.: P07.05

HELICOBACTER PYLORI, BUT NOT GASTRIN ARE ASSOCIATED WITH THE DEVELOPMENT OF COLONIC NEOPLASMS

M. Selgrad, J. Bornschein, A. Kandulski, J. Weigt, T. Wex and P. Malfertheiner
Department of Gastroenterology, Hepatology and Infectious Diseases, Magdeburg, Germany

Objectives: Recent studies have suggested that *Helicobacter pylori* (*H. pylori*) constitutes a risk for the development of colonic neoplasia. Hypergastrinemia can be induced by chronic *H. pylori* infection and gastrin can act as putative promoter of colorectal carcinogenesis. Aim of the study was to assess whether *H. pylori* infection and/or increased serum gastrin levels are associated with the occurrence of colonic neoplasms.
Methods: We prospectively included 377 consecutive patients with a minimum age of 50 years that underwent colonoscopy. *H. pylori* infection status was determined by serology. Serum gastrin levels were measured in fasting state by commercially available assays.
Results: In *H. pylori* infected patients (n = 126; 33.4%), the overall occurrence of colonic neoplasms was more frequent compared to *H. pylori* negative patients (n = 251; 66.6%) (OR = 2.87, 95% CI: 1.84–4.48). *H. pylori* infection occurred more frequently in patients with hyperplastic polyps (OR = 2.28, 95% CI: 1.07–4.90) and adenomas presenting with low grade intraepithelial neoplasia (IEN) (OR = 2.25, 95% CI: 1.38–3.66). Due to the low number of patients with high grade IEN or invasive colorectal adenocarcinoma (n = 14), attributable risk for these neoplastic lesions could not be assessed. Hypergastrinemia was not associated with an increased risk for occurrence of any colonic neoplasms and there was no difference in serum gastrin levels between *H. pylori* positive (mean, 17.67 pg/mL) and *H. pylori* negative (mean, 20.21 pg/mL) patients.
Conclusions: *H. pylori* infection is associated with an increased risk for colonic neoplasm. Interestingly, serum-gastrin levels do not confer an attributable risk for the development of colorectal neoplasms.

Abstract no.: P07.06

PREDICTIVE FACTORS OF TREATMENT RESPONSE AFTER *HELICOBACTER PYLORI* ERADICATION IN LOW GRADE MALT LYMPHOMA

H. Moon, H. Jeong and J. Sung
Chungnam National University Hospital, Daejeon, Korea

Background/Aims: *Helicobacter pylori* is associated with low grade gastric MALT lymphoma in 72–98% of cases. *Helicobacter pylori* eradication induces remission in most patients with low grade gastric MALT lymphoma. However, 10–20% of low grade gastric MALT lymphomas are unresponsive to *Helicobacter pylori* eradication treatment. The aims of this study were to analyze effect of eradication of *Helicobacter pylori* on early stage low grade gastric MALT lymphoma and to define the predictive factors of lymphoma regression.
Methods: From 2002 to 2010, 53 *Helicobacter pylori* infected patients with modified Ann Arbor stage I_{E1} gastric MALT lymphoma were included in this study, their medical records were reviewed. All patients were treated by *Helicobacter pylori* eradication. Tumor response was evaluated by endoscopic and histologic findings.
Results: Forty eight patients (90%) achived complete remission after *Helicobacter pylori* eradication therapy. Seven patients (13%) including two patients relapsed needed a second-line treatment after eradication. Mean follow-up period was 30 months (range, 12–60 months). There was no significant association in age, sex, endoscopic appearance, lifestyle (smoking, drinking), co-morbidities between treatment response group and non-response group. But, The patients with distal tumor (97.7%) had a higher complete remission rate than proximal (60%) ($p = 0.002$). Also, the patients (97%) not taking medication had a higher complete remission rate than taking the drug (21%) ($p = 0.05$).
Conclusions: *Helicobacter pylori* eradication as single therapy may be appropriate for early stage gastric MALT lymphoma. The proximally located MALT lymphoma, or if the patients are taking any medication may be necessary to carefully follow-up.

Abstract no.: P07.07

HYPERMETHYLATION OF TUMOR SUPPRESSOR GENES IN CANCEROUS AND NON CANCEROUS GASTRIC MUCOSA

Y. Talebkhan,* M. Esmaili,* S. Eybpoosh,† S. Saberi,* N. Salarieh,* A. Oghalaie,* A. Abdirad,‡ M. Eshagh Hosseini,§ M. Mohagheghi* and M. Mohammadi*
*Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran; †Research Center for Modeling in Health, Institute for Future Studies in health, Kerman University of Medical Sciences, Kerman, Iran; ‡Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran; §Endoscopy Clinic, Amiralam Hospital, Tehran University of Medical Sciences, Tehran, Iran

Introduction: Promoter hypermethylation is an important mechanism of gene silencing. Several aberrant methylations have been reported in the promoter region of tumor suppressor genes (including p16, DAPK and E-cad) in association with gastric carcinogenesis.
Methods: The association of promoter hypermethylation of p16, DAPK and E-cad was assessed through melting curve analysis amongst 104 Iranian GC cases in comparison with 37 non ulcer dyspeptic (NUD) subjects. Tumoral and non tumoral tissues of gastric cancer patients and inflamed and normal tissues of NUD subjects were assessed. Demographic data (including smoking habits) and Hp status were obtained by patient interviews and biopsy-based tests (culture, urease test and histology) respectively.
Results: Our statistical analysis revealed that hypermethylation of p16 gene had an increasing linear trend ranging from normal gastric tissue towards inflamed tissues in NUD subjects, to non-tumoral tissue and finally tumoral tissues of GC cases ($p < 0.05$). Hypermethylation of p16 gene increased the risk of GC development in both cardia/noncardia subsites and intestinal/diffuse subtypes by 3–4 folds, which remained significant after data adjustment. This magnitude of risk was comparable to those of convincing risk factors such as Hp infection and smoking and was not confounded by the latter two risk factors, as adjustment for these factors did not significantly reduce the risk impact (OR = 3.5; 95% CI = 1.3–9.4).
Conclusion: Our data recommends further analysis of p16 gene hypermethylation as an indicator of histopathological changes ranging from inflammation to gastric cancer. Analysis of larger populations will better ascertain the role of p16 hypermethylation in gastric carcinogenesis.

Abstract no.: P07.08

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION AND ITS GENETIC HETEROGENEITY IN AUTOIMMUNE ATROPHIC CHRONIC GASTRITIS PATIENTS

S. Zanussi, M. Casarotto, G. Basaglia, R. Tedeschi, S. Giacomini, V. Canzonieri, V. De Re, S. Maiero, R. Cannizzaro and P. De Paoli
National Cancer Institute, IRCCS, Aviano, Italy

Patients with autoimmune atrophic chronic gastritis (AACG) show high risk to develop precancerous lesions, dysplasia and carcinoids. Due to the cross-reactiv-

ity of gastric parietal cell antibodies (GPCab) with *Helicobacter pylori* (HP), a central role of the bacterium in the pathogenesis of this disease has been hypothesized.

We evaluated the association AACG and HP infection, and its genetic heterogeneity aiming to find hints for its contribution to the pathogenetic process.

Twenty-one subjects with histological and/or functional AACG diagnosis (GPCab positivity and/or GastroPanel) were submitted to serum HP-IgG evaluation by ELISA and to culture in HP selective medium (N = 34 biopsies). Ten to 12 single colonies/biopsy, representing the possible HP genetic heterogeneity in the stomach, were analysed for the presence of CagA, CagE, VirB11, mapping into CagIsland, and VacA and Hom genes.

Previous or active HP infection was demonstrated in 13/21 patients (61.9%). Six out of 21 subjects (28.6%) showed cultivable HP, 14/21 (66.6%) had a heterogeneous gastric microbiota, which prevented HP isolation in six cases. Five patients showed poor CagIsland genes heterogeneity: two presented an intact CagIsland, one showed one gene deletion in 80% of the substrains, two carried three gene deletions in 91% and 100% of the substrains. One patient presented many deletions: cagA, 80%, cagE, 60%, virB11, 40%. An higher frequency of HomA than homB was detected (67.7% vs 32.3%); vacA-vacuolating and -not-vacuolating genotype was found in 42.2% and in 57.8% of the substrains, respectively.

AACG was associated to previous or active HP infection in a relative high proportion of cases. HP was isolable with difficulty, showed an homogeneous CagIsland profile, with variable presence of virulence factors. The possible contribution of an altered gastric microbiota to the pathogenesis and evolution of AACG is an intriguing hypothesis and deserves further studies.

Abstract no.: P07.09

THE DYNAMICS OF PEPSINOGEN LEVELS IN A CAUCASIAN POPULATION WITHIN A 3-YEAR PERIOD

P. Janovic,[*,†,‡] K. Funka,[*,†] I. Kikuste,[*,†] A. Lapina,[†] I. Vilkoite,[*,†] D. Rudzite,[†] E. Cine,[*] A. Lejnieks,[†,‡] I. Polaka,[*] I. Daugule[*] and M. Leja[*,†]

*University of Latvia, Riga, Latvia; [†]Riga East University Hospital, Riga, Latvia; [‡]Riga Stradins University, Riga, Latvia

Aims: Pepsinogen (PgI) and the ratio between PgI and pepsinogen II (PgI/PgII) detection is used as an indirect marker for atrophy in the corpus of the stomach mucosa. The aim of the study was to evaluate the dynamics of pepsinogen test results within a 3-year period in a Caucasian population.

Material and Methods: Patients with decreased Pg levels at the time of initial sampling, were invited to undergo repeated plasma sampling and upper endoscopy with biopsy work-up according to updated Sydney system in average 3 years after the initial blood sample. PgI and PgII were measured in plasma simultaneously in the initial sample and the follow-up sample (Eiken Chemical Co., Japan). The initial selection of the cases was made based on a cross-sectional population-based study in Latvia.

Results: Plasma samples from 107 cases were available for the analysis (45 men; the mean age was 62). The mean PgI level was 33.1 in the initial sample and 32.2 in the follow -up sample, no statistical difference was revealed ($p = 0.61$). The mean PgI/PgII was 2.0 and 2.2, respectively ($p = 0.06$).

In the group of patients with corpus atrophy (according to histology; 41 patients altogether) the mean PgI was 23.9 initially and 21.2 at the control ($p = 0.42$); mean PgI/II was 1.35 initially, and 1.51 at the control ($p = 0.21$).

Conclusion: Our data show that initially PgI or PgI/PgII levels are relatively stable and do not changesubstantially during a 3 year period neither in the entire patient sample nor in patients with corpus atrophy.

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Abstract no.: P07.10

TREATMENT OUTCOME FOR GASTRIC MALT LYMPHOMA ACCORDING TO *HELICOBACTER PYLORI* INFECTION STATUS: A SINGLE CENTER EXPERIENCE

J. Jhi, G. Kim, G. Song, B. Lee, T. Kim and H. Lee

Pusan National University Hospital, Busan, Korea

Background/Aims: *Helicobacter pylori* (*H. pylori*) eradication therapy has been used as the first-line treatment for *H. pylori*-positive gastric mucosa-associated lymphoid tissue (MALT) lymphoma. However, the management strategy for *H. pylori*-negative MALT lymphoma is still controversial. Therefore, the aim of this study was to examine the success rate of each treatment option for *H. pylori*-positive and *H. pylori*-negative gastric MALT lymphomas.

Methods: A total of 57 patients with gastric MALT lymphoma between December 2000 and June 2012 were enrolled in the study. Treatment responses were compared between *H. pylori*-positive and *H. pylori*-negative gastric MALT lymphomas.

Results: Of the 57 patients, 43 patients (75%) had *H. pylori* infection. Forty-eight patients underwent *H. pylori* eradication as a first-line treatment, and complete remission was achieved in 31/39 (80%) patients with *H. pylori*-positive MALT lymphoma and in 5/9 (56%) patients with *H. pylori*-negative MALT lymphoma; no significant difference was observed between the groups ($p = 0.135$). The other treatment modalities, including radiation therapy, chemotherapy, and surgical operation, were effective irrespective of *H. pylori* infection, with no significant difference in treatment response between *H. pylori*-positive and *H. pylori*-negative MALT lymphomas.

Conclusions: *H. pylori* eradication therapy may be considered as a first-line treatment regardless of *H. pylori* infection status.

P08 Oesophageal and extradigestive diseases

Abstract no.: P08.01

ASSOCIATION BETWEEN *HELICOBACTER PYLORI* AND NONALCOHOLIC FATTY LIVER DISEASE IN THE GENERAL POPULATION

S. Kang, D. Kim, J. Kim and S. Cho
Seoul National University Hospital Gangnam Center, Seoul, Korea

Introduction: Association between *Helicobacter pylori* infection and nonalcoholic fatty liver disease (NAFLD) is poorly characterized. The aim of this study was to investigate the association between *H. pylori* positivity with *cagA* status and NAFLD in the large, national, general population.
Methods: The Third National Health and Nutrition Examination Survey (NHANES) from 1988 to 1994 was utilized. NAFLD was defined by ultrasonographic detection of hepatic steatosis without other known liver diseases. Antibodies to *H. pylori* and *cagA* of participants were measured using the *H. pylori* IgG and anti-*cagA* IgG ELISA.
Results: Among total of 11 808 participants who had result of both ultrasonography and *H. pylori* serology, prevalence of NAFLD was 22.9%. The prevalence of NAFLD was higher in *H. pylori* positive subjects (33.5 ± 1.79%) than in negative subjects (26.1 ± 1.65%, $p < 0.001$). Compared with *cagA* positive group, participants with negative *cagA* had higher prevalence of NAFLD (31.1 ± 2.30% vs 36.4 ± 2.37%, $p < 0.001$). Overall participants with NAFLD had higher prevalence of *H. pylori* positivity in multivariable analysis (Odds ratio [OR]: 1.17; 95% confidence interval [CI]: 0.95–1.43) with marginal significance. With regard to presence of *cagA* protein, *H. pylori* and *cagA* positivity was not associated with NAFLD (OR: 1.05; 95% CI: 0.81–1.37) but, *cagA* negative *H. pylori* positivity was significantly associated with NAFLD in multivariable analysis (OR: 1.30; 95% CI: 1.01–1.67).
Conclusions: The prevalence of NAFLD was higher in *H. pylori* positive subjects than in negative subjects. Especially, *cagA* negative *H. pylori* positivity was significantly associated with NAFLD, independent of other known factors in the general population.

Abstract no.: P08.02

ASSESSMENT OF THE ASSOCIATION BETWEEN *HELICOBACTER PYLORI* INFECTION AND OSTEOPENIA IN JAPANESE HEALTHY ADULTS

D. Chinda,* T. Shimoyama,* M. Matsuzaka,* K. Danjo,† S. Nakaji* and S. Fukuda*
*Hirosaki University Graduate School of Medicine, Hirosaki, Japan; †Medical Corporation LYRE Mizoguchi Hospital, Shizuoka, Japan

Background and Aims: Osteoporosis in women is usually caused by the decrease of estrogen production, but the etiology is not clear in men. A Western study showed high prevalence of CagA-positive *H. pylori* infection in male patients with osteoporosis. Therefore, we examined whether *H. pylori* infection is associate with loss of bone density in Japanese healthy adults.
Methods: Three hundred and seventy-nine male and 631 female adults who attended mass survey in 2005 were studied. *H. pylori* stool antigen and serum anti-*H. pylori* antibodies were measured. The bone density was measured as calcaneal osteo sono-assessment index using quantitative ultrasound. Adjusted odds ratio having osteopenia was calculated by logistic regression analysis using age, BMI, smoking, alcohol consumption, periodical exercise, latest educational level, and *H. pylori* infection as independent variables.
Results: In single variate analysis, *H. pylori* infection was significantly associated with osteopenia both in male and female. However, the adjusted OR of *H. pylori* infection for osteopenia was 1.90 ($p = 0.29$, 95% CI: 0.58–6.19) in male and 0.88 ($p = 0.61$, 95% CI: 0.55–1.42) in female. In contrast, age was significantly associated with osteopenia and calculated OR in male and female was 1.05 ($p < 0.001$, 95% CI: 1.03–1.07) and 1.06 ($p < 0.001$, 95% CI: 1.04–1.08), respectively.
Conclusion: No significant association was found between *H. pylori* infection and osteopenia while age was a significant risk for the developing osteopenia in this series of subjects.

Abstract no.: P08.03

IMPACT OF *HELICOBACTER PYLORI* INFECTION ON ALZHEIMER'S DISEASE SEVERITY: A MOUSE MODEL STUDY

C. Roubaud-Baudron,*,†,‡ C. Varon,*,† S. Pietropaulo,§,¶ L. Chambonnier,*,† A. Buissonnière,*,† Y. Cho,§,¶ V. Fénelon,†,** L. Blaszczyk,†,** N. Macrez,†,†† F. Mégraud*,† and N. Salles*,†,‡
*INSERM U853, Bordeaux, France; †Université Bordeaux Segalen, Laboratoire de Bactériologie, Bordeaux, France; ‡Pôle de gérontologie clinique, CHU Hôpitaux de Bordeaux, Bordeaux, France; §CNRS UMR 5287, Bordeaux, France; ¶Université Bordeaux 1, Bordeaux, France; **INSERM U862, Bordeaux, France; ††CNRS UMR 5293, Bordeaux, France

Helicobacter pylori infection seems to play a critical role in extragastric diseases including Alzheimer's dementia (AD). Chronic *H. pylori* infection could worsen AD lesions via atherosclerosis and inflammation. Thus, our aim was to infect AD transgenic (Tg) mice and their wild type (WT) ittermates with bacteria from two different Helicobacter species in order to explore their cerebral and behavioural lesions.
Two groups of Tg mice (APPswe + PS1dE9) and their WT littermates were infected with *H. pylori* SS1 (n = 60) or H. felis (n = 60) or left uninfected (n = 60) and sacrificed after 4, 6 and 10 months. For the two studies, brain and stomach specimens were processed to detect cerebral amyloid plaques (thioflavine S stain), astroglial and microglial cells (immunochemistry anti-GFAP and anti-Iba-1, respectively) and gastric lesions (hematoxylin and eosin stain). Spatial memory, social interaction and anxiety were tested before sacrifice.
Although we found no behavioural or histological effect after 4 months of *H. pylori* infection, an association was observed after 6 months of infection, with an increased number of amyloid plaques and neuroinflammation in Tg mice but without a significant difference regarding behavioural experiments. Altogether, Tg mice were more cognitively impaired than the WT littermates. M10 results are currently in progress.
After epidemiological arguments supporting an AD – *H. pylori* association, this murine model provides the first evidence of the impact of *H. pylori* infection on the brain.

Abstract no.: P08.04

LONG TERM RESULTS OF ERRADICATION OF *HELICOBACTER PYLORI* ON CHRONIC IDIOPATIC URTICARIA

C. Senosiain, M. Maroto, M. García-Sánchez, C. Martín de Argila, L. Crespo, J. Foruny and D. Boixeda
H.U. Ramón y Cajal, Madrid, Spain

Background: *Helicobacter pylori* (HP) is the main bacteria implicated in gastric pathology, which has also been related to extradigestive manifestations, such as chronic idiopathic urticaria (CIU).
Objective: To analyse long term effects of HP erradication on CIU.
Patients and Methods: Prospective study of 63 patients with CIU treated for HP erradication. HP infection was assesed by the (13)C-Urea breath test and a confirmatory test (culture or anatomical pathology). First line treatment was based on a combination of a proton pump inhibitor (PPI), amoxicilin and charithromycin. In case of failure, patients were treated with PPI, charithromycin, metronidazol and bismute. If second failure, a third treatment was based on antibiogram. Dermatological and digestive symptoms were assesed before and after erradication.
Results: Eighty patients were recluted, from which 63 were finally included in the study. The mean age was 44 (SD 14) years, with 73% of women. Initial urticarial symptoms were: pruritus (71.4%), wheals (70%), angioedema (33.3%), orophaynx edema (14.3%) and dermographism (12.7%). Digestive symptoms (66%) were: heartburn (43%), epygastralgia (35%), metheorism (31.7%), dyspepsia (23.8%), constipation (9.5%), belching (6.4%), nausea (6.3%), vomits (3.2%), regurgitation (3.2%), dysphagia (1.6%).
HP erradication was achieved after 1, 2, 3 or 5 treatments in 76%, 17%, 5% or 1.6% respectively. The mean time of follow-up was 126 (SD 40.9) months. At the end, 33% of patients were asymptomathic, 40% referred partial recovery, 22% found no changes in symptoms and only one patient worsenned.

Conclusions: *H. pylori* erradication has a benefitial long-term effect on CIU (73%), which suggests a plausible pathogenic relation between this bacteria and this entity. In conclusion, every patient with CIU and *H. pylori* should be treated with an erradication therapy.

Abstract no.: P08.05

THE PRESENCE OF *HELICOBACTER PYLORI* (*H. PYLORI*), BUT NOT GASTRITIS ACTIVITY, ARE ASSOCIATED WITH IRON DEFICIENCY MARKERS IN ADOLESCENT GIRLS WITH NORMAL HEMOGLOBIN LEVEL

S. Tereshchenko, N. Gorbacheva and L. Lapteva
Medical Research Institute for Northern Problems, Russian Academy of Medical Sciences, Krasnoyarsk, Russian Federation

The pathophysiologic mechanisms involved in the relationship between *H. pylori* infection and iron deficit are not established well.
Aim: To determine either the presence of *H. pylori* solely or *H. pylori*-related changes in gastric mucosa are associated with iron iron deficiency markers in adolescent girls.
Methods: Hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), red blood cell distribution width (RDW), serum iron, and ferritin levels were studied in 94 adolescent girls aged 12–18. Endoscopic examinations were performed and two biopsy specimens, one from the antrum and one from the corpus, were used for rapid urease test (Helpil-test, AMA, Russia). According to test the girls were grouped as *H. pylori* negative (group 1, n = 38), *H. pylori* positive with low urease activity (group 2, n = 35) and *H. pylori* positive with high urease activity (group 3, n = 21). Histopathologic examinations were performed and gastritis was graded according to the updated Sydney histologic scoring system The Kruskal-Wallis and Mann-Whitney tests were used.
Results: In all three groups the hemoglobin levels were >120 g/L. We have not found any distinctions between Hb, RDW, serum iron, and ferritin levels in assigned groups according with *H. pylori* status. Ht levels was lower in *H. pylori* positive adolescents (p1-2 = 0.041) and erythrocyte MCVs have progressively decreased with increasing urease activity (p1-2 = 0.068; p1-3 = 0.03; p2-3 = 0.033). Mononuclear infiltration of gastric mucosa was associated with lower levels of serum iron ($p = 0.08$) and MCV ($p = 0.02$). There were no any distinctions in iron deficiency markers according histopathologic changes of gastritis activity (according neutrophil infiltration).
Conclusion: Thus, our findings more likely support the hypothesis of ferrum sequestration by antral *H. pylori* than causative role of *H. pylori*-related changes in gastritis mucosa leading to iron deficiency.

Abstract no.: P08.06

HELICOBACTER PYLORI COLONIZATION AND PREECLAMPSIA: THE GENERATION R STUDY

W. J. den Hollander,* S. Schalekamp-Timmermans,* I. L. Holster,* V. W. Jaddoe,* G. I. Perez-Perez,† M. J. Blaser,† E. A. P. Steegers* and E. J. Kuipers*
*Erasmus MC University Medical Center, Rotterdam, The Netherlands; †New York Langone Medicine Center, New York, NY, USA

Introduction: Preeclampsia (PE) is characterized by endothelial dysfunction and related hypertension and coagulative disorders. Although the exact pathogenesis is still unknown, certain infectious agents seem to play a role. *Helicobacter pylori* (*Hp*) has been reported to induce platelet aggregation, and we therefore hypothesized that this bacterium could be associated with PE.
Aims and Methods: The aim was to assess the association between *H. pylori* colonization and PE. We measured IgG anti-*Hp* and CagA-antibodies in serum of pregnant women of the Generation R study, a population-based prospective cohort study. Delivery and medical records were retrieved for identification of subjects with PE, which were defined according to standard criteria. Information on demographics, education, and maternal risk factors was collected by questionnaires. Odds ratios (OR) of PE for *Hp* colonization were calculated using logistic regression analyses after adjustment for potential confounders.
Results: Serum of 6348 pregnant women was analyzed (mean age 29.7 ± SD 5.3). In total, 2923 women were *Hp* positive (46%) and 1028 of them were CagA-positive (35%). For 132 women pregnancy was complicated with PE (2.1%). *Hp* colonization rate in women with PE was 56% compared to 44% in subjects without PE ($p = 0.02$). CagA-positivity rate was 20% in women with and 16% in women without PE ($p = 0.30$). Adjusted for potential confounding effects, women colonized with *Hp* were more likely to develop PE (final OR 1.53; 95% CI 1.04–2.26). CagA-positivity was not associated with PE.
Conclusion: Our data demonstrate that *Hp* colonization in pregnant women is associated with PE. *Hp* may be involved in different inflammatory mechanisms, which might potentially affect the pathogenesis of PE. Understanding and further validation of this association may contribute to effective intervention (e.g. *Hp* eradication treatment) for reducing morbidity and mortality from this disease.

Abstract no.: P08.07

ASSOCIATION OF *HELICOBACTER PYLORI* INFECTION AND GASTRIC MUCOSAL ATROPHY TO SERUM LEVEL OF SELENIUM IN HEALTHY ADULTS

S. Sato,* T. Shimoyama,* Y. Okada,* K. Danjo,† S. Nakaji‡ and S. Fukuda*
*Department of Gastroenterology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; †Medicar Corporation LYRE Mizoguchi Hospital, Shizuoka, Japan; ‡Department of Social Medicine, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

Backgrounds: Trace elements are essential components for wound healing and maintenance of immune systems. Selenium has been shown to have anti-aging and anti-carcinogenetic effects. However, few studies have shown the association between *Helicobacter pylori* (*H. pylori*) and serum level of selenium. We examined the association between *H. pylori* infection and serum level of selenium in healthy subjects considering gastric mucosal atrophy.
Methods: Subjects were 330 males and 541 females aged 26–83 years old who attended mass survey. Serum level of selenium and pepsinogens (PGs), antibodies to *H. pylori* were measured. Stool samples were also obtained to measure *H. pylori* antigen. *H. pylori* status was defined positive or negative when the results of both serology and stool antigen were concordant. Gastric mucosal atrophy was considered positive if both PG I < 70 μg/L and PG I/II < 3.0 were observed.
Results: Serum level of selenium was lower in *H. pylori* infected subjects (207.5 ± 16.9 μg/L) comparing with non-infected subjects (235.8 ± 34.2) who were born 1970s ($p < 0.01$). In subjects with *H. pylori* infection born in 1950s, serum level of selenium was 239.7 ± 37.2 μg/L in subjects with gastric mucosal atrophy and 254.3 ± 37.0 μg/L in subjects without atrophy.
Conclusion: *H. pylori* infection and gastric mucosal atrophy may associate with lower serum selenium concentration among relatively younger population.

P09 Paediatric issues

Abstract no.: P09.01

MATERNAL *HELICOBACTER PYLORI* COLONIZATION IS NOT ASSOCIATED WITH ASTHMA SYMPTOMS, AIRWAY INFLAMMATION AND AIRWAY RESISTANCE IN THEIR CHILDREN UNTIL THE AGE OF 6 YEARS: THE GENERATION R STUDY

W. J. den Hollander,* A. M. M. Sonnenschein-Van der Voort,* I. L. Holster,* L. Duijts,* J. C. de Jongste,* V. W. Jaddoe,* G. I. Perez-Perez,† M. J. Blaser,† H. A. Moll* and E. J. Kuipers*

*Erasmus MC University Medical Center, Rotterdam, The Netherlands; †New York Langone Medical Center, New York, NY, USA

Background: The declining prevalence of *Helicobacter pylori* (*Hp*) in Western countries has been suggested to be a factor involved in the increase of childhood asthma. Although mother to child transmission is thought to be a major *Hp* transmission route, no data are available whether maternal *Hp* colonization is associated with childhood asthma.
Aim and Methods: We aimed to investigate whether maternal *Hp* status is associated with asthma in their child. Antibodies against *Hp* and CagA were measured in pregnant women, participating in the Generation R study, a population-based prospective cohort study. Data on childhood asthma included wheezing at the age of 1–4 years, and at the age of 6 years physician-diagnosed asthma, FeNO (fractional exhaled nitric oxide), a marker for eosinophilic airway inflammation, and interrupter resistance (Rint), a measure of airway resistance. Multivariate analyses were adjusted for potential confounders.
Results: In total 5739 mothers (mean age 30.3 ± SD 5.0) and their children (M/F; 2861/2878) were included in these analyses. Of those, 2375 (41%) were *Hp*-positive, from which 834 (35%) were CagA-positive. Univariate analyses revealed following results between children of *Hp*-positive versus negative mothers: wheezing at 4 years of age (14.0 vs 11.9% [$p = 0.06$]), physician-diagnosed asthma (7.0 vs 5.4% [$p = 0.08$]), FeNO median (7.80 ppb [2.5–97.5th percentile 2.75–33.35] vs 7.20 ppb [2.91–30.00] [$p < 0.01$]), and Rint (Z-score) mean (0.90 ± 2.98 vs 1.11 ± 3.12 [$p = 0.05$]). Multivariate analyses revealed no associations between maternal *Hp* seropositivity and childhood wheezing at different ages, physician-diagnosed asthma (OR 1.18; 95%CI 0.85, 1.64), Rint (*Z*-score change −0.17; −0.41, 0.07) or FeNO (0.02; −0.04, 0.07). CagA positivity did not result in significant differences in outcome.
Conclusion: This large prospective study revealed no associations between maternal *Hp* colonization and asthma, FeNO, or Rint until the age of 6 years.

Abstract no.: P09.02

EVALUATION OF MSI-1, CD44 AND CD105 AMONG CHILDREN WITH *H. PYLORI* GASTRITIS

M. Ugras,* M. Tosun† and F. Aktepe‡

*Yeditepe University, Istanbul, Turkey; †Department Histology Embriology, Medical Faculty, Kocatepe University, Afyonkarahisar, Turkey; ‡Pathology Department, Medical Faculty, Kocatepe University, Afyonkarahisar, Turkey

Aim: We aimed to search the effect of *H. pylori* on gastric stem cells and gastric mesenchymal stem cells among children.
Material and Method: The gastric biopsy materials of *H. pylori* positive children were retrospectively studied. *H. pylori* gastritis was graded according to Sydney classification. Stem cell evaluation was made immunohistochemically. The immunopositive cells were counted with Image J Image Analysis Software. For statistical analysis SPSS for Windows 16.0 program was used and Oneway ANOVA and Tukey Posthoc tests were used for analysis.
Results: In the evaluation of slides, it was determined that the number of Msi-1, CD105 and CD44 immunopositive mesenchymal stem cells were increased parallel to increased *H. pylori* density. In statistical analysis; Msi-1 positive cell number were not statistically different between HP(−) and HP (+) ($p = 0.308$), but different between HP(++) and (+++) groups (respectively $p = 0.037$, 0.003). On the other hand, although there was no difference between HP (+) and (++) groups ($p = 0.102$), there was significant difference between HP(+) and HP(+++) groups ($p = 0.013$), there was no significant difference between HP (++) and (+++) groups ($p = 0.166$) In the evaluation of CD44 positive mesenchymal stem cells' number, albeit the number of these cells were increased parallel to HP density, there was no significant difference between all groups ($p > 0.05$ for all). In the evaluation of CD105 positive mesenchymal stem cells, the number of these cells were significantly higher than other three groups ($p = 0.000$ for all). On the other hand, there was no significant difference between HP(−), (+) and (++) groups ($p > 0.05$ for all).
Conclusion: All markers were seen to be elevated. MSi-1 increase can be suggested compensatory. *H. pylori* causes increase in both CD44 and CD105 positive cells.

Abstract no.: P09.03

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN JAPANESE CHILDREN

M. Okuda,* S. Kikuchi,† T. Osaki,‡ J. Ueda,† K. Maekawa,* Y. Lin,† S. Kamiya‡ and Y. Fukuda*

*Department of General Medicine and Community Health Science, Hyogo Medical College of Medicine, Sasayama, Japan; †Department of Public Health, Aichi Medical University School of Medicine, Nagakute, Japan; ‡Department of Infectious Diseases, Kyorin University School of Medicine, Mitaka, Japan

Objectives: The aim of this study is to estimate prevalence of *H. pylori* infection in children using stool antigen or antibody tests in a rural area of Japan.
Methods: Subjects were children from six junior high school, seven elementary schools, six kindergartens, and three nursery schools in Sasayama-city. In 2010, 1299 children aged 0–8 years and in 2011, 1909 aged 0–11 years and in 2012, 1222 aged 12–15 years (junior high school students) were asked to give stool samples, and 689 (53%), 835 (44%) and 337 (28%) children were participated, respectively. *H. pylori* stool antigen was performed using Test-Mate pylori Antigen EIA (Wakamoto Pharmaceutical, Tokyo, Japan). According to the manufacturer's instruction, cutoff value was decided at 0.1, which was also validated by PCR and UBT. *H. pylori* antibody tests were performed using URINELISA (Otsuka Pharmaceuticals Co, Ltd, Tokyo, Japan) and/or E-Plate "Eiken" *H. pylori* antibody (EIKEN CHEMICAL Co, Ltd, Tokyo, Japan). Furthermore we evaluated serum pepsinogen I, II and I/II ratio in 206 children.
Results: In 2010 and 2011, stool antigen positive % (sample size) were 1.9% (689) and 1.8% (835) respectively in total. Antibody positive rate was 4.2% (337) in children aged 12–15 years.
Conclusions: In Japan, *H. pylori* prevalence was 0.0–3.7% in those aged 0–5 years, 1.0–3.3% in 6–8 years, 1.5–4.9% in 9–11 years and 4.2% in 12–15 years.

Abstract no.: P09.04

EPSTEIN BARR VIRUS AND *HELICOBACTER PYLORI* CO-INFECTION ARE POSITIVELY ASSOCIATED WITH SEVERE GASTRITIS IN PEDIATRIC PATIENTS

E. M. Fuentes-Pananá, M. Cardenas-Mondragón, R. Carreón-Talavera, M. Camorlinga-Ponce, A. Gomez-Delgado and J. Torres

Instituto Mexicano del Seguro Social, Mexico City, Mexico

H. pylori infection is acquired during childhood and causes a chronic inflammatory response in the gastric mucosa, which is considered the main risk factor to acquire gastric cancer (GC) later in life. More recently, infection by Epstein-Barr virus (EBV) have also been associated with GC. The role of EBV in early inflammatory responses and its relationship with *H. pylori* infection remains poorly studied. Here, we assessed whether EBV infection in children correlated with the stage of gastritis and whether co-infection with *H. pylori* affected the severity of inflammation.
Three hundred and thirty-three pediatric patients with non-atrophic gastritis were studied; gastric biopsies were taken and inflammation graded according to the Sydney system; peripheral blood was drawn and antibodies against EBV (IgG and IgM anti-VCA) and *H. pylori* (IgG anti-whole bacteria and anti-CagA) were measured in sera. We found that children infected only by EBV presented mild mononuclear (MN) and none polymorphonuclear (PMN) cell infiltration, while those infected by *H. pylori* presented moderate MN and mild PMN. In contrast, patients co-infected with both pathogens were significantly associated with severe gastritis. Co-infection of *H. pylori* CagA+/EBV+ had a stronger association with severe MN (PR 3.0) and PMN (PR 7.2) cells than cases with single *H. pylori* CagA+ infection.
Co-infection with EBV and *H. pylori* in pediatric patients is associated with severe gastritis. Even single infections with *H. pylori* CagA+ strains are associated with mild to moderate infiltration arguing for a cooperative effect of *H. pylori* and EBV in the gastric mucosa and revealing a critical role for EBV

previously un-appreciated. This study points out the need to study both pathogens to understand the mechanism behind severe damage of the gastric mucosa, which could identified children with increased risk to present more serious lesions later in life.

Abstract no.: P09.05

DOES *HELICOBACTER PYLORI* PROTECTS AGAINST EOSINOPHILIC ESOPHAGITIS IN CHILDREN?

Y. Elitsur,* B. Alabd Alrazzak* and Y. Demetieva†
*Marshall University School of Medicine, Huntington, WV, USA; †Emmanuel College, Boston, MA, USA

The rate of Eosinophilic esophagitis (EoE) in children is increasing while the rate of *Helicobacter pylori* (Hp) infection has been decreasing in children from developed countries. EoE is closely related to allergy, while Hp infection is closely related to poor hygienic conditions. Interestingly, in countries with poor hygienic conditions allergy is low (hygienic theory) and Hp infection is high. The opposite epidemiological data may suggest a "protective" effect between-both diseases. The relationship between Hp infection and EoE has never been investigated in the pediatric population.

Aim: To investigate the relationship between Hp infection and EoE in children.

Material and Methods: A retrospective analysis of first diagnostic endoscopy (2007–2012) was performed. Chronological data and histologic diagnoses were collected. Biopsies from the esophagus and stomach were available in all charts irrespective of mucosal appearance. Hp diagnosis was determined by histology. EoE diagnosis was established according to clinical guideline (Liacouras 2011).

Results: A total of 966 charts were available for review. The mean age and M: F ratio was 11.3 years and 1:1.18, respectively. Esophagitis, idiopathic gastritis, EoE, and Hp infection was detected in 268 (28%), 480 (50%), 62 (6%), and 31 (3%) charts, respectively. The association between Hp infection and esophageal eosinophilis, gastritis, and EoE is described in the following Table.

Conclusion: No significant association (Phi Coefficient) was noted between Hp infection and EoE disease (−0.024). Positive association was found between Hp infection and gastritis (0.183) and between Hp infection and GER (0.294). We hypothesize that the low rate of EoE and Hp infection in our population is responsible for the lack of association between both diseases.

Table 1 Correlation of *H. pylori* infection with EoE

	EoE	Gastritis	GER
Count (%)	1 (3)	32 (100)	31 (97)
Fisher exact	0.716	<0.0001	<0.0001
Phi coefficient (*p*-value)	−0.024 (0.461)	0.183 (<0.0001)	0.294 (<0.0001)

Abstract no.: P09.06

HELICOBACTER PYLORI ERADICATION RATE IN SLOVENIAN PAEDIATRIC POPULATION, 2011–2012

S. Jeverica,* A. Levanič,† L. Gangl Žvikar,‡ U. Dolinar* and M. Homan§
*Institute for Microbiology and Immunology, Ljubljana, Slovenia; †Departement of Paediatrics, Ptuj General Hospital, Ptuj, Slovenia; ‡Departement of Paediatrics, Slovenj Gradec General Hospital, Slovenj Gradec, Slovenia; §Department of Gastroenterology, Hepatology and Nutrition, University Medical Centre Ljubljana, University Children's Hospital, Ljubljana, Slovenia

Backgrounds: Antimicrobial resistance is the leading cause for the *Helicobacter pylori* (HP) eradication failure. High clarithromycin resistance was detected in adult population in Slovenia. No such data were available for children so far. It is recommended to use guided approach in paediatric population if resistance to clarithromycin exceeds 20%. Herein, we present the HP eradication rate of guided therapy among Slovenian children in 2011–2012.

Methods: Retrospective analysis of all children who underwent guided therapy for HP eradication in three Slovenian general hospitals and the University Medical Centre was performed. Demographic characteristics, primary resistance rates, duration/composition of therapy, and eradication rate were analysed.

Results: Total of 50 patients were included in the analysis: 66.0% (n = 33) female and 34.0% (n = 17) male, 3–17 years of age, mean age 11.4 years 37 patients had the test-of-cure result available, one patient had refused to be treated and 12 were lost to follow up. Primary resistance rate for clarithromycin, metronidazole, levofloxacin, amoxicillin and tetracycline were 22.0%, 10.4%, 0.0%, 0.0%, and 0.0%, respectively. 59.5% (n = 22) of patients received clarithromycin-based triple therapy. Mean duration of the treatment was 12.8 days. Successful eradication, as confirmed by urea breath test or stool antigen test, 2 months after the therapy, was achieved in 94.6% (n = 35) patients.

Conclusions: The clarithromycin resistance among Slovenian children is high (22.0%), which warrants the guided approach for the initial HP eradication therapy selection. HP eradication rate using guided approach is excellent (94.5%) and far exceeds the primary HP eradication rate among adults using non-guided approach.

Abstract no.: P09.07

QUALITY OF LIFE AND *H. PYLORI* GASTRITIS AMONG CHILDREN

M. Ugras,* O. Guraksin,† O. Uneri‡ and A. Vitrinel*
*Department of Pediatrics, Medical Faculty, Yeditepe University, Istanbul, Turkey; †Department of Pediatrics, Medical Faculty, Kocatepe University, Afyon, Turkey; ‡Bakirkoy Pschiatry Teaching Hospital, Istanbul, Turkey

Helicobacter pylori (*H. pylori*) is a Gram-negative bacteria that colonizes gastric mucosa and is the important etiologic agent for peptic ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue lymphoma. Benefits of the eradication of *H. pylori* have been reported from patients presenting with these clinical conditions. However, the improvement in symptoms in patients with functional dyspeptic disorders after *H. pylori* eradication remains controversial. The Pediatric Quality of Life Inventory (PedsQL) is a modular instrument that measures health related quality of life, and investigates the physical and psychosocial functioning, unrelated to health, of children 2–18 years old. In this study, the objective was to evaluate the quality of life of the children with and without gastritis.

Methods: The parents and patients fullfilled the Ped QL before and 2 months after the endoscopic procedure. Children with no abdominal complaint made up the control group. The PedQL and Sydney score of the children who underwent endoscopy was evaluated.

Results: Forty-two children aged 7–17 made up the *H. pylori* gastritis group: Group 1. Group 2 represents the control grup with 25 children aged between 8 and 16 years. Total score in group 1 before endoscopy by parents was 75.11 ± 15.72, children was 75.76 ± 17.14, after endoscopy by parents 85.13 ± 10.49, by children was 86.03 ± 10.33. Total score in control group by parents was 81.31 ± 13.13 and by children was 83.60 ± 13.11. PedsQL was similar in children with *H. pylori* after endoscopy and control group. There was statistically significant difference between total score before and after endoscopy reported by children, and not significant but some difference reported by parents.

Conclusion: *H. pylori* may have some effect in the quality of life of children.

Abstract no.: P09.08

THE EFFECT OF *HELICOBACTER PYLORI* AND GASTRITIS ON MEAN PLATELET VOLUME AMONG CHILDREN

M. Ugras,* A. Vurmaz,† T. Koken,† C. Tokyol,‡ J. Coban,§ I. Dogan¶ and A. Vitrinel*

*Department of Pediatrics, Yeditepe University Hospital, Istanbul, Turkey; †Biochemistry Department, Medical Faculty, Kocatepe University, Afyonkarahisar, Turkey; ‡Pathology Department, Medical Faculty, Kocatepe University, Afyonkarahisar, Turkey; §Biochemistry Department, Yeditepe University Hospital, Istanbul, Turkey; ¶Pathology Department, Yeditepe University Hospital, Istanbul, Turkey

Helicobacter pylori (*H. pylori*) causes gastritis, peptic ulcer disase and gastric cancer. The extragastric manifestations are increasing as our scientific interventions become more detailed. *H. pylori* causes chronic inflammation and relations with some diseases such as atherosclerosis is attributed to inflammation. Mean platelet volume (MPV) is suggested to be a marker of inflammation. We aimed to evaluate any relation with *H. pylori* gastritis and MPV levels among children. MPV levels of children with *H. pylori* gastritis (Group 1), children with *H. pylori* negative gastritis (Group 2) and control groups were evaluated. There was statistically significant difference between group 1 and group 2; group 1 and group 3; and group 2 and group 3. There was negative corelation between MPV and inflammation scored histopathologically. We suggest that gastritis caused by *H. pylori* causes an inflammatory responce and effects MPV levels among children.

Table 1 Mean platelet volumes in each group

	MPV (fL)			
	Minimum	Maximum	Mean ± SD	*p*
Group 1 (n:37)	8.44	11.30	9.7965 ± 0.71848	<0.005[a]
Group 2 (n:20)	9.70	11.40	10.3400 ± 0.63445	<0.005[b]
Group 3 (n:24)	8.60	9.80	9.2500 ± 0.42834	<0.005[c]

MPV, mean platelet volume.
[a]Statistical signifgicance between group 1 and group 2 ($p < 0.005$).
[b]Statistical signifgicance between group 1 and group 3 ($p < 0.005$).
[c]Statistical signifgicance between group 2 and group 3 ($p < 0.005$).

P10 Diagnosis

Abstract no.: P10.01

VOLATILE ORGANIC COMPOUND IN THE BREATH TO DIFFERENTIATE BETWEEN GASTRIC CANCER AND BENIGN CONDITIONS

M. Leja,*,†,‡ H. Amal,§ K. Funka,*,‡ I. Lasina,* R. Skapars,*,† G. Ancans,*,† I. Liepniece-Karele*,† and H. Haick§

*University of Latvia, Riga, Latvia; †Riga East University Hospital, Riga, Latvia; ‡Digestive Diseases Cente GASTRO, Riga, Latvia; §TECHNION – Israel Institute of Technology, Haifa, Israel

Background: The possibility to identify new breath compounds as a fingerprint of a different groups with different clinical gastric conditions by the GC-MS analysis will open a new venue that guide the development of a new, tailor-made sensors for breath analysis of gastric cancer.

Methods: Two alveolar exhaled breath samples were collected from 364 volunteers and were analyzed using gas chromatography coupled with mass spectrometry (GC-MS) in order to identify the chemical composition of the breath samples. In the near future the same samples will be analyzed using nanomaterial-based sensors in order to study the feasibility of a novel method in oncology based on breath analysis for identifying gastric diseases.

Findings: Seventeen substances in the breath were identified and could be associated with gastric cancer, gastric ulcer, gastric dysplasia and less severe gastric conditions. Eleven VOCs were found significantly in a higher concentration in the cancerous group when compared to the control group and this indicates that the breath of cancer patients have a different and unique fingerprints.

Interpretation: These data suggest that certain groups of VOCs may be preferentially associated with the specific gastric condition. Some of these compounds have logical\clinical explanations, other cannot yet be easily understood.

Abstract no.: P10.02

USEFULNESS OF DUAL PRIMING OLIGONUCLEOTIDE (DPO)-PCR KIT FOR DETECTION OF *HELICOBACTER PYLORI* AND ITS RESISTANCE TO CLARITHROMYCIN

J. S. Han, K. H. Suh, S. Y. Kim, S. W. Jung and S. W. Lee

Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea

Background: Antimicrobial resistance to clarithromycin has been growing concern in the treatment of *Helicobacter pylori (H. pylori)*. The use of traditional culture-based methods for determination of clarithromycin resistance in *H. pylori* is time-consuming and has difficulty in clinical setting. Our aim was to investigate the performance of the dual priming oligonucleotide (DPO)-PCR kit named Panplex™ ClaR-*H. pylori* Detection in clinical setting.

Methods: From January 2012 to February 2013, untreated patients who performed endoscopy and biopsy and showed positive *H. pylori* via DPO-PCR kit were investigated. They were treated and *H. pylori* eradication rate was assessed 4–5 weeks later.

Results: During study period, DPO-PCR kit was performed in 222 untreated patients and 120 patients (54.1%) showed positive *H. pylori* infection. Among 120 strains with positive *H. pylori,* 34 strains (28.3%) were clarithromycin-resistance: A2142G mutation in 18 strains and A2143G mutation in 16 strains. Ninety-eight patients received eradication therapy and 82 patients performed test to confirm the results of eradication. Various regimens were used and the eradication rate was 95.1% (per protocol). In mutant strains, the eradication rate of triple therapy including PPI, amoxicillin, clarithromycin was 66.7% and the eradication rate of triple therapy including PPI, amoxicillin, metronidazole or quadruple therapy containing bismuth was 100%.

Conclusion: DPO-PCR kit is a practical method for the detection of *H. pylori* infection and clarithromycin resistance. Tailored therapy according to clarithromycin resistance using DPO-PCR kit improves cure rates.

Abstract no.: P10.03

ANALYSIS OF CAGA SEROTYPES WITH *HELICOBACTER PYLORI* MULTIPLEX SEROLOGY

A. Michel,* S. Wang,† W. Zhang,† M. Kist,‡ G. Figueroa,§ B. Dondog,* H. Suzuki¶ and M. Pawlita*

*Infection and Cancer Program, German Cancer Research Center (DKFZ), Heidelberg, Germany; †Department of Pathology, Tangdu Hospital, The Fourth Military Medical University, Xi'an, China; ‡National Reference Center for Helicobacter pylori, Institute of Medical Microbiology and Hygiene, University of Freiburg, Freiburg, Germany; §Laboratory of Microbiology, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile; ¶Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan

Chronic *H. pylori* infection is associated with severe gastrointestinal disease including cancer. Antibodies to different antigens have been identified as risk markers. Among them cytotoxin associated antigen A (CagA) has been widely investigated. However its pathogenic role is still controversial in populations of different geographical regions.

CagA is translocated intracellularly where it is phosphorylated at EPIYA sites. Phosphorylated CagA binds and activates phosphatase SHP2 that induces cell growth and motility. Western and East-Asian *H. pylori* strains are distinguished by EPIYA site sequence variation. East-Asian CagA has stronger SHP2 binding affinity and thereby higher biological activity.

We recombinantly expressed peptides with the CagA-EPIYA motifs of Western strain G27 and East-Asian strain F32 and included both into *H. pylori* multiplex serology, a bead-based assay (Luminex) that allows simultaneous and quantitative detection of antibodies against 15 different *H. pylori* proteins.

We analysed *H. pylori* antibody positive sera from Germany (n = 191), China (n = 140), Japan (n = 117), Chile (n = 308) and Mongolia (n = 499). Among CagA seropositives, single positivity to Western CagA was 93.6% in Mongolia, 84.0% in Chile and 81.3% in Germany. Single positivity to East-Asian CagA was 82.9% in Japan. Double positivity and single positivity to the opposing Western or East-Asian CagA was <2.2% in all populations. China showed a mixed population with single positivity of 11.1% to Western and 63.2% to East-Asian CagA.

CagA serotype analysis embedded in *H. pylori* multiplex serology might be suited to further compare CagA antibody responses associated with gastrointestinal disease presentations.

Abstract no.: P10.04

C13-UREA BREATH TEST SKIPPING CITRIC ACID HAS LOW SPECIFICITY FOR DIAGNOSING *H. PYLORI* INFECTION

X. Calvet Calvo,*,†,‡ M. Ramírez-Lázaro,*,†,‡ S. Lario,*,†,‡ J. Sánchez-Delgado,*,†,‡ A. Montserrat,*,†,‡ A. Casalots,§ D. Suarez,¶ R. Campo,*,†,‡ F. Junquera,*,†,‡ E. Brullet,*,†,‡ I. Sanfeliu** and F. Segura††,‡‡,§§

*Digestive Diseases Department, Hospital de Sabadell, Institut Universitari ParcTaulí, Sabadell, Spain; †Departament de Medicina, Universitat Autònoma de Barcelona, Sabadell, Spain; ‡CIBEREHD, Instituto de Salud Carlos III, Barcelona, Spain; §Servei de Patología, UDIAT-CD, Institut Universitari Parc Taulí, Universitat Autònoma de Barcelona, Sabadell, Spain; ¶Unitat d'Epidemiologia i Avaluació, Fundació Parc Taulí, Universitat Autònoma de Barcelona, Sabadell, Spain; **Laboratori de Microbiologia, UDIAT-CD, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0018), Sabadell, Spain; ††Servei de Malalties Infeccioses, Hospital de Sabadell, Institut Universitari Parc Taulí; ‡‡Departament de Medicina, Universitat Autònoma de Barcelona; §§Spanish Network for the Research in Infectious Diseases (REIPI RD06/0018), Sabadell, Spain

Background and Aims: In a previous study, UBiT-100 mg, (Otsuka, Spain) – a commercial 13C-urea breath test omitting citric acid pretreatment – had a high rate of false positive results. The aim of the present study was to validate these results in a new independent cohort of patients.

Methods: Dyspeptic patients (n = 272) were prospectively enrolled. UBiT was performed according to manufacturer's recommendations. *H. pylori* infection was determined combining culture, histology and rapid urease test. UBiT sensitivity, specificity, positive and negative predictive values and its 95% confidence intervals were calculated.
Results: UBiT showed a false positive rate of 17%. The test sensitivity was 100% (95% CI: 99.6–100%), the specificity 83.1% (95% CI: 76.1–90.1%) and the positive and negative predictive values 86.2% and 100% respectively.
Conclusions: UBiT suffers from a high rate of false positive results and suboptimal specificity; UBT protocols skipping citric acid pre-treatment should be revised.

Abstract no.: P10.05

MONOCLONAL ANTIBODY AGAINST ALKYLHYDROPEROXIDE REDUCTASE (HP 1563) OF *HELICOBACTER PYLORI* AND ITS ANTIGENICITY

T. Mohammadian,* H. Amini† and M. Paknejad†
*Department of Microbiology, Faculty of Beasic Sciences, Islamic Azad University, shahr-e Qods-Branch, Qods, Iran; †Department of Medical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Introduction: Development of a stool antigen immunoassay to detect *Helicobacter pylori* infection requires monoclonal antibody against the specific antigen. Alkylhydroperoxide reductase (AhpC) of *Helicobacter pylori* 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. The aims of this study were to prepare a monoclonal antibody against AhpC.
Methods: The isolation and purification of AhpC from *H. pylori* were attempted by various techniques including ammonium sulfate precipitation, dialysis, preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroelution. Furthermore mice were immunized intraperitoneally with homogenized gel containing the AhpC band of protein extract of *H. pylori* in sodium dodecyl sulfate- polyacrylamide gel electrophoresis. The monoclonal antibody was produced using the hybridoma technique.
Results: One-dimensional preparative gel electrophoresis allows a single and short purification step, the high-resolution capacity of this technique leads to a high level of purity of the enzyme and consequently to a very high specificity of the antibody. The high specificity of antibody was identified by immune blotting in which the antibody reacted with the purified AhpC and whole cell protein extract from *H. pylori* in addition to the intact cells of *H. pylori*.
Conclusion: This approach is simple, time and cost-saving for preparation of monoclonal antibody against AhpC of *H. pylori*.

Abstract no.: P10.06

IS GENOTYPE HELICODR METHOD EFFICIENT ENOUGH TO DETECT *HELICOBACTER PYLORI* IN COMPARISON WITH TRADITIONAL PHENOTYPIC METHODS?

T. Becerikli,* Ö. Yılmaz,* E. Demiray-Gürbüz,* M. Soytürk,† S. Sarıoğlu,‡ H. Ellidokuz§ and İ. Şimşek†
*Department of Medical Microbiology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; †Department of Gastroenterology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; ‡Department of Pathology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; §Department of Medical Informatics and Biostatistics, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey

Aim: To assess effectiveness of GenotypeHelicoDR to detect *H. pylori* and determination of clarithromycin and levofloxacin susceptibility with regard to culture and E-test.
Methods: Antrum and corpus biopsy specimens from 58 patients with dyspepsia (40F, 18M; 41.1 ± 13.5 years) were studied by GenotypeHelicoDR, culture and E-test. *H. pylori* positivity was defined according to at least two positivity of culture, histopathology and RUT. PCR was performed among 11 of 21 patients' discrepant results for clarithromycin susceptibility and PCR products (898 bp) sent for sequencing to detect point mutations.
Results: Fifty-one (87.9%) patients were *H .pylori* positive according to our criteria. Fifty (86.2%) of them were culture positive. Thirty-four (68%), 35 (70%) patients were susceptible; 10 (20%), 9 (18%) were resistant; 6 (12%), 6 (12%) patients' antrum and corpus presented a double population of susceptible and resistant bacteria by E-test for clarithromycin and levofloxacin, respectively. All patients were *H. pylori* positive by GenotypeHelicoDR; 36 (62.1%), 33 (56.9%) were susceptible; 5 (8.6%), 11 (19%) were resistant; 17 (29.3%), 14 (24.1%) presented a double population for clarithromycin and levofloxacin, respectively. Phenotypic and genotypic profiles of resistance to clarithromycin and levofloxacin were different. Among sequence analyses of 11 patients' strains, 10 of them were correlated with GenotypeHelicoDR. Three new mutations (C2136T, C2310A, C2428T) were determined in three patients' sequence analyses conferring wild type for known main three mutations associated with clarithromycin resistance and E-test results were also resistant for clarithromycin.
Conclusion: Although GenotypeHelicoDR didnot correlate enough with culture and E-test in our setting, three new mutations were incidentally detected through discordant results. This method is a rapid, culture independent with the ability to study directly from biopsy and also has capability for detection of mixed infections including both wild type and resistant *H. pylori* strains.

Abstract no.: P10.07

RAPID IN-OFFICE *H. PYLORI* SEROLOGIC TEST: A USEFUL TOOL IN AREAS WITH HIGH *H. PYLORI* PREVALENCE?

G. M. Martins,* B. S. F. Sanches,† L. D. Moretzsohn† and L. G. V. Coelho†
*Santa Casa de Belo Horizonte, Belo Horizonte, Brazil; †Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Introduction: Although test-and-treat strategy has been recommended in Brazil, there are few alternatives to provide simple and cheap tests to diagnose *H. pylori* (HP) infection in primary care setting.
Aim: To determine the performance of a rapid, in-office immunochromatographic serologic test for the detection of HP infection in Brazilian patients.
Methods: One hundred and ninety-five dyspeptic patients, never treated to HP infection, underwent upper endoscopy in addition to antrum and corpus biopsies to search HP by histology and urease test. A rapid serologic test (Abon Biopharm, Hangzhou, China) was performed immediately before the endoscopy, using capillary blood obtained by a fingerstick. If the specimen contained HP antibodies, a colored line appeared after 10 minute in the test line region of the strip. To be included in the study the results of histology and urease tests had to be concordant (gold standard). Sensitivity, specificity, positive and negative predictive values and accuracy of the serologic test were determined.
Results: Fourteen patients presented discordant histology and urease tests and 13 patients failed to show a control colored line in the test strip, and were excluded. Then, 168 patients, 68% female, mean age 39 (18–74) years, 52% HP positive, were included. Serology showed a sensitivity of 86.4% and specificity of 98.8%. The positive and negative predictive values of the test were 99.2% and 79.6%, respectively; the accuracy was 90.7% (considering a 65% HP prevalence in Brazil).
Conclusion: The rapid serologic test provided an accurate diagnosis of HP infection, showing a high positive predictive value. Therefore, could be an appropriate option for the initial diagnosis of HP infection in the context of test-and-treat strategy in areas with high HP prevalence.

Abstract no.: P10.08

COMPARISON OF ENDOSCOPIC FINDINGS FROM NIGERIAN PATIENTS WITH GASTRODUODENAL DISEASES WITH *H. PYLORI* VIRULENCE FACTORS

M. A. Fowora,* U. Breithaupt,† A. Otegbayo,‡ A. Akere,‡ C. Onyekwere,§ D. Ndububa,¶ O. A. Lesi,** F. Abdulkareem,** E. E. Anomneze,†† A. T. Seriki,‡‡ A. Adeleye,‡‡ E. Omonigbehin,* B. Moses,* M. Ijeh,* S. Muller,§§ R. Haas† and S. I. Smith*
*Nigerian Institute of Medical Research, Lagos, Nigeria; †Max von Pettenkofer Institute, Munich, Germany; ‡University College Hospital, Ibadan, Nigeria; §Lagos State University Teaching Hospital, Lagos, Nigeria; ¶Obafemi Awolowo University Teaching Hospitals Complex, Ile Ife, Nigeria; **Lagos University Teaching Hospital, Lagos, Nigeria; ††Health Gate Clinics, Lagos, Nigeria; ‡‡University of Lagos, Lagos, Nigeria; §§LMU, Munich, Germany

Background: *Helicobacter pylori* is the causative agent of gastritis, ulcer and is a risk factor in the development of gastric cancer (GC) (Blaser et al. 1995). The study is aimed at comparing the endoscopic findings from isolated *H. pylori* in Nigeria with their virulence factors.
Methods: A total of 83 isolates of *H. pylori* were screened for their virulence factors using PCR for cagA, vacA and dupA genes and compared with their

stomach endoscopic findings. Endoscopic findings included: (1) normal mucosa, (2) superficial mucosal lesions, (3) duodenal ulcers (DU) and (4) polyp depression.

Results: Isolates from subjects with superficial mucosal lesions were the highest (50.6%) then normal endoscopic findings (36.1%), ulcer (9.6%) and polyp (2.1%). From ulcer subjects, all were positive for cagA, vacAs1m1, 80% positive for the dupA genes. Those with normal mucosa showed cagA (82.1%), vacA s1b (86.2%), m1 (72.4%) and dupA2 (34.5%).

Discussion: The results show that majority of the isolates were positive for the virulence genes irrespective of the endoscopic findings. This is the first report about the presence of dupA genes in Nigerian isolates, with majority being dupA2. Only two (2.4%) of the isolates were positive for both dupA1 and dupA2 and were from subjects presenting with superficial mucosal lesion. The dupA gene is a risk marker for DU development and a protective factor against gastric cancer (GC), there was no GC case in our study.

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Abstract no.: P10.09

IMPACT OF CHANGES IN *H. PYLORI* INFECTION RATE AND UPPER GASTROINTESTINAL ENDOSCOPE FINDINGS IN JAPAN

T. Kawai,* H. Sugimoto,* M. Fukuzawa,* M. Fukuzawa,† C. Kusano,† T. Gotoda† and F. Moriyasu†

*Endoscopy Center, Tokyo Medical University Hospital, Tokyo, Japan; †Gastroenterology and Hepatology, Tokyo Medical University Hospital, Tokyo, Japan

Background: In this study, we investigated changes in the prevalence of *Helicobacter pylori* infection and the relationship between *H. pylori* infection and endoscopicfindings and diagnoses.

Subjects and Methods: The subjects were 419 patients who underwent *H. pylori* serological test and endoscopy.

Endoscopic diagnoses available were reflux esophagitis (RE), red streaking (RS), fundic polyps (FP), hyperplastic polyps (HP), erosive gastritis (EG), gastric xanthoma (GX), endoscopic erythematous/exudative gastritis (EE/EG), nodular gastritis (NG), gastric ulcer (GU), and duodenal ulcer (DU).

Results: The overall *H. pylori* infection rate was 33.7% (141/419). Infection rates by age group were: 20–29; 15.7% (11/70), 30–39; 28.0% (42/150), 40–49; 34.3% (49/143), and 50–59; 69.6% (39/56). The proportions of the endoscopic diagnoses RE, RS, EG, FP, HP, GX, EE/EG,NG, GU and DU were 23.4%, 39.9%, 14.4%, 8.6%, 0%, 0%, 0%, 0%, 1.1% and 1.4%,respectively, in *H. pylori* (−) subjects. In the *H. pylori* (+) subjects, these were 11.4%, 5.0%, 14.9%, 0.7%, 4.3%, 5.0%, 22%, 4.3%, 13.4%, and 21.9%.

Conclusions: *H. pylori* infection rates were considerably reduced in comparison to previous reports in Japan. Clear differences were also seen in the endoscopic findings according to thepresence of *H. pylori* infection.

Abstract no.: P10.10

IMPRINT FISH (I-FISH): A NOVEL APPROACH IN FISH TECHNIQUE FOR *HELICOBACTER PYLORI* INDEPENDENT FROM PARAFFIN-EMBEDDED TISSUE SECTION

Y. Erzurumlu,* T. Becerikli,† Ö. Yılmaz,† M. Soytürk‡ and I. Şimşek‡

*Department of Biochemistry, Faculty of Pharmacy, Ege University, İzmir, Turkey; †Department of Medical Microbiology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; ‡Department of Gastroenterology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey

Aim: To develop a rapid FISH method to detect *H. pylori* and determination of clarithromycin resistance simultaneously apart from paraffin-embedded-tissue-sections.

Methods: Imprint slides of fresh/frozen gastric biopsy specimens were specially prepared on poly-L-lysine coated slides. Before the preparation of slides for gastric biopsy FISH, biopsies were quite minced with french press in PBS to obtain well homogenate. Slide preparations were performed during cultivation. After heat-fixation, chemical fixation performed using formaldehyde and ethanol. Slides were washed with 1X PBS. For dehydration, slides were incubated in 50%, 80%, 96% ethanol, respectively. Hybridization steps were performed according to commercially available kit procedure (BACTfish™, Hungary) for both I-FISH and directly gastric biopsy FISH. Slides were visualized by fluorescence microscopy. I-FISH was confirmed with culture, E-test, gastric biopsy and paraffin-embedded-tissue-section FISH.

Results: I-FISH and gastric biopsy FISH allowed the detection of whole bacteria and determination of clarithromycin susceptibility. Fixation steps were successfully achieved which are very important for I-FISH technique and also gastric biopsy mincing step is very important to obtain homogenous specimen. I-FISH was concordant with other techniques which were performed for confirmation. Conclusion

I-FISH and gastric biopsy FISH are rapid, timesaving, paraffin-embedded-tissue-section and culture independent techniques. These FISH techniques result nearly in 1.5–2 hours although it takes 2–10 days for culture and several days to get paraffin-embedded-tissue-sections from Pathology Department. Imprint approach can be applied as a point-care-testing during endoscopy and could be used instead of conventional culture and antimicrobial susceptibility testing for clarithromycin when a quick decision is necessary for patients with treatment failure.

Abstract no.: P10.11

EVALUATION OF A NOVEL CHEMILUMINISCENT INMUNOASSAY FOR THE DETECTION OF *HELICOBACTER PYLORI* ANTIGEN AND THEIR CORRELATION WITH IMMUNOCHROMATOGRAPHIC RAPID TEST IN STOOLS SAMPLES FROM ADULTS PATIENTS

J. Vargas,*,† M. López-Sánchez,* M. Pabón,‡ E. Lamas,‡ M. Parra* and M. Castro-Fernández†,‡

*Unit of Infectious Diseases and Microbiology, Valme University Hospital, Seville, Spain; †CIBERehd, Seville, Spain; ‡Unit of Clinical Management of Digestives Diseases, Valme University Hospital, Seville, Spain

Background: The diagnosis of infection with *H. pylori* can be achieved by non-invasive test. The ^{13}C Urea Breah Test (UBT) remains the best method. Alternatively can be used a stool antigen test (SAT).

Aim: Evaluate a new test LIAISON *H. pylori* SA (CLIA) (DiaSorin, Italy). We analized the correlation with inmunochromatographic RAPID Hp StAR test (IC) (Oxoid, UK), the actual assay used in clinical routine en our lab. The comparison was made by using UBT (BreathTek, Otsuka) as "gold standard".

Methods: We present preliminary results of a study that continues. It includes a total of 103 dyspeptic patients (57 women) with a median age of 40 years. The *H. pylori* status was based on the results of UBT (cutoff >2.4 DOB). Fecal samples were collected simultaneously and two SATs were conducted as described above. The statistical analysis were made with SPSS v19.0.

Results: In 50 patients (48.5%) the UBT was positive. Of them, CLIA and IC were positive in 36 (36.9%). In 53 cases with negative UBT, one patient had a positive result for both SATs, one patient was positive by CLIA and one patient was positive by IC. The sensitivity, specificity, positive and negative predictive value for both SATs was 72%, 96.2%, 94.7% and 78.5% respectively. The agreement between UBT and both SATs was good (κ = 0.69). The comparison between SATs showed a very good correlation between them (κ = 0.87). There were six discordant results.

Conclusion: The CLIA is a useful method for the diagnosis. Both SATs show high specificity, but the CLIA provides an interpretation more objective of the results and its automation is better for laboratory processing. The sensitivity observed lead us to an individual assessment of the discordant cases between UBT and SATs.

Abstract no.: P10.12

FISH-BASED METHOD FOR IN VIVO DETECTION OF *H. PYLORI* IN GASTRIC MUCOSA USING ADVANCED LNA PROBES

S. Fontenete,*,†,‡ N. Guimarães,*,†,‡ M. Leite,† P. Madureira,§,¶ C. Figueiredo,†,** J. Wengel‡ and N. F. Azevedo*

*LEPAE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal; †IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; ‡Nucleic Acid Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark; §ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal; ¶IBMC, Institute for Molecular Biology and Cell Biology, Porto, Portugal; **FMUP, Faculty of Medicine of Porto University, Porto, Portugal

In the last years, there have been several attempts to improve the diagnosis of infection caused by *Helicobacter pylori*. Fluorescence in situ hybridization (FISH) is one of the techniques used to detect *H. pylori* infection but it requires biopsies

from the human stomach. Thus, the development of an in vivo FISH-based method to allow the direct detection and visualization of this bacterium within the human body, without the need of removing the biopsy, would significantly reduce the time of analysis and allow the diagnostic to be performed during endoscopy. Using locked nucleic acid (LNA) and 2′-O-methyl RNA (2′OMe) nucleotides, we developed a new FISH-based methodology for the detection of *H. pylori* under conditions mimicking the human gastric microenvironment. First, a phosphothioate LNA/2′OMe probe was designed and synthesized using standard phosphoramidite chemistry. FISH hybridization was then successfully performed on slides and in suspension at 37°C during 30 minutes using only urea and sodium chloride in a range of acidic pH. The efficiency of hybridization was quantitatively analyzed by measuring fluorescence intensity by microscopy and flow cytometry. The sensitivity and specificity of the FISH method was maintained even at very low pH conditions. Therefore, this approach is promising and might be used in vivo in the future in combination with a confocal laser endomicroscope for *Helicobacter pylori* visualization. Future work will focus on assessing the toxicity of the method and on using animal models as a proof-of-concept of the method in vivo.

Abstract no.: P10.13

COMPARATIVE EVALUATION OF EFFICIENCY OF HELIC AMMONIA BREATH TEST FOR DIAGNOSTICS OF *H. PYLORI* INFECTION IN GASTRIC MUCOUS

M. R. Konorev, M. E. Matveenko and E. N. Komleva
Medical University, Vitebsk, Belarus

Objectives: To determine the efficiency of HELIC Ammonia Breath Test or HELIC ABT (AMA, Russia) for diagnostics of *H. pylori* (Hp) in gastric mucous at patients with dyspepsia syndrome.
Materials and Methods: Blind, diagnostic, cross-section study of 68 patients (the mean age 37.2 ± 15.2 years, 21 men and 47 women) with application of Latin square (table 2 × 2) for HELIC ABT was carried out. Examination of *H. pylori* infection in gastric mucosa by Giemsa and rapid urease test (HELPYL-test, AMA, Russia) were used as comparison methods. Examination of *H. pylori* from five parts of the gastric mucosa (two biopsy specimens from the body, one biopsy sample from the corner, two biopsies from the antrum part of the stomach) was carried out.
Results: Of estimation of efficiency HELIC ABT (examination of *H.pylori* by Giemsa and rapid urease test as comparison methods respectively): sensitivity (Se) – 0.9; 0.95; specificity (Sp) – 0.91; 0.83; prevalence (P) – 0.5; 0.7; test accuracy (TA) – 0.9; 0.92; negative predictive value (−PV) – 0.91; 0.9; positive predictive value (+PV) – 0.9; 0.94; positive likelihood ratio (LR+) – 10.0; 5.6; negative likelihood ratio (LR−) – 0.1; 0.06. Reproducibility of the method is 0.94.
Conclusion: HELIC Ammonia Breath Test have high clinical efficiency for diagnostics *H. pylori* in gastric mucous (Se – 0.9; Sp – 0.91; TA – 0.9; Reproducibility – 0.94). HELIC Ammonia Breath Test recommended for use in therapeutic practice as a screening method.

Abstract no.: P10.14

EVALUATION OF STOOL ANTIGEN TEST ON INITIAL DIAGNOSIS AND CONTROL TEST OF *HELICOBACTER PYLORI* ERADICATION

B. S. F. Sanches,* G. M. Martins,† H. A. F. Pinto,* A. C. D. Meira,* E. J. Alves,* O. R. Trindade,* B. D. C. V. Cota* and L. G. V. Coelho*
*Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; †Santa Casa de Belo Horizonte, Belo Horizonte, Brazil

There are few non-invasive alternatives to detect *H. pylori* (HP) infection in Brazil.
Aim: To evaluate the performance of a rapid, office-based, immunochromatographic stool antigen test (SAT) for the detection of HP infection, before and after treatment, in Brazilian patients.
Methods: One hundred participants, never treated to HP infection, and 101 patients previously treated for HP infection were studied. All participants were submitted to 13C-urea breath test (UBT) (gold standard) and SAT (Abon Biopharm, Hangzhou, China) as follow: a diluted stool sample was dispensed into the sample port of the test device and the appearance of a colored line after 10 minute in the test line region of the strip indicates a positive result.
Results: Among the 100 naïve participants (56% female, mean age 41 years, 35% HP positive) SAT showed a sensitivity of 94.3% (95% CI: 0.814–0.984) and a specificity 78.5% (95% CI: 0.670–0.867). The positive and negative predictive value were 89% and 88%, respectively. The accuracy was 88.7%. Among the 101 post-treatment patients (59% female, mean age 55 years, 26.7% HP positive) SAT showed a sensitivity of 77.8% (95% CI: 0.592–0.894) and a specificity of 81% (95% CI: 0.707–0.884). The positive and negative predictive values were 88.4–66.3%, respectively. The accuracy was 79%. In all analysis we considered a 65% HP prevalence in Brazil.
Conclusions: In regions with high HP prevalence, the rapid, office-based, immunochromatographic SAT constitutes a reasonable non-invasive test to initial diagnosis of HP infection. However, it is not useful as a control test of HP eradication.

Abstract no.: P10.15

DIAGNOSIS OF *H. PYLORI* INFECTION IN ROUTINE CLINICAL PRACTICE IN RUSSIA

D. S. Bordin, O. I. Berezina, V. A. Kim, Y. A. Orobey and N. B. Patrushev
Central Research Institute of Gastroenterology, Moscow, Russian Federation

Background: *H. pylori*-associated diseases are important health problem in Russia. Diagnosis of infection should be carried out in accordance with clinical guidelines, but physicians don't always follow these recommendations.
Aim: To evaluate the diagnostic methods for *H. pylori* used in routine clinical practice.
Methods: This is first part of multicenter prospective observational program. Four hundred and seventeen patients (168 male, 249 female, age 44) with *H. pylori*-associated diseases were observed.
Results: For detection of *H. pylori* invasive and non-invasive methods were used in 65.7% (rapid urease test – 30%, histology – 14.6%, cytology – 21.1%) and 47.7% (urea breath test – 18%, antibodies in blood – 26.1%, antigen stool test – 3.6%) patients accordingly. Only in 13.4% patients were used two or three methods. Follow-up after treatment was performed in 387 patients (92.8%). For confirmation of eradication invasive and non-invasive tests were applied in 29.7% (rapid urease test – 15.3%, histology – 4.9%, cytology – 9.5%) and 74.1% (urea breath test – 37.2%, antibodies to *H. pylori* – 17.8%, stool test – 19.1%) patients accordingly. Two methods were used in 3.9%. Errors in eradication monitoring have been identified in 71%. In 62.3% control was performed <4 weeks after treatment. In 17/8% were used antibodies to *H. pylori*.
Conclusions: For identification of *H. pylori* in routine clinical practice invasive tests are used more often. On the contrary for eradication control non-invasive methods are used more often. Errors in eradication control were observed in 71%. Monitoring the routine management of patients with *H. pylori* infection and educational programs for physicians is needed.

Abstract no.: P10.16

COMPARISON OF TWO SEROLOGICAL TESTS TO DETERMINE THE CAGA STATUS IN DYSPEPTIC LATVIAN PATIENTS

D. Rudzite,*,†,‡ I. Daugule,† G. Moisejevs,*,†,‡ M. Gerhard,§ G. Göttner,¶ T. Zacher,¶ S. A. Krotov,** I. Davidjuka,* D. Janciauskas,†,†† I. Liepniece-Karele,*,†,‡‡ S. Isajevs,*,† I. Lasina,† K. Funka,†,§§ I. Kikuste,*,† A. Vanags,§§ I. Tolmanis‡,§§ and M. Leja*,†,§§
*Riga East University Hospital, Riga, Latvia; †University of Latvia, Riga, Latvia; ‡Riga Stradins University, Riga, Latvia; §Institut für Medizinische Mikrobiologie, Immunologie und Hygiene, Technische Universität, Munich, Germany; ¶Mikrogen Diagnostik, GmbH, Munich, Germany; **Vector Best, Novosibirsk, Russian Federation; ††Lithuanian University of Health Sciences, Kaunas, Lithuania; ‡‡Academic Histology laboratory, Riga, Latvia; §§Center of Digestive Diseases "GASTRO", Riga, Latvia

Introduction: Clinical outcomes of *Helicobacter pylori* (Hp) infection are associated both with host and bacterial virulence factors. It has been shown that presence of cytotoxin-associated gene (*cagA*), responsible for CagA protein production, is associated with more severe clinical outcome such as atrophic gastritis and adenocarcinoma. Aim of the study was to find out if there is interrater agreement between two methods for detection of Hp anti-CagA antibodies: enzyme-linked immunosorbent assay (ELISA) and immunoblot.
Materials and Methods: Sera from 303 patients (median of age 61 years, males/females 112/191) with dyspeptic symptoms coming for diagnostic gastroscopy were collected. IgG immunoblot (Mikrogen Diagnostik, Germany) and total IgM, IgA and IgG ELISA (Vector-BEST, Russia) were used to detect Hp

anti-CagA antibodies according to the manufacturer's instructions. Inter-rater agreement was calculated as kappa (k) value.
Results: A significant inter-rater agreement between both test systems ($\kappa = 0.592$, $p = 0.043$) for anti-CagA detection was observed. As shown in table immunoblot system identified extra 59 positive cases.

	Anti-CagA (Immunoblot)		
	Negative	Positive	Total
Anti-CagA (ELISA)			
Negative	88	59	147
Positive	2	154	156
Total	90	213	303

Conclusions: Both test system has good inter-rater agreement, but immunoblot maybe considered as more sensitive test system for detection of CagA status. Further research comparing the data for Hp infection status and clinical outcome is necessary. Questionable remains time period in which anti-CagA disappear after eradication of HP.
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Abstract no.: P10.17

ALMAGATE INTERFERENCE IN BREATH TEST RESULTS FOR THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION. ALMATEST STUDY

C. Pons Vilardell,*,† S. Maisterra,† S. Salord,† À. Pla,† D. Asensio,‡ F. J. Fernández,‡ G. Traveria§ and G. Roura§
*Hospital de Bellvitge, Hospitalet de Llobregat, Spain; †CAP Just Oliveres, Hospitalet de Llobregat, Spain; ‡Almirall, S.A., Barcelona, Spain; §Adknoma Health Research, S.L., Barcelona, Spain

Introduction: The Urease Breath Test (UBT) is a non-invasive test widely used by physicians for its accuracy in detecting *Helicobacter pylori*, however, the commonest reason for false negative tests is breath testing too soon after a course of antibiotics, bistmuth or omeprazole.
Objective: To determine whether administration of almagate interferes with UBT for the diagnosis of HP infection.
Subjects and Methods: Pilot, unicentre, observational, postauthorization study with consecutive sampling in a primary care medical centre in Spain. Thirty patients aged over 18 years who were treated with almagate prior to completion of the UBT and who were not treated with antibiotics or proton pump inhibitors in the previous 30 days were included. The data were collected by the investigator at a baseline visit, during which the UBT was performed. If a UBT negative result was obtained, almagate was withdrawn for at least 30 days, after which the UBT was performed again to detect possible false negatives.
Results: We analyzed data from 27 patients (70.4% female). Mean age was 56.9 ± 17.4 years. 92.6% of patients had dyspepsia, 40.7% abdominal pain and 37.0% heartburn. 59.3% of patients were suspected of having HP infection. 22.2% of patients had a history of duodenal ulcer and 11.1% had gastric ulcer and MALT lymphoma. Regarding the main objective, 51.9% of patients had a negative UBT at baseline visit, which was confirmed at the following visit in 100.0% of cases.
Conclusions: Almagate does not interfere with the result of UBT for the diagnosis of HP infection.

Abstract no.: P10.18

PROBE4PYLORI®: A NEW KIT FOR THE RAPID DETECTION OF *H. PYLORI* AND ASSOCIATED CLARITHROMYCIN RESISTANCE IN GASTRIC BIOPSIES

L. Cerqueira,* R. M. Fernandes,* R. M. Ferreira,† M. Oleastro,‡ F. Carneiro,†,§,¶ C. Brandão,§,** P. Pimentel-Nunes,§,** M. Dinis-Ribeiro,§,** C. Figueiredo,†,§ C. W. Keevil,†† M. J. Vieira* and N. F. Azevedo*,‡‡
*IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal; †IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; ‡National Institute of Health Dr. Ricardo Jorge, Avenida Padre Cruz, Lisboa, Portugal; §Faculty of Medicine of the University of Porto, Porto, Portugal; ¶Centro Hospitalar São João, Department of Pathology, Porto, Portugal; **Department Gastroenterology, Portuguese Oncology Institute Porto, Porto, Portugal; ††Environmental Healthcare Unit, School of Biological Sciences, University of Southampton, Southampton, UK; ‡‡LEPAE, Faculty of Engineering, University of Porto, Porto, Portugal

In this study an evaluation of Probe4Pylori®, a previously established PNA-FISH diagnostic test for *H. pylori* clarithromycin resistance in paraffin embedded gastric biopsies was performed and compared against culture followed by E-test and PCR. The Probe4Pylori® showed very promising results with values of sensitivity (80%) and specificity (approximately 90%) for the patients harboring clarithromycin-resistant *H. pylori*. Due to the fact that different biopsies from the same patient were used for culture and for molecular methods, the relatively low value of sensitivity can be explained by the heterogeneous distribution of *H. pylori* cells in the stomach, as for some patients only one of the biopsies contained *H. pylori*. Furthermore, the results from Probe4Pylori® did not seem to be affected by previous treatments with antimicrobials and proton pump inhibitors. It is also the only method tested here that allows direct visualization of different *H. pylori* strains (resistant and susceptible). Future application of this kit will hopefully engage the administration of more adequate therapies to eradicate this bacterium.

Abstract no.: P10.19

THE HISTOLOGY AND RAPIDE UREASE TEST ASSOCIATION: A GOOD ALTERNATIVE OF UBT IN THE CONTROL OF POST THERAPEUTIC *H. PYLORI* ERADICATION

M. Boudjella, A. Tebaibia, F. Mouffok, Y. Saadaoui, N. Chiali, M. Lahcene and N. Oumnia
Algerian Laboratory Reaserch on Helicobacter, Algeria, Algeria

Aim: To evaluate performance of Histology and Rapid Urease Test (RUT) association in the control of *Helicobacter pylori* (*H.pylori*) post therapeutic eradication.
Patients and Methods: In this prospective study, *H.pylori* testing was performed in 120 consecutive adult dyspeptic patients (mean age: 33.8; NUD: 53, DU: 96) not using proton pomp inhibitors or antibiotics during the 4 weeks before testing. Each patient had had histology, RUT and UBT. *H.pylori* was defined when UBT was positive and was absent when UBT was negative. All patients have been treated by different triple therapies. Twelve weeks after the end of therapy, they were reevaluated by the three tests. *H.pylori* eradication was confirmed by the negativity of UBT.
Results: Sensitivity of UBT, histology, RUT were respectively 98.3% (95%CI: 90–99), 83.5% (95%CI: 74.5–90),75.3% (95%CI: 65.3–83.2).Their positive predictive value (PPV) were respectively 91% (95%CI: 80.6–96.3), 100% (95%CI: 94–100), 97.3% (95%CI: 89.8–98.3). Sensitivity of histology and RUT association was 91% (95%CI: 80.6%–96.3). Its PPV was 98.3% (95%CI: 90–99).
Conclusions: After treatment, the sensitivity of histology and RUT association was high while its PPV was good. Therefore, it can be a good alternative to UBT in the control of *H.pylori* post treatment eradication.

Abstract no.: P10.20

BREATH AMMONIUM TEST IN DIAGNOSTIC OF *HELICOBACTER PYLORI* INFECTION

N. V. Baryshnikova,*,† L. N. Belousova,‡ A. S. Smirnova,*,† E. E. Tsopanova,‡ I. A. Loeva,‡ O. Y. Orlov† and E. A. Pavlova†

*St-Petersburg State Medical University n.a. I.P. Pavlov, St-Petersburg, Russian Federation; †St-Petersburg State Budget Healthcare Institution "City Hospital of Saint Venerable Martyr Elizabeth", St-Petersburg, Russian Federation; ‡North-West State Medical University n.a. I.I. Mechnikov, St-Petersburg, Russian Federation

Background: A breath test with C13 urea is not common in use in Russian gastroenterology practice. It is important to make an alternative breath tests to diagnose *Helicobacter pylori* infection. The aim: to compare results of different methods of diagnostics of *H. pylori* infection with estimation of efficacy of breath ammonium test.

Materials and Methods: Forty-two patients with dyspepsia were under supervision. To all patients the gastroscopy with a biopsy from stomach body and antrum and a complex of diagnostic methods for infection verification were made. Four diagnostic methods were used: breath ammonium test ("Helic-test", Association of Medicine and Analytic, St-Petersburg), histological method (by dr. Antonov P.V., St-Petersburg), polymerase chain reaction (PCR) with detection of *ureC, ureI, cagA* genes (Laboratory "Diagnostic", St-Petersburg), the breath test with C13 urea (the analysis of samples of exhaled air was made in Italy in "Spectra-2000" laboratory). Samples of exhaled air were transported to Italy one time a month. Results

By "Helic-test" the positive result was received in 50% of patients. By histological method *H. pylori* was defined in 48% and by PCR – in 50% of patients. Unexpected there were results of breath urease test with C13 urea: 26% of positive results.

Conclusions: Breath ammonium test shows a high efficacy in comparison to histological method and PCR and can be recommended to use in diagnostic of *H. pylori* infection. Low percentage of positive results of breath urease test with C13 urea is probably connected with long process of transportation. Therefore, it is necessary to avoid long storage of samples.

Abstract no.: P10.21

A NOVEL LATERAL FLOW TEST STRIP (LFTS) FOR THE DETECTION OF *HELICOBACTER PYLORI* INFECTION

B. A. Salih, C. Karakus, Z. Ulupinar and F. Akbas

Fatih University, Istanbul, Turkey

The diagnoses of *Helicobacter pylori* infection is done invasively by endoscopy. Non-invasive tests such as the ELISA and LFTS can help in diagnoses by detecting serum antibodies. *H. pylori* cagA gene an important virulent factor encodes an immunodominant CagA protein. The available kits generally uses bacterial lysate for the detection of such factor thus the demand for a pure recombinant CagA (rCagA) protein will have an impact on the detection processes. Our aim is to develop a LFTS using rCagA and monoclonal antibodies for the detection of anti-CagA antibodies in patient sera. The cagA 5′ conserved region of the gene was cloned and a rCagA of 67 kDa was obtained. The rCagA was then conjugated to gold nanoparticles and placed onto the conjugate pad. A non-conjugated rCagA was immobilized on the test line. Anti-CagA monoclonal antibodies were immobilized on the control line. The addition of a sample drop onto the sample pad lead to a lateral flow of the sample fluid containing anti-*H. pylori* antibodies toward the conjugate pad where it bound to the antigen coated on gold particles. The complex then flow to the test line where it bound to the immobilized antigen and resulted in a red color line. The flow of the sample fluid continued toward the control line where the remaining antigen coated gold particles bound to the immobilized anti-CagA monoclonal antibodies and gave a red color line. This test shows a very promising tool that aid in the diagnoses of *H. pylori* infection.

P11 Clinical trials and novel treatments

Abstract no.: P11.01

SECOND-LINE RESCUE THERAPY WITH LEVOFLOXACIN AFTER FAILURE OF TREATMENT TO ERADICATE *HELICOBACTER PYLORI* INFECTION: TIME TRENDS IN A SPANISH MULTICENTER STUDY OF 1300 PATIENTS

J. P. Gisbert,* A. Perez-Aisa,† F. Bermejo,‡ M. Castro-Fernandez,§ P. Almela,¶ J. Barrio,** A. Cosme,†† I. Modolell,‡‡ F. Bory,§§ M. Fernandez-Bermejo,¶¶ L. Rodrigo,*** J. Ortuño,††† P. Sanchez-Pobre,‡‡‡ S. Khorrami,§§§ A. Franco,¶¶¶ A. Tomas,**** I. Guerra,‡ E. Lamas,§ J. Ponce††† and X. Calvet††††

*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Agencia Sanitaria Costa del Sol, Malaga, Spain; ‡Hospital de Fuenlabrada, Madrid, Spain; §Hospital de Valme, Sevilla, Spain; ¶Hospital General de Castellon, Castellon, Spain; **Hospital Rio Hortega, Valladolid, Spain; ††Hospital de Donostia, San Sebastian, Spain; ‡‡Consorci Sanitari de Terassa, Barcelona, Spain; §§Hospital del Mar, Barcelona, Spain; ¶¶Hospital San Pedro de Alcantara, Caceres, Spain; ***Hospital Central de Asturias, Oviedo, Spain; †††Hospital La Fe, Valencia, Spain; ‡‡‡Hospital Clinico San Carlos, Madrid, Spain; §§§Hospital Son Espases, Mallorca, Spain; ¶¶¶Hospital 12 de Octubre, Madrid, Spain; ****Hospital General de Cataluña, Barcelona, Spain; ††††Hospital de Sabadell, Barcelona, Spain

Background: Second-line bismuth-containing quadruple therapy is complex and frequently induces adverse effects. A triple rescue regimen containing levofloxacin is a potential alternative; however, resistance to quinolones is rapidly increasing.

Aim: To evaluate the efficacy and tolerability of a second-line triple regimen containing levofloxacin in patients whose *Helicobacter pylori* eradication treatment failed and to assess whether the efficacy of the regimen decreases with time.

Methods: Design: Prospective multicenter study. Patients: Patients in whom treatment with a regimen comprising a PPI, clarithromycin, and 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 using the 13C-urea-breath test 4–8 weeks after therapy. Compliance/tolerance: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

Results: The study sample comprised 1315 consecutive patients (mean age: 49 ± 15 years, 59% female, 31% peptic ulcer) of whom 96% took all medications correctly. Per-protocol and intention-to-treat eradication rates were 74.5% (95%CI = 72–77%) and 73.2% (95%CI = 71–76%). Efficacy (intention-to-treat) was 76% in the year 2006, 68% in 2007, 70% in 2008, 76% in 2009, 73% in 2010, 71% in 2011, and 75% in 2012. In the multivariate analysis, none of the studied variables (including diagnosis and year of treatment) were associated with success of eradication. Adverse effects were reported in 19% of patients, most commonly nausea, metallic taste, myalgia, and abdominal pain.

Conclusion: Ten-day levofloxacin-containing therapy is an encouraging second-line strategy, providing a safe and simple alternative to quadruple therapy in patients whose previous standard triple therapy has failed. The efficacy of this regimen remains stable with time.

Abstract no.: P11.02

EMPIRICAL RESCUE THERAPY AFTER *H. PYLORI* TREATMENT FAILURE. A 15-YEAR SINGLE CENTER STUDY OF 650 PATIENTS

J. P. Gisbert, J. L. Gisbert and S. Marcos

La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain

Aim: To evaluate the efficacy of different "rescue" therapies empirically prescribed during 15 years to 650 patients in whom at least one eradication regimen had failed to cure *H. pylori* infection.

Methods: Design: Prospective single-center study. Patients: Consecutive patients in whom at least one eradication regimen had failed. Intervention: The first eradication treatment always included a standard triple therapy (PPI-clarithromycin-amoxicillin). Rescue regimens included: 2nd- or 3rd-line: PPI (standard dose b.i.d.), amoxicillin (1 g b.i.d.) and levofloxacin (500 mg b.i.d.) for 7–10 days; or bismuth quadruple therapy with PPI (standard dose b.i.d.), bismuth (120 mg q.i.d.), tetracycline (500 mg q.i.d.) and metronidazole (250 mg/q.i.d.); 4th-line: PPI (standard dose b.i.d.), amoxicillin (1 g b.i.d.) and rifabutin (150 mg b.i.d.) for 10 days. Antibiotic susceptibility was unknown (rescue regimens were chosen empirically). Outcome: Eradication was defined as a negative 13C-urea breath test 4–8 weeks after completing therapy.

Results: Six hundred and fifty patients were included (65% females, mean age 50 years, 81% functional/noninvestigated dyspepsia, 19% peptic ulcer): 2nd-line (513 patients), 3rd-line (110 patients), and 4th-line (27 patients). Compliance with 2nd, 3rd and 4th-line regimens was 93% in all cases. Adverse effects were reported by 35%, 30%, and 37% of the patients receiving 2nd, 3rd, and 4th-line regimens. Overall, *H. pylori* cure rates with 2nd, 3rd, and 4th-line rescue regimens were 75% (95%CI = 71–78%), 75% (66–83%), and 59% (39–80%). Specifically, 2nd-line levofloxacin-containing triple therapy cured 79% of patients, and 3rd-line bismuth-quadruple therapy cured 71%. Cumulative *H. pylori* eradication rate with four successive treatments was 99.5%.

Conclusion: It is possible to construct an overall treatment strategy to maximize *H. pylori* eradication, based on the administration of four consecutive empirical regimens; thus, performing bacterial culture even after a second or third eradication failure may not be necessary in clinical practice.

Abstract no.: P11.03

EFFICACY OF CLARITHROMYCIN-SUSCEPTIBILITY BASED TAILORED *HELICOBACTER PYLORI* ERADICATION TREATMENT MAINTAINING ACID SECRETION FOR A FULL 24 HOUR

M. Sugimoto, T. Uotani, S. Sahara, H. Ichikawa and T. Furuta

Hamamatsu University School of Medicine, Hamamatsu, Japan

Background: The eradication rate of *Helicobacter pylori* has gradually decreased for two reasons: increased prevalence of CAM-resistant strains and acid inhibition during treatment. However, the optimum regimen of PPIs is unknown at present. We therefore assessed acid inhibitory effects of multiple PPI dosing methods and efficacy of CAM-susceptibility-based eradication by maintaining acid secretion for a full 24-h.

Methods: [Study 1] Using pH monitoring, we evaluated the efficacy of multiple dosing regimens with the rabeprazole (40 mg sid, 20 mg bid, and 10 mg qid). [Study 2] As the tailored regimen based on CAM-susceptibility, patients infected with CAM-sensitive *H. pylori* were treated with rabeprazole 10 mg qid, AMPC 500 mg qid and CAM 400 mg bid for 1 week, and with CAM-resistant were treated with rabeprazole/AMPC and MNZ 250 mg bid for 1 week (n = 153).

Results: [Study 1] Respective median 24-h pH for rabeprazole 40 mg sid, 20 mg bid, and 10 mg qid were 4.8 (3.6–6.4), 5.7 (4.1–7.4), and 6.6 (4.9–8.4). Increasing the dosing times effectively increased pH throughout a 24-h period. Rabeprazole 10 mg qid maintained pH >4 for 24 hour in CYP2C19 rapid metabolizers (RMs). [Study 2] The intention-to-treat eradication rate in tailored regimen group was 96.7% (95%CI: 92.5–98.9%, 148/153). The eradication rates in the rabeprazole/AMPC/CAM and rabeprazole/AMPC/MNZ regimen were 95.3% and 98.3%, respectively.

Discussion: Four-times daily dosing of rabeprazole 10 mg achieved potent acid inhibition in CYP2C19 RMs, suggesting its potential usefulness in patients refractory to PPI treatment. Using this PPI qid for all patients made CAM-susceptibility-based eradication treatment effective, with an eradication rate exceeding 95%.

Abstract no.: P11.04

ANTIBIOTIC SUSCEPTIBILITY -GUIDED TREATMENT OF *HELICOBACTER PYLORI* INFECTION. A SYSTEMATIC REVIEW AND META-ANALYSIS

S. L. Gongora,* X. Calvet Calvo,†,‡,§ V. García-Hernando,* I. Puig¶,** and J. P. Gisbert§,††

*Unitat Docent Parc Taulí, Facultat de Medicina, Universitat Autònoma de Barcelona, Sabadell, Spain; †Corporació Sanitaria Parc Taulí, Sabadell, Spain; ‡Departament de Medicina Universitat Autònoma de Barcelona, Barcelona, Spain; §Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Madrid, Spain; ¶Digestive, Diseases Unit, Althaia Xarxa Assistencial Universitaria de Manresa, Manresa, Spain; **Universitat Internacional de Catalunya, Barcelona, Spain; ††Servicio de Aparato Digestivo, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa, Madrid, Spain

Background: Antibiotic-resistant *Helicobacter pylori* strains are becoming increasing prevalent and the cure rate of standard triple therapy has been reported to be unacceptably low in many settings. In this context, using susceptibility-guided therapies has been proposed as a valid alternative to standard treatments.

Aims: To evaluate the efficacy and feasibility of culture-guide antimicrobial treatment in a systematically review and to compare it to empirical treatment using a meta-analysis.
Methods: A systematic search was performed in PubMed. Randomized controlled trials and non-randomized clinical trials reporting sensitivity guided treatment of *H. pylori* were selected.
Results: Eighteen articles were included in the review and nine in the different meta-analyses. The meta-analysis of first-line therapies showed that the culture-guided therapy was superior to empiric triple therapy (cure rates 87% vs 73.3%, OR = 2.89, 95%CI: 1.73–4.81). Empirical therapy consisted mainly in 7–10-day triple therapies. In addition, all the studies randomized the patients after endoscopy and culture, and the applicability of culture-guided therapy in clinical practice remains unclear. For second line therapy, empiric treatment was not different from culture-guided treatment (59% vs 80% OR = 2.06, 95%CI: 0.38–11.04) although the power of the comparison was limited by the reduced number of patients. Finally, cure rates of culture-guided third-line or rescue therapy were generally poor. However, a study combining susceptibility-guided treatment and long quadruple therapies obtained 94% global cure rates.
Conclusions: The current evidence gives a weak support to the recommendation of using treatment guided by resistances at any stage of *H. pylori* therapy. Comparative efficacy and applicability of culture-guided treatments with the currently recommended first-line quadruple therapies remains unknown. The combination of susceptibility determination and 10-day quadruple therapies for rescue treatment achieved promising results and deserves further investigation.

Abstract no.: P11.05

FOURTH-LINE RESCUE THERAPY WITH RIFABUTIN IN PATIENTS WITH THREE *H. PYLORI* ERADICATION FAILURES

J. P. Gisbert,* M. Castro-Fernandez,† A. Perez-Aisa,‡ A. Cosme,§ J. Molina-Infante,¶ L. Rodrigo,** I. Modolell,†† J. L. Cabriada,‡‡ J. L. Gisbert,* E. Lamas,† S. Marcos* and X. Calvet§§
*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Hospital de Valme, Sevilla, Spain; ‡Agencia Sanitaria Costa del Sol, Malaga, Spain; §Hospital de Donostia, San Sebastian, Spain; ¶Hospital San Pedro de Alcantara, Caceres, Spain; **Hospital Central de Asturias, Oviedo, Spain; ††Consorci Sanitari de Terassa, Barcelona, Spain; ‡‡Hospital de Galdakao, Vizcaya, Spain; §§Hospital de Sabadell, Barcelona, Spain

Background: In some cases, *Helicobater pylori* infection persists even after three eradication treatments.
Aim: To evaluate the efficacy of an empirical forth-line rescue regimen with rifabutin in patients with three eradication failures, extending the experience of an ongoing multicenter study.
Methods: Design: Multicenter, prospective study. Patients: In whom the following three eradication treatments had consecutively failed: 1st treatment: PPI + clarithromycin + amoxicillin; 2nd treatment: quadruple therapy (PPI + bismuth + tetracycline + metronidazole); 3rd treatment: PPI + amoxicillin + levofloxacin. Intervention: In patients failing these three regimens, a 4th regimen with rifabutin (150 mg b.i.d.), amoxicillin (1 g b.i.d.) and a PPI (standard dose b.i.d.) was prescribed for 10 days. Outcome: Eradication was confirmed using the 13C-urea breath test 4–8 weeks after therapy. Compliance and tolerance: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.
Results: One-hundred and twenty-five patients (mean age 50 years, 41% males, 26% peptic ulcer/74% functional dyspepsia) were included. Compliance: eight patients did not take correctly the medication (in six cases due to adverse effects). Per-protocol and intention-to-treat eradication rates were 56% (95% CI = 46–65%) and 54% (44–63%). Adverse effects were reported in 42 (34%) patients, the most frequent being: nausea/vomiting (severe in two cases), asthenia/anorexia, abdominal pain, diarrhoea, fever, metallic taste, myalgia, hypertransaminasemia, leucopoenia (<1500 neutrophils, two cases), thrombopoenia (<150 000 platelets, two cases) headache, and aphthous stomatitis. Myelotoxicity resolved spontaneously in all cases.
Conclusion: Even after three 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.

Abstract no.: P11.06

LEVOFLOXACIN, METRONIDAZOLE, AND LANSOPRAZOLE TRIPLE THERAPY COMPARED TO QUADRUPLE THERAPY AS A SECOND-LINE TREATMENT OF *HELICOBACTER PYLORI* INFECTION IN KOREA

G. Kim,* B. Lee,* H. Lee,* G. Song* and S. Seol†
*Pusan National University School of Medicine, Busan, Korea; †Inje University College of Medicine, Busan, Korea

Background/Aims: Several rescue therapies have been recommended to eradicate *Helicobacter pylori* (*H. pylori*) infection in patients with first-line eradication therapy failure, but they still fail in more than 20% of the cases. The aim of this study was to evaluate the efficacy and safety of levofloxacin, metronidazole, and lansoprazole triple therapy compared to quadruple therapy as a second-line treatment of *H. pylori* infection.
Methods: A total of 123 patients who failed first-line triple therapy for *H. pylori* infection were randomly assigned to two groups: (1) levofloxacin, metronidazole, and lansoprazole (LML) for 7 days, and (2) tetracycline, bismuth subcitrate, metronidazole, and lansoprazole (quadruple) for 7 days. Successful eradication was defined as a negative ^{13}C-urea breath test 8 weeks after completion of treatment.
Results: Of the 123 patients, 56 were enrolled in the LML group, and 57 were in the quadruple group. According to intention-to-treat analysis, the infection was eradicated in 38/56 (67.9%) patients in the LML group and 48/57 (84.2%) in the quadruple group ($p = 0.042$). Per-protocol analysis showed successful eradication in 38/52 (73.1%) patients from the LML group and 48/52 (92.3%) from the quadruple group ($p = 0.010$). There were no significant differences in the adverse effects experienced by the patients in either treatment group.
Conclusions: LML therapy is less effective than quadruple therapy as a second-line treatment of *H. pylori* infection. Therefore, quadruple therapy would be considered as the primary second-line strategy for patients experiencing first-line *H. pylori* therapy failure in Korea.

Abstract no.: P11.07

RANDOMISED CLINICAL TRIAL COMPARING SEQUENTIAL AND CONCOMITANT THERAPIES FOR *HELICOBACTER PYLORI* ERADICATION IN ROUTINE CLINICAL PRACTICE

A. G. McNicholl,* A. C. Marin,* J. Molina-Infante,† M. Castro-Fernandez,‡ J. Barrio,§ J. Ducons,¶ X. Calvet,** C. de la Coba,†† M. Montoro,‡‡ F. Bory,§§ A. Perez-Aisa,¶¶ M. Forne,*** M. Ramas,* R. Millan,‡ P. Araguren,¶ P. Garcia-Iglesias,** B. Belloc,‡‡ X. Bessa,§§ E. Sainz,*** J. L. Gisbert,* E. Lamas,‡ E. Marcos,* M. I. Moreno,††† F. Abad-Santos††† and J. P. Gisbert*
*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Hospital San Pedro de Alcantara, Caceres, Spain; ‡Hospital de Valme, Sevilla, Spain; §Hospital Rio Hortega, Valladolid, Spain; ¶Hospital Clinico, Zaragoza, Spain; **Hospital de Sabadell, Barcelona, Spain; ††Hospital de Cabueñes, Gijon, Spain; ‡‡Hospital San Jorge, Huesca, Spain; §§Hospital del Mar, Barcelona, Spain; ¶¶Agencia Sanitaria Costa del Sol, Malaga, Spain; ***Hospital Muta de Terrassa, Barcelona, Spain; †††Clinical Pharmacology, La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain

Objectives: No trial has compared non-bismuth quadruple "sequential" and "concomitant" regimens in settings with increasing clarithromycin rates. The study aims were to compare the effectiveness and safety of these therapies for *Helicobacter pylori* treatment.
Methods: Prospective randomised clinical trial in 11 Spanish hospitals. Patients naïve to eradication therapy with non-investigated/functional dyspepsia or peptic ulcer disease were included. Patients were randomised (1:1) to sequential (omeprazole [20 mg/12 hour] and amoxicillin [1 g/12 hour] for 5 days, followed by 5 days of omeprazole [20 mg/12 hour], clarithromycin [500 mg/12 hour] and metronidazole [500 mg/12 hour]), or concomitant treatment (same drugs taken concomitantly for 10 days). Eradication was confirmed with 13C-urea breath test or histology 4 weeks after treatment. Adverse events (AEs) and compliance were evaluated with questionnaires and residual medication count.
Results: Three hundred and thirty-eight consecutive patients were randomised. Mean age was 47 years, 60% were women, 22% smokers and 20% had peptic ulcer. Concomitant and sequential eradication rates were, respectively, 87% versus 81% by intention-to-treat ($p = 0.15$) and 91% versus 86% ($p = 0.13$) per protocol. Respective compliances were 83% versus 82%. Treatment-emergent AEs were reported in 59% of patients (no differences found

between treatments). AEs were mostly mild (60%), and average length was 6.1 days, causing discontinuation only in 12 patients. In the multivariate analysis, concomitant treatment showed an OR of 1.5 towards better eradication rate in a borderline significance CI (95% CI, 0.9–2.8).
Conclusions: Non-bismuth quadruple "concomitant" therapy led to a non-statistically significant advantage (5%) over "sequential" therapy, coming closer to 90% cure rates. Both therapies showed an acceptable safety profile. Current data suggest that a 10-day "concomitant" regimen for first-line treatment may be the most efficient strategy for the eradication of *H. pylori* infection.

Abstract no.: P11.08

TRENDS OF SECOND-LINE ERADICATION THERAPY FOR *HELICOBACTER PYLORI* IN JAPAN: A MULTICENTER STUDY IN THE TOKYO METROPOLITAN AREA

D. Asaoka,*,† A. Nagahara,*,† T. Matsuhisa,†,‡ S. Takahashi,†,§ K. Tokunaga,†,§ T. Kawai,†,¶ K. Kawakami,†,** H. Suzuki,†,†† M. Suzuki,†,‡‡ T. Nishizawa,†,‡‡ N. Kurihara,†,§§ M. Ito,†,¶¶ H. Sasaki,*,† F. Omata,†,*** S. Mizuno,†,††† A. Torii,†,‡‡‡ T. Ohkusa,†,§§§ T. Mine†,¶¶¶ and N. Sakaki†,****
*Department of Gastroenterology, Juntendo University, School of Medicine, Tokyo, Japan; †Tokyo HP Study Group, Tokyo, Japan; ‡Department of Gastrointestinal Endoscopy, Tama-Nagayama University Hospital of Nippon Medical School, Tokyo, Japan; §Third Department of Internal Medicine, Kyorin University School of Medicine, Tokyo, Japan; ¶Endoscopy Center, Tokyo Medical University, Tokyo, Japan; **Department of Gastroenterology and Hepatology, Tokyo Medical University, Tokyo, Japan; ††Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan; ‡‡Division of Gastroenterology, National Hospital Organization Tokyo Medical Center, Tokyo, Japan; §§Department of Surgery, Nerima General Hospital, Tokyo, Japan; ¶¶Department of Gastroenterology, Yotsuya Medical Cube, Tokyo, Japan; ***Department of Internal Medicine, St. Luke's International Hospital, Tokyo, Japan; †††Department of Gastroenterology and Hepatology, Nihon University, School and Medicine, Tokyo, Japan; ‡‡‡Torii Medical Clinic, Tokyo, Japan; §§§Department of Gastroenterology and Hepatology, The Jikei University Kashiwa Hospital, Chiba, Japan; ¶¶¶Department of Gastroenterology, Tokai University School of Medicine, Kanagawa, Japan; ****Foundation for Detection of Early Gastric carcinoma, Tokyo, Japan

Background: In Japan, the eradication rate of first-line therapy for *H.pylori* with a PPI, amoxicillin (AMPC) and clarithromycin (CAM) has been decreasing because of a high prevalence of CAM resistance. A possible decrease of the eradication rate for second-line therapy with a PPI, AMPC and metronidazole (MNZ) is of concern. The aim of this study is to assess the trends in second-line eradication therapy for *H.pylori* in Japan.
Materials and Methods: We accumulated data retrospectively on patients administered second-line eradication with a PPI, AMPC and MNZ for 1 week who failed first-line eradication therapy with a PPI, AMPC and CAM in Japan from 2007 through 2011. Trends for second-line eradication rates in modified intention-to-treat analyses were investigated. Second-line eradication rates were categorized by three PPIs (rabeprazole (RPZ), lansoprazole (LPZ) or omeprazole (OMZ)) and evaluated.
Results: We accumulated data on 1373 patients. The overall second-line eradication rate was 92.4%. Second-line eradication rates in 2007, 2008, 2009, 2010 and 2011 were 97.7%, 90.6%, 94.5%, 91.8% and 91.8%, respectively, with no significant trends revealed. Second-line eradication rates categorized by three PPIs for the entire 5-year period were 91.6%, 93.4% and 92.4% (RPZ, LPZ and OPZ, respectively) with no significant differences among the three PPIs.
Conclusions: There were no significant trends in the second-line eradication rates and the rates remained consistently high. From the viewpoint of high prevalence of CAM resistance in Japan, triple therapy with PPI, AMPC and MNZ may be a better strategy for first-line therapy compared to triple therapy with PPI, AMPC and CAM.

Abstract no.: P11.09

A RANDOMISED STUDY COMPARING 10 DAYS CONCOMITANT AND SEQUENTIAL TREATMENTS FOR THE ERADICATION OF *HELICOBACTER PYLORI*, IN A HIGH CLARITHROMYCIN RESISTANCE AREA

S. D. Georgopoulos,* E. Xirouchakis,* E. Zampeli,† I. Grivas,‡ C. Spiliadi,§ M. Sotiropoulou,† F. Laoudi,* P. Kasapidis‡ and S. Michopoulos†
*Athens Medical, P. Faliron Hospital, P. Faliro, Athens, Greece; †Alexandra General Hospital, Athens, Greece; ‡Central Clinic of Athens, Athens, Greece; §Athens Medical, Amarousion Hospital, Amarousion, Athens, Greece

Aims: Our study compares the effectiveness and safety of quadruple non-bismuth "concomitant" and "sequential" regimens for *H. pylori* eradication in a high clarithromycin resistance area.
Patients and Methods: This is a prospective randomized clinical trial in three participating centers from Greece. Up to now we have included 90 *H. pylori* positive patients, without previous eradication attempt, with functional dyspepsia or peptic ulcer disease. All patients had a positive CLO-test and/or histology and/or culture. They were randomized to receive either sequential (esomeprazole 40 mg and amoxicillin 1 g bid for 5 days, followed by 5 days of esomeprazole 40 mg, clarithromycin 500 mg and metronidazole 500 mg), or concomitant treatment (all drugs taken concomitantly for 10 days). Eradication was confirmed by 13C-urea breath test or histology 4–6 weeks after treatment. Adverse events and adherence to treatment were evaluated.
Results: Forty-five patients (22F/23M, aged 18–81, mean 55 years, 25.5% smokers, 21.4% with ulcer disease) allocated to concomitant and 45 (20F/25M, aged 24–94, mean 50.8 years, 28.5% smokers, 23.2% with ulcer disease) to sequential treatment. Eradication rates were, respectively, 89% versus 82% by intention to treat (p = ns) and 93% versus 84% (p = ns) per protocol. Adherence to treatment was overall 98% (95%CI 95.9–99.6) and comparable among treatments. Treatment related side effects were reported in 45% of patients, without differences among treatment arms. Only one patient under sequential experienced severe abdominal distension.
Conclusions: Concomitant treatment has a non-statistically significant advantage (9%) over sequential therapy and was the only one overcoming 90% per protocol in a high clarithromycin resistance area. Both regimens were well tolerated and safe for the patients.

Abstract no.: P11.10

NON-BISMUTH QUADRUPLE (CONCOMITANT) THERAPY FOR ERADICATION OF *H. PYLORI*: STANDARD VERSUS OPTIMIZED (14-DAY, HIGH-DOSE PPI) REGIMEN

J. P. Gisbert,* Y. Harb,† J. Molina-Infante,‡ I. Modolell,§ R. Anton,¶ F. Bermejo,** M. Barenys,†† A. Perez-Aisa,‡‡ L. Rodrigo,§§ J. Ortuño,¶¶ A. Cosme,*** P. Almela,††† J. Alcedo,† M. Fernandez-Bermejo,‡ A. C. Marin* and A. G. McNicholl*
*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Hospital de Barbastro, Huesca, Spain; ‡Hospital San Pedro de Alcantara, Caceres, Spain; §Consorci Sanitari de Terrassa, Barcelona, Spain; ¶Hospital Clinico, Valencia, Spain; **Hospital de Fuenlabrada, Madrid, Spain; ††Hospital Viladecans, Barcelona, Spain; ‡‡Agencia Sanitaria Costa del Sol, Malaga, Spain; §§Hospital Central de Asturias, Oviedo, Spain; ¶¶Hospital La Fe, Valencia, Spain; ***Hospital de Donostia, San Sebastian, Spain; †††Hospital General, Castellon, Spain

Background: Non-bismuth quadruple "concomitant" regimen, including a PPI, amoxicillin, clarithromycin and a nitroimidazole for 10 days, is increasingly used as first-line treatment. Optimizing this regimen by increasing the PPI dose and the extending to 14 days may further increase its efficacy.
Aim: To evaluate the efficacy and tolerability of a standard and optimized "concomitant" regimens.
Methods: Design: Prospective multicenter study. Patients: Consecutive patients infected by *H. pylori*. Treatment: In a first phase, patients received a standard concomitant therapy (group A): PPI at standard dose b.i.d., amoxicillin 1 g

b.i.d., clarithromycin 500 mg b.i.d. and metronidazole 500 mg b.i.d. for 10 days. In a second phase, patients received an optimized concomitant therapy (group B): the same regimen but with esomeprazole 40 mg b.i.d. and for 14 days. Outcome: Eradication was confirmed with 13C-urea breath test 4–8 weeks after therapy. Compliance/tolerance: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

Results: Three hundred and forty-eight consecutive patients were included (mean age 50 years, 57% females, 19% peptic ulcer and 81% dyspepsia): 236 in group A (standard) and 112 in group B (optimized). Compliance with treatment was 95% and 98% in groups A and B. Per-protocol eradication rates in groups A and B were 88% (95%CI = 83–92%) and 93% (88–98%). Respective intention-to-treat cure rates were 86% (81–91%) and 93% (88–98%) ($p < 0.05$). Adverse effects (most of them mild) were reported in 38% of patients in group A and in 55% in group B ($p < 0.01$), the most common being: metallic taste, diarrhoea, vaginal candidiasis (these last three were more frequent in group B), nausea/vomiting, abdominal pain, and aphthous stomatitis.

Conclusion: Fourteen-day high-dose PPI non-bismuth quadruple "concomitant" regimen is highly effective for *H. pylori* eradication, achieving cure rates >90%. This optimized regimen seems to be more effective than the standard one.

Abstract no.: P11.11

SECOND-LINE RESCUE TRIPLE THERAPY WITH LEVOFLOXACIN AFTER FAILURE OF NON-BISMUTH QUADRUPLE "SEQUENTIAL" OR "CONCOMITANT" TREATMENT TO ERADICATE *H. PYLORI* INFECTION

J. P. Gisbert,* J. Molina-Infante,† A. C. Marin,* G. Vinagre,† J. Barrio‡ and A. G. McNicholl*

*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Hospital San Pedro de Alcantara, Caceres, Spain; ‡Hospital Rio Hortega, Valladolid, Spain

Background: Non-bismuth quadruple "sequential" and "concomitant" regimens, including a PPI, amoxicillin, clarithromycin and a nitroimidazole, are increasingly used as first-line treatments for *Helicobacter pylori* infection. Eradication with rescue regimens may be challenging after failure of key antibiotics such as clarithromycin and nitroimidazoles.

Aim: To evaluate the efficacy and tolerability of a second-line levofloxacin-containing triple regimen (PPI-amoxicillin-levofloxacin) in the eradication of *H. pylori* after non-bismuth quadruple containing treatment failure.

Methods: Design: Prospective multicenter study. Patients: In whom a non-bismuth quadruple regimen, administered either sequentially (PPI + amoxicillin for 5 days followed by PPI + clarithromycin + metronidazole for five more days) or concomitantly (PPI + amoxicillin + clarithromycin + metronidazole for 10 days) had previously failed. Intervention: levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.) and PPI (standard dose b.i.d.) for 10 days. Outcome: Eradication was confirmed with 13C-urea breath test 4–8 weeks after therapy. Compliance and tolerance: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

Results: One hundred and ten consecutive patients were included (mean age 50 years, 59% females, 13% peptic ulcer and 87% dyspepsia): 37 after "sequential", and 73 after "concomitant" treatment failure. All patients complied with treatment. Overall, intention-to-treat and per-protocol *H. pylori* eradication rates were 73.6% (95% CI = 65–82%) and 75% (66–84%). Respective per-protocol cure rates for "sequential" and "concomitant" failure regimens were 83% and 71%. Adverse effects were reported in 8 (7.3%) patients; all of them were mild (metallic taste, nausea/vomiting, abdominal pain, diarrhoea, asthenia, vaginal candidiasis, and aphthous stomatitis).

Conclusion: Ten-day levofloxacin-containing triple therapy constitutes an encouraging second-line strategy in patients with previous non-bismuth quadruple "sequential" or "concomitant" treatment failure.

Abstract no.: P11.12

AN UPDATE ON SECOND-LINE TREATMENTS EFFICACY WHEN STANDARD TRIPLE THERAPY WITH PPI, AMOXICILLIN AND CLARITHROMYCIN HAS FAILED IN *HELICOBACTER PYLORI* ERADICATION

A. C. Marin, A. G. McNicholl and J. P. Gisbert

La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain

Background: Standard triple therapy with PPI-amoxicillin-clarithromycin is used against *Helicobacter pylori* infection but it fails in ≥20% of patients.

Aim: To conduct a meta-analysis of studies assessing the efficacy of re-treatments after a PPI-amoxicillin-clarithromycin first-line eradication failure.

Methods: *Selection of studies:* Studies reporting efficacy were used for generic inverse variance, and randomized clinical trials (RCT) for meta-analyses. Inclusion criteria: studies treating *H. pylori*-positive patients after PPI-amoxicillin-clarithromycin failure. Studies were excluded if confirmation of eradication was made only by serology, PCR or polyclonal stool antigen test, or if second-line treatment was selected depending on antibiotic susceptibility. *Search strategy:* Bibliographical searches were performed in PubMed up to April 2013. Also abstracts from international congresses were included (Digestive Disease Week and European Helicobacter Study Group). *Data synthesis:* Intention-to-treat eradication rate.

Results: Thirty-five RCT (with 27 different comparisons) met inclusion criteria, but there was not enough information to perform a formal meta-analysis. The efficacy of second-line treatments was analyzed by inverse variance, as shown on the table. Re-treatment with PPI-metronidazole-amoxicillin seemed to be the most effective. When PPI-levofloxacin-amoxicillin was prescribed for 10 days instead of 7, a higher eradication rate was achieved (84% vs 69%). Sensitivity analyses excluding abstracts confirmed the robustness of these results.

Conclusion: PPI-metronidazole-amoxicillin and 10-day PPI-levofloxacin-amoxicillin are the best options as rescue treatments when PPI-amoxicillin-clarithromycin first-line therapy has failed in *H. pylori* eradication.

Second-line treatment	Number of studies (patients)	Eradication rate (95%CI)	*p*-value	I^2 (%)
PPI-levofloxacin-amoxicillin	20 (2709)	75 (0.70–0.80)	<0.001	88
PPI-bismuth-tetracycline -metronidazole	40 (3402)	77 (0.74–0.81)	<0.001	86
PPI-metronidazole -amoxicillin	23 (1611)	87 (0.84–0.91)	<0.001	75
ranitidine-bismuth-citrate-tetracycline-nitroimidazole	6 (358)	76 (0.64–0.88)	<0.001	86

Abstract no.: P11.13

GISTAR STUDY DESIGN

M. Leja,* I. Upmace,* J. Y. Park,† M. Plummer† and R. Herrero†

*University of Latvia, Riga, Latvia; †International Agency for Research on Cancer, Lyon, France

Objectives: Currently no ideal preventive modalities are available for reducing gastric-cancer caused mortality in organized population-based application. The primary objective of the study is to determine if *H. pylori* screening followed by eradication of positive subjects and endoscopic follow-up of those with serological evidence of atrophic gastritis reduces mortality from gastric cancer in middle-aged people in high-risk areas.

Methods: The GISTAR study is a multicentre randomized study of *H. pylori* eradication and pepsinogen testing for prevention of gastric cancer mortality. Here, we report the study design.

Results: Altogether 30 000 individuals aged 40–64 years will be enrolled, providing 90% study power to detect at least 35% reduction in gastric cancer mortality at 15 years of follow-up; initially a pilot study by enrolment approximately 3000 individuals is planned. Participants will be randomly allocated to one of two groups. In the active investigation/management group those positive for *H. pylori* will be offered eradication therapy, individuals with decreased pepsinogen will be invited for endoscopy. The control group will receive standard health care. The primary endpoint for our trial will be the mortality difference from gastric cancer between the groups at 15 years or when enough cases accumulate to demonstrate a statistical difference.
Conclusions: The study is expected to provide valuable information on the utility for reduction in gastric cancer mortality of: (1) *H. pylori* eradication in adults on a population-basis, including among subjects who may already have pre-malignant lesions; (2) pepsinogen testing in screening settings.

Abstract no.: P11.14

THE EFFICACY OF HYBRID AND SEQUENTIAL THERAPIES AS FIRST-LINE TREATMENT FOR *HELICOBACTER PYLORI*

D. Oh,* D. Lee,* K. Kang,* Y. Park,* C. Shin,* N. Kim,* H. Yoon* and H. Jung†
*Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, Korea; †Seoul National University College of Medicine, Seoul, Korea

Background/Aims: Recent prospective studies have shown sequential therapy has not achieved 85% *H. pylori* eradication rates in Korea. The aim of this study was to assess the efficacy of hybrid therapy as first-line treatment for *H. pylori* eradication.
Method: From December 2012 to April 2013, A total 103 (mean age 56.6, male 37, female 66) patients who proven *H. pylori* infection were randomized to received either 14 day-Hybrid therapy (rabeprazole 20 mg b.i.d. and amoxicillin 1 g b.i.d. for 14 days plus clarithromycin 500 mg b.i.d. and metronidazole 500 mg b.i.d. for the remaining 7 days) or 14 day-sequential therapy (rabeprazole 20 mg b.i.d. and amoxicillin 1 g b.i.d. for the first 7 days, followed by rabeprazole 20 mg b.i.d., clarithromycin 500 mg b.i.d., metronidazole 500 mg b.i.d. for the remaining 7 days). Outcome of eradication was evaluated by the 13C-UBT at least 4 weeks later after cessation of treatment.
Result: One hundred and three patients (58 patients in the hybrid group and 45 patients in the sequential group) completed the study. The eradication rates of hybrid treatment group and sequential treatment group were 77.6% (45/58) (95% CI = 66.9–88.3%) and 75.6% (34/45) (95% CI = 63.1–88.1%) by intention-to-treat analysis ($p = 0.809$). By the per-protocol, eradication rates were 84.6% (44/52) (95% CI = 74.8–94.4%) and 79.1% (34/43) (95% CI = 70.0–91.2%) ($p = 0.483$). There were no significant between-group differences in compliance and discontinuation due to severe side-effects.
Conclusion: Fourteen day-hybrid therapy failed to achieve significantly higher eradication rates than 14 day-sequential therapy. Both of them cannot achieve over 85% of eradication rate. So, further studies are needed to find alternative first-line treatment for better eradication rate for Korean population.

Abstract no.: P11.15

META-ANALYSIS OF SEQUENTIAL VERSUS STANDARD TRIPLE THERAPY FOR *HELICOBACTER PYLORI* ERADICATION

O. P. Nyssen,* A. G. McNicholl,* F. Megraud,† V. Savarino,‡ G. Oderda,§ C. Fallone,¶ L. Fischbach,** F. Bazzoli†† and J. P. Gisbert*
*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Bactériologie-Enfants, Hôpital Pellegrin, Bordeaux, France; ‡Dipartimento di Medicina Interna e Specialita Mediche, Genova, Italy; §Paediatric Endoscopy Units, Universita del Piemonte Orientale, Novara, Italy; ¶Medicine, McGill University Health Centre, Montreal, QC, Canada; **Department of Epidemiology, University of North Texas Health Science Center, Fort Worth, TX, USA; ††Dipartimento di Medicina Interna e Gastroenterologia, Bologna, Italy

Background: Sequential therapy (SEQ) has been suggested as a new first-line treatment option to replace the standard triple therapy (STT), where eradication rates have declined.
Aim: To conduct a meta-analysis of studies comparing SEQ versus STT for *H. pylori* eradication.
Methods: Selection of studies: randomized controlled trials comparing SEQ (10 days) and STT (at least 7 days) for the eradication of *H. pylori*. Search strategy: bibliographical searches in electronic databases, and manual search of abstracts from Congresses, were conducted up to January 2013. Data synthesis: intention-to-treat eradication rate.
Results: We included 28 randomized controlled studies with a total of 8387 patients (3931 in SEQ and 4456 in STT). The overall analysis showed that SEQ was significantly more effective (83.7% SEQ versus 74.6% STT in the intention-to-treat analysis; OR = 2.02; 95%CI = 1.54–2.64; $p < 0.001$). Results were highly heterogeneous ($I^2 = 79\%$) and 10 studies were unable to demonstrate differences between therapies. Subgroup analyses suggested that patients with clarithromycin resistance and/or taking esomeprazole-rabeprazole could benefit more from the SEQ. However there were no differences when STT lasted 14 days. Although, overall, mean eradication rate with SEQ was over 80%, a tendency towards lower efficacy with this regimen was observed in the more recent studies (weighted linear regression per year −0.02 [−2% per year] in SEQ versus −0.005 [−0.5% per year] in STT), and in studies performed outside Italy (OR 1.38 versus 4.09).
Conclusion: The meta-analysis demonstrated that SEQ is more effective than STT lasting <14 days. Nevertheless, the apparent advantage of sequential treatment seems to be decreasing overtime; therefore further and continuous assessment is needed before a generalized change in all settings is recommended for first line *H. pylori* treatment.

Abstract no.: P11.16

SHOULD THE NATIONAL STRATEGY FOR THE FIRST-LINE TREATMENT OF *H. PYLORI* INFECTION BE CHANGED?

M. Katicic,* T. Filipec Kanizaj,* B. Skurla,* M. Prskalo,* A. Mrzljak,* N. Sobocan,* T. Bradic,* T. Borcic,* A. Skrtic,* V. Maricic† and V. Plecko‡
*University Hospital Merkur, Zagreb, Croatia; †Pliva Hrvatska d.o.o., Zagreb, Croatia; ‡University Center Rebro, Zagreb, Croatia

Background: Randomized controlled trials (RCT) and meta analyses have shown, in countries with primary resistance of *H. pylori* to clarithromycin higher than 20%, the Sequential treatment as the first-line therapy to be superior in efficacy than classic triple therapy.
Aim: To evaluate whether the National Strategy for the first-line treatment of *H. pylori* infection in the Republic of Croatia should be changed.
Methods: In a period between 2008 and 2010, at the Croatian Reference Centre for *H. pylori* infections, covering the northern and western areas of Croatia, 269 patients were treated by 10-days triple therapy (128 by IPP-A-Cl; 141 by IPP-M-Cl). Primary resistance to macrolides was 23.9% and to metronidazole – 37.9%. In a period between 2011 and 2013, 53 patients were treated with sequential therapy. Primary resistance to macrolides over this period was nearly similar – 22.2%, and 37.9% to metronidazole.
Results: Success of triple therapy in the first period was unsatisfactory: IPP-A-C: 103/128 = 80.5%: IPP-A-M: 110/141 = 78% (PP analysis). The success of sequential treatment in the last period was 50/53 = 94.3% (PP analysis), which was statistically significantly higher than in the previous period (IPP-A-C vs Sequential $p < 0.02$; IPP-A-M vs Sequential $p < 0.01$). Similar results were achieved at another Centre covering the area of south Croatia.
Conclusion: The Croatian Consensus Conference on *H. pylori* infection was held at the beginning of 2013. In our opinion, the results stated above and literature data lend support to our conclusion that, in the absence of culture results, Sequential therapy should be used as the standard first-line treatment for *H. pylori* infection in our country.

Abstract no.: P11.17

OPTIMIZED TRIPLE THERAPY FOR *HELICOBACTER PYLORI* ERADICATION: PRELIMINARY RESULTS

J. Molina-Infante,* A. J. Lucendo,† T. Angueira,† I. Modolell,‡ B. Gomez-Rodriguez,§ J. Gomez-Camarero,¶ A. C. Marin,** A. G. McNicholl** and J. P. Gisbert**
*Hospital San Pedro de Alcantara, Caceres, Spain; †Hospital General de Tomelloso, Ciudad Real, Spain; ‡Consorci Sanitari de Terrassa, Barcelona, Spain; §Hospital Sagrado Corazon, Sevilla, Spain; ¶Hospital Gregorio Marañon, Madrid, Spain; **La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain

Introduction: Standard triple therapy for *Helicobacter pylori* infection is not recommended when its efficacy is lower than 80%. Updated data show that this is the case for most areas in Spain. Whether optimizing triple therapy (e.g.

increasing the PPI dose or extending the treatment duration) might result in acceptable cure rates remains currently unknown.
Aim: To assess the efficacy, compliance and safety of an optimized triple therapy, defined by a 14-day duration and high-dose acid suppressive therapy.
Methods: Consecutive *H. pylori* positive patients, naïve to eradication therapy, with non-investigated/functional dyspepsia or peptic ulcer disease, were treated with esomeprazole 40 mg/12 hour, amoxicillin 1 g/12 hour and clarithromycin 500 mg/12 hour over 14 days. Eradication was confirmed with 13C-urea breath test or histology 8 weeks after treatment. Adverse events and treatment compliance were evaluated with questionnaires and residual medication count.
Results: Up to now, 79 patients have been included in this multicenter study (most of the sample size [n = 62, 78%] came from a single center). Mean age was 49 years, 60% were females, 29% were smokers, and 27% had peptic ulcer. Eradication rates were 90% (95%CI = 83–97%) by per-protocol and 87% (81–95%) by intention-to-treat analysis. Seventy-two patients (91%) were fully compliant with therapy. Adverse events were reported by 58% of patients. By far, the most common side effect was metallic taste (43%), followed by diarrhea (15%) and epigastralgia (11%). No severe adverse events were reported.
Conclusions: Preliminary results of this ongoing study show that an optimized 14-day high-dose PPI triple therapy might lead to higher eradication rates (90%) than those previously reported in Spain, with favorable tolerance and compliance.

Abstract no.: P11.18

THIRD-LINE RESCUE THERAPY WITH BISMUTH-CONTAINING QUADRUPLE REGIMEN AFTER FAILURE OF TWO TREATMENTS (WITH CLARITHROMYCIN AND LEVOFLOXACIN) TO ERADICATE *HELICOBACTER PYLORI* INFECTION

J. P. Gisbert,* A. Perez-Aisa,† L. Rodrigo,‡ J. Molina-Infante,§ I. Modolell,¶ F. Bermejo,** M. Castro-Fernandez,†† R. Anton,‡‡ B. Sacristan,§§ A. Cosme,¶¶ J. Barrio,*** Y. Harb,††† M. Gonzalez-Barcenas,† M. Fernandez-Bermejo,§ A. Algaba,** A. C. Marin* and A. G. McNicholl*
*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Agencia Sanitaria Costa del Sol, Malaga, Spain; ‡Hospital Central de Asturias, Oviedo, Spain; §Hospital San Pedro de Alcantara, Caceres, Spain; ¶Consorci Sanitari de Terrassa, Barcelona, Spain; **Hospital de Fuenlabrada, Madrid, Spain; ††Hospital de Valme, Sevilla, Spain; ‡‡Hospital Clinico, Valencia, Spain; §§Hospital San Pedro, Logroño, Spain; ¶¶Hospital de Donostia, San Sebastian, Spain; ***Hospital Rio Hortega, Valladolid, Spain; †††Hospital de Barbastro, Huesca, Spain

Background: *Helicobacter pylori* eradication therapy with a proton pump inhibitor, clarithromycin, and amoxicillin fails in a considerable number of cases. A rescue therapy with PPI-amoxicillin-levofloxacin still fails in more than 20% of patients.
Aim: To evaluate the efficacy and tolerability of a bismuth-containing quadruple regimen in patients with two consecutive *H. pylori* eradication failures.
Methods: Design: Prospective multicenter study. Patients: In whom a first treatment with PPI-clarithromycin-amoxicillin and a second with PPI-amoxicillin-levofloxacin had failed. Intervention: A third eradication regimen with a PPI (standard dose b.i.d.), bismuth subcitrate (120 mg q.i.d. or 240 mg b.i.d.), tetracycline (from 250 mg t.i.d. to 500 mg q.i.d.) and metronidazole (from 250 mg t.i.d. to 500 mg q.i.d.) was prescribed for 7–14 days. Outcome: Eradication was confirmed using the 13C-urea breath test 4–8 weeks after therapy. Compliance and tolerance: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.
Results: Two-hundred and forty-four patients (mean age 50 years, 57% females, 20% with peptic ulcer disease and 80% with uninvestigated or functional dyspepsia) were initially included, and two were lost to follow-up. Ninety-seven percent of patients complied with the protocol. Per-protocol and intention-to-treat eradication rates were 66.1% (95% confidence interval, 60–72%) and 65.6% (59–72%). Adverse effects were reported in 21% of the patients, the most common being nausea (11%), abdominal pain (11%), metallic taste (8%), asthenia (8%), vomiting (6%), and diarrhoea (7%); all of them were mild.
Conclusion: A bismuth-containing quadruple regimen is an acceptable third-line strategy and a safe alternative after two previous *H. pylori* eradication failures with standard clarithromycin-containing and levofloxacin-containing triple therapies.

Abstract no.: P11.19

LIPOSOMES AS DRUG DELIVERY SYSTEMS AGAINST *HELICOBACTER PYLORI*

M. Gomes,* P. Lehours,† M. Gaspar,‡ M. Colla Carvalheiro‡ and F. F. Vale*,§
*Faculdade de Engenharia, Universidade Católica Portuguesa, Sintra, Portugal; †Université Bordeaux Segalen, INSERM U853, Bordeaux, Portugal; ‡IMed.UL. Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; §Centro de Estudos do Ambiente e do Mar (CESAM/FCUL), Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Liposomes are nanospheres of phospholipids organized into bilayers, which are able to incorporate therapeutic agents irrespectively of molecular weight, electric charge or solubility. The rational of using liposomal formulations instead of free agents is based on complementary or cumulative mechanisms of action: liposomes can act as solvent of insoluble agents, alter their biodistribution, increase cell interaction and decrease the toxicity of the active agents. Moreover liposomes can be superficially labeled in such a way that they can be directed to specific targets. This strategy has been already carry out with success against Gram-negative pathogens such as *Pseudomonas aeruginosa* and *Klebsiella* spp. Liposomes may be useful for the delivery of new drugs against *H. pylori* that would not reach this bacterium without this encapsulation, due to drugs that are not stable in gastric acid and/or that cannot cross the cell wall of Gram-negative bacteria. To understand the most suitable liposome composition for drug delivery against *H. pylori* we have tried different compositions of liposomes with L-α-Phosphatidylethanolamine-N-(lissamine rhodamine B sulfonyl) (Rh-PE) that is a fluorescent phospholipid probe. This compositions were tested against different *H. pylori* strains stained with DAPI and observed with fluorescence microscopy to observe if there is superposition of both signals and this way verify if the liposome interacts with *H. pylori*. The most recent results of different liposome composition tested against *H. pylori* will be presented.

Abstract no.: P11.20

SEQUENTIAL VERSUS CLASSICAL TRIPLE TREATMENT STUDY IN A GREEK POPULATION

V. Ntouli, S. Brakas, C. Zeglinas, S. Charalampopoulos, S. Labrinakos, G. Michalopoulos and C. Tzathas
Tzaneio General Hospital of Piraeus, Piraeus, Greece

Introduction: Maastricht consensus IV data raises serious questions for *H. pylori* eradication in Greek population regarding the effectiveness of triple treatment as well as first-line treatments with metronidazole.
Aim: Presentation of the preliminary results of a first-line treatment prospective study of Sequential Treatment (ST) for eradication of *H. pylori* versus Classical Triple Treatment (CTT).
Method: One hundred and fifty-two *H. pylori* positive out-patients were randomized to receive a 10 day regiment either with:
1. CTT: PPIs × 2, Clarithromycin 500 mg × 2, Amoxicillin 1 g × 2,
2. 90 patients (average age: 54.41 years, M:40, F:50) or
ST: PPIs × 2 plus:
Amoxicillin 1 g × 2, for the first 5 days andClarithromycin 500 mg × 2 and Tinidazole 500 mg × 2 for the next 5 days
Sixty-two patients (average age: 51.84 years, M:29, F:33)
All patients underwent eradication test with UBT at least 6 weeks after completion of treatment. Results were analyzed by ITT (intension-to-treat) και PP (per-protocol) analysis and were compared with x^2 (ϒates correction) and logistic regression analysis.
Results: There is a statistical significance among the eradication percentages of ST and CTT ($P < 0.001$).ST has a 6.6 greater possibility to eradicate *H. pylori* compared to CTT.

	Total patients	Lost to control	Eradicated	Eradicated % PP	Eradicated % ITT	Not eradicated	Not eradicated % PP	Not eradicated % ITT
CTT	90	4	53	61,62%	58,88%	33	38,37%	36,66%
ST	62	4	53	91,37%	85,48%	5	8,62%	8,06%

Conclusion: ST should be the eradication regiment of choice for Greek *H. pylori* positive patients regarding the international criteria for *H. pylori* eradication.

Abstract no.: P11.21

EFFICACY OF SUBSTITUTIVE STRATEGY BASED ON TRITHERAPIES USING METRONIDAZOLE AND CLARITHROMYCIN IN ERADICATION OF *HELICOBACTER PYLORI* INFECTION

M. Boudjella, A. Tebaibia, F. Moffok, Y. Saadaoui, R. Larras, M. Lahcene and N. Oumnia
I Algerian Laboratory Research on Helicobacter, Algeria, Algeria

Aim: To evaluate the efficacy of substitutive strategy based on metronidazole and clarithromycin regimens in the eradication of *H.pylori* infection.
Methods: From January 2002 to March 2009, we collected in a prospective, randomized, double-blind and unicentric study data of 129 patients infected with Hp. They were divided in two groups and treated in first line with one of the two following regimens: OAM10 (Omeprazole: 2 × 20 mg + Amoxicillin: 2 × 1 g + Metronidazole: 3 × 500 mg; 10 days) or OAC7 (Omeprazole: 2 × 20 mg + Amoxicillin: 2 × 1 g + Clarithromycin: 3 × 500 mg; 7 days). In second line, patients initially treated by the OAC 7 association were on OAM10 triple therapy and those treated with OAM10 received OAC7 regimen. The Control of eradication was performed 12 weeks after treatment. The eradication of the infection was attested by the negativity of four tests: UBT, RUT + histology and culture.
Results: In ITT, respective eradication rates of first-line anti *H. pylori* OAM10 and OAC7 regimens were 74.2% and 68.7%. PP rates were respectively 80.7% and 75.4%. In patients whom received second-line treatment, the eradication rate in the OAM10 group was 66% in ITT and 71% in PP. *H. pylori* infection cure was achieved in 57% of cases (in ITT and PP) in the OAC7 group. After two consecutive treatments, the cumulative rates of *H. pylori* eradication in the OAM10 group (second line) were 82% and 91.6%, respectively in ITT and PP. In the OAC7 group (second line), the ITT and PP eradication rates were respectively 80.6% and 94.3%.
Conclusion: The substitutive strategy has provided high rates of eradication of *Helicobacter pylori* infection. Which encourages to treat patients with more effective regimens in order to reach a cumulative eradication rate of 100%.

Abstract no.: P11.22

EFFICACY OF SECOND-LINE ANTI *HELICOBACTER PYLORI* TRIPLE THERAPY CONTAINING METRONIDAZOLE AND CLARITHROMYCIN

M. Boudjella, A. Tebaibia, F. Mouffok, Y. Saadaoui, M. Lahcene and N. Oumnia
Algerian Laboratory Reasearch on Helicobacter, Algeria, Algeria

Aim: To evaluate the efficacy of 2 second-line regimens, containing omeprazole (O), amoxicillin (A), metronidazole (M) and clarithromycin (C), in the eradication of *H. pylori*.
Methods: It was a prospective and unicentric study with two groups of patients. From January 2007 to March 2009, we enrolled 120 patients in whom had failed one of the three following anti *H. pylori* first line triple therapy: OAM10, OAM7 and OAC7 (men: 41, mean age: 38.3 years, gastritis: 92, Duodenal ulcer: 28). In the second line, patients initially treated by OAC7 have been treated by OAM10 triple therapy (Omeprazole 2 × 20 mg + Amoxicillin: 2 × 1 g + Metronidazole: 3 × 500 mg; 10 days). Those treated in the first line by OAM7 and OAM10 received OAC7 regimen (Omeprazole: 2 × 20 mg+ Amoxicillin: 2 × 1 g + Clarithromycin: 2 × 500 mg, 7 days). The Control of eradication was performed 12 weeks after treatment. The success was attested by the negativity of four tests: UBT, RUT + histology andculture.
Results: In ITT, the eradication rates with OAM10 and OAC7 regimens were respectively 58.3% and 48.3%. In PP, they were 61.4% and 50%. In patients treated by OAM10, the eradication rates for métronidazole- susceptible strains and métronidazole-resistant strains was respectively 64.3% versus 51 % in ITT and 69.2% versus 55.5% in PP. In patients receiving OAC7, the eradication rates for clarithromycin susceptible strains and clarithromycin-resistant strains were 68% versus 25% in ITT and PP. No other factor which have been evaluated, such as age, sex, type of pathology (gastritis vs UD), was predictive of treatment failure.
Conclusion: In our study, modest results have been obtained with second-line clarithromycin and metronidazole anti *H. pylori* triple therapies. It is there fore imperative to conduct clinical trials in Algeria evaluating another first-line regimens in order to minimize initial failure rate.

Abstract no.: P11.23

PROGRESS OF THE FIRST-LINE ERADICATION RATE OF *H. PYLORI* USING TRIPLE THERAPY-MULTI CENTER STUDY IN TOKYO METROPOLITAN AREA

T. Kawai,* S. Takashi,† k. Tokunga† and N. Sakaki‡
*Endoscopy Center, Tokyo Medical University Hospital, Tokyo, Japan; †3rd Department of Internal Medicine, Kyorin University of Medicine, Tokyo, Japan; ‡Foundation for Detection of Early Gastric Cancer, Tokyo, Japan

Purpose: *H.pylori* infection is amost important factor of developing gastric cancer. In 2013 eradication therapyof *H.pylori* was applied for not only peptic ulcer but also chronic gastritis by Japanese government. In Japan only triple therapy (PPI + AMPC + CAM) 7 days was applied for first line *H.pylori* eradication regimen. In this study we investigate theprogress of this first line eradication rate from 1995 to 2012.
Subjects and Methods: The eradication rate of triple therapy (PPI/AC) was collected from 15 Hospitals in Tokyo metropolitan area. The evaluation of eradication was conducted by urea breath test. The cut off value is <2.5‰.
Results: The eradication rates (ITT/PP) were 80.0%/82.9% (1995, N = 145), 81.5%/85.9% (1996, N = 135), 78.3%/84.4% (1997, N = 138), 82.5%/87.1% (1998, N = 188),78.5%/79.5% (2001, N = 242), 71.2%/72.9% (2002, N = 208), 67.8%/70.5% (2003, N = 183), 75.6%/84.6% (2004, N = 131),56.4%/70.5% (2005, N = 110), 70.5%/75.8% (2006, N = 271), 67.4%/82.0% (2007, N = 135), 64.0%/76.3% (2008, N = 261), 60.5%/74.3% (2009, N = 329), 66.5%/78.8% (2010, N = 370), 71.1%/79.5% (2011, N = 498), 69.0%/75.9% (2012, N = 494) respectively.
Conclusion: Recently the eradication rate of first-line triple therapy decreased compared to that before 2000, but decrease in eradication rate is not noticeable after 2001. (Institution: Tokyo MedicalUniversity (Kawai T & kawakami), Kyorin University School of Medicine (Takahashi S & Tokunaga K, Juntendou University of Medicine (Nagahara A, Asaoka D), Tama-Nagayama University Hospital of Nippon Medical School (Matsuhisa T), Keio University School of Medicine (Masaoka T, Suzuki H), National Hospital Organization Tokyo Medical Center (Nishizawa T, Suzuki M) Nerima General Hospital (Kurihara N), Yotsuya Medical Cube (Ito M), St. Luke's International Hospital (Omata F), Nihon University, School and Medicine (Mizuno S), Torii medical clinic (Torii A), The Jikei University Kashiwa Hospital (Okusa T), Tokai University School of Medicine (MIne T), Foundation for Detection of Early Gastric Cancer Sakaki N)).

Abstract no.: P11.24

COMPARISON OF THE EFFICACY OF 10 DAY-TRIPLE THERAPY-BASED, BISMUTH-CONTAINING QUADRUPLE THERAPY WITH SEQUENTIAL THERAPY OF *HELICOBACTER PYLORI*

B. S. Moon,* S. W. Yi,* H. C. Lim* and Y. C. Lee†
*Yongin Severance Hospital, Yonsei University, Seoul, Korea; †Severance Hospital, Yonsei University, Seoul, Korea

Background/Aim: In recent studies of the first-line therapy, the eradication rate is decreasing and as another option for *Helicobacter pylori* (HP) eradication, triple therapy-based, bismuth-containing quadruple therapy (TBQ) is tried. The aim of this study was to compare the efficacy of 10-day TBQ therapy with sequential therapy for HP eradication.
Methods: From 2009 to 2013, 165 patients with HP infections allocated two groups. TBQ therapy group: lansoprazole (LPZ) 30 mg *bid,* Amoxicillin (AMX) 1000 mg *bid,* clarithromycin (CLA) 500 mg *bid,* metronidazole (MTZ) 500 mg *tid,* and tripotassiumdicitrato-bismuthate 600 mg *bid* for 10 days. Sequential therapy group: LPZ 30 mg *bid,* AMX 1000 mg *bid* for the first 5 days, followed LPZ 30 mg *bid,* CLA 500 mg *bid,* MTZ 500 mg *tid* for 5 days. The eradication of HP was assessed by urea breathing test and the side effects were assessed after 4 weeks.
Results: The eradication rate of TBQ therapy group was higher than sequential therapy group in ITT analysis 74.6% (45/60) versus 72.4% (76/105) ($p = 0.852$) and in PP analysis 76.3% (45/59) versus 73.1% (76/104) ($p = 0.848$). The side effects including taste alteration (10.0% vs 5.3%), nausea (5.0% vs 10.5%), black stool (5.0% vs 0%), and the withdrawal was not significantly different between two groups (1/105 vs 1/60, $p = 0.747$).
Conclusion: TBQ therpy for 10 days was effective to eradicate HP as first line therapy with mild and moderate side effects in Korea.

Abstract no.: P11.25

HELICOBACTER PYLORI FIRST-LINE TREATMENT WITH BISMUTH-CONTAINING QUADRUPLE THERAPY, AND SECOND-LINE LEVOFLOXACIN-BASED RESCUE OPTION, IN PATIENTS ALLERGIC TO PENICILLIN

J. P. Gisbert,* J. Barrio,† I. Modolell,‡ J. Molina-Infante,§ A. Perez-Aisa,¶ J. L. Gisbert,* M. Fernandez-Bermejo§ and S. Marcos*

*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Hospital Rio Hortega, Valladolid, Spain; ‡Consorci Sanitari de Terrassa, Barcelona, Spain; §Hospital San Pedro de Alcantara, Caceres, Spain; ¶Agencia Sanitaria Costa del Sol, Malaga, Spain

Background: In patients allergic to penicillin, a PPI-clarithromycin-metronidazole triple therapy is the most frequently recommended regimen. However, this regimen may be relatively ineffective in areas of high clarithromycin resistance such as Spain, where a bismuth-containing quadruple therapy may be preferred. Furthermore, *H. pylori* eradication is a challenge in patients allergic to penicillin who have failed a first-eradication trial.

Aim: To assess the efficacy and safety of *H. pylori* first-line treatment with a bismuth-containing quadruple therapy, and of a second-line levofloxacin-based rescue option, in patients allergic to penicillin.

Methods: Design: Prospective multicenter study including consecutive patients allergic to penicillin. Intervention: PPI (standard dose b.i.d.), bismuth subcitrate (120 mg q.i.d.), tetracycline (doxycycline 100 mg b.i.d. or oxytetracycline 500 mg q.i.d.) and metronidazole (500 mg t.i.d.) was prescribed for 10 days. Second-line treatment with PPI (standard dose b.i.d.), clarithromycin (500 mg b.i.d.) and levofloxacin (500 mg b.i.d.) was administered for 10 days. Outcome: Eradication was confirmed by 13C-urea-breath test 4–8 weeks after therapy. Compliance/tolerance: Compliance was determined through questioning and recovery of empty medication envelopes. Adverse effects were evaluated by means of a questionnaire.

Results: Twenty-four patients allergic to penicillin received first-line treatment (PPI-bismuth, tetracycline-metronidazole). Mean age 54 years, 71% females, 17% peptic ulcer/83% functional dyspepsia. All patients complied with treatment. Adverse effects were reported in three patients (12%; metallic taste, nausea/vomiting, and diarrhoea), all of them mild. Eradication rate (both per-protocol and intention-to-treat) was 75% (95%CI = 53–90%). Ten patients received second-line therapy (PPI-clarithromycin-levofloxacin). Compliance was 100%, and two patients reported adverse effects (20%; metallic taste, and nausea, all mild). Eradication rate (both per-protocol and intention-to-treat) was 60%.

Conclusion: Allergic to penicillin *H. pylori* infected patients may be treated, as a first-line option, with a bismuth-containing quadruple therapy (that is, PPI-bismuth-tetracycline-metronidazole), which seems to be a better option than PPI-clarithromycin-metronidazole. A levofloxacin-based regimen (together with a PPI and clarithromycin) represents a second-line rescue option in the presence of penicillin allergy.

Abstract no.: P11.26

DETECTION RATES OF CLARITHROMYCIN RESISANCE AND ERADICATION RATES OF *HELICOBACTER PYLORI* INFECTION: EFFICACY OF PANPLEX™ CLAR-*H. PYLORI* DETECTION

H. Jung, J. Moon, K. Park, M. Kwon, S. Jeong, Y. Park, W. Yoon, J. Kim, S. Ryu, Y. Kim and S. Seol

Inje University, Seoul, Korea

Background/Aims: The clarithromycin resistance of *Helicobacter pylori* (*H. pylori*) rises rapidly, leads to failure of *H. pylori* eradication. Panplex™ ClaR-*H. pylori* Detection test identifies A2142G and A2143G point mutation of 23S rRNA gene. These mutations trigger clarithromycin resistance. The purpose of this study is to detect clarithromycin resistance and *H. pylori* eradication rates.

Methods: From March 2012 to February 2013, we examined CLO test and Panplex™ ClaR-*H. pylori* Detection in peptic ulcer, mucosa-associated lymphoid tissue lymphoma and early gastric cancer at Seoul Paik Hospital. We investigated *H. pylori* positive rates according to the both tests. We studied *H. pylori* eradication rates by urea breath test 6 weeks after *H. pylori* eradication regimens (LA, lansoprazole 30 mg qid/amoxicillin 500 mg qid in clarithromycin-resistant patients, LAC, lansoprazole 30 mg bid/amoxicillin 1000 mg bid/clarithromycin 500 mg bid in clarithrmycin-susceptable patients) for 14 days.

Results: Total 176 cases were evaluated. *H. pylori* positive rate by Panplex™ PCR was 63.1% (111 of 176) and by CLO test was 55.7% (98 of 176). Concordance rate between both tests was 90.3%. In *H. pylori* positive patients by Panplex™ PCR, clarithromycin-resistant (A2142G or A2143G point mutation) patients were 23 (20.7%). Overall 64 patients received *H. pylori* eradication therapy. Eradication rate in clarithromycin-resistant 16 patients using LA regimen was 50%, in clarithromycin-susceptible 48 patients using LAC regimen was 91.5%.

Conclusions: LAC triple therapy in clarithromycin-susceptible patients by Panplex™ ClaR-*H. pylori* showed high *H. pylori* eradication rate, but LA dual therapy in clarithromycin-resistant patients reported low eradication rate.

Abstract no.: P11.27

HELICOBACTER PYLORI-BINDING BIOMATERIALS AS ALTERNATIVE TREATMENT FOR GASTRIC INFECTION

I. C. Gonçalves,*,†,‡ M. Fernandes,* A. Magalhães,† I. V. Rodrigues,*,‡ C. A. Reis†,§ and C. L. Martins*,¶

*INEB, Instituto de Engenharia Biomédica, Porto, Portugal; †IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; ‡Faculdade de Engenharia, Universidade do Porto, Porto, Portugal; §Faculdade de Medicina, Universidade do Porto, Porto, Portugal; ¶Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Introduction: Chitosan, a polymer with bacteriostatic properties, has been used as drug carrier to the stomach due to its mucoadhesiveness. However, its high solubility in low pH restricts its use for gastric applications, making crosslinking a requirement. This work studies the effect of genipin crosslinked chitosan microspheres in binding *Helicobacter pylori* (*H. pylori*) and preventing/removing its adhesion to gastric cells.

Methods: Microspheres were produced by extruding chitosan into a sodium triphosphate pentabasic solution and crosslinking with genipin for different times. Microspheres size and morphology were visualized by scanning electron microscopy and optical microscopy (OM). Crosslinking was assessed by time-lapse fluorescence microscopy and infrared spectroscopy. Stability in simulated gastric fluid was evaluated by OM and microspheres gastrointestinal transit tested in vivo in C57BL/6 mice. *H. pylori* strains with different adhesins were ^{35}S- or FITC-labeled (evaluation by beta-luminescence or confocal microscopy, respectively). The capacity of microspheres to bind *H. pylori* and to avoid or remove *H. pylori* adhesion to a gastric carcinoma cell line (MKN45) was evaluated in pH 2.6 and 6.

Results/Conclusion: Chitosan microspheres ($d = 170 \pm 15$ μm) crosslinked with 10 mmol/L/1 h genipin are stable in acidic pH and remain in the stomach of C57BL/6 mice for 2 hour. *H. pylori* adhere to chitosan microspheres in pH2.6 and 6, independently of the bacterial strain. In both pH the addition of chitosan microspheres, before or after pre-incubation with *H. pylori*, decreased *H. pylori* adhesion to gastric cells. Chitosan microspheres appear as alternative/complementary treatment for *H. pylori* gastric infection.

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Abstract no.: P11.28

CHANGES OF ERADICATION RATES OF 7 AND 14 DAYS OF *HELICOABACTER PYLORI* INFECTION IN KOREA

S. Park

St. Mary's Hospital, Seoul, Korea

Background/Aims: Proton-pump inhibitor (PPI)-based triple therapy is the recommended first-line and second-line treatment for *Helicobacter pylori (H. pylori)* infection. The eradication rates of *Helicobacter pylori* were decreased in Korea. The aims of this study were to evaluate the recent 3-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 2010 to May 2012, eradication rates in 353 patients with *H. pylori* infection who received the first-line therapy for 7 (PAC7, 102 patients) or 14 days (PAC14, 251 patients) were evaluated retrospectively. Seventy patients who failed to the first-line therapy seven or 14 days were prescribed same first line regimens for 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 2010 to 2012 were decreased 80.4%, 77.1% and 71.4%, respectively. Fourteen days eradication rates were 86.5%, 81.3%, and 74.6%, respectively. The eradication rates of repeated first-line regimens group was 67.1% (47/70) in first line treatment failure group.

Eradication rates of the PAC14 were higher than those of the PAC7 group (81.3% vs 77.5%). Annual eradication rates from the year 2010 to 2012 were 84.4%, 80.2%, and 73.91% consecutively. There is a linear by linear association of decreasing trend of eradication rates during the past five years ($p < 0.001$).

Conclusions: Annual *Helicobacter 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.

Abstract no.: P11.29

QUADRUPLE THERAPY WITH PPI, BISMUTH, JOSAMYCINE AND AMOXICILLIN. PRELIMINARY RESULTS OF THE OBSERVATIONAL PROGRAM

D. S. Bordin, O. I. Berezina, V. A. Kim, N. B. Patrushev and Y. A. Orobey
Central Research Institute of Gastroenterology, Moscow, Russian Federation

Background: The four-component first line anti-helicobacter therapy including proton pump inhibitor (PPI), bismuth, macrolide and amoxicillin recommended by Russian national guidelines. Fourteen-member macrolide clarithromycin is used within eradication therapy more often. Josamycine is the 16-member macrolide that is also recommended for eradication therapy in Russia.

Aim: To evaluate rate of *H. pylori* eradication and number of adverse effects in patients, treated by quadruple therapy (PPI, bismuth, josamycine and amoxicillin) in routine clinical practice.

Methods: This is part of multicenter prospective observational non-comparative program. Quadruple therapy were prescribed for 417 patients (168 male, 249 female, median age 44) with *H. pylori* -associated diseases (chronic gastritis, peptic ulcer). Control of eradication was performed strictly in accordance to clinical guidelines only in 112 (26.8%) patients, which were included in analysis.

Results: Overall eradication rate was 89% (100/112). Eradication rates of the 7- 10- 12- and 14-day therapy were 87.5% (7/8), 94.4% (67/71), 100% (1/1) and 78.1% (25/32) accordingly.

Scheme tolerability was evaluated by physicians as good in 84% of patients, satisfactory in 12.5% and non-satisfactory in 3.5%. Adverse effects were reported only in 4 (3.5%) patients: nausea and vomiting (1), nausea (1), skin rash (2).

Conclusions: Quadruple first line scheme including proton pump inhibitor, bismuth, josamycine and amoxicillin demonstrates good eradication rate. Reporting of adverse reactions in routine clinical practice is very low.

Abstract no.: P11.30

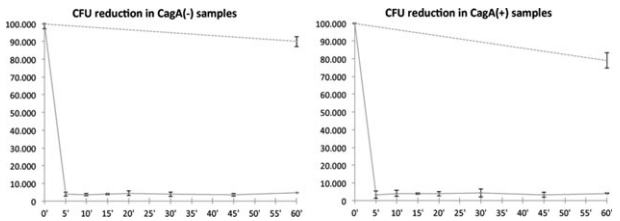
DESIGNING AN ANTIMICROBIAL PHOTODYNAMIC THERAPY AGAINST *H. PYLORI*: FROM FUNDAMENTAL CONCEPTS TO IN-VITRO ACHIEVEMENTS

M. Calvino-Fernandez,* A. G. McNicholl,† S. Benito-Martinez,* X. Calvet,‡ T. Parra-Cid,* D. Garcia-Fresnadillo§ and J. P. Gisbert†
*Unidad de Investigacion, Hospital de Guadalajara and CIBEREHD, Guadalajara, Spain; †La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; ‡Hospital de Sabadell, Barcelona, Spain; §Facultad de Ciencias Quimicas, Universidad Complutense, Madrid, Spain

Introduction: Antimicrobial Photodynamic Therapy (APDT) is based on the photocatalytic generation of reactive oxygen species, such as singlet oxygen, by using harmless visible light and a (photo) sensitising dye. Singlet is a short lived and powerful oxidising species that can inactivate microorganisms in the presence of eukariotic cells without damaging them. Singlet oxygen sensitisers are innocuous at photochemically active doses (micro- to milli-molar range).

Aim: To evaluate APDT against *H. pylori*, in vitro, using a new photosensitising material (PSM) based on a ruthenium (II) complex covalently bound to micrometric glass beads.

Methods: Five *H. pylori* isolates (classified according to cagA genotype, and metronidazole-clarithromycin resistance) have been used. Assays were performed by duplicate, at bacterial concentrations of 5 × 104 and 105 CFU/mL in TSB and by continuous shaking. Each strain was placed in three wells: A: bacteria, B: bacteria + P (1–3 mg) and C: bacteria + PS (1–3 mg), and was incubated in the dark or illuminated with a blue LED (20–25 mW). Aliquots were taken each 30′ until 2 hour, cultured onto TSA for 2–3 days and colonies were counted.



Results: Data from CFU is shown in the figure, compared to non-illuminated samples or with irradiated samples without PSM. It was also confirmed that DNA is a molecular target for oxidant species released during APDT (evaluated by alkaline gel electrophoresis after endonuclease III incubation, ureC and cagA RT-PCR, and bacterial fingerprint). Results were independent of cagA gene and antibiotic resistances.

Conclusions: Antimicrobial Photodynamic Therapy might constitute a complementary or an alternative treatment for *H. pylori* eradication in the gastro-duodenal tract, particularly in patients infected with antibiotic resistant strains.

Abstract no.: P11.31

USEFULNESS OF *HELICOBACTOR PYLORI* ERADICATION THERAPY INCLUDING SITAFLOXACIN FOR PATIENTS WITH PENICILLIN ALLERGY

S. Sahara,* M. Sugimoto,* T. Uotani,* H. Ichikawa,* T. Yamada,* S. Osawa,† K. Sugimoto* and T. Furuta‡
*First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan; †Department of Endoscopic and Photodynamic Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan; ‡Center for Clinical Research, Hamamatsu University School of Medicine, Hamamatsu, Japan

Background: The Maastricht IV/Florence Consensus Report recommended a PPI-clarithromycin-metronidazole combination as a first-line treatment in patients with penicillin allergy. However, there was no evidence for patients with penicillin allergy in Japan. We think that insufficient acid inhibition during treatment is a risk factor, as insufficient acid inhibition makes antimicrobial agents unstable and leads to their degradation in stomach. Our aim was to investigate the efficacy of PPI-metronidazole-sitafloxacin triple therapy considering acid inhibition for patients with penicillin allergy.

Methods: As preliminary trial, a total of 10 *H. pylori*-positive Japanese patients with penicillin allergy received the triple therapy consisting of rabeprazole 10 mg qid, metronidazole 250 mg bid and sitafloxacin 100 mg bid for 1 week, irrespective of CYP2C19 genotype status and bacterial resistance to antibiotics. The presence of bacterial resistance to antibiotics was examined by MIC method using the strain of tissue culture. After 2 months of eradication, ^{13}C urea breath test was performed.

Results: Bacterial resistances to AMPC, CAM, MNZ, LVFX in five patients who performed culture test were 0%, 40%, 40%, 80%, respectively. Four patients were naive for treatment and five patients were treated once. CYP2C19 genotypes were RM in five patients, IM in 4, PM in 1. Eradication rate was 100% (95% CI: 74.1–100%). An adverse event was experienced diarrhea during the treatment in only one patient.

Conclusions: This PPI-metronidazole-sitafloxacin triple therapy overcoming CYP2C19 genotypes was useful for Japanese patients with penicillin allergy. Sitafloxacin might be one of selection in patients infected with LVFX-resistant *H. pylori*.

Abstract no.: P11.32

COMPARISON WITH ERADICATION RATES OF MOXIFLOXACIN CONTAINING TRIPLE THERAPY AS SECOND LINE TREATMENT FOR *HELICOBACTER PYLORI* INFECTION ACCORDING TO FIRST LINE REGIMEN

K. Kang,* D. Lee,* D. Oh,* N. Kim,* Y. Park,* C. Shin,* H. Yoon* and H. Jung†
*Department of Internal medicine, Seoul National University Bundang Hospital, Seongnam-si, Korea; †Department of Internal medicine, Seoul National University College of Medicine, Seoul, Korea

Background: There was a controversy the efficacy of eradication with moxifloxacin based triple therapy as second line treatment for *Helicobacter pylori* infection. And most of published papers focused on patients failed to treat with standard triple therapy. So, we investigated the efficacy of moxifloxacin as second line therapy and the eradication rates of that according to previous first-line regimen.
Method: A total of 305 patients who were failed to eradicate with first line treatment received 14 days moxifloxacin containing triple therapy (moxifloxacin 400 mg q.d, amoxicillin 1000 mg b.i.d, rabeprazole 20 mg b.i.d). As first line treatment, they were prescribed 7 day-standard triple therapy (n = 186), 10 day-bisthmus containing quadruple therapy (n = 32), 7 day-concomitant therapy (n = 40) and 14 day-sequential therapy (n = 47). Primary outcome was the eradication rate by intention-to treat (ITT) and per-protocol (PP) analysis.
Result: The eradication rate of moxifloxacin based triple therapy as second line was 64.6% (95% CI 59.1–69.8) by ITT, and 74.1% (95% CI 68.5–79.0) by PP. And the peptic ulcer patients had higher eradication rate than non-ulcer group ($p = 0.042$). ITT and PT according to first regimen were 66.7/77.0% (95% CI 59.6–73.0/69.9–82.8) in standard triple group, 62.5/69.0% (95% CI 45.3–77.1/50.8–82.7) in bisthmus containing quadruple group, 62.5/69.4% (95% CI 47.0–75.8/53.1–82.0) in concomitant group and 59.6/70.0% (95% CI 45.3–72.4/54.6–81.9) in sequential group. There was no significant difference between groups ($p = 0.600$).
Conclusion: Two-week moxifloxacin based triple therapy as second line did not show expected level for the primary outcome. The group treated with moxifloxacin after failure of standard triple therapy had highest rate of eradication, but there was no statistical significance in the efficacy among the first line regimens.

Abstract no.: P11.33

DIVIDING HIGH-DOSE PPI/SITAFLOXACIN-BASED TRIPLE *HELICOBACTOR PYLORI* ERADICATION THERAPY IS USEFULNESS AS THIRD-LINE THERAPY IN JAPANESE: PRELIMINARY STUDY

T. Uotani,* M. Sugimoto,* H. Ichikawa,* S. Sahara* and T. Furuta†
*First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan; †Center for Clinical Research, Hamamatsu University School of Medicine, Hamamatsu, Japan

Background: The Maastricht IV report recommends that the treatment of *Helicobacter pylori* should be guided by antimicrobial susceptibility testing whenever possible, after failure of second-line treatment. In Japanese guideline for third-line *H. pylori* eradication therapy, PPI/amoxicillin/levofloxacin or high-dose PPI/amoxicillin is recommended. However, eradication rates are not so excellent, lower than 75%. Recently, usefulness of potent acid inhibition during treatment by four-times-daily dosing of PPI and sitafloxacin (STFX) with low MIC to *H. pylori* was suggested. Therefore, we aimed to confirm the effect of 4-times-daily dosing of PPI and STFX for third-line treatment as preliminary study.
Methods: A total of 12 *H. pylori*-positive Japanese patients were received one of two regimens: (1) rabeprazole 10 mg qid, Ampicillin 500 mg qid and STFX 100 mg bid (RAS) (n = 8), (2) rabeprazole, metronidazole 250 mg bid and STFX (RMS) (n = 4) for 1 week as third-line treatment. After 2 months of eradication, 13C urea breath test was performed.
Results: Bacterial resistances to AMPC, CAM, MNZ, LVFX in 12 patients who performed culture test were 0%, 83.3%, 50%, 16.7%, respectively. Seventy-five percent of the subjects treated with third-line eradication were CYP2C19 rapid metabolizers (RMs). Total eradication rate was 91.7% (95%CI: 61.5–99.8) in ITT analysis. That in RAS is higher than RMS (RAS: 100% and RMS: 75%, respectively). Diarrhea as side effect was observed in the subject of 16.7%.
Conclusions: This PPI-STFX-based triple therapy with potent acid inhibition was useful for Japanese patients received third-line treatment. STFX might be one of selection in patients infected with LVFX-resistant *H. pylori*.

Abstract no.: P11.34

EFFICACY OF *LACTOBACILLUS REUTERI* IN THE TREATMENT OF *HELICOBACTER PYLORI* INFECTION

M. P. Dore,*† M. Cuccu,* M. Massidda,* C. Rocchi,* S. Soro,* G. Marras* and D. Y. Graham†
*Dipartimento di Medicina Clinica e Sperimentale Clinica Medica, University of Sassari, Sassari, Italy; †Baylor College of Medicine, Houston, TX, USA

Introduction: Probiotics have been proven to be useful in the treatment of several gastrointestinal diseases. In addition, they may compete directly with *H. pylori*, possibly through the inhibition of adherence, as well as by producing metabolites and antimicrobial molecules. *Lactobacillus reuteri* (*L. reuteri*) is able to inhibit *H. pylori* in vitro and in vivo and theoretically may cure the infection. The aim of our study was to examine the efficacy of *L. reuteri* for treatment of *H. pylori* infection.
Materials and Methods: This was a "two stage trial". In the first step 22 patients were enrolled. *H. pylori* infection was defined as the presence of the bacteria on gastric biopsies and a positive 13C-UBT. Treatment consisted of *L. reuteri* (DSM 17938) 108 cfu plus pantoprazole 20 mg twice a day for 4 weeks. Eradication was established by a negative 13C-UBT 4–6 weeks post-therapy. Compliance was considered good if at least 90% of the total number of the pills were taken. There was no pharmacutical company sponsor.
Results: Twenty-one patients completed the study (mean age; 52, 36% men). *L. reuteri* treatment cured 14% (3/21; 95% CI = 0.30–36) of infected patients Per Protocol analysis. In five patients a reductions of Delta 13C-UBT value >50% was registered. Compliance was excellent (100%). No side effects were recorded.
Conclusions: *L. reuteri* may have a potential role as an alternative for *H. pylori* eradication especially for patients where antibiotic therapy is contraindicated. More studies will be needed to ascertain whether duration, amount of cfu, or administration timing could improve eradication rate.

Abstract no.: P11.35

GELATINE TANNATE SUPPLEMENTATION REDUCES ANTIBIOTICS ASSOCIATED SIDE-EFFECTS OF ANTI-*HELICOBACTER PYLORI* FIRST-LINE THERAPY

G. Gigante, G. Caracciolo, M. Campanale, V. Cesario, V. Bove, G. Cammarota, G. Gasbarrini and A. Gasbarrini
Catholic University Rome, Policlinico Gemelli, Rome, Italy

Treatment outcome for HP depends on the class of antibiotic administered, dosages used, therapy duration, bacterial resistance and patients'compliance. Gastrointestinal side-effects during therapy include diarrhea, nausea, taste distortion, stomatitis and bloating, and are major determinants for a lack of compliance. Gelatine tannate (GT) has been shown to be efficacious in treating acute diarrhea in infant.
To explore whether Gelatine Tannate 500 mg t.d supplementation is efficacious in preventing diarrhea and GI side effects related to anti-*H. pylori* standard first line eradication therapy with esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and amoxicillin 1 g b.d. Open label no-controlled trial, proof of concept. Forty (23F/17M, mean age 40 ± 15) HP-positive patients were enrolled for triple therapy with GT for 14 days (GT-group, GTg). Each patient was required to complete a validated daily diary (slightly modified from DeBoer et al.) for 4 weeks. We referred to our previous published study ("Bacillus clausii therapy to reduce side-effects of anti-*Helicobacter pylori* treatment: randomized, double-blind, placebo controlled trial" E.C. Nista, A Gasbarrini et al, Aliment Pharmacol Ther 2004; 20: 1181–1188), as a control population (CT).
Occurrence of nausea was 10 % in GTg at 1 week, way lower compared to 50 % of CT. At 2 weeks, incidence of nausea was respectively 5% in GTg and 30% in CT. Patients experienced diarrhoea only in 5% at 1 week and 2.5% at 2 weeks in GTg compared to 30% and 10 % of CT. The incidence of epigastric pain, vomiting, constipation and skin rash were absent in GTg, as well as in CT.
Data suggest that the efficacy of GT is particularly visible in the first week of treatment while antibiotics are co-administered and the higher incidence of side-effects is usually registered.

Abstract no.: P11.36

THE RELATIONSHIP BETWEEN PSYCHOSOMATIC DISORDERS AND METABOLIC PROCESSES OF THE GASTRODUODENAL ZONE IN HP-POSITIVE PEPTIC ULCER PATIENTS

I. N. Skrypnyk,* A. Gopko* and N. Kharchenko†
*Ukrainian Medical Stomatological Academy, Poltava, Ukraine; †National Medical Academy of the Postgraduate Education n.a. P.L.Shupik, Kyiv, Ukraine

The aim was to assess the relationship between the degree of psychosomatic disorders in peptic ulcer of the duodenum (PUD) pts and metabolic processes in the mucosa of the gastroduodenal zone (GDZ).

The study involved 127 HP-positive PUD pts, mean age 37.8 ± 4.8 years. Psychosomatic disorders were assessed on the basis of analysis of questionnaires by tests of mental adaptation to stress effects, stress resistance, anxiety scale and quality of life of patients. In order to assess the function of mucus formation GDZ content determined N-acetylneuraminic acid (NANA) and fucose concentration in serum and their excretion in the urine. Activity of oxidative stress measured by MDA-reagents, hydrogen peroxide, antioxidant defense – for the activity of superoxidedysmutase (SOD).

In PUD pts established growth NANA content in blood serum, which correlated with physical ($r = -0.56$) and psychological ($r = -0.72$) quality of life and has a direct relationship with anxiety ($r = +0.64$). One of the manifestations of lesions of the mucous membrane GDZ is fucoproteins metabolism, which is increase in serum. The level of excretion in urine had a relationship with anxiety, stress resistance level ($r = -0.49$; $r = -0.55$) and correlation with the psychological quality of life index ($r = -0.71$). In PUD pts increased degree of oxidative stress, which confirmed the likely growth of MDA-reagents and hydrogen peroxide. The established correlation between the studied parameters and the degree of stress resistance ($r = -0.62$; $r = -0.58$), anxiety ($r = -0.66$; $r = -0.82$) and direct contact with the psychological index quality of life ($r = +0.69$; $r = +0.78$).

Metabolic processes in the mucosal barrier in HP-positive PUD pts depend on the degree of anxiety, stress resistance, psychological and emotional adaptation, affecting the quality of life of pts.

Abstract no.: P11.37

A LOWER QUALITY OF LIFE IN PATIENTS WITH FUNCTIONAL BOWEL DISORDERS COMPARED TO THOSE WITH GASTRIC DYSPEPSIA

G. Gigante,* G. Caracciolo,* V. Cesario,* M. Camapnale,* V. Bove,* V. Ojetti,* G. Gasbarrini† and A. Gasbarrini*
*Catholic University Rome, Rome, Italy; †Fondazione Italiana Ricerca in Medicina, Bolagna, Italy

A low health-related quality of life (HRQL) was reported in subjects with functional gastrointestinal disorders such as functional bowel disorders (FBDs), and gastric dyspepsia (GD). FBDs, such as lactose intolerance, small intestinal bacterial overgrowth, alterations of gastrointestinal transit time, could be investigated with hydrogen breath test (H_2BT). The first non-invasive test to investigate GD is urea breath test (C^{13}UBT), to exclude *HP* infection. Short Form Health Status Survey (SF36) demonstrated internal consistency, construct validity and concurrent validity when applied to patients (pts) with significant bowel dysfunction.

To compare, by SF36, HRQL of pts affected by FBDs or GD.

We enrolled, from the Gastroenterology unit of Policlinico Gemelli in Rome, 96 pts (61F/35M; age mean 40 ± 15 years). Fifty pts underwent H_2BT and 46 C^{13}UBT; HRQL was analyzed based on SF36 scores and component summary scores.

Mean values of all items analyzed by SF36 were lower compared to general Italian population (nv 50). Both groups were homogeneus for sex and age. A significant lower Mean Mental Health Index (MHI) was observed in FBDs compared with GD: 33.9 ± 11.7 versus 45.1 ± 8.9 (mean difference −11.2, 95% CI −18.4, −4.0; $p = 0.004$). Moreover a significantly higher Physical Health Index (PHI) was observed for FBDs compared with GD: 49.0 ± 8.1 versus 42.1 ± 8.9 (mean difference 6.9, 95% CI 1.0, 12.8; $p = 0.024$).

GI diseases which can be analyzed with BT are related with lower SF36 scores. Our preliminary results suggest that pts affected by FBDs have a lower MHI and an higher PHI compared with those with GD. This difference is probably due to the fact that pts who undergo C^{13}UBT often have a organic disease below, while pts with FBDs have a more complex phisiopathological framework, in which may be implicated an alterated perception of disease.

Abstract no.: P11.38

HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH FUNCTIONAL GASTROINTESTINAL DISORDERS

G. Gigante,* V. Bove,* V. Ojetti,* G. Cammarota,* G. Gasbarrini† and A. Gasbarrini*
*Catholic University Rome, Rome, Italy; †Fondazione Italiana Ricerca in Medicina, Bologna, Italy

A low health-related quality of life (HRQL) was reported in subjects with functional gastrointestinal disorders (FGDs). Lactose intolerance, other sugars intolerances, small intestinal bacterial overgrowth, alterations of gastrointestinal transit time, dyspepsia are very common in clinical practice, and could be investigated with breath test (BT) analysis. The Short Form Health Status Survey (SF36) demonstrated internal consistency, construct validity and concurrent validity when applied to patients (pts) with significant bowel dysfunction. To assess, by SF36, the impact of FGDs on HRQL of pts that perform BT. We enrolled, from the Gastroenterology Unit of Policlinico Gemelli in Rome, 96 pts (61F, 35M; age mean 40 ± 15 years) with FGDs who underwent H2BT and/or C13 Urea BT. HRQL was analyzed on SF36 scores and component summary scores. For each item, the mean difference and 95% confidence interval (CI) with corresponding two-tailed *p*-values between the pts and the general population were calculated by means of a *z*-test. All items analyzed by SF36 were lower compared to the normal values for the general Italian population. Mean Mental Health Index (MHI) and Physical Health Index (PHI), the two main scores of SF 36, were both under the normal values for the general population: MHI 39.1 ± 11.8 (nv 50, mean difference −10.9, 95% CI −15.0, −6.7; $p < 0.001$); PHI 45.8 ± 9.0 (nv 50, mean difference −4.2, 95% CI −7.4, −1.1; $p = 0.010$).Pts submitted to BT showed a lower SF36 scores compared to general population. Therefore FGDs have a significant impact on HRQL of pts in particular this kind of pts showed a significant reduction of MHI. SF36 could provide a useful adjunct to current methods of evaluating treatment outcomes for FGDs, and potentially other disorders.

Abstract no.: P11.39

HELICOBACTER PYLORI ERADICATION IN THE ELDERLY PATIENTS: THE ERADICATION RATES AND ABNORMAL GASTROINTESTINAL RESPONSES

J. Chung, Y. Seok and J. Kim
Department of Internal Medicine, Sahmyook Medical Center, Seoul, Korea

Background/Aims: *Helicobacter pylori* (*H. pylori*) infection is closely related with a wide range of gastrointestinal disease. One-week triple therapy is currently considered as the gold standard for the treatment of *H. pylori* for all ages. Because of increasing life expectancy, the demand for the eradication of elderly are also increased. But abnormal gastrointestinal responses are major limitations in elderly patients.

The aim of our study was to evaluate the eradication rates and identify the abnormal response rates between the younger and the elderly patients.

Methods: Four hundred and twelve patients with *H. pylori* infection between January 2011 and April 2013 were included (mean age: 46.7 years; range 17-83). Among 412 patients, 65 (16%) patients were older than 70 years.

After 1 week of *H. pylori* eradication triple therapy (Pantoprazole 40 mg, clarithromycin 500 mg, amoxicillin 1 g bid), we evaluated the eradication rates and abnormal gastrointestinal responses (diarrhea, bloating, constipation, abdominal pain, borborygmus, flatulence, stool frequency, belching and nausea) and severities at 1 and 4 weeks after completion of treatment.

Results: The overall eradiactaion rate was 77.9 %. The eradication rate was higher in the younger age group,but not statistically significant (82.3% vs 75.9%; $p = 0.06$). The incidence of abnormal gastrointestinal response rates were similar (14.1% vs 15.3%; $p = 0.09$), but high grade (severe) abnormal responses were more notified at the elderly group (7.56% vs 11.9%, $p = 0.006$) at the first week after eradication.

Conclusions: In elderly patients, *H. pylori* eradication rates were not inferior to younger age group. Except some high grade abnormal gastrointestinal response, Overall abnormal responses rate are similar in the both group. So If we carefully monitor the abnormal response, we should strongly consider the eradication in the elderly group.

Abstract no.: P11.40

SECOND-LINE RESCUE THERAPY WITH MOXIFLOXACIN AFTER FAILURE OF TREATMENT TO ERADICATE *HELICOBACTER PYLORI* INFECTION

J. P. Gisbert,* L. Ferrer,† T. Angueira,‡ B. Gomez,§ I. Modolell,¶ J. Molina-Infante,** J. L. Dominguez-Jimenez,†† J. Ortuño,‡‡ A. Perez-Aisa,§§ P. Almela,¶¶ A. C. Marin,* E. Medina,† A. J. Lucendo‡ and A. G. McNicholl*

*La Princesa University Hospital, IP and CIBEREHD, Madrid, Spain; †Hospital General Universitario, Valencia, Spain; ‡Hospital General de Tomelloso, Ciudad Real, Spain; §Clínica Sagrado Corazón, Sevilla, Spain; ¶Consorci Sanitari de Terrassa, Barcelona, Spain; **Hospital San Pedro de Alcantara, Caceres, Spain; ††Hospital Alto Guadalquivir, Jaen, Spain; ‡‡Hospital La Fe, Valencia, Spain; §§Agencia Sanitaria Costa del Sol, Madrid, Spain; ¶¶Hospital General, Castellon, Spain

Background: Second-line bismuth-containing quadruple therapy is complex and frequently induces adverse effects. A triple rescue regimen containing levofloxacin is a potential alternative for *Helicobacter pylori* eradication. However, resistance to quinolones is rapidly increasing and may jeopardize its future efficacy. Moxifloxacin, a new generation quinolone, may be less affected by quinolone resistance than levofloxacin.

Aim: To evaluate the efficacy and tolerability of a second-line triple regimen containing moxifloxacin in patients whose previous *H. pylori* eradication treatment failed.

Methods: Design: Prospective multicenter study. Patients: Patients in whom a standard triple therapy (PPI, clarithromycin, and amoxicillin) or a non-bismuth quadruple therapy (PPI, clarithromycin, amoxicillin and metronidazole) had failed. Intervention: Moxifloxacin (400 mg o.d.), amoxicillin (1 g b.i.d.), and esomeprazole (40 mg b.i.d.) for 14 days. Outcome: Eradication was confirmed using the 13C-urea-breath test 4–8 weeks after therapy. Compliance/tolerance: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

Results: Up to now, 37 patients have been consecutive included (28 after standard triple therapy, and nine after non-bismuth quadruple therapy failure). Mean age was 47 ± 15 years, 51% were women, and 24% had peptic ulcer. Almost all patients (95%) took all medications correctly. Per-protocol and intention-to-treat eradication rates were 77% (95%CI = 62–92%) and 73% (95%CI = 57–89%). Cure rates were similar after standard triple therapy and non-bismuth quadruple therapy failure (78% and 75% by per-protocol, respectively). Adverse effects were reported in 27% of patients, most commonly abdominal pain (11%), diarrhoea (11%), metallic taste (5.4%), asthenia (5.4%), and nausea/vomiting (2.7%); all but one were mild (one patient presented severe vomiting, forcing treatment discontinuation).

Conclusion: Fourteen-day moxifloxacin-containing therapy is an effective and safe second-line strategy in patients whose previous standard triple therapy or non-bismuth quadruple therapy has failed.

Abstract no.: P11.41

SEQUENTIAL TREATMENT AGAINST *H. PYLORI* INFECTION IN A GREEK POPULATION-A MULTIVARIANT ANALYSIS

V. Ntouli, C. Zeglinas, S. Brakas, S. Charalampopoulos, S. Labrinakos, G. Michalopoulos and C. Tzathas

Tzaneio General Hospital of Piraeus, Piraeus, Greece

Introduction: In Greece high clarithromycin and metronidazole resistance plus unavailability of the alternative first-line treatments make sequential treatment (ST), the realistic first-line choice for *H. pylori* eradication.

Aim: Presentation of preliminary results of a prospective study using as first-line the ST in a Greek population and search for possible factors affecting successful *H. pylori* eradication.

Method: Sixty-two outpatients (Average Age:51.84 years), diagnosed as H.P-positive, were interviewed using a preformed questionnaire, recording sex, age, smoking, alcohol consumption, NSAID and long-term PPI intake, symptoms, anemia, upper GI personal history and family history (FH) of gastric Ca.

All received 10-day ST: PPIs × 2 with: Amoxicillin 1 g × 2, the first 5 days and Clarithromycin 500 mg × 2 plus Tinidazole 500 mg × 2, the next 5 days.

Six to eight weeks after ending of treatment, all patients received UBT and compliance control.

Results were analyzed by ITT (intension-to-treat) και PP (per-protocol) analysis and were compared with x^2 (ϒates correction) and logistic regression analysis.

Results: Sixty-two treated patients. Four lost to eradication control.

Fifty-three eradicated and five not eradicated H.P.

PP: 91.37%- ITT: 85.48%.

Eradicate	♂/♀	Age 45≥/45<	Smoking −/+/ex	Alcohol −/+	NSAID −/+	Dyspepsia −/+	GOR −/+
+	25/28	34/19	23/17/13	49/4	48/5	18/35	20/33
−	2/3	5/0	3/0/2	3/2	5/0	1/4	2/3

Eradicate	Anemia −/+	Gastric ulcer −/+	Duodenal ulcer −/+	PPIs −/+	FH Ca −/+
+	37/16	50/3	42/11	47/6	50/3
−	3/2	3/2	5/0	3/2	5/0

Statistical analysis revealed a reverse correlation between patient's age and the probability to eradicate H.P. ($p = 0.028$).

Conclusion: ST used on a Greek population as first-line eradication of H.P. meets international requirements and overcomes resistance and availability problems. Of factors studied, only younger age of treated patients seems to increase probability of eradication.

Abstract no.: P11.42

EFFICIENCY DIFFERENT OF PPI'S IN ERADICATION OF *H. PYLORI*

I. Paliy, S. Zaika, A. Piddubetska and S. Pareek

Vinnytsya National Pirogov Memorial Medical University, Vinnytsya, Ukraine

Introduction: The problem of the effectiveness of eradication of *Helicobacter pylori* (H.p.) remains relevant to modern gastroenterology. Considering the pharmacokinetic characteristics of different generations of proton pump inhibitors (PPIs), possible differences between omeprazole, pantoprazole, lansoprazole and rabeprazole in the successful eradication of H.p. should be expected.

Objective: To study the differences in action of omeprazole, pantoprazole, rabeprazole and lansoprazole in eradication of H.p. with clarithromycin and amoxicillin.

Materials and Methods: The study based on 273 results of respiratory urease tests, which were carried out to monitor the effectiveness of eradication of H.p. Respiratory urease test was performed 4 weeks after the completion of eradication. All studies were performed on the infrared isotope analyzer IRIS.

Depending on the purpose of study results of respiratory tests were divided into groups. Group of omeprazole was 100 studies, pantoprazole – 66, lansoprazole – 53, rabeprazole – 54.

For eradication of H.p. patients received omeprazole 0.02 g b.i.d, pantoprazole – 0.04 g b.i.d., lansoprazole – 0.03 g b.i.d, 0.02 g of rabeprazole b.i.d with clarithromycin in doses of 0.5 g b.i.d. and 1.0 g of amoxicillin b.i.d. The duration of treatment was 7 days.

The results of the study: The effectiveness of eradication of H.p. in the omeprazole group was 86%, pantoprazole – 81.8%, lansoprazole – 84.9%, rabeprazole – 83.3%. Comparing groups of omeprazole-pantoprazole, omeprazole-lansoprazole, omeprazole- rabeprazole, pantoprazole- lansoprazole, pantoprazole- rabeprazole, lansoprazole-rabeprazole, no significant difference observed ($p > 0.05$) between the groups.

Conclusion: Omeprazole, pantoprazole, lansoprazole and rabeprazole in standard doses are equally effective for eradication H.p.when used in combination with amoxicillin and clarithromycin.

Abstract no.: P11.43

EFFICACY OF AMYTRIPTILINE IN IMPROVING INTESTINAL PERMEABILITY AND QUALITY OF LIFE IN PATIENTS WITH GASTROINTESTINAL DISORDERS

G. Gigante,* G. Caracciolo,* V. Bove,* V. Ojetti,* G. Ianiro,* G. Cammarota,* G. Gasbarrini† and A. Gasbarrini*

*Catholic University Rome, Rome, Italy; †Fondazione Italian Ricerca in Medicina, Bologna, Italy

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder (GFD). In the pathogenesis are implicated alterations of GUT microbiota, motility, intestinal immunity and inflammation, and recently was given a role to in intestinal permeability. The aim of our study was to evaluate the efficacy of amitriptyline to improve the quality of life and intestinal permeability. We enrolled 20 pts (10F, 10M; age mean 38 ± 4 years) with IBS according to Roma III criteria.

Patients underwent to intestinal permeability with chromium EDTA test. Quality of life was analyzed by SF36. We administered amytriptiline 10 mg po twice daily for 30 days. At the end of treatment we repeated intestinal permeability test and SF36. All items analyzed by SF36 were lower compared to the normal values for the general Italian population. Mean Mental Health Index (MHI) and Physical Health Index (PHI), the two main scores of SF 36, were both under the normal values for the general population: MHI 39.1 ± 11.8 (nv 50, mean difference −10.9, 95% CI −15.0, −6.7; $p < 0.001$); PHI 45.8 ± 9.0 (nv 50, mean difference −4.2, 95% CI −7.4, −1.1; $p = 0.010$). All patient had increase of intestinal permeability to chromium EDTA test at baseline. The results of chromium EDTA after therapy was: 18 patients within normal limits and two upper normal limits. In two patients in which intestinal permeability was not normalized, Sf36 test was altered but slightly improved (MHI from 39 ± 2 to 45 ± 2, PHI from 45 ± 1 to 48 ± 1). In 18 patients in which intestinal permeability was normalized, Sf36 test was in normal limits. Amytriptiline is effective in improving quality of life and intestinal permeability in IBS patients. This result may demonstrate the role of the GUT brain axis in the pathogenesis of IBS.

Abstract no.: P11.44

OPTIMAL TIMING OF THE START OF ERADICATION THERAPY FOR *HELICOBACTER PYLORI* IN PATIENTS HOSPITALIZED DUE TO PEPTIC ULCER BLEEDING

H. Yoon, D. Lee, N. Kim, Y. Park, C. Shin, K. Kang and D. Oh
Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Korea

Objective: It is controversial when *Helicobacter pylori* (*H. pylori*) eradication therapy should be started in the patients with peptic ulcer bleeding (PUB). The aim of this study was to compare *H. pylori* eradication rates in the patients with PUB according to the timing of the start of eradication therapy.
Methods: Clinical data about adults hospitalized due to PUB were retrospectively collected. The *H. pylori* eradication rates of the patients who started eradication therapy during hospitalization and the patient who did in the outpatient department were compared.
Results: Between 2003 and 2012, a total of 232 patients with PUB were evaluated for *H. pylori* infection by histology and/or rapid urease test. Of these patients, 53.7% (127/232) was confirmed *H. pylori* infection status before discharge. Among the patients who discharged before confirming *H. pylori* infection status, 13.3% (14/105) was lost to follow-up. Among the patients found to be *H. pylori*-positive in the outpatient department, 41.4% (12/29) did not receive eradication therapy. There was no significant difference in *H. pylori* eradication rate between the patients who received eradication therapy during hospitalization and the patients who did in the outpatient department (intention-to-treat: 68.8% (53/77)/60% (12/20), $p = 0.594$; per-protocol: 82.8% (53/64)/80% (12/15), $p = 0.723$).
Conclusions: There was no difference in *H. pylori* eradication rate according to the timing of the start of eradication therapy in patients hospitalized due to PUB. However, because many patients who discharged before confirming *H. pylori* infection lose an opportunity for eradication therapy, it is suggested to confirm *H. pylori* infection and start eradication therapy before discharge in the patients with PUB.

Abstract no.: P11.45

EFFECTIVENESS OF SEQUENTIAL THERAPY VERSUS TRIPLE THERAPY: PROSPECTIVE STUDY ABOUT 596 CASES

I. Derrouich, H. Krami, I. Errabih, F. Z. Daoudi, N. Benzzoubeir, L. Ouazzani and H. Ouazzani
Service de Mdecine B. CHU Ibn Sina, Rabat, Morocco

Introduction: Various therapy regimens have been tested in the eradication of *Helicobacter pylori* (HP) and this pilot study was designed to evaluate the effectiveness and tolerability of sequential therapy versus triple therapy.
Material and Methods: It's a prospective and randomized study in the Medicine B department (CHU Ibn Sina Rabat, Morocco) during 18 months from December 2011 to may 2013. Five hundred and ninety-six patients infected with HP were included, HP status was assessed by histology, 306 patients have received the sequential treatment (IPP + Amoxicillin for 05 days followed by IPP + Clarithromycin + Metronidazole for 05 days), 290 patients have received the triple therapy (IPP + Amoxicillin + Metronidazole for 14 days), after 04 weeks, HP was checked by biopsy in 10%, the urea breath test in 66%, the search for Ag in stool in 10% and by serology in 4%. The statistical analysis was made on SPSS18software.
Results: The mean age was 43.13 years (326 females, 270 males). HP was present at (+++) in 53%, at (++) in 38% et at (+) in 9%. Eradication rate was 93.6% in patients who received sequential therapy and 73% in those who received triple therapy, the difference was statistically significant ($p = 0.001$). The test of superiority showed that sequential therapy was more effective than triple therapy ($p = 0.0005$). Compliance with the medication and tolerability were similar in the two groups.
Conclusion: Our study have showed that sequential therapy have more effectiveness than triple therapy without impact on patient compliance.

Abstract no.: P11.46

"GASTROPANEL" APPLICATION EXPERIENCE IN EXAMINING PATIENTS WITH STOMACH DISEASES

O. Ignatenko, N. Batpenov and E. Boretskaya
Scientific Research Institute of Traumatology and Orthopedics, Laboratory of Clinical Immunology, Astana, Kazakhstan

With the help of "Gastropanel" it is possible to assess the condition and functional activity of the whole mucous coat of stomach by blood testing. This research method is non-invasive and is considered to be comfortable for the patient. It should be noted that "Gastropanel" in comparison with gastroscopy and biopsy examination, is a more sensitive method of research which allows to diagnose considerably smaller changes of the structure and functional activity of the gastric mucosa.
In our laboratory there were examined 82 patients with different stomach complaints: dyspepsia phenomena, epigastric pains, epigastric burning and nausea. It was detected that 75.3% of the patients had antibodies to *H. pylori*. In assessing the level of gastrin-17 and pepsinogen -1, the low level of these markers (19.7% and 14.8% respectively) was registered.
Medical backgrounds were studied and diagnostic gastroscopy tests were conducted in this group of patients. The patients made complaints about heartburn, epigastric pain, and so on. With the help of the testing board "Gastropanel" we detected and revealed atrophic gastritis in the gastric antrum in 46.2% of the patients, 79.2% of whom had *H. pylori* and 20.7% of whom had none.
We carried out diagnostic gastroscopy in 25 patients having the symptoms of atrophic gastritis with the occurrence of *H. pylori*. According to the gastroscopy data atrophic gastritis was confirmed in 23 patients and only two patients showed a normal stomach mucous membrane.
Thus, there appear to be sufficient reasons for the usage of the diagnostic "Gastropanel" as an expedient and appropriate non-invasive diagnostic method of revealing illnesses in the stomach mucous membrane. It is also a reliable method of the primary differential diagnostics of functional dyspepsia and serious organic pathology requiring the implementation of supplementary examination and treatment.

P13 Drug resistance

Abstract no.: P13.01

UPDATE ON THE EFFICACY OF TRIPLE THERAPY FOR *HELICOBACTER PYLORI* INFECTION AND CLARITHROMYCIN RESISTANCE RATES IN SPAIN (2007–2012)

J. Molina-Infante* and J. P. Gisbert†
*Hospital San Pedro de Alcantara, Caceres, Spain; †Hospital La Princesa, Madrid, Spain

Introduction: Triple therapy, which remains the standard treatment for *Helicobacter pylori* infection, should be discouraged when its efficacy is lower than 80% or when clarithromycin resistance rates are above 15–20%. Available data in Spain until 2008 have shown borderline effectiveness of triple therapy (≈80%) and borderline clarithromycin resistance rates (≈15%).
Aim: To update the available evidence on the efficacy of triple therapy and clarithromycin resistance rates in adults in Spain over the last 6 years.
Methods: A literature search (2007–2012) was conducted in Medline and the abstracts books of the annual meetings of several Spanish gastroenterological and microbiological congresses. The search terms were "*Helicobacter pylori*", "Spain" and "clarithromycin". Studies were selected if they included a triple therapy consisting of a proton pump inhibitor with clarithromycin and amoxicillin or if they analyzed *H. pylori* clarithromycin susceptibility in treatment-naïve patients.
Results: There were five articles and nine abstracts (3147 patients) on triple therapy, which showed a mean *H. pylori* cure rate of 70.8% (95% CI = 66–76%). When stratified by the duration of therapy, the mean cure rates were 68.8% (60–76%) for 7-day regimens and 71.8% (68–78%) for 10-day regimens. Regarding clarithromycin resistance rates, four articles and five abstracts (1709 patients) revealed a mean resistance rate of 18.3% (13–22%).
Conclusions: The efficacy of standard triple therapy seems to be unacceptable in recent studies conducted in Spain, possibly associated with higher than previously reported clarithromycin resistance rates.

Abstract no.: P13.02

PREVALENCE OF PRIMARY RESISTANCE OF *HELICOBACTER PYLORI* TO CLARITHROMYCIN (CLA) AND LEVOFLOXACIN (LF) IN SOUTHERN SPAIN

J. Navarro-Jarabo,* F. Fernandez-Sanchez,* N. Fernandez-Moreno,* F. Casado,† A. Hervás,‡ H. Pallarés,§ C. Rodriguez,¶ J. Puente,** A. Pérz-Aísa* and N. Montiel*
*Agencia Sanitaria Costa del Sol, Marbella, Spain; †Hospital Clínico San Cecilio, Granada, Spain; ‡HRU Reina Sofía, Córdoba, Spain; §Hospital Juan Ramón Jimenez, Huelva, Spain; ¶Hospital Puerta del Mar, Cadiz, Spain; **Agencia Sanitaria Alto Guadalquivir, Andújar, Spain

Prevalence of Primary Resistance of *Helicobacter pylori* to Clarithromycin (CLA) and Quinolones (Q) in Southern Spain
Introduction: The outcomes in the eradication of Hp with clarithromycin-based triple therapy depend primarily on the resistance of Hp to antibiotics. Maastricht III Consensus Conference recommends the implementation of loco-regional surveillance programs for primary resistance of Hp to CLA. In our geographical area (Southern Spain) there are no data. The aim of this study is to determine the prevalence of the primary resistance of Hp to CL and LF in our area
Material and Methods: Multicentric cross sectional study in six hospitals located in different cities in Andalusia. Patients included were from both sexes, aged 18–80 years. Patients previously treated for Hp eradication were excluded. Resistance of Hp to CLA and LF was assessed by determining mutations by PCR: mutations of the gen 23s rRNA define CLA-R and mutations of the gen gyrA define LF-R
Results: Four hundred and five gastric samples were collected. CLA-R was detected in 73 patients (18%); CI95%:14 158–21 892. LF-R was detected in 54 patients (13.3%); CI95%:9899–16 767. Heteroresistance was detected for both antibiotics: CLA 37/73 (50.6%) and LF 28/54 (51.8%). Variability for CLA-R was detected between centers, ranging from 10% to 27% with statistical significance (p 0.04). However there were no differences for LF-resistance (p 0.5)
Conclusions: In Southern Spain there are high rate of primary CLA-R and LF-R. There is a wide variability in the rate of CLA-R. More studies are necessary to assess the clinical impact of the Hp -heteroresistance

Abstract no.: P13.03

NATIONWIDE SURVEY ANTIBIOTIC RESISTANT STRAINS OF *HELICOBACTER PYLORI* INFECTION IN THAILAND

R. Vilaichone,*,† P. Gumnarai,†,‡ T. Ratanachu-ek§ and V. Mahachai†,¶
*Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani, Thailand; †National Gastric Cancer and Helicobacter pylori Research Center, Bangkok, Thailand; ‡Department of Biochemistry, Thammasat University Hospital, Pathumthani, Thailand; §Department of Surgery, Rajavithi Hospital, Bangkok, Thailand; ¶Gastroenterology Unit, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand

Objective: The aim of this study was to survey the antibiotic resistant pattern of *H. pylori* infection in different geographical locations in Thailand and to determine factors associated with antibiotic resistance.
Methods: A total of 3964 dyspeptic patients who underwent upper endoscopy from different regions (North, Northeastern, Central and Southern) of Thailand during January 2004–December 2013 were enrolled in this study. Two antral gastric biopsies were obtained for culture and susceptibility tests were performed using E-test.
Results: One thousand and three hundred and fifty patients (34.1%) were infected with *H. pylori* identified by rapid urease test. E-test for amoxycillin (AMX), clarithromycin (CLR), metronidazole (MNZ), and tetracyclin (TET) was successful in 400 isolates and levofloxacin (LVX), ciprofloxacin (CIP) was successful in 208 isolations. The endoscopic findings demonstrated 329 gastritis patients and 71 peptic ulcer patients. The prevalence of antibiotic-resistant *H. pylori* was AMX 5.2%, TET 1.7 %, CLR 3.7%, MNZ 36%, CIP 7.7%, LVX 7.2% and multi-drugs 11%. In CLR resistant strains, age >40 years was significantly higher than age <40 years (4.7% vs 0%; p-value < 0.05). Furthermore, the prevalence of metronidazole resistant in Southern region was significantly higher than Northeastern region (66.7% vs 33.3%; p-value < 0.05).
Conclusion: Prevalence of *H. pylori* infection has decreased in all regions of Thailand. The prevalence of metronidazole resistant strain was highest in Southern region of Thailand and remains the most common antibiotic resistant strains in Thailand whereas clarithromycin resistance has markedly declined in recent years. Age >40 years might be a predictor for clarithromycin resistant strain in Thailand.

Abstract no.: P13.04

ERADICATION OF *HELICOBACTER PYLORI* INFECTION USING NEW TREATMENT STRATEGIES IN A HIGH-RESISTANT CLARITHROMYCIN POPULATION IN SOUTHERN SPAIN

M. L. González-Bárcenas,* J. M. Navarro-Jarabo,* M. C. García Gavilán,* N. Fernández-Moreno,* M. A. Romero-Ordoñez,* F. M. Fernández Cano,* F. Rivas,* J. P. Gisbert† and A. Pérez Aisa*
*Agencia Sanitaria Costa del Sol, Marbella, Spain; †Hospital Universitario de la Princesa, Madrid, Spain

Introduction: Eradication rates of *Helicobacter pylori* (Hp) infection have decreased primarily due to an increase of resistance to clarithromycin. In our geographical region, this resistance is higher than 15%, limiting our use of classical triple therapy. Recent recommendations change the first-line treatments in areas where resistance is over 15–20%.
Objective: To determine the eradication rate achieved of Hp infection in a population with high- resistance levels to clarithromycin by treating with 14-day triple therapy, concomitant therapy, and sequential therapy.
Method: Prospective analysis of naïve patients infected by Hp treated with one of the following: optimized 14-day triple therapy (amoxicillin 1 g/12 hour, clarithromycin 500 mg/12 hour, esomeprazol 40 mg/12 hour), concomitant therapy (clarithromycin 500 mg/12 hour, metronidazole 500 mg/12 hour, amoxicillin 1 g/12 hour and esomeprazol 40 mg/12 hour) during 10 and 14 days, and sequential therapy (5-days: PPI/12 hour and amoxicillin 1 g/12 hour, followed by 5-days: PPI/12 hour, clarithromycin 500 mg/12 hour and metronidazole 500 mg/12 hour). Eradication was tested using the C13 Urea breath test.
Results: Thirty-nine patients (64.1%women, 35.9%men, mean age of 51.54 (±14.38) were initially included (23.1% smokers, 7.7% uninvestigated dyspepsia, 59% functional dyspepsia, 28.2% peptic ulcer, 5.1% other diagnosis). 33.3% received a 14-day classical triple therapy regimen (optimized), 41% were treated with a 10-day concomitant therapy, 17.9% received a 14-day concomitant therapy, and 7.7% followed a sequential regimen. Eradication was achieved in 33/37 (89.2%). Adverse effects were observed in 28.2% of our patients (12.8% diarrhea, 10.3% metallic taste, 10.3% nausea).

Conclusions: Our preliminary results show that the Hp infection should be treated with these new treatment strategies in high clarithromycin-resistant populations. Because this is extracted from a small population, further studies are needed.

Abstract no.: P13.05

PREVALENCE OF CYP2C19 POLYMORPHISMS IN A SUBGROUP OF *HELICOBACTER PYLORI* POSITIVE (HP+) GREEK PATIENTS

S. Lycousi,* N. Mathou,† K. D. Paraskeva,† A. Giannakopoulos,† F. Artemaki,* E. Evgenidou,† S. Papasavvas,† E. Platsouka* and J. A. Karagiannis†
*Microbiology Department, "Konstantopoulio" Hospital, Athens, Greece; †Gastroenterology Unit, "Konstantopoulio" Hospital, Athens, Greece

Background and Aim: Proton pump inhibitors (PPI's) related differences in HP eradication are partly due to CYP2C19 polymorphisms.Their prevalence, correlation with antibiotic resistance molecular tests and role in eradication treatment regimes has not been studied in HP+ Greek patients.
Patients and Methods: One hundred twenty-three patients undergone upper GI endoscopy for various GI symptoms and 59 were tested (+) for HP infection. Molecular genetic test is available to identify HP (GenoType Helico DR Test-HAIN). A multiplex PCR and DNA strip hybridization were performed for resistance to clarithromycin (significant mutation of 23S gene -positions 2146 and 2147) and fluoroquinolones (gyr A gene-codons 87 and 91). Fifty-nine HP+ patients genotyped for CYP2C19*2 and *3 alleles. The CYP2C19*2*3 allele was genotyped by Real-Time PCR method using the Light Mix Kit human CYP2C19*2 and CYP2C19 *3 (TIB MOLBIOL) in Light Cycler 480 (Roche Diagnostic).
Results: Heterozygous extensive metabolizers (HetEM, *2/*1) were 27/59 patients (45.7%). Only two patients (3.38%) were poor metabolizers (PM, *2/*2). There were no *3/*1 or *3/*2 type patients. Eleven patients were homozygous extensive metabolizers (HomEM, wild type, *1/*1) and three patients were poor metabolizers (PM, *2/*2) from the clarithromycin resistant HP+ patients group (13/59, 22%). The three HP+ patient who were resistant to fluoroquilolones were HetEM (*2/*1). Eradication with 14 days regime (PPI + clarithromycin + amoxycilin) was near 96%.
Conclusions: More epidemiological data in Greek population are needed to establish the real prevalence of the CYP2C19 polymorphisms which, combined with the antibiotic resistant molecular test could be useful for difficult to treat patients.

Abstract no.: P13.06

EFFECT OF METRONIDAZOLE RESISTANCE IN THE EFFICACY OF AMOXICILLIN AND METRONIDAZOLE PLUS A PPI (PAM) REGIMEN FOR TREATING *H. PYLORI* (*HP*) INFECTION. A SYSTEMATIC REVIEW AND META-ANALYSIS

M. Baylina Melé,* I. Puig del Castillo,† X. Calvet,*‡ H. Cohen,§ C. Dacoll§ and J. P. Gisbert‡,¶
*Corporació Sanitària Parc Taulí, Sabadell, Spain; †Althaia Xarxa Assistencial Universitària de Manresa, Manresa, Spain; ‡Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spain; §Clínica de Gastroenterología, Facultad de Medicina, Montevideo, Uruguay; ¶Hospital Universitario de La Princesa, Madrid, Spain

Introduction: The influence of metronidazole resistance on the efficacy of PAM triple therapy is not well known. Previous reviews observed that PAM efficacy increases in fourteen-day treatments, when antibiotics are given three times a day (t.i.d.), and when high-dose PPIs are used. The present study aimed to systematically review the effect of metronidazole resistance in PAM efficacy depending on the above-mentioned factors.
Methods: A systematic review was performed in PubMed and the ISI web of knowledge for articles published until May 2013 reporting eradication rates according to metronidazole sensitivity in naïve *Hp* patients treated with PAM. Main analysis compared PAM efficacy in metronidazole-sensitive versus metronidazole-resistant strains. Measure effect was risk difference (RD). A sub-analysis was planned for patients receiving 14-day treatments, high- dose PPIs or t.i.d. antibiotics.
Results: Twenty five studies (32 treatment arms) with 1523 patients were included. Overall cure rates were 0.80; 95% CI: 0.78–0.82. *Hp* was successfully eradicated in 1155 of 1298 patients harbouring metronidazole-sensitive strains (0.89; 95%CI: 0.87–0.91) and in 368 of 610 with metronidazole-resistant strains (0.60; 95%CI: 0.56–0.64). RD was 0.30; 95%CI: 0.26–0.34. However, in the studies using 14-day schedules (eight studies, nine arms, 644 patients), RD difference decreased to 0.22; 95%CI: 0.15–0.28. Not enough data were available for the remaining sub-analyses.
Conclusions: PAM treatment is 30% more effective in metronidazole-susceptible than in metronidazole-resistant strains; administering PAM for 14 days increases its efficacy in resistant strains. Further studies are needed to explore the usefulness of high-dose PPIs and resistance effect for improving PAM efficacy.

Abstract no.: P13.07

EVALUATION OF CLARYTHROMYCIN RESISTANCE AMONG IRANIAN HP ISOLATES BY E-TEST AND REAL TIME PCR

M. Hakemi Vala,* H. Goudarzi,* M. Sarie,† M. Gholami* and A. Ardalan‡
*Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of; †Milad Hospital, Tehran, Iran, Islamic Republic of; ‡Shahid Beheshti University, Tehran, Iran, Islamic Republic of

Introduction: The importance of Hp infection is related to gastric carcinoma and Mucosal associated lymphoma. Usually a triple therapy included two antibiotics plus bismuth sulphate is prescribe for treatment. In most cases treatment failure is related to clarythromycin resistant. Therefore, there is an urgent need to evaluate resistant rate among domestic Hp strains. Based on the key role of clarythromycin in treatment, the aims of this study was evaluation of clarithromycin resistance among Iranian Hp isolates by E-test and real time PCR.
Materials and Methods: Eighty biopsy samples were detected from endoscopy candidates who refer to Milad and Fayazbakhsh hospitals during 2011–2012. All samples were homogenized immediately after they received and cultured on two supplemented blood agar with and without antibiotics (SXT, Vancomycin and amphotricin B). All plates incubated at microaerophil condition and 37°C for 72 hour.
Further Hp identification was done by gram straining and biochemical tests like oxidase and catalase. DNA extraction was done by boiling method. All presumptive Hp colonies were confirmed for ureC detection by PCR method.
Frequency of Clarythromycin resistant was determined by E-test method based on CLSI standards. Among resistant Hp strains with MIC ≥1 mg/L, point mutation profile were determined by real-time PCR assay.
Results: From 80 biopsy samples 20 positive culture were detected and confirmed by biochemical and molecular PCR methods. 21.7% Hp strains showed Clarythromycin resistant phenotype after E- test. Only A 2143G point mutation at melting temperature 54.7°C was detected among resistant strains by real-time PCR method.
Conclusion: Based on the previous reports of clarythromycin resistant in Iran which was 17% versus to 21.7% in recent study, it seems clarythromycin resistant rate is increasing and macrolide drugs must prescribe by precaution.
Key words: *Helicobacter pylori*, clarythromycin, Real time PCR

Abstract no.: P13.08

THE EFFECT OF EMPIRICAL RESCUE THERAPY FOR TWO OR MORE CONSECUTIVE *H. PYLORI* ERADICATION FAILURES

D. Oh,* D. Lee,* K. Kang,* Y. Park,* C. Shin,* N. Kim,* H. Yoon* and H. Jung†
*Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, Korea; †Seoul National University College of Medicine, Seoul, Korea

Background/Aims: The eradication rate of first and second-line therapies have been decreasing progressively due to increasing antimicrobial resistance. After two or more consecutive *H. pylori* eradication failures, clinicians have faced the dilemma of determining which of the following therapy would be the most appropriate. The aim of this study was to elucidate clinical course and treatment strategies of refractory *H. pylori* infection.
Materials and Method: From 2003 to 2012, total 123 (mean age 63.9) patients who had experienced at least two consecutive *H. pylori* eradication failures were enrolled at the Seoul National University Bundang Hospital. Efficacy of different rescue regimens was compared by confirming of eradication rate. Antibiotic susceptibility test for *H. pylori* was not done in all cases.
Result: The clinical & endoscopic findings were as follows: 84 patients (67.7%) – erosive or atrophic gastritis and functional dyspepsia, 15 patients (12.1%) – gastric ulcer, six patients (4.8%) – duodenal ulcer, 10 patients (8.1%) – DU + GU. There was no significant difference in the eradication rate between each rescue regimens. Eradication rates with the 3rd, 4th and

5th-line rescue regimens were 51.6% (62/120), 48.7% (20/41), and 20.0% (2/10), respectively. Finally, cumulative *H. pylori* eradication rate with the 3~7th rescue regimens (mean 3.51 times) was 73.0% (84/115). The cumulative incidence rate of gastric cancers did not differ between the eradicated group and failed group (mean observation period: 42.9 months).
Conclusion: Even with the consecutive treatments of refractory *H. pylori* infection using empirical regimens, *H. pylori* eradication rate was gradually declining. Finally, cumulative overall eradication rate could not achieve over 75%. Now that repeated empirical treatment without culture cause a significant limitation for effective eradication in the future, we should consider careful treatment strategies in refractory *H. pylori* infection.

Abstract no.: P13.09

A LOCAL AUDIT OF ERADICATION RATES FOR FIRST-LINE TRIPLE THERAPY FOR *HELICOBACTER PYLORI* AND DETECTION OF MULTI-RESISTANT INFECTION

S. M. Smith,* G. Holleran,*,† C. O'Morain* and D. McNamara*,†
*Trinity College Dublin, Dublin, Ireland; †Adelaide & Meath Hospital Tallaght, Dublin, Ireland

Introduction: The urea breath test (UBT) demonstrates significant accuracy for initial diagnosis of *Helicobacter pylori* infection and eradication confirmation following treatment.
Aim: To assess local eradication rates of first-line triple therapy in *H. pylori*-infected individuals using the UBT database at Tallaght Hospital.
Methods: Antibiotic use and treatment success were retrospectively investigated in individuals who tested positive for *H. pylori* using the Carbon-13 UBT (cut-off = 4.0 delta over baseline) over a 6-month period in 2012.
Results: In all, 22.4% (587 of 2620) of UBTs in the investigated timeframe were positive for *H. pylori*. Three hundred and thirty positive patients were selected from the local area for further investigation, 81.2% (n = 268) of which were referred from primary care and 18.8% (n = 62) from the gastroenterology clinic. Of the patients referred for a post-treatment test, first-line treatment information was obtained from patients or clinicians for 24.5% (n = 81) of individuals. The most commonly prescribed antibiotics were clarithromycin and amoxicillin (86.4%; n = 70), amoxicillin and metronidazole (8.6%; n = 7), clarithromycin and metronidazole (3.7%; n = 3) and clarithromycin and tetracycline (1.3%; n = 1). Eradication rates were as follows; clarithromycin and amoxicillin (82.9%; n = 58), amoxicillin and metronidazole (71.4%; n = 5), clarithromycin and metronidazole (66.7%; n = 2), and clarithromycin and tetracycline (100%; n = 1). Eradication was unsuccessful in 7.4% (n = 6) of patients following two or more courses of antibiotics.
Conclusions: Therapy involving metronidazole was below the recommended intention-to-treat rate of 80%. Additionally, 7.4% of patients failed to respond to multiple regimens, implying multi-resistant infection. Local antimicrobial surveillance and testing is warranted to guide clinicians in their therapeutic choice.

Abstract no.: P13.10

PRIMARY AND SECONDARY ANTIMICROBIAL RESISTANCE OF *HELICOBACTER PYLORI* (HP) CLINICAL ISOLATES FROM GREEK ADULT PATIENTS

B. Martinez-Gonzalez,* P. Psarrakos,† S. Georgopoulos,‡ S. Michopoulos,§ P. Kasapidis,¶ D. N. Sgouras† and A. F. Mentis†
*Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece; †Hellenic Pasteur Institute, Athens, Greece; ‡Gastroenterology and Hepatology Department, Athens Medical, P. Faliron General Hospital, Athens, Greece; §Gastroenterology Unit, Alexandra Hospital, Athens, Greece; ¶Department of Gastroenterology, Central Clinic of Athens, Athens, Greece

The increasing prevalence of antimicrobial resistant *Helicobacter pylori* strains is a major cause of treatment failure. We evaluated primary and secondary resistance of *Hp* strains from Greek patients to amoxicillin-(AMO), metronidazole-(MET), tetracycline-(TET), clarithromycin-(CLA) and levofloxacin-(LEV). Furthermore, we determined *Hp* gene mutations associated with CLA and LEV resistance.
The study enrolled 266 *Hp* isolates from adult patients (age 52.3 ± 14.3), 160 of which had undergone at least one failed course of treatment and 106 that have not received any previous eradication therapy or PPIs. *Hp* strain susceptibility was assessed by the E-test method, according to the 3rd European Multicentre Study Group, adopting the MIC breakpoints for AMO and LEV (>0.5 mg/L), CLA and TET (>1 mg/L) and MET (>8 mg/L). Presence of genetic mutations were determined by Real-Time PCR (Light Cycler, Roche) in CLA-resistant and by sequencing analysis of *Hp gyrase A* gene in LEV-resistant strains.
No resistance to AMO or TET was detected. Primary resistance levels to MET and CLA were determined at 37.7% (40/106) and 23.6% (25/106), respectively. High levels of secondary resistance to MET (50.6%, 81/160) and CLA (72.3%, 114/160) were also observed. Primary (11.3%) and secondary (15.6%) LEV resistance was observed in isolates that were also resistant to MET and/or CLA. The predominant mutations correlated with CLA-resistance were A2143G and A2142G in *23S rRNA* gene and the Asn87Lys mutation in *gyrA* gene for LEV-resistant strains.
Antimicrobial susceptibility testing should be considered, prior to the selection of the proper antibiotic scheme, in order to achieve greater eradication rates in Greece.

Abstract no.: P13.11

EFFICACY OF SEQUENTIAL THERAPY AND CVADRUPLE THERAPY AS FIRST-LINE REGIMENS IN *HELICOBACTER PYLORI* ERADICATION

A. Singeap,*,† A. Trifan,*,† C. Cojocariu,*,‡ I. Girleanu,* C. Sfarti*,† and C. Stanciu†,§
*University of Medicine and Pharmacy, Iasi, Romania; †"St. Spiridon" Hospital, Center of Gastroenterology and Hepatology, Iasi, Romania; ‡"St. Spiridon" Hospital, Center of Gastroenterology and Hepatology, Iasi, Austria; §Romanian Academy, Iasi, Romania

Background: *H. pylori* is an important pathological factor for gastritis, ulcer, gastric carcinoma and gastric MALT lymphoma. Eradication of infection depends of patient compliance and resistance of bacteria to antibiotics. In the regions with high resistance to clarithromicin, first treatment choice are sequential or cvadruple therapy.
Aim: To evaluate the efficacy of sequential and cvadruple therapy as first-line regimens for *H. pylori* eradication in patients with actual documented infection, untreated previously.
Patients and Methods: We studied the efficacy and tolerance of two eradication regimens, administered to two groups of patients with actual infection (proven by respiratory test, fecal antigen or biopsy): A-sequential therapy with PPI double dose + Amoxicillin 1 g × 2/day 5 days followed by PPI double dose + Clarithromicin 0.5 g × 2/day + Metronidazol 0.5 g × 2/day 5 days, B-cvadruple therapy with PPI double dose + Tetracyclin 0.5 g × 4/day + De-Nol 120 mg × 4/day + Metronidazol 0.25 g × 4/day, 10 days. We analysed also the secondary effects by questionning the patients.
Results: Forty-eight patients were treated. In group A (26 patients), eradication was tested in 22 patients and was obtained in 18 patients (ITT 69%, PP 84%). In group B (22 patients), eradication was tested in 19 patients and was obtained in 18 patients (ITT 72%, PP 86%).
Adverse events appeared in A – 15% cases, B – 18% cases, with no influence on finalizing the treatment.
Conclusions: The efficacy of both eradication treatments proposed as first-line regimens is high. The eradication rate was superior to triple therapy (70–80% in studies) but lower that similar regimens metaanalysis.

Abstract no.: P13.12

AN OVERVIEW OF ANTIBIOTIC PHENOTYPIC RESISTANCES TO *HELICOBACTER PYLORI* (HP) IN SOUTHERN ITALY: MAY TIGECYCLINE BE THE "DRUG OF THE FUTURE?

R. Monno, C. Capolongo, F. Giorgio, G. Losurdo, A. Di Leo and E. Ierardi
University of Bari, Bari, Italy

Background and Aim: Antibiotic resistances to Hp represent a worldwide growing problem and resistant strains are often treated successfully with "rescue therapies". However, none of them reaches a 100% eradication rate. Therefore, aims of this study were: (1) an overview of phenotypic resistances of Hp to conventional antibiotics in our geographic area; (2) the evaluation of a novel antibiotic, i. e. tigecycline, efficacy.
Materials and Methods: Biopsy samples of gastric body and antrum from 127 positive patients were analyzed for bacterium culture and antibiogram by E-test. Breakpoints were considered according to EUCAST 2011–12 MIC values.
Results: Among all the patients 50 had assumed two or more eradicating therapies, 26 had never been treated and no data about previous therapies were available for the remaining 51. The prevalence of resistances for each antibiotic is reported in the table.

Multiple resistances were seen in the 43% of resistant strains. Compared to tetracycline MIC50 and MIC90 values, tigecycline showed a stronger activity (0.032 vs 0.064 μg/mL and 0.125 vs 0.38 μg/mL, respectively).
Conclusions: At the moment tigecycline do not show any resistance in vitro and could be "the drug of the future". However, the need of parenteral administration and the limitation of its use in severe hospital infections, are a real handicap at the moment.

Table 1 Prevalence of antibiotic resistances in the three groups of studied patients

	Treated (50) (%)	Untreated (26) (%)	Unknown (51) (%)
Clarithromycin	40.28	2.63	24.7
Levofloxacin	11.8	2.73	12.97
Metronidazole	28.6	6.77	18.85
Amoxicillin	18.9	2.7	6.8
Tetracycline	8.3	0	4.2
Tigecycline	0	0	0

Abstract no.: P13.13

IS DOXYCYCLINE A VALID ALTERNATIVE AS RESCUE THERAPY FOR MULTIPLE-ANTIBIOTIC RESISTANT *HELICOBACTER PYLORI* IN A SOUTH-EUROPEAN COUNTRY?

N. M. Almeida,* J. M. Romãozinho,* M. M. Donato,† C. Luxo,‡ O. Cardoso,‡ M. A. Cipriano,* C. Marinho* and C. M. R. Sofia*
*Coimbra University Hospital, Coimbra, Portugal; †Faculty of Medicine, Coimbra, Portugal; ‡Faculty of Pharmacy, Coimbra, Portugal

Background: Antimicrobial resistance of *Helicobacter pylori* (Hp) is increasing and treatment of multiple-resistant strains is challenging, mainly in countries where tetracycline, furazolidone and bismuth salts are unavailable.
Aim: This study aimed to compare the efficacy and safety of a triple therapy regimen containing doxycycline for treatment of multiple resistant Hp.
Patients and Methods: Prospective unicentric study, involving 14 adult patients (female-11; mean age-50.4 ± 11.6 years) referred to Hp eradication due to dyspepsia (64.4%), iron-deficient anemia (21.4%) or gastroesophageal reflux needing chronic therapy with proton pump inhibitors (14.2%). A median of two unsuccessful attempts (1–6) of Hp eradication was performed in all of them. They were submitted to upper digestive endoscopy with biopsies for histological and microbiologic characterization. Minimum inhibitory concentration determination, using E-test method, revealed Hp resistance to metronidazole, clarithromycin, levofloxacin and sensitivity to amoxicillin and tetracycline. A triple treatment regime with 13 days of Pantoprazol 80 mg bid and 10 days of Amoxicillin 1 g 12/12 hour + Doxycycline 100 mg 12/12 hour was prescribed. Compliance, adverse events and final result, determined by 13C Urea Breath Test (UBT), were registered.
Results: Adverse effects (nausea, vomiting, abdominal pain) developed in four patients but treatment suspension was necessary in only one of them. UBT 8–10 weeks after treatment was positive in all patients.
Conclusions: Doxycycline, an antibiotic of tetracycline group, presented by some authors as a potential rescue therapy in multiple resistant Hp infections, is not valid as an alternative treatment in our country.

Abstract no.: P13.14

ANTIBIOTIC RESISTANCE OF *HELICOBACTER PYLORI* IN KAZAN, RUSSIA

R. Abdulkhakov,* E. Filipova,* A. Khalikova,* S. Abdulkhakov,*,† L. Akhmetova,* D. Khakimova,* O. Pozdeev,*,‡ L. Morozova,*,‡ A. Safin§ and I. Akhmetov*
*Kazan State Medical University, Kazan, Russian Federation; †Kazan Federal (Volga region) University, Kazan, Russian Federation; ‡Kazan State Medical Academy, Kazan, Russian Federation; §Republican Clinical Hospital № 2, Kazan, Russian Federation

Objective: To study *H. pylori* prevalence in different age groups and *H. pylori* susceptibility to antibiotics used in eradication therapy.
Materials and Methods: *H. pylori* samples isolated from biopsies of gastric mucosa of patients undergoing upper GI endoscopy were examined. *H. pylori* was detected using histology, bacteriological method and PCR. Disc diffusion method was used to determine *H. pylori* antibiotic susceptibility. Sensitivity to two drugs (metronidazole and levofloxacin) was evaluated.
Results: The prevalence of *H. pylori* infection and antibiotic susceptibility was investigated in 308 patients, 198 women and 110 men. The presence of *H. pylori* was determined based on PCR, bacteriology and histology. *H. pylori* was detected in 53% (n = 129) of patients using PCR, in 55% (n = 135) by bacteriology and in 55% (n = 135) of patients by histology.
Prevalence of *H. pylori* infection among 10–19 years old patients (n = 9) was 44%; in 20–29 years old (n = 60) – 47%; 30–39 years old (n = 68) – 59%; 40–49 years old (n = 44) – 47%; 50–59 years old (n = 62) – 58%; 60–69 years old (n = 31) – 48%; 70–79 years old (n = 14) – 42%; 80–89 years old (n = 3) – 33%.
The overall resistance to metronidazole and levofloxacin was 53%, and 5%, respectively.
Conclusions: The revealed prevalence of *H. pylori* infection was 53%. High *H. pylori* infection rate was revealed among all age groups with maximal rate in 30–39 and 50–59 years old group. High level of resistance to metronidazole was detected however levofloxacin was found to be quite effective against *H. pylori* in examined region.

Abstract no.: P13.15

PRIMARY AND SECONDARY *HELICOBACTER PYLORI* METRONIDAZOLE RESISTANCE: IMPACT ON THE EFFICACY OF 10 DAYS OMEPRAZOLE, AMOXICILLIN AND METRONIDAZOLE TRIPLE THERAPY

M. Boudjella, T. Amar, Y. Saadaoui, F. Mouffok, C. Nabil, M. Lahcene and N. Oumnia
Algerian Laboratory Research on Helicobacter, Algeria, Algeria

Aim: To evaluate the impact of primary and secondary *Helicobacter pylori* (*H. pylori*) resistance to metronidazole on the efficacy of 10 days omeprazole, amoxicillin and metronidazole triple therapy.
Patients and Methods: In a prospective and unicentric study, 122 *H.pylori* positive patients (men: 41, mean age: 38.3 years, gastritis: 100, Duodenal ulcer: 28) have been treated between September 2004 and June 2010 by OAM10 regimen (Omeprazole: 2 × 20 mg + Amoxicillin: 2 × 1 g + Metronidazole: 3 × 500 mg; 10 days). Sixty-two among them (group 1) have received this combination therapy as first-line regimen. The others 60 patients (group 2) received OAM10 as second line regimen after failure of clarithromycin based regimen. The métronidazole resistance was tested using E Test (cut-off Resistance = 8 μg/m). The Control of eradication was performed 12 weeks after treatment. The therapeutic success was attested by the negativity of four tests: UBT, RUT + histology and culture.
Results: The primary and secondary metronidazole resistance rates were respectively 37% and 65.2%. Eradication rates of *H. pylori* in group 1 and group 2 was respectively 74% and 58.3 % of cases in ITT and 81% versus 61.4% in PP. In group 1, métronidazole-resistance lowerd efficacy by 15.5% in ITT (82.3% vs 66.7; $p = 0.5$) and 5.4% in PP (82.3% vs 76.9%; $p = 0.9$). In group 2, this resistance reduce efficacy by 14.3% in ITT (64.3% vs 50%; $p = 0.4$) and by 13.7% in PP (69.2% vs 55.5%, $p = 0.4$). There was no statistically significant difference between primary and secondary resistance in their impact on the efficacy of OAM10 regimen as well in ITT then in PP.
Conclusion: In our study, *H.pylori* metronidazole resistance haven't significantly reduces the efficacy of OAM10 as well in first line than in second line. This is an argument for retreating patients with another regimen containing this antibiotic.

Abstract no.: P13.16

CHANGE IN ANTIBIOTIC RESISTANCE OF *HELICOBACTER PYLORI* STRAINS

B. S. Moon,* B. R. An,* H. J. Kim,* S. W. Yi,* H. C. Lim* and Y. C. Lee†
*Yongin Severance Hospital, Yonsei University, Seoul, Korea; †Severance Hospital, Yonsei University, Seoul, Korea

Background/Aim: The eradication rate of *Helicobacter pylori* (HP) has been decreased due to increased antibiotic resistance in Korean patients recently. The aims of this study were to investigate the prevalence of primary antibiotic resistance for recent 4 years.
Materials and Methods: One hundred and sixty-five HP strains were isolated from culture of endoscopic biopsy specimen in Yongin Severance Hospital. Sev-

enty-one patients were enrolled from 2009 to 2010, and 94 patients from 2011 to 2012. The susceptibility of HP strains to clarithromycin, amoxicillin, metronidazole, tetracycline, metronidazole and levofloxacin was assessed using agar gel diffusion method.

Results: The resistance to clarithromycin and metronidazole significantly were increased from 5/71 (7.0%) to 15/94 (16.0 %) ($p = 0.082$) and from 32/71 (45.1%) to 53/94 (56.4%) ($p = 0.150$), respectively. Resistance to amoxicillin and levofloxacin was slightly declined from 2/71 (5.6%) to 2/94 (2.1%) ($p = 0.216$) and from 19/71 (26.8) to 21/94 (22.3%) ($p = 0.512$), respectively. Resistant strains to tetracycline were not found. Dual resistant strains to clarithromycin and metronidazole were increased from 0% (0/71) to 9.6% (9/94) ($p = 0.079$). Among 66 cases who could be to be assessed HP eradication of primary therapy, 75.8% (50/66) were cured. HP eradication rates were lower with clarithromycin resistant strains in 42.9% (3/7) than clarithromycin susceptible strains 79.6% (47/59) ($p = 0.053$).

Conclusion: HP resistant to clarithromycin and metronidazole in 2011–2012 was higher than in 2009–2010. The eradication rates were lower in clarithromycin-resistant strains.

Abstract no.: P13.17

PREDICTING FACTORS FOR *HELICOBACTER PYLORI* ERADICATION FAILURE IN STANDARD TRIPLE THERAPY

A. Singeap,*,† A. Trifan,*,† C. Cojocariu,*,† I. Girleanu,* C. Sfarti*,† and C. Stanciu†,‡

*University of Medicine and Pharmacy, Iasi, Romania; †"St. Spiridon" Hospital, Center of Gastroenterology and Hepatology, Iasi, Romania; ‡Romanian Academy, Iasi, Romania

Background: A higher *H. pylori* eradication rate is an actual objective, due to its important pathogenic role and justified by its variable antibiotic susceptibility.

Aim: To evaluate the predicting factors of *H. pylori* eradication failure using standard triple therapy.

Patients and Methods: We verified *H. pylori* eradication by respiratory test, fecal antigen or biopsy in patients addressed to the Ambulatory of Center of Gastroenterology and Hepatology, which effectuated previously eradication therapy, by standard triple therapy. We correlated the eradication failure with age, gender, indication of eradication (dyspepsia, gastritis, ulcer), side effects, compliance, previously antibiotics administrations.

Results: Fifty-two patients which declared previous eradication treatment were tested. Actual infection was documented in 18 patients (failure rate 34.6%). In univariate analysis, we found a non-significant correlation with age, gender, indication of eradication, side effects; as factors influencing but also in a non-significant manner, we found: the provenience of the prescription (GP or specialist) and previous antibiotics administrations; non-compliance was significantly ($p = 0.005$) correlated with eradication failure.

Conclusions: The only factor significantly correlated with eradication failure was non-compliance; other influencing factors, but in a non-significant manner, were: the provenience of the prescription and previous antibiotic administrations.

Abstract no.: P13.18

MULTIRESISTANCE IN *H. PYLORI*: AN EVALUATION FOR CLARITHROMYCIN RESISTANT STRAINS ISOLATED FROM A PATIENT POPULATION OF ISTANBUL, TURKEY

P. Yuksel,* H. Bahar Tokman,* R. Caliskan Algingil,* E. O. Sevuk,† F. Kalayci,* B. K. Bolek,† A. Kurt,* O. Yilmazli,* O. Akgul,* B. A. Salih,† Y. Z. Erzin,‡ K. Bal‡ and B. S. Kocazeybek*

*Department of Medical Microbiology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; †Department of Biology, Fatih University, Istanbul, Turkey; ‡Department of Gastroenterology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey

Rising clarithromycin resistance of *H.pylori* increased today the recommendation of tetracyclin, metronidasole or levofloksasin based triple or quadruple therapies however multiresistant *H.pylori* strains influences negatively the eradication efforts. The aim of this study was to detrmine the prevalence of multiresistance in clarithromycin resistant *H. pylori* strains isolated from gastric biopsies of patients with peptic ulcer and gastritis, living in Istanbul which can be accepted as a prototyp city of Turkey wıth eurasian geographic features.

Clarithromycin resistance of *H. pylori* isolates was determined by E test (Liophilchem,Italy) and point mutations responsible from this resistance was determined by Real Time PCR using Clarithromycin resistance kit (AnDiaTec, ROCHE).The minimal inhibition concentration (MIC) of tetracyclin, metronidasole and levofloxacin for these 22 *H. pylori* strains were detrmined by E test.and susceptibilities were defined according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The ATCC 43504 strain of *H. pylori* was used as reference strain.

A total of 22 clarithromycin resistant *H. pylori* isolates were identified from gastric biopsies of patients.The clarithromycin resistance was due to a point mutation of A2144G and A2143G .Co-resistance was identified in 54.5% of these strains and the most common two co-resistant phenotyp were clarithromycin/metronidasole and clarithromycin/levofloxacin resistant phenotyps with a rate of 45.4% and 27.2 % respectively.Additionnaly a clarithromycin/metronidasole/levofloxacin co-resistance was determined in 18.1% of our strains.

According to the presence of multiresistant strains, empirical treatment including clarithromycin, metronidazole or levofloxacin will stay ineffective in our region in the near future. Performing the susceptibility test and treatment of the patient according to the susceptibility report could be effective for a succesfull treatment and for redusing the increasing resistance of *H. pylori*.

Abstract no.: P13.19

MUTATIONS IN THE 23S RRNA GENE OF CLARITHROMYCIN-RESISTANT *HELICOBACTER PYLORI* STRAINS FROM LOWER SILESIA, POLAND

A. Bińkowska,* M. M. Biernat,* J. Grabińska,* Ł. Łaczmański† and G. Gościniak*

*Department of Microbiology, Wroclaw Medical University, Wroclaw, Poland; †Department of Endocrinology and Diabetology, Wroclaw Medical University, Wroclaw, Poland

Objectives: The eradication rate of *Helicobacter pylori* is decreasing due to antibiotic resistance, mainly to clarithromycin. The prevalence and the relationship between mutations in this gene and clarithromycin susceptibility was studied.

Materials and Methods: The study was performed on 42 *H. pylori* strains isolated from children and adults diagnosed for *H. pylori* infection. Analyzed strains were divided in three groups: first consisted of 14 strains resistant to clarithromycin from children, the second was composed of 14 strains resistant to clarithromycin from adults and control group consisted of 14 susceptible strains. The clarithromycin sensitivity was determined by gradient diffusion (E-test) assay. The 23S rRNA gene amplified by PCR, was further analyzed for point mutations responsible for clarithromycin resistance by sequencing analysis.

Results: In first group, the A2143G mutation was identified in 71% (n = 10) whereas A2142G mutation in 14% (n = 2) of strains. Among strains from adults, the A2143G mutation was detected in 50% (n = 7) and A2142G mutation in 21% (n = 3) of strains. *H. pylori* strains from children 14.3% (n = 2) and from adults 29% (n = 4) presented other mutations of the 23S rRNA gene at positions T2182C, G2223A or C2244T. No potential mutations were detected in strains from control group.

Conclusion: Our results suggest that susceptibility to clarithromycin is predicted by detection of mutations at positions 2143 and 2142 of the 23S rRNA gene whereas mutation A2143G is the most prevalent in this study. Other point mutations of the 23S rRNA gene could be also responsible for resistance to clarithromycin.

Abstract no.: P13.20

ANTIMICROBIAL RESISTANCE OF *H. PYLORI*: DATA FROM ISTANBUL AND ITS SUBURBS

H. Bahar Tokman,* H. Cakan,† R. Caliskan Algingil,* E. O. Sevuk,‡ P. Yuksel,* A. Kurt,* B. K. Bolek,‡ F. Kalayci,* O. Yilmazli,* O. Akgul,* B. A. Salih‡ and B. S. Kocazeybek*

*Department of Medical Microbiology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; †Department of Microbiology, Faculty of Forensic Science, Istanbul University, Istanbul, Turkey; ‡Department of Biology, Fatih University, Istanbul, Turkey

Surveillance of antimicrobial resistance patterns of *H. pylori* can provide for our cauntry a general guidlines in the effort to eradicate *H. pylori*. The aim of this sudy was to evaluate, in a patient population of Istanbul and its suburbs of Turkey which has western (Asia) and eastern (Europe) geographic features, the prevalence of resistance of *H. pylori* to amoxicillin, clarithromycin, metronidazole, tetracycline and levofloxacin.

Ninety-eight *H. pylori* strains were isolated between February 2012 and January 2013 from patients coming from the Asian and European sides of Istanbul. MICs of amoxicillin, clarithromycin, metronidazole, tetracycline and levofloxacin were determined by E test (Liophilchem, Italy). Susceptibilities were defined according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standarts Institute (CLSI). The ATCC 43504 strain of *H. pylori* was used as control .The point mutations responsible from the clarithromycin resistance was determined by Real Time PCR using Clarithromycin resistance kit (AnDiaTec -ROCHE).According to EUCAST and CLSI breakpoints, all of our isolates were found susceptible to amoxicillin (MIC ranges, <0.016–0.032 mg/L) and tetracycline (MIC ranges, <0.016–0.032 mg/L). 37.5% (36/98) of our isolates were found resistant to clarithromycin (MIC ranges, <0.016–>256 mg/L), 35.5% (34/98) resistant to metronidasole (MIC ranges, <0.016–>256 mg/L) and 40.8 % (40/98) resistant to levofloxacin (MIC ranges, <0.008–256 mg/L).The clarithromycin resistance was due to a point mutation of A2144G and A2143G. Our *H. pylori* strains isolated from an euasian population of Istanbul and suburbs showed an increased clarithromycin, metronidasole and especially fluoroquinolone resistance. This situation will discourage the clinician to use a treatment containing these antibiotics. We belive that adaptation of new treatment strategies according to local resistance patterns of *H. pylori* will be useful in the near future.

Abstract no.: P13.21

PREVALENCE OF *HELICOBACTER PYLORI* RESISTANCE TO METRONIDAZOLE, CLARITHROMYCIN, AMOXYCILLIN, TETRACYCLINE, ERYTHROMYCIN AND NITROFURANTON IN MONGOLIA

J. Sarantuya,* B. Mandkhai,† M. Enkh-Ulzii‡ and N. Bira*
*Health Science University of Mongolia, Ulaanbaatar, Mongolia; †Ach Medical Institute, Ulaanbaatar, Mongolia; ‡Shastins Clinical Hospital, Ulaanbaatar, Mongolia

Backround: The resistance of *H. pylori* to the recently available antibiotic treatment regimens has been a growing problem. Therefore aim of study was to determine the prevalence of antibiotic resistance among *H. pylori* strains isolated from Mongolians and to provide a new molecular method easily detect mutations predictive of clarithromycin resistance in *H. pylori*.
Method: All 262 samples of gastric biopsies were obtained during upper gastrointestinal endoscopy from the patients referred for the exploration of clinical gastritis. All urease positive samples were cultured according to standard microbiological procedures. The *H. pylori* strains were grown under microaerophilic conditions on selective Pylori agar. *H. pylori* antibiotic sensitivity was examined using Etest method. In addition, the mutations of the corresponding gene were studied by GenoType HelicoDR DNA strip testing.
Result: Total of 262 gastric biopsy specimens, 63.3% (166) were confirmed to have gastric *H. pylori* infection by CLO test. We have successfully obtained 68.6% (114) pure *H. pylori* isolates. The overall *H. pylori* Etest antibiotic resistance rates were 52.8% for clarithromycin, 67.3% for metronidazole, 33.4% for amoxicillin, 40% for tetracycline, 26.7% for erythromycin and 13.3% for nitrofuranton. Both resistances were significantly higher in female than in male patients. GenoType HelicoDR test result for detection of 14 clarithromycin resistance clinical strains. Overall, the most frequent mutation was A2147G (MUT3 profile), observed in five strains, followed by D91N (MUT1) in only one strain.
Conclusion: The prevalence of *H. pylori* infection increased among Mongolian population. In the present study, *H. pylori* metronidazol-resistant strains are more frequently found in Mongolians and clarithromycin-resistant strains frequently have mutations in the 23S rRNA gene.

Abstract no.: P13.22

THE STANDARD TRIPLE THERAPY WAS NOT EFFECTIVE FOR PATIENTS WHO HAD 23S RIBOSOMAL RNA MUTATED *HELICOBACTER PYLORI*

M. Jung and J. Lee
Deparment of Internal Medicine, Kyungpook National University School of Medicine, Daegu, Korea

Background and Aims: Proton pump inhibitor and two types of antimicrobial agents, amoxicillin, and clarithromycin have been widely used in the eradication of *Helicobacter pylori* (*H. pylori*). However, antibiotic resistant strain has been rapidly increased as an important factor for the failure of eradication. Previously, special attention was given to the mechanism of clarithromycin resistance, and mutations of A2143G or A2144G in the bacterial 23S ribosomal RNA (23S rRNA) gene were well established with the relation of clarithromycin resistance.
Methods: Patients, who was diagnosed chronic gastritis, peptic ulcer disease or gastric epithelial neoplasm, was examined by *H. pylori* PCR and mutation at 23S ribosomal RNA (23S rRNA). Positive *H. pylori* PCR with or without 23S rRNA was eradicated by standard triple therapy with amoxicillin, proton pump inhibitor, and clarithromycin. We try to evaluate the predictor of *H. pylori* eradication as 23S rRNA mutation.
Results: Two hundred eighty seven patient was studied. *H. pylori* PCR positive was 139/287 (48.4%). 23S rRNA mutatation was 34/139 (24.5%). The eradication rate of *H. pylori* for the point mutated group was 30.4% (7/23), and significantly lower than those of wild type group which are 63.8% (51/80), respectively ($p = 0.005$)
Conclusion: When 23S rRNA point mutation was positive, the standard triple therapy was not effective. We should consider the alternative regimen for the 23S rRNA point mutation group. Next we try to evaluate the standard quadruple therapy against the point mutation group.

P14 Immunity, Animal Models & Vaccines

Abstract no.: P14.01

HARNESSING GENOMICS AND IMMUNOINFORMATICS TO IDENTIFY IMMUNOGENIC *H. PYLORI* SEQUENCES FOR VACCINE DEVELOPMENT

S. F. Moss,* S. Zhang,* J. Aponte,* J. Desrosiers,† F. Terry,‡ L. Fast,*,† W. Martin,‡ A. De Groot†,‡ and L. Moise†,‡

*Brown University, Providence, RI, USA; †University of Rhode Island, Providence, RI, USA; ‡Epivax Inc., Providence, RI, USA

Background: After 25 years of effort, the development of an *H. pylori* vaccine for humans has stalled. The availability of multiple *H. pylori* genomes coupled with advances in immunoinformatics now permits novel approaches to rational epitope selection for multivalent T cell vaccine design.

Aim: To comprehensively characterize peripheral T cell response to a panel of conserved *H. pylori* MHC class II epitopes.

Methods: Ninety *H. pylori* MHC class II epitopes predicted to be highly immunogenic based upon consensus strain sequence analysis and MHC II binding assays (Ardito et al, Immunome Res 2011) were synthesized. Their ability to induce 24-hour recall responses in peripheral blood mononuclear cells (PBMCs) from 15 HP+ and 15 HP− subjects was evaluated by IFN-gamma ELISPOTS. Cytometric bead array (CBA) cytokine multiplex assays were performed in 24-hour culture supernatants from five representative HP+ and five HP− cases

Results: Individual peptides stimulated significantly higher IFN-gamma ELISPOT values in PBMCs from HP+ subjects. The average number of spot-forming cells/million PBMCs was 10.4 ± 1.5 in HP+ versus 1.89 ± 0.2 in HP− ($p < 0.0001$). Significantly elevated levels of TNF-alpha, IL2, IL4, IL6, and IL10 were noted in HP+ cases compared with HP- whereas IL17A expression was no different between groups.

Conclusion: Computational mining of the *H. pylori* genomes has identified a panel of immunogenic consensus sequences of peptides that have now been validated as human MHC class II epitopes in IFN-gamma ELISPOT assays. This panel can be utilized as the basis to formulate a multi-epitope T-cell vaccine.

Abstract no.: P14.02

NOVEL *HELICOBACTER* SPECIES ISOLATED FROM ASIAN MICE INDUCE TYPHLOCOLITIS IN C57BL/6 IL10$^{-/-}$ MICE

Z. Shen, Y. Feng, S. Muthupalani, L. E. Cheaney, C. A. Kaufman and J. G. Fox

Massachusetts Institute of Technology, Cambridge, MA, USA

Helicobacter species infections have been reported worldwide in numerous colonies of laboratory mice. Select enterohepatic *Helicobacter* species (EHS) cause inflammatory bowel disease (IBD), colon cancer, hepatitis and hepatocellular carcinoma in mice. These findings have prompted screening for EHS in mouse health monitoring protocols. A novel EHS was isolated from the stomach and intestines of clinically normal mice received from three Asian institutes. The novel EHS was microaerobic, grew at 37 and 42°C, was catalase and oxidase positive, but urease negative. It is most closely related to the16S rRNA gene of *H. muridarum* (98%); and to the *rpo*B gene of *H. hepaticus* (90%). The novel EHS has in vitro CDT activity; its *cdt*B gene sequence has 83% identity with that of *H. hepaticus*. When the organism was inoculated into C57BL/6 IL10$^{-/-}$ mice, it was cultured from the stomach, colon and cecum of infected mice at 6 and 10 weeks post infection. The cecum had the highest colonization levels by quantitative PCR. The histopathology of the lower bowel was characterized by moderate to severe inflammation, with associated mild edema, epithelial defects and mild to moderate hyperplasia and dysplasia. Inflammatory cytokines IFNγ, TNFα, IL17 and iNOS were significantly up-regulated in the cecal tissue of infected mice. These results demonstrate that novel EHS can induce IBD in IL10 -/- mice and highlight the importance of identifying *Helicobacter* spp. especially when they are introduced from outside colonies from different geographic locations. Exclusion of EHS from mouse colonies will reduce the risk of compromising research results.

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P15 Probiotics

Abstract no.: P15.01

NUTRACEUTICAL TREATMENT AGAINST *HELICOBACTER PYLORI* INFECTION?

A. M. Santos,* M. T. Lopes,* T. Pereira,† P. Chaves,† M. Oleastro,‡ J. Machado‡ and A. Guerreiro*

*CEDOC-Faculdade de Ciências Médicas, Lisboa, Portugal; †Instituto Português de Oncologia Dr. Francisco Gentil, Lisboa, Portugal; ‡Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal

Helicobacter pylori induces gastric mucosa inflammation leading to gastritis and peptic ulcer disease. A wide variety of nutraceuticals are known to possess anti-inflammatory properties.

Aim: To study the effect of two nutraceuticals (curcumin and synbiotic 2000®) in prevention of gastric mucosa inflammation on chronic *H. pylori* experimental infection.

Materials and Methods: Sixty C57BL/6 mice were inoculated with SS1 – *H. pylori* strain by gavage. After infection confirmation by ^{13}C-urea breath test mice where then treated with either PBS, curcumin (10 mg/mouse) or synbiotic 2000® (50 mg/mouse), three times per week. Five mice from each group were euthanized at week 6, 18, 27 and 57. Gastric samples were removed for histology (H-E) and immunohistochemistry analysis.

Results: All the 60 mice were Hp positive by ^{13}C-urea breath test and immunohistochemistry. In the PBS group the gastric mucosa inflammation was present in 40% of mice at week 6 and 18, in 75% at week 27 and in 100% of mice at week 57. In the curcumin group there was no mucosa inflammation at week 6 and 18. At week 27, 55% of mice presented mucosa inflammation and at week 57, 57% of mice had mucosa inflammation. In the synbiotic group gastric mucosa inflammation was also not present at week 6 and 18. At week 27 and 57 the percentage of mice with gastric mucosa inflammation was 32% and 71% respectively. The treatment with either curcumin or synbiotic significantly decreased the gastric mucosa inflammation at all time-points.

Conclusions: These results suggest the therapeutic usefulness of both nutraceuticals in reducing the gastric inflammation during chronic experimental mice *H. pylori* infection.

The supplementation of diet in humans with curcumin or synbiotic 2000® may be a novel therapeutic approach against gastric inflammation induced by Hp infection.

Abstract no.: P15.02

ADDITION OF DIFFERENT PROBIOTICS TO THE STANDARD ERADICATION THERAPY OF *HELICOBACTER PYLORI* INFECTION: COMPARATIVE ANALYSIS OF EFFICACY

N. V. Baryshnikova,* Y. P. Uspenskiy* and A. N. Suvorov†

*St-Petersburg State Medical University n.a. I.P.Pavlov, St-Petersburg, Russian Federation; †Science Research Institute of Experimental Medicine RAMS, St-Petersburg, Russian Federation

Aim: To analyze the efficacy of addition of different probiotics to the standard eradication therapy in patients infected with *H. pylori*.

Methods: One hundred and fifteen patients with *H. pylori* were divided into four groups. All patients received standard eradication therapy (omeprasol 20 mg, amoxicillin 1000 mg, claritromicin 500 mg twice a day during 7 days). 1st group (23 patients) in addition received *Enterococcus faecium strain L-3* three grages three times a day during 1 month. 2nd group (32 patients) – received *Bacillus subtilis* contain probiotic 2 capsules two times a day during 1 month. 3rd group (40 patients) – probiotic with *Bifidobacterium bifidum* MF 20/5 10^7, *Bifidobacterium longum* SP 07/3 10^7, *Lactobacillus gasseri* PA 16/8 10^7 one tablet once a day during 1 month. 4th group (20 patients) received only standard eradication therapy. Detection of *H. pylori* was made by rapid urease test, gistological method, polymerase chain reaction to all patients before treatment and 1.5–2 month after the end of therapy. Bacteriological analysis of excrements was made to evaluate changes in colon microflora. Statistical estimation was performed in Statistica 6.0 for Windows XP. Efficacy of *H. pylori* eradication was estimated by intention to treat criteria.

Results: Eradication rate in 1st group was 75% ($p < 0.05$), in 2nd group – 72% ($p < 0.05$), in 3rd group – 82% ($p < 0.01$ in comparison to 4th group) and in 4th group – 60%. In 4th group were detected increase of opportunistic bacteria in colon and decrease of *Bifidobacteria* and *Lactobacilli*. Disorders in colon microflora content were no found in other groups.

Conclusion: Usage only a standard eradication therapy is not quite effective. Addition of probiotics to the standard treatment significantly improves eradication rate and safety of treatment.

Abstract no.: P15.03

FLOROLACT DURING ANTIHELICOBACTER THERAPY

B. D. Starostin and G. Starostina

SPb GBUZ City Policlinic № 38, Gastroenterological Department, Saint-Petersburg, Russian Federation

Purpose: To evaluate the impact of Florolakt on eradication *Helicobacter pylori* (H.p.) and adverse events during standard sequential antihelicobacter (anti-H.p.) therapy.

Materials: In first group (n = 73) patients with duodenal ulcer or gastric ulcer, chronic gastroduodenitis associated with H.p. received the standard version of sequential therapy: pantoprazole (sanpraz), amoxicillin (flemoxin), clarithromycin (fromilid), tinidazole plus additionally prebiotic florolakt 1 envelope × 2 times a day. Patients in second group (n = 43) received only the same anti-H.p. regime without florolakt. It was evaluated eradication H.p. (per protocolum – PP and intention to treat – ITT) and adverse events related to the anti-H.p. regime. Prebiotic Florolact contains fructooligosaccharides, gum arabic, and lactitol, which have a synergistic prebiotic effect.

Results: In first group using florolakt eradication H.p. was observed in 90% (ITT) and 94% (PP). In second group during anti-H.p. regime without florolakt eradication H.p. Seventy-nine percent ITT ($p > 0.05$) and 87% PP ($p > 0.05$). The emergence of antibiotic-associated diarrhea observed respectively in first group 2.7% against 13.9% in second group (ITT) – ($p < 0.05$) and respectively in 2.9% versus 16.2 % ($p < 0.05$) – PP. Other adverse events (nausea, abdominal discomfort, changes in taste) in first group with adjuvant therapy by florolakt were observed in 16% versus 26% in second group, who were not taking florolakt ($p < 0.05$). Total adverse events in second group were met more than two times higher than in first group.

Conclusions: The introduction of prebiotic florolakt significantly reduces the incidence of adverse events and increased the eradication *H.pylori*.

Abstract no.: P15.04

CAN PROBIOTICS INCREASE THE SUCCESSFUL ERADICATION OF *HELICOBACTER PYLORI* INFECTION IN KOREA?

M. Choi, S. Park, Y. Kim, D. Koh, S. Kae, J. Lee and H. Jang

Dongtan Sacred Heart Hospital, Hallym University College of Medicine, Hwaseong-si, Gyeonggi-do, Korea

Background/Aims: This study was performed to evaluate whether the addition of probiotics to proton pump inhibitor-based triple therapy increases the likelihood of successful *Helicobacter pylori* (*H. pylori*) eradication.

Methods: We retrospectively reviewed 218 patients who undertaken *H. pylori* eradication therapy between March 2010 and February 2012. These patients were classified three groups, (A) PPI-based 7-day triple therapy, (B) the same triple therapy plus probiotics for 7-day, and (C) the same triple therapy plus probiotics for 14-day. We compared eradication rates of three group. ^{13}C-urea breath test was performed at 4 weeks after completion of the therapy to confirm the successful eradication.

Results: The total eradication rate of these patients was 71.1% (155/218). By per protocol analysis, *H. pylori* eradication rates for the groups A, B, and C were 64.4% (67/104), 80.8% (42/52), and 74.2% (46/62) respectively ($p = 0.086$). The eradication rate of the group B was significantly higher than that of the group A ($p = 0.036$). In subgroup analysis, *H .pylori* eradication rate of the probiotics group (B and C) was 77.2% (88/114), which was significantly higher than that of the non-probiotics group (A) ($p = 0.038$). Regardless of adding probiotics, the eradication rate of group C which was treated for 14 days was not significantly higher than that of group A and B which was treated for 7 days (74.2% vs 69.9%, $p = 0.525$).

Conclusions: The addition of probiotics to conventional triple therapy increases the eradication rate of *H. pylori* in this study, regardless of duration of eradication treatment.

Abstract no.: P15.05

MONGOLIAN PROBIOTIC INHIBITION AGAINST *H. PYLORI*

J. Sarantuya,* B. Mandkhai,† N. Bira* and J. Dugersuren‡
*Health Science University of Mongolia, Ulaanbaatar, Mongolia; †Ach Medical institute, Ulaanbaatar, Mongolia; ‡Mongolian Veterinary Institute, Ulaanbaatar, Mongolia

Background: *Helicobacter pylori* is a highly prevalent pathogen considered as an aetiological factor for gastroduododenal ulcers, and a risk factor for gastric adenocarcinoma and lymphoma in humans.The evidence that some strains of Lactobacillus and Bifidobacterium are able to inhibit *H. pylori* growth through the release of bacteriocins or organic acids. Therefore, it is important to in vitro study develop low-cost, large-scale, alternative probiotic to the at-risk population to prevent or decrease *H. pylori* colonization.
Methods: Eighteen samples of gastric biopsies were obtained during upper gastrointestinal endoscopy from the patients referred for the exploration of clinical gastritis. All samples were cultured according to standard microbiological procedures. An in vitro disk diffusion assay was employed to assess the lactic acid bacteria LBO1, 2, 3, 4, 6, 7 cells and cell free supernatants (CFS) and bifidobacteria BFO1, BFO4 anti- *H. pylori* activity.
Results: Ability of LBO1 strain to inhibit growth of *H. pylori* is 55.5% [95% CI 32.5–78.4], LBO2 88.8% [95% CI 74.2–103.3], LBO3 50% [95% CI 26.9–73.0] and LBO4 38.8% [95% CI 32.5–78.4]. Then LBO 6 and LBO7 strains had no inhibitory activity against *H. pylori*. Average inhibition zone is 11.6 mm for LBO1, 11.3 mm for LBO2, 10.2 mm for LBO3 and 10.5 mm for LBO4.
Inhibitiory activity of Lactobacillus CFS1 against *H. pylori* accounts for 61.1%, CFS2 for 72.2%, and CFS3 for 33.3%, while CFS4 inhibits only HP78 strain. CFS6 and CFS7 were both Lactobacillus LBO cultures. Average inhibition zone is 10 for CFS1, 11.3 mm for CFS2 and 10.3 mm for CFS3.
Bifidobacterium BFO1 strain was 83.3% inhibition activity. But BFO4 was not inhibit against all *H. pylori* strains.
Conclusion: Lactobacillus LBO2 and Bifidobacterium BFO1 strains were isolated from Mongolian traditional fermented milk product were obtained more inhibition against *H. pylori* strains other Lactobacillus LBO and Bifidobacterium BFO strains.

Abstract no.: P15.06

DIFFERENTIATED APPROACHES TO THE ADMINISTRATION OF PRE- AND PROBIOTICS TO THE PEPTIC ULCER PATIENTS ON THE BACKGROUND OF ANTIHELICOBACTERIAL THERAPY

I. N. Skrypnyk,* G. Maslova* and N. Kharchenko†
*Ukrainian Medical Stomatological Academy, Poltava, Ukraine; †National Medical Academy of the Postgraduate Education n.a. P.L.Shupik, Kyiv, Ukraine

In accordance with Maastricht Consensus-IV the presence of peptic ulcer (PU) of duodenum is a clear indication for carrying out antihelicobacterial therapy (AHBT). In our investigation it has been established that after prescribing AHBT 20% of PU pts have irritable bowel syndrome (IBS), associated with intestinal dysbiosis (ID) of stages I–II.
The aim of this investigation is to evaluate the effectiveness of using pre- and probiotics in the complex therapy of PU pts as means for primary and secondary prevention of ID, appearing on the AHBT background.
One hundred and fifteen PU HP-positive pts have been examined, they being administered AHBT: Pantoprasole, Clarithromycin and Amoxicillin in usual dosages for 10 days.
In 100% of PU pts after the treatment the healing of the ulcer has been noted, 89.6% has had eradication of HP. Twenty-two PU pts has been administered additionally to AHBT biosynthetic lactic acid, 22 pts with obstipation prescribed Lactulose, it permitting to prevent ID in 100% cases.
24 PU pts and IBS associated with ID I-II with obstipation the administration of Lactulose in combination with AHBT assisted to the disappearance of ID in 100%. At the same time the administration of biosynthetic lactic acid to 32 PU pts having ID fully put away the presence of ID in 77% cases. Fifteen PU pts having ID at the absence or sharp decrease of bifidobacterium titre in faeces have been administered Lactulose in combination with bifidobacterial yoghurt, it resulting in disappearance of dysbiosis in 86.7% cases.
At administrating AHBT the PU pts must also have pre- or probiotics with the aim of the primary prevention of ID or its treatment. Lactulose or Lactulose in combination with bifidobacterial yoghurt may be of the most beneficial effect in relation of the intestinal microbiocenosis normalization.

Abstract no.: P15.07

A COMBINATION OF PRE- AND PROBIOTICS, VITAMINS, MINERALS, ANTIOXIDANTS AND ANTI-INFLAMMATORY AGENTS AS A NEW THERAPEUTIC APPROACH FOR INCREASED INTESTINAL PERMEABILITY

G. Ianiro,* V. Ojetti,* E. Gaetani,* L. Laterza,* F. Scaldaferri,* F. Beghella Bartoli,* S. De Martino,* G. Dinoi,* G. Gigante,* G. Cammarota,* G. Gasbarrini† and A. Gasbarrini*
*Division of Intern, Department of Internal Medicine, Division of Gastroenterology, Catholic University School of Medicine, Rome, Italy; ‡"Ricerca in Medicina" ONLUS Foundation, Rome, Italy

Background: Impairment of the intestinal barrier has been involved in the pathogenesis of several diseases. Atrophic gastritis is a common consequence of a long-term untreated *H. pylori* infection, and may lead to small intestinal bacterial overgrowth, that is a cause of impaired intestinal permeability. To date, there is no standard treatment for increased intestinal permeability.
Aim and Methods: Our aim was to assess the efficacy of a dietary complement containing pre- and probiotics, vitamins, minerals, antioxidants and anti-inflammatory agents in ameliorating the increased intestinal permeability and global state of health in subjects with IBS without constipation. In this proof-of-concept study, a combination of pre- and probiotics (*L. acidophilus* NCFM, B lactis Bi-07), vitamins, minerals, antioxidants and anti-inflammatory agents (Nutrimonium®) was added to the common diet of 33 subjects with D-IBS or M-IBS and increased intestinal permeability (measured with 51CrEDTA Test) for 60 days. After the treatment, each patient repeated the intestinal permeability test with 51CrEDTA. Global health state with a slightly modified version of EQ-5D VAS (0–100 mm) was evaluated at baseline and after the treatment.
Results: All the patients completed the treatment. Five patients were unable to repeat the 51CrEDTA Test and were withdrawn from the study. In the remaining 28 patients, the mean 51CrEDTA score was respectively 7.43 (SD: ±2.75) at baseline and 5.93 (SD: ±2.7333) after the treatment ($p = 0.089$). Mean EQ-5D VAS was respectively 40 (SD: ±14.86) at baseline and 64.61 (SD: ±9) after the treatment ($p < 0.0001$).
Conclusions: Restoring the impaired gut barrier may be challenging, especially since there are several therapeutic targets to hit, as gut microbiota, intestinal mucus, intestinal immune cells, tight-junction function, et cetera. A multimodal approach, with a combination of different healing agents, seems to be effective both in improving intestinal permeability and in ameliorating symptoms. Since this study has many limitations, further, randomized controlled trials, with an adequate sample size, are needed to confirm these results.

P16 Other Helicobacters

Abstract no.: P16.01

ORAL GLUTAMINE SUPPLEMENTATION REDUCES *HELICOBACTER SUIS* – RELATED GASTRIC PATHOLOGIES IN AN EXPERIMENTAL PIG MODEL

E. De Bruyne,* B. Flahou,* F. Pasmans,* A. Smet,* S. Millet,† R. Ducatelle* and F. Haesebrouck*

*Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; †Institute for Agricultural and Fisheries Research (ILVO), Melle, Belgium

Helicobacter (*H.*) *suis* is a zoonotic bacterium causing gastritis and reduced daily weight gain in pigs. A metabolic profiling study has shown that *H. suis* depletes glutamine (Gln) from the bacterial culture medium. *H. suis* -glutamyltranspeptidase plays an important role in the depletion of this nutrient by conversion to glutamate, which can subsequently be taken up and used as an energy source by *H. suis*. Gln depletion may however have consequences for the health of epithelial cells as well as lymphocytes. Therefore, we aimed at investigating the effect of orally supplemented Gln on *H. suis*-linked gastric pathologies in a pig model. Pigs experimentally infected with *H. suis* were divided in three groups: one receiving a standard diet and two groups receiving a Gln supplemented diet (1% or 4%). Animals were sacrificed 4 weeks after infection. Histopathological examination revealed a chronic gastritis in the antrum of *H. suis*-inoculated pigs receiving the standard diet. Providing the Gln 1% diet did not result in lower gastritis scores (2.2 vs 2.4 for the standard diet). However, pigs belonging to the Gln 4% group showed a pronounced reduction of gastric lymphocytic infiltration (average gastritis score 1.2 vs 2.4). In both Gln-supplemented groups, a significant downregulation of IL-8 expression ($p = 0.001$) could be observed. These results show that Gln supplementation may be helpful in reducing gastric pathological changes evoked by an *H. suis* infection in pigs.

Abstract no.: P16.02

HELICOBACTER SPECIES ISOLATED FROM THE FECES OF SWINE USED FOR BIOMEDICAL RESEARCH

Z. Shen, Y. Feng, L. Moody and J. G. Fox

Massachusetts Institute of Technology, Cambridge, MA, USA

Swine have proven to be particularly effective in biomedical research for human diseases because of their genetic makeup, and being closely aligned with humans. Atypical *H. canadensis* has been isolated from swine feces in Denmark and The Netherlands. In this study, feces from 44 swine used for biomedical research in two different U.S. institutes were screened for Helicobacter species. All the fecal samples were positive for helicobacter using helicobacter genus specific primers; 84% of the samples were positive with *H. canadensis* species specific PCR. Using microaerobic conditions ($N_2$80; $H_2$10; $CO_2$10) feces were plated on CVA and blood agar after filtration through a 0.65 μm filter; four different *Helicobacter* species were isolated. 16S rRNA gene and *hsp60* gene sequence analysis indicated that these four species were *H. canadensis, H. equorum, Helicobacter sp. flexispira* taxon 6 and a novel *Helicobacter* spp., each of which was distinguishable by RFLP analysis. The *H. canadensis* and *H.equorum* strains isolated from the U. S. swine are similar to the strains isolated from European farms in biochemical profiles, EM morphology and 16S rRNA gene sequence homology. Beside *Helicobacter* species, *C. coli, C. jejuni, C. hyointestinalis, C. lanienae* and *C. mucosalis* were also isolated from swine feces screened in this survey. In summary, pigs used in biomedical research have a high prevalence of helicobacter and campylobacter infections. *Helicobacter* spp. and *Campylobacter* spp. may differ in pigs from different sources; however most pigs are colonized with multiple *Helicobacter* spp. and *Campylobacter* spp. which may pose a risk of zoonotic transmission for personnel using pigs in biomedical research.

Abstract no.: P16.03

GENOTYPIC DESCRIPTION OF A NOVEL *HELICOBACTER SUD-CETORUM* ISOLATED FROM WILD DOLPHIN STRANDED IN WESTERN AUSTRALIA

C. Tay, T. Perkins, F. Thirriot, E. Chua and B. Marshall

University of Western Australia, Nedlands, WA, Australia

Helicobacter sud-cetorum, was cultured from the various location of the main and pylorus stomachs of a wild, stranded spotted dolphins (Stenella attenuata). High quality draft genomes of five isolates (two from the main and three from the pylorus stomach) were sequenced using illumina MiSeq platform. Similar to *H. pylori*, the GC content of the genome is about 40%. The 1 million 150 bp paired end reads were denovo assembled into 27 contigs by CLC genomic benchwork and the genome size was estimated to be 1.4 mbp. Due to the high nucleotide diversity, only 15% of the reads were mapped to the two published H. cetorum strains (strain 00-7128 and 99-5656) and to two *H. pylori* strains (strain 26695 and J99). At least 1400 genes were annotated by Rapid Annotation using Subsystem Technology (RAST). This H. sud-cetorum strain were shown presence of the vacA gene; presence of two divergent ureAB genes (one in a urease cluster with all the accessory genes and one locating elsewhere); absence of tlpABC chemotaxis genes; and absence of cagPAI. Nucleotide-level pair-wise comparison of the flagella system between the H. sud-cetorum (strain ATFT1), two H. cetorum (strain 00-7128 and 99-5656), and five *H. pylori* (strain 26695, J99, G27, P12, and Puno120) suggested that they were conserved. The basal body genes were about 80% similar across the strains; the hook genes were about 70% similar; the biosynthetic and regulatory genes were about 77% similar; and the filament genes were about 60% similar. This study discuss the micro-evolution between the five isolates cultured from different location of the stomach and for the first time described the unique features of H. sud-cetorum genome.

Abstract no.: P16.04

HELICOBACTER CANIS COLONIZATION IN SHEEP: A ZOONOTIC LINK

A. G. Swennes,* M. L. Turk,* E. Trowel,* C. Cullin,* J. S. Pang,* Z. Shen,* F. E. Dewhirst† and J. G. Fox*

*Massachusetts Institute of Technology, Cambridge, MA, USA; †The Forsyth Institute, Cambridge, MA, USA

Enterohepatic *Helicobacter* spp. (formerly classified as *Flexispira rappini*) have been identified in sheep and associated with abortion. Because sheep-origin *Helicobacter* spp. have not been extensively characterized, a fecal culture-based survey of several New England sheep flocks was undertaken. Sheep feces were collected in sterile *Brucella* broth containing 10% glycerol. Samples were plated on 5% sheep blood agar and CVA agar and cultured at 37°C under microaerobic conditions in vented jars containing N2, H2, and CO2 (80:10:10). *Helicobacter*-positive samples were identified by colony morphology, phase-contrast microscopy, Gram-negative staining, and *Helicobacter* genus-specific 16S rRNA PCR. From 1 flock, *Helicobacter* spp. were cultured from six of 23 (26%) sheep, and their putative species identity and clonality was confirmed by RFLP and REP-PCR. DNA was subsequently extracted from five pure isolates for 16S rRNA sequencing. BLASTn alignment confirmed isolates shared 99% identity with *Helicobacter canis*. Isolates were subjected to biochemical testing and compared to *H. canis* strains NCTC-12740 (human-origin), ATCC-51401 (dog-origin), MIT 98-153 (cat-origin) and MIT 99-7633 (rhesus macaque-origin). Although *H. canis* has not been associated with a specific ovine disease syndrome, it has been associated with canine hepatitis, canine and feline diarrhea, and feline intestinal adenocarcinoma. *H. canis'* role in hepatic and intestinal disease is given further plausibility by extensive prior experimental enterohepatic *Helicobacter* spp. use in mouse inflammation and neoplasia models. *H. canis* has also been isolated from humans with bacteremia or gastroenteritis and has been associated with Crohn's disease and hepatitis. In all human cases, patients had a history of dog or cat contact, suggesting zoonotic transmission. This study identifies sheep as new and potentially important *H. canis* reservoir host, via direct zoonotic transmission or indirect transmission by dogs or cats. Interspecies transmission of enterohepatic *Helicobacter* species, particularly *H. canis*, merits continued study.

Abstract no.: P16.05

AVAILABILITY OF A SPECIFIC SYNTHETIC ANTIGEN OF *HELICOBACTER HEPATICUS* FOR SEROLOGICAL DIAGNOSIS

T. Shimoyama,* R. Takahashi,† I. Mizuki,‡ Y. Fukuda† and S. Fukuda*

*Department of Gastroenterology, HIrosaki University Graduate School of Medicine, Hirosaki, Japan; †General Medicine and Community Health Science of Sasayama Medical Center, Hyogo College of Medicine, Nishinomiya, Japan; ‡Department of Internal Medicine, Fuyoukai Murakami Hospital, Aomori, Japan

Background and Aim: We had developed a monoclonal antibody HRII-51 which has high specificity for *H. hepaticus*. MAb HRII-51-immunoreactant was molecular weight of 15 kDa (HH-15). Purification of HH-15 is so complicated

and thus we examined the possibility of synthesized HH-15 for serological diagnosis of *H. hepaticus* infection.
Methods: HH-15 antigen was synthesized by Fmoc solid phase chemistry (synthetic antigen HH-15). Direct sandwich ELISA was prepared in which synthetic antigen HH-15 was immobilized on ELISA plates. Specificity and sensitivity of this ELISA was examined using sera obtained from mice inoculated with *H. hepaticus* (n = 22), *H. bilis* (n = 11) *and H. pylori* (n = 10). Sera from mice inoculated with *H. hepaticus* were also tested after absorption with *H. hepaticus* cell lysate.
Results: After the absorption with *H. hepaticus* cell lysate, OD value of sera from mice inoculated with *H. hepaticus* decreased significantly ($p < 0.01$). A cut-off value to define *H. hepaticus* seropositivity was defined as the mean OD value plus 2-fold standard deviation of these absorbed sera. By using this cut-off value, specificity and sensitivity of synthetic antigen HH-15-based ELISA in mice inoculated *Helicobacter* spp. were estimated as 90.5% (19/21) and 72.7% (16/22).
Conclusion: Synthetic histone-like DNA binding protein of *H. hepaticus* (HH15) would be a useful ligand for the diagnostic test of *H. hepaticus* infection.

Abstract no.: P16.06

EFFECT OF *HELICOBACTER PYLORI* ERADICATION ON SERUM LIPID LEVELS

S. Song, W. Kim, C. Kwon, K. Ko, S. Hong and P. Park
CHA Bundang Medical Center, CHA University, Sungnam-si, Korea

Background/Aims: *Helicobacter pylori* infection was associated with the pathogenesis of atherosclerosis. However, the relationship between *Helicobacter pylori* infection and serum lipid levels remains controversial. The aim of this study was to determine whether eradication of *Helicobacter pylori* affects serum lipid levels.
Methods: A total of 311 subjects who underwent health check-up in Bundang CHA hospital between January 2008 and January 2013 were investigated retrospectively. All subjects underwent gastroduodenoscopy, and *Helicobacter pylori* infection was diagnosed by a rapid urease test or histology. Serum total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), and fasting blood glucose were measured at baseline and after 2 months in all subjects.
Results: In the study we included 151 subjects who received successful treatment for *Helicobacter pylori* eradication and 160 control subjects who did not receive the treatment. No significant differences in TC, HDL, LDL, TG and fasting blood glucose were found between two groups at baseline. TC and LDL levels were decreased significantly in subjects who received successful eradication therapy ($p < 0.05$) whereas fasting blood glucose, TG and HDL levels were not significantly changed.
Conclusion: TC and LDL levels were decreased after *Helicobacter pylori* eradication, TC and HDL levels were not. *Helicobacter pylori* eradication may effects on the TC and LDL levels.
Keywords: *Helicobacter pylori* eradication, cholesterol

Abstract no.: P16.07

PRESENCE OF *HELICOBACTER SUIS* AND LACTOBACILLUS STRAINS IN STOMACHS OF PIGS WITH AND WITHOUT ULCER OF THE PARS OESOPHAGEA

L. R. Almeida,* L. B. Alvim,* P. S. Costa,* A. M. A. Nascimento,* Á. C. Nunes,* D. M. M. Queiroz,† G. A. Rocha† and S. B. Moura*
*Instituto de Ciências Biológicas/UFMG, Belo Horizonte, MG, Brazil; †Faculdade de Medicina/UFMG, Belo Horizonte, MG, Brazil

Ulcer of the *pars oesophagea* is a prevalent and serious problem in swine production. Data about the association of the disease and *Helicobacter suis* are still controversial. Although gastric *Lactobacillus* has been isolated from healthy pigs, there is no study comparing the strains of *Lactobacillus* in stomachs with and without ulcer. Therefore, bacterial load and diversity of *Lactobacillus* strains as well as the presence of *H. suis* was investigated in stomachs with (n = 13) and without (n = 10) ulcer of the *pars oesophagea* from slaughtered pigs. Antrum, corpus and *pars oesophagea* biopsies, obtained with 5 mm biopsy punches, were used for *Helicobacter* and *H. suis* nested PCRs and for quantitative *Lactobacillus* culture on MRS agar. Repetitive sequence-based PCR using $(GTG)_5$ primer was employed to differentiate *Lactobacillus* isolates. Attempts to culture *H. suis* were also made as described by Baele et al (2008). *H. suis* primary culture was obtained from the gastric mucus of four from eight stomachs with ulcer that were urease-positive. Specific PCRs for *Helicobacter* genus and *H. suis* were positive in 20 (86.9%) animals and did not associate with ulcer of the *pars oesophagea* ($p > 0.05$). The number of *Lactobacillus* isolates was significantly greater in the antrum and corpus of stomachs without ulcer ($p < 0.001$ for both). The dendogram based on $(GTG)_5$-PCR fingerprint analysis of the isolates showed unique and distinct profiles between the groups. These results may be helpful in choosing probiotics as an alternative strategy for prevention or treatment of gastric ulcer in swine.
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Abstract no.: P16.08

A PRELIMINARY REPORT OF *HELICOBACTER* LIKE ORGANISMS IN GASTRIC MUCOSA OF SPANISH HORSES

A. A. Morales and A. Mendez
Department of Anatomy and Comparative Anatomic Pathology, College of Veterinary Medicine, University of Cordoba, Cordoba, Spain

The aim of this study was to report the presence of *Helicobacter* infection in Spanish horses. Were studied eight horses with a history of acute abdominal crisis (colic) and gastric ulcers syndrome in the Department of Anatomy and Comparative Anatomic Pathology, College of Veterinary Medicine. University of Cordoba, Spain. All horses were necropsies. Samples of gastric tissue were collected. The tissue samples fixed in formalin were processed by conventional histological techniques. Additionally, the special staining procedure of Warthin-Starry. Of the eight horses studied, 25% Grade 0 Epithelium is intact throughout; no hyperemia, no hyperkeratosis (yellowish color, sloughing). Grade 1 Mucosa is intact but there are areas of hyperemia and/or hyperkeratosis 12.5%. Grade 2 Small, single or multi-focal erosions or ulcers 25%. Grade 3 Large, single or multi-focal ulcers, or extensive erosions and sloughing 25%. Grade 4 Extensive ulcers, with areas of deep submucosal penetration 12.5%. Using the Warthin-Starry special stain, spiral-shaped bacteria only in the gastric mucosa glandular were found in 6/8 (75%), Grade 1: 17%, Grade 2: 33.3%, Grade 3: 33.3%, Grade 4: 16.6%. To conclude, we detected the presence of *Helicobacter* Like Organisms in the gastric mucosa with acute abdominal crisis (colic) and Gastric Ulcer Syndrome of Spain horses.

Abstract no.: P16.09

RECURRENCE OF GASTRIC POLYPS AFTER ENDOSCOPIC REMOVAL WITH OR WITHOUT *HELICOBACTER PYLORI* ERADICATION

S. Y. Kim, J. S. Han, K. H. Suh, S. W. Jung and S. W. Lee
Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea

Background: Gastric polyps are not rare finding during endoscopic examination. Evidence indicates that eradication of *Helicobacter pylori (H. pylori)* leads to disappearance of hyperplastic polyps in the stomach. However, little is known about the effect of the eradication of *H. pylori* on the recurrence of hyperplastic or other gastric polyps following endoscopic removal. The aim of the study was to evaluate the recurrence rate of gastric polyps after endoscopic removal and the association between the polyp recurrence and *H. pylori* eradication.
Methods: The medical records of patients diagnosed with gastric polyps between January 2008 and December 2009 were reviewed. Gastric polyps were removed by biopsy or polypectomy according to the size of polyps. *H. pylori* infection and the success of eradication were assessed by endoscopic biopsy or urea breath test. At follow-up endoscopy, recurrence of gastric polyps was investigated.
Results: Of the 248 patients with gastric polyps, 134 patients who underwent the follow-up endoscopy at least 2 months later after removal of polyps were analyzed. Among 58 patients with *H. pylori* infection, 41 received the eradication therapy. During mean follow-up for 29.5 months, two patients showed the recurrence of gastric polyp after *H. pylori* eradication and no recurrence was observed in patients who did not receive the eradication therapy. There was no significant difference in recurrence rate of gastric polyps between patients with and without *H. pylori* eradication.
Conclusion: The eradication of *H. pylori* did not reduce the recurrence of gastric polyp following endoscopic removal of gastric polyps.

Abstract no.: P16.10

CLINICAL SIGNIFICANCE OF *HELICOBACTER SUIS* IN IDIOPATHIC PARKINSONISM

S. M. Dobbs,*,†,‡ R. J. Dobbs,*,†,‡ A. Smet,§ C. Weller,* A. Charlett,*,¶ I. Bjarnason,† A. J. Lawson,** M. A. A. Ibrahim,†† D. Taylor,*,‡ C. Blaecher,§ F. Pasmans,§ R. Ducatelle,§ B. Flahou§ and F. Haesebrouck§

*Pharmaceutical Science, King's College London, London, UK; †Department of Gastroenterology, King's College Hospital, London, UK; ‡Maudsley Hospital, London, UK; §Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; ¶Statistics Unit, Centre for Infectious Disease Surveillance and Control, Public Health England, London, UK; **Laboratory of Gastrointestinal Pathogens, Public Health England, London, UK; ††Diagnostic Immunology Laboratory, King's College and St Thomas's Hospitals, London, UK

Background: *Helicobacter suis* has an adverse profile in idiopathic parkinsonism (IP): – All-cause (age-at-diagnosis-/gender-adjusted) mortality was eight-times greater where *H. suis* was detected in archived gastric-biopsy DNA-extracts (6/19) than where it was not (2/40 (one lost to follow-up)). With *H. suis*-positivity, but not *H. pylori,* platelet count tended to be lower and volume was higher, implying destruction. B-cell count was lower with both. A U-turn in brady/hypokinesia was described (*Helicobacter* 2005;10:267–75) in an IP-patient after eradicating a spiral *Helicobacter,* now identified as *H. suis*. Here, we describe a second case (CC). We investigate whether *H. suis* (unlike *pylori*) does not 'guard' against small-intestinal-bacterial-overgrowth, which is common in, and deleterious to, IP (Gut Pathogens 2012;4:12).

Methods: Subsequent to gastric-biopsy culture for *H. pylori,* and (if negative) PCR targeting 16S rRNA and *vacA,* DNA-extracts from the above 60 IP-probands, with 20 follow-up samples, were examined for *H. suis* using a *ureA*-based species-specific qPCR.

Results: After *H. pylori* eradication (n = 30), peak-hydrogen in a 2-hour lactulose-hydrogen-breath-test was 2.5 (95% CI: 1.2, 4.8) times higher than before (n = 25) ($p = 0.01$), *H. suis*-status having no additional effect. CC's gastric biopsy (*H. pylori*-negative) and blood were *H. suis*-positive. After tetracycline/clarithyromycin/PPI, chronic profuse sweating ceased and marked reduction in facial swelling (mask-like face) was sustained over 6 months.

Conclusions: Excess mortality and a case of presumed systemic-infection indicate need for prospective study of *H. suis* in IP. Peak-hydrogen was not influenced by *H. suis*-status after allowing for effect of *H. pylori* eradication: factors other than overgrowth may determine excess mortality.

Abstract no.: P16.11

THE DETECTION OF *HELICOBACTER* SPECIES DNA IN POLISH PATIENTS WITH INFLAMMATORY BOWEL DISEASES – PRELIMINARY STUDY

M. M. Biernat,* A. Bińkowska,* E. Poniewierka,† K. Neubauer,† R. Kempiński,† J. Grabińska* and G. Gościniak*

*Department of Microbiology, Wroclaw Medical University, Wroclaw, Poland; †Department of Gastroenterology and Hepatology, Wroclaw Medical University, Wroclaw, Poland

Inflammatory bowel diseases are diseases of unkown etiology. It has been speculated that they are result of activation of the immune response to an unknown antigen. Microorganisms of the genus *Helicobacter* could be this unknown antigen. The aim of this study was to determine the presence of *Helicobacter* species in patients with inflammatory bowel diseases.

Material and Methods: The study was performed on 47 colon biopsies and serum samples taken from 22 patients with Crohn disease and from 25 patients with ulcerative colitis. The 16S rRNA gene of Helicobcater spp. was amplified by PCR and analyzed by sequencing analysis. Anti-*H. pylori* IgG antibodies detection was performed by ELISA.

Results: Among analyzed colon biopsies, 18 (38%) was positive for *Helicobacter* 16S rRNA gene. *H. pylori* was detected in 12 (25%) patients, whereas other *Helicobacter* spp. in 6 (12%) patients. In patients with Crohn disease *H. pylori* genetic material was detected in 3 (6%) biopsies whereas in patients with ulcerative colitis in 9 (19%). Other *Helicobacter* species were detected in 2 (4%) patients with Crohn disease and in 4 (8%) with ulcerative colitis. Anti-*H. pylori* IgG antibodies were detected in 10 (45%) of serum samples of patients with Crohn disease and in 11 (44%) of patients with ulcerative colitis.

Conclusions: Our results show that microorganisms of the genus *Helicobacter* spp. might play a role in pathogenesis of inflammatory bowel diseases. Further studies, especially culture of these bacteria, are needed to establish the relationship between presence of *Helicobacter* spp. and inflammatory bowel diseases.

P18 *H. pylori* and Gastric Cancer

Abstract no.: P18.01

EFFECT OF LONG TERM USE OF PROTON PUMP INHIBITOR ON ATROPHIC GASTRITIS AND GASTRIC CANCER: A FIVE-YEAR LONGITUDINAL COHORT STUDY IN SOUTH KOREA

S. Kim, S. Kang, K. Hong, J. Kim and H. Jung
Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea

Objectives: Gastric cancer is second most common malignancy and *H.pylori* infection is widespread among adults in Korea. Proton pump inhibitors (PPIs) are broadly used and study for an association between PPIs therapy and gastric cancer (GC) are limited. We evaluated the effect of long term therapy of PPIs on atrophic gastritis (AG) and GC in Korea.
Methods: We identified all adult patients took a prescription of PPIs among those who visited the Seoul National University Hospital from January 1, 2005 to December 31, 2009. We reviewed total 23 385 patients who were exposed to PPIs and performed gastroduodenoscopy. The patients were divided into three groups according to the treatment duration (group 1: <3 months; group 2: 3–12 months; group 3: >12 months) and followed from the time to take the first prescription of PPIs until the last visit. The development of GC was defined as diagnosis after more than 1 year from the first PPIs prescription day. Relative risks (RR) were calculated using Cox proportional hazards models.
Results: Total 445 patients diagnosed as AG and 100 patients as GC during average 18 563 and 19 171 person-years of follow-up, respectively. Longer duration of PPIs therapy was associated with increased risk of AG (RR: 1.29; 95% CI: 1.04–1.59 in group 2, RR: 1.39; 95% CI: 1.01–1.92 in group 3, $p = 0.02$), after adjusted for age, gender, helicobacter infection, and co-morbidities. When we divided patients into two groups according to helicobacter infection, these association was stronger and clear in helicobacter non infected group. GC development showed increased tendency in longer duration of PPI groups but it was not significant association with the duration of PPI use.
Conclusions: The long term use of PPI is associated with the development of atrophic gastritis. However, it was not associated with the development of gastric cancer.

Abstract no.: P18.02

THE ROLE OF AUTOPHAGY IN *HELICOBACTER PYLORI*-RELATED GASTRIC CANCER

N. Castano Rodriguez,* N. O. Kaakoush,* K. Goh,† K. Fock,‡ D. Forman§ and H. Mitchell*
*The University of New South Wales, Sydney, NSW, Australia; †University of Malaya, Kuala Lumpur, Malaysia; ‡Changi General Hospital, Singapore, Singapore; §International Agency for Research on Cancer, Lyon, France

Introduction: Autophagy, a degradation pathway in which cytoplasmic content is engulfed and degraded by the lysosome, plays a pivotal role in inflammation and immunity. Because defects in autophagy lead to increased susceptibility to infection, we investigated the role of autophagy in *Helicobacter pylori*-related gastric cancer (GC).
Methods: Gene expression of 84 molecules involved in autophagy was assessed through quantitative real-time PCR in gastric epithelial cells (AGS) challenged with *H. pylori* GC026, a highly virulent strain (CagA+, VacA s1m1+) isolated from a GC patient. Further, polymorphisms in *ATG16L1* (rs2241880) and *IGRM* (rs13361189, rs4958847) were detected by MALDI-TOF mass spectrometry in 304 ethnic Chinese individuals (86 non-cardia GC cases/218 controls with functional dyspepsia).
Results: Autophagy might be particularly impaired by highly virulent *H. pylori* strains as molecules involved in the induction (*ULK1*, *Beclin1*, *AMBRA1*, *UVRAG*, *VPS15*, *WIPI1*) and maturation (*ATG5*, *ATG12*, *ATG16L1*, *ATG16L2*, *GABARAP*, *ATG4C*) of autophagosomes showed significant down-regulation in *H. pylori* GC026-challenged AGS cells. Remarkably, *IGRM* showed decreased expression levels in *H. pylori* GC026-challenged AGS cells (fold-regulation: −9.64, *p*-value: 0.016), a gene encoding an IFN-inducible GTPase that stimulates oxidative, autophagic, membranolytic, and inflammasome-related antimicrobial activities. Further, in Chinese individuals, *ATG16L1*-rs2241880 increased the risk of GC (OR: 1.85, 95% CI: 1.10–3.11) while *IGRM*-rs4958847 was protective (OR: 0.30, 95% CI: 0.11–0.84).
Discussion: Highly virulent *H. pylori* strains might evade/exploit autophagy for survival and replication, a mechanism that would lead to perpetuation of the infection and subsequent chronic inflammation in the host. *ATG16L1*-rs2241880 and *IGRM*-rs4958847 are novel polymorphisms associated with GC in Chinese.

Abstract no.: P18.03

GENETIC POLYMORPHISMS AND EXPRESSION OF GENES INVOLVED IN THE NOD-LIKE RECEPTORS SIGNALLING PATHWAY AND *HELICOBACTER PYLORI*-RELATED GASTRIC CANCER

N. Castano Rodriguez,* N. O. Kaakoush,* K. Goh,† K. Fock,‡ D. Forman§ and H. Mitchell*
*The University of New South Wales, Sydney, NSW, Australia; †University of Malaya, Kuala Lumpur, Malaysia; ‡Changi General Hospital, Singapore, Singapore; §International Agency for Research on Cancer, Lyon, France

Introduction: NOD-like receptors (NLRs) form inflammasomes, intracellular multiprotein complexes critical for generating mature pro-inflammatory cytokines. Here, we investigated for the first time the role of genetic polymorphisms and expression of genes involved in the NLR signalling pathway in *Helicobacter pylori*-related gastric cancer (GC).
Methods: A case-control study was conducted to determine host genetic polymorphisms in 310 ethnic Chinese (87 non-cardia GC cases and 223 controls with functional dyspepsia). Gene expression of 84 molecules involved in the NLRs signalling pathway was assessed through quantitative real-time PCR in THP-1 cells challenged with two *H. pylori* strains, GC026 (GC) and 26695 (gastritis).
Results: Fifty polymorphisms were detected by PCR, real-time PCR and MALDI-TOF mass spectrometry. Three polymorphisms showed significant associations with GC (*NLRP3*-rs10754558, *CARD8*-rs11672725, *CARD8*-rs12984929). On multivariate analysis, *CARD8*-rs11672725 remained a risk factor even after adjustment (OR: 4.80, 95%CI: 1.39–16.58). Statistical analyses assessing the joint effect of *H. pylori* infection and the selected polymorphisms revealed strong associations with GC (*CARD8*-rs10405717, *CARD8*-rs12984929, *NLRP3*-rs12079994, *NLRP3*-rs3806265, *NLRP3*-rs4612666, *CASP1*-rs2282659, *CASP1*-rs530537, *CASP1*-rs61751523 and *NLRP12*-rs2866112). Gene expression analyses showed down-regulation of *NLRP12* and *NLRX1* and up-regulation of *PTGS2* upon exposure to both *H. pylori* strains, however, *H. pylori* GC026 showed the greatest changes. Despite down-regulation of molecules involved in early stages of the NLR signalling pathway, persistent up-regulation of *NF-kB* in *H. pylori* GC026-challenged cells was observed.
Discussion: NLRs are clearly involved in GC. Detection of associations between molecules involved in innate immunity and *H. pylori* -related GC has the potential to provide insights into targeted treatment in genetically-susceptible individuals.

Abstract no.: P18.04

ROLE OF IQGAP1 IN *HELICOBACTER PYLORI*-INDUCED EPITHELIAL TO MESENCHYMAL TRANSITION OF GASTRIC EPITHELIAL CELLS

E. Bessède,*,† C. Staedel,†,‡ L. Chambonnier,*,† F. Mégraud*,† and C. Varon*,†
*INSERM U853, Bordeaux, France; †Université de Bordeaux, Bordeaux, France; ‡INSERM U869, Bordeaux, France

IQGAP1 is a scaffolding protein involved in the regulation of cadherin-based cell adhesion of epithelial cells. This protein plays a crucial role in maintaining the integrity of cell/cell junctions, and deregulation of its expression has been described in gastric adenocarcinoma. The oncogenicity of *Helicobacter pylori* is mainly associated with the CagA oncoprotein, which alters epithelial integrity by disrupting cadherin-based cell/cell junctions. We have shown that this is accompanied by an epithelial to mesenchymal transition (EMT), leading to the emergence of cells with cancer stem cell (CSC) properties (Bessède *et al.*, under revision/review?; Baud *et al.*, 2013). The aim of our project was to determine the role of IQGAP1 in response to *H. pylori*-induced EMT in vitro.
Co-culture experiments on gastric epithelial cell lines with *H. pylori* CagA positive or negative mutant strains were performed, and the expression of IQGAP1 and EMT markers was evaluated. Secondly, inhibition of IQGAP1 by siRNA was performed to evaluate the consequences on *H. pylori*-induced morphological changes, EMT and acquisition of CSC properties. Results reveal that *H. pylori* infection induced morphological changes and a hummingbird phenotype associated with a delocalization of IQGAP1 from cell/cell junctions. Furthermore, they were associated with an upregulation of IQGAP1 expression, both in a

CagA dependent manner. Preliminary results suggest that IQGAP1 may participate in *H. pylori*-induced EMT.
In conclusion, these results suggest that IQGAP1 could be a target of *H. pylori* and CagA and may participate in *H. pylori*-induced cell/cell junctions disruption, EMT and gastric disease.

Abstract no.: P18.05

SEROLOGIC SCREENING OF GASTRIC CANCER RISK USING RECOMBINANT *HELICOBACTER PYLORI* TIP-α PROTEIN

S. Saberi,* Y. Talebkhan,* M. Esmaili,* P. Hassanpour,* M. Bababeik,* M. Doozbakhshan,* A. Oghalaie,* M. Eshagh Hosseini,† M. Mohagheghi‡ and M. Mohammadi*

*Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of; †Endoscopy Clinic, Amiralam Hospital, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; ‡Cancer Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of

Introduction: *H. pylori* infection induces chronic inflammation associated with induction of pro-inflammatory cytokines, such as tumor necrosis factor-α, the key cytokine in tumor promotion. One of the TNF-α inducing protein (Tip-α) gene family in *H. pylori* genome is Tip-α, which acts as a carcinogenic factor by induction of TNF-α gene expression. We have, therefore, evaluated serum antibodies against this protein as a candidate risk marker of gastric cancer.
Methods: Fifty eight *H. pylori* sero-positive (27 nonulcer dyspepsia and 31 gastric cancer) patients were screened. Tip-α genes of an Iranian and 26695 *H. pylori* strains were amplified, cloned and expressed in E. coli under IPTG induction. Partial sequencing and restriction digestion analysis was used for identity confirmation. The recombinant proteins were used in a western blotting technique to screen patients' sera using HRP-conjugated anti-human antibodies as secondary antibodies. Data analysis was performed using STATA ver.10.
Results: There was a 97% sequence homology between Tip-α gene of Iranian *H. pylori* strain and 26695. Sero-reactivity to the rTip-α was significantly higher (62.8%) among gastric cancer patients than those with NUD (37.2%) ($p = 0.019$). The presence of serum antibodies to the recombinant antigen increased the risk of gastric cancer by 4.6 folds (OR = 4.6; 95%CI = 1.3–17.0). Adjustment for the potential confounders of age and gender amplified the risk by more than 30% (OR = 6.1; 95%CI = 1.5–25.2).
Conclusion: Our preliminary data support a predictive role for serum antibodies against Tip-α protein in non-invasive screening of gastric cancer risk amongst Iranian *H. pylori* infected subjects. Larger sample sizes are, however, required before drawing a firm conclusion.

Abstract no.: P18.06

RISK FACTORS OF MULTIPLE GASTRIC NEOPLASMS AFTER ENDOSCOPIC RESECTION

J. Lim, S. Kim, J. Choi, J. Im, J. Kim and H. Jung

Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea

Background: To clarify risk factors for metachronous or synchronous gastric neoplasm.
Methods: We reviewed medical records of patients who had endoscopic resection for gastric high-grade adenomas or early gastric cancers between April 2005 and February 2011. Metachronous neoplasm was defined as high-grade adenoma or carcinoma developing more than one year after endoscopic resection at another site in the stomach. Second neoplasm found within one year was defined as synchronous neoplasm. We reviewed following parameters: age, sex, *H. pylori* status, size and pathologic type of primary neoplasm, gastric atrophy and intestinal metaplasia.
Results: Among 1044 subjects, 45 had metachronous neoplasms and 56 had synchronous neoplasms. In univariate analysis, male gender, age ≥65, antral location, absence of *H. pylori*, <4.5 cm of size, atrophy and intestinal metaplasia were related to multiple gastric neoplasms. In multivariate analysis, antral location (OR1.638, 95%CI: 1.028–2.609), absence of *H. pylori* (OR1.559, 95%CI: 1.010–2.406), intestinal metaplasia (OR6.765, 95%CI: 1.638–27.947) were revealed to be independent risk factors of multiple gastric neoplasm. For metachronous neoplasm, age of 65 or more (OR2.091.95% CI: 1.082–4.041) and absence of *H. pylori* (OR2.374, 95%CI: 1.216–4.636) were found to be independent risk factors. However, only intestinal metaplasia was an independent risk factor of synchronous neoplasm. *H. pylori* eradication did not affect the incidence of multiple gastric neoplasms.
Conclusion: Among endoscopically resected gastric neoplasms, lesions located in the antrum and accompanied by intestinal metaplasia without *H. pylori* infection were likely to develop synchronous or metachronous neoplasms. For lesions with these characteristics, short term follow-up and meticulous endoscopic evaluation are recommended.

Abstract no.: P18.07

ERADICATION RATES OF *HELICOBACTER PYLORI* INFECTION FOR STOMACH CANCER PATIENTS WHO UNDERGO SUBTOTAL GASTRECTOMY

K. Kang,* D. Lee,* D. Oh,* N. Kim,* Y. Park,* C. Shin,* H. Yoon* and H. Jung†

*Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam-si, Korea; †Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea

Introduction: The eradication rate of *Helicobacter pylori* with standard triple treatment showed to decrease worldwide. So, many authors are introducing various regimens. We investigated the eradication rate and trend of it using standard triple regimen as first-line anti-*Helicobacter pylori* treatment on patients who were experienced subtotal gastrectomy for adenocarcinoma. Also, we looked into efficacy of bismuth containing quadruple regimen as rescue therapy.
Method: From January 2004 to December 2010, a total of 430 patients with *H. pylori* infection after receiving subtotal gastrectomy for adenocarcinoma were treated with 7 days-standard triple therapy (amoxicillin 1000 mg b.i.d, clarithromycin 500 mg b.i.d, esomeprazole 20 mg b.i.d). We retrospectively analyzed overall eradication rate and trend of it using ITT (Intention To Treatment) and PP (Per-Protocol). As same way, we assayed efficacy of 10 days-bismuth containing quadruple treatment (tripotassium dicitrato bismuth 300 mg q.i.d, tetracyclin 500 mg q.i.d, metronidazole 500 mg t.i.d, esomeprazole 20 mg b.i.d) as rescue therapy.
Result: The overall eradication rates were 81.0% (95% CI, 77.2–84.3) and 88.3% (95% CI, 85.0–91.0) by ITT and PP. The annual eradication rate from year 2004 to 2010 were 89.4%, 95.4%, 85.2%, 89.7%, 85.5%, 86.5% and 87.3% by PP. There was no decreasing tendency and no statistical significant of the eradication rate ($p = 0.588$). Twenty-eight patients treated with bismuth containing quadruple therapy as rescue regimen, only two of them were failed. PP was 92.8% (95% CI 77.3–98.0).
Conclusion: The postoperative eradication rate of *H.pylori* infection using standard triple therapy did not show satisfied result. But it had higher rate than it of other eradicated indications not having operation in Korea. And bismuth containing quadruple treatment after failure of first line therapy had high efficacy.

Abstract no.: P18.08

PREDICTING VALUE OF SEROLOGICAL BIOMARKERS FOR GASTRIC CANCER IN RUSSIA: A RETROSPECTIVE COHORT STUDY

A. V. Belkovets, O. V. Reshetnikov, S. A. Kurilovich, T. G. Openko, J. I. Ragino and S. K. Malyutina

Institute of Internal Medicine, Novosibirsk, Russian Federation

Background: Gastric cancer (GC) morbidity and mortality rates in Russia are greatly higher comparing other countries. Atrophic gastritis (AG) is the most important risk predictor for GC.
The aim of the study was to assess the value of biomarkers of AG for early detection of GC in Siberian population. General population sample was surveyed in Novosibirsk in 2003–2005 (10 000 subjects aged 45–69 years). Each serum sample was deeply frozen and stored. In 2012 this database was compared with the data of the Population Cancer Registry. For each case of GC, an appropriate control case was selected at the ratio 1:2 matching the area of residence, sex and age. One hundred and sixty-one serum samples (54 – GC group and 107 – control group) were available for the analysis using a panel of serum biomarkers "Gastropanel" (Biohit, Finland). The standard criteria for the diagnosis of AG were used according to manufacturer's instructions.
Results: Indicators of gastric atrophy (OR; 95% CI) were associated with GC for PGI (2.5; 1.2–5.4), PGII (8.8; 1.8–43.1), and PGI/PGII ratio (3; 1.4–6.4), but neither for G-17 (0.7, 0.4–1.6), nor for the presence of antibodies to *H. pylori* (1; 0.2–1.5).

Conclusions: Noninvasive set of serological biomarkers was confirmed as an informative and non-expensive tool for the early detection of GC in population-based retrospective cohort survey in Siberian population. Low levels of PGI, PGII, and PGI/PGII ratio were proven as the most valuable prognostic factors. Decreased level of G-17 as a single index did not significantly predict the risk of GC.

Abstract no.: P18.09

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN PATIENTS WITH PRECANCEROUS LESIONS OF STOMACH IN A HIGH-INCIDENCE REGION OF KOREA

S. Chung, C. Lee, M. Kim, S. Kang and M. Park
Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Korea

Background: Chronic atrophic gastritis (AG), intestinal metaplasia (IM) and epithelial dysplasia (ED) were confirmed precancerous lesions of stomach. Most of these conditions are correlated with long-term *Helicobacter pylori* (*H. pylori*) infections. We aimed to determine the prevalence of *H. pylori* infection among patients with precancerous conditions in a high-incidence region of Korea.
Method: We performed a cross-sectional case–control study of 2832 subjects undergoing both upper endoscopy and *H. pylori* serology (IgG antibody positivity) during health Check-up. Patients with biopsy proven precancerous lesions were subgrouped as follows: chronic atrophic gastritis (AG, n = 975), intestinal metaplasia (IM, n = 387) and epithelial dysplasia (ED, n = 156). GC (n = 270) were also included. As control populations, those with normal endoscopic finding were investigated. The odd ratio (OR) of precancerous lesions among those with antibody positivity were estimated by logistic regression. Family history of gastric cancer (GC), smoking and alcohol were added to the model.
Results: The percent of *H. pylori* positivity was 42% among controls and steadily increased among those with AG (69%), IM (76%) and ED (82%), before falling to 58% among those with GC, with nonsignificant variation by sex, age, alcohol, and cigarette smoking habits. The OR of IM/ED associated with *H. pylori* positivity was 2.4 (1.6–9.2).
Conclusions: Precancerous lesions of stomach are associated with high percentage of *Helicobacter pylori* infection. The findings suggest that *H. pylori* infection contributes to the process of gastric carcinogenesis, particularly during the early stages, in this high-risk area of GC.

Abstract no.: P18.10

PREOPERATIVE PROGNOSTIC VALUE OF POSITRON EMISSION TOMOGRAPHY-CT (PET-CT) IN SURGICALLY RESECTED GASTRIC CANCER

S. Choi and J. Jang
Dong-A University College of Medicine, Busan, Korea

Background/Subjective: Apart from the diagnostic value of Positron Emission Tomography-Computed Tomography (PET-CT), the prognostic value of pretreatment PET-CT was not commonly evaluated in gastric cancer, so we investigated its preoperative prognostic value.
Method: Retrospectively we collected 107 cases of gastric cancer patients who had underwent surgical treatment after being observed FDG uptake by preoperative PET-CT at our center from April 2007 to December 2010. Among firstly enrolled 107 patients, cases of follow up losses (13), palliative resection (5), neoadjuvant chemotherapy (1), unrelated death (1) were excluded and finally total 87 patients were evaluated. Follow up duration was defined as period from operation to date that patients were examined last imaging study such as PET-CT or CT, and the median follow up duration was 34.2 ± 14.8 months until June 2012. FDG uptake values were observed based on maximal standardized uptake value (SUVmax) varied by patients' weight. In order to find correlation of SUVmax with recurrence, Kaplan Meier's survival analysis with log rank test and cox proportional hazard model were performed with using SUVmax cutoff value defined from ROC curve.
Result: Significant difference of T staging ($p < 0.001$) and N staging ($p < 0.001$) were observed between recurrence group and non-recurrence group in patients' baseline characteristics, but SUVmax was not showed strong difference between two groups (p-value: 0.116). Significant statistical difference was observed in Kaplan Meier's survival analysis with log rank test (p-value: 0.035) between high SUVmax group and low SUVmax group which separated by SUVmax cutoff value 5.6. However, in multivariate analysis with cox proportional hazard model revised for age, sex, T-staging, N-staging, the SUVmax did not showed statistical significance in correlation with recurrence (SUVmax classification by cutoff value : p-value 0.436).
Conclusion: High SUVmax on PET-CT is not independent risk factors to predict poor outcomes of surgically resected gastric cancer.

Abstract no.: P18.11

THE TREND IN THE ERADICATION RATES OF FIRST-LINE THERAPY FOR *HELICOBACTER PYLORI* INFECTION AND RISK FACTORS OF ERADICATION THERAPY

S. Kim, M. Park, S. Park, W. Moon, J. Cheon, H. Kwon, K. Ku, C. Yoo, J. Kim, G. Lee, S. Song and Y. Choi
Department of Internal Medicine, Kosin University College of Medicine, Busan, Korea

Background/Aims: The standard triple therapy which was combined with proton pump inhibitor (PPI), amoxicillin and clarithromycin was widely used for *Helicobacter pylori* (*H. pylori*) infection in Korea. However, the recent trend of the eradication rates in *H. pylori* using first-line triple therapy has been infrequently reported. The aims of this study were to evaluate the trend of the *H. pylori* eradication rates in single center during recent ten years and to identify the risk factors relating the failure of eradication therapy.
Methods: From January 2003 to December 2012, *H. pylori* eradication rates in 1413 patients who diagnosed with *H. pylori* infection and received 7 days triple therapy were investigated according to years, demographic and clinical factors retrospectively. *H. pylori* eradication was confirmed by ^{13}C-urea breath test, rapid urease test or histopathological examination at least 4 weeks after the completion of triple therapy.
Results: The overall *H. pylori* eradication rate was 84.9%. The annual eradication rates from the year 2003 to 2012 were 93.5%, 80.0%, 87.2%, 88.5%, 92.0%, 88.3%, 85.7%, 84.1%, 83.7% and 78.8% consecutively by per-protocol analysis. The eradication rate in first-line triple therapy was decreased during the recent ten years ($p = 0.015$). Multivariate analysis showed that female (OR 1.69; 95% CI 1.12–2.55) and smoking (OR 1.61; 95% CI 1.05–2.47) were associated with the failure of *H. pylori* eradication therapy.
Conclusions: The effect of first-line triple therapy for *H. pylori* infection has decreased during recent ten years, which suggests that the antibiotic resistant *H. pylori* has increased against the therapy based on the combination of PPI, amoxicillin and clarithromycin. Therefore, other variable first-line therapies might be considered for *H. pylori* eradication in the near future.

Abstract no.: P18.12

CAGA ASSOCIATION WITH GASTRIC CANCER DEVELOPMENT RISK

L. Vanaga,* I. Daugule,* G. Moisejevs,*,†,‡ D. Rudzite,*,†,‡ S. Krotov,§ D. Janciauskas,*,¶ I. Liepniece-Karele,*,** S. Isajevs,*,† A. Sivins,*,† I. Kikuste,*,† G. Ancans,*,†,‡ K. Funka,*,†† I. Lasina,* I. Tolmanis,‡,†† A. Vanags†† and M. Leja*,†,††
*University of Latvia, Riga, Latvia; †Riga East University Hospital, Riga, Latvia; ‡Riga Stradins University, Riga, Latvia; §Vector BEST, Novosibirsk, Russian Federation; ¶Lithuanian University of Health Sciences, Kaunas, Lithuania; **Academic Histology laboratory, Riga, Latvia; ††Center of Digestive Diseases "GASTRO", Riga, Latvia

Introduction: *Helicobacter pylori* (HP) infection is known risk factor for development gastric cancer. One of the most studied virulence factors is cytotoxin associated gene (cagA). The aim of this study was to investigate whether CagA seropositivity is associated with risk of development gastric cancer.
Methods: Gastric cancer group included patients with histolopathologicaly proven disease (n = 219, median of age – 66, males/females 92/127) and control group was represented by dyspeptic patients (n = 191, median of age – 56, males/females 67/124) having no evidence of gastric mucosa atrophy, evaluated according to Operative Link for Gastritis Assessment (OLGA) with score 0-I. HP seropositivity was determined using anti-HP IgG (Biohit, Finland) and anti-CagA IgG, IgM and IgA (Vector BEST, Russia). Statistical test used – χ^2.
Results: Rate of HP seropositivity among gastric cancer patients was significantly highar compared to control group (77.2 % vs 64.4%, $p = 0.03$). Compared to control group, gastric cancer patients had significantly higher rate of anti-CagA seropositivity (44% vs 58.9%, OR = 1.8, CI 95% = 1.23–2.70, $p = 0.002$).
Conclusions: Having HP infection positive to CagA virulence factor significantly increases risk of development of gastric cancer. However gastric cancer

development process is very complex and interaction of host and HP factors should be studied precisely.

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Abstract no.: P18.13

THE EFFECT OF H.P ERRADICATION FOR METACHRONOUS GASTRIC CANCER AFTER ENDOSCOPIC RESECTION: A SYSTEMATIC REVIEW AND METAANALYSIS

S. Kim, Y. Cho, Y. Jo, Y. Park, S. Ahn and B. Son
Eulji Hospital of Eulji University, Seoul, Korea

Recently Endoscopic resection (ER) which includes EMR and ESD has been established as a less invasive treatment of EGC.the metachronous gastric cancer (MGC) risk of remnant stomach after ER has been major concern to gastrometerologists.

Methods: A literature search was done to identify all relevant studies that compared ESD with EMR. The Pubmed, Medline, and Web of Science databases were searched systematically for all articles published between 1 January 2000 and 31 December 2011. A meta-analysis was done of all the studies of metachronous gastric cancers after ER; include EMR and ESD. The primary end point was to investigate the risk of metachronous gastric cancer in the natural course after ER in EGC. Secondary endpoints were the charicteristics of metachronous gastric cancer; age, sex, *Helicobactor pylori* infection status, the lesion size, location, histology.

Results: Five randomized studies were identified. From the refereneces of five present studies. the histological factor of MGC occurrence was high at the primary lesion showed poorly differentiated and signet ring cell carcinoma. Also, the proportion of undifferentiated histology in of multiple gastric cancers was high. In the five studies, the two studies have studied correlation between *H. pylori* and occurrence of multiple gastric cancers ER. In the Meta-analysis we cannot conclude whether *H. pylori* eradication could decrease the occurrence of MGC ($p = 0.128$). The time interval of endoscopic detection of MGC being treated with EMR was 36.28 months. The annual incidence of metachronous cancer was about 3–4%.

Conclusion: As there have been many studies of residual gastric cancer, there have been few reports of MGC after ER for EGC. Also, it is not enough to show the characteristics and predicting factors of MGC lesions and impact of h.p erradicationfor prevention of MGC after ESD.

Abstract no.: P18.14

INHIBITION OF ADHESION AND INVASION OF *HELICOBACTER PYLORI* IN HUMAN GASTRIC ADENOCARCINOMA CELLS BY ORIGANUM MINUTIFLORUM ESSENTIAL OIL

F. Özen, F. Y. Ekinci and M. Korachi
Biotechnology Department, Yeditepe University, Istanbul, Turkey

Essential oils (EOs) are volatile, natural, organic constituents of aromatic plants. Origanum minutiflorum EO has high antimicrobial activity against a wide range of clinical and foodborne pathogens due to the presence of bioactive compounds, mainly carvacrol/thymol. *Helicobacter pylori*, which colonizes the stomach of more than half of the world's population, plays a crucial role in the pathogenesis of peptic ulcer and gastric cancer. *H. pylori* infects by adhering to gastric epithelial cells and, upon colonization, releases cytotoxins.

This study aimed at investigating the ability of O. minutiflorum EOs to inhibit the adhesion/invasion potential of *H. pylori* J99 upon human gastric cancer AGS cells (gastric adenocarcinoma, ATCC CRL 1739). EO was extracted by the hydro-distillation method using a clevenger apparatus. In a standard gentamicin assay containing 1/8000–1/40 000 (v/v) dilutions of O. minutiflorum EO, the absence of elongation of AGS cells (hummingbird phenotype, by scanning electron microscopy, SEM) was used as indicative of inhibition by the EO of *H. pylori* infection.

SEM results showed that presence of EO extract with a concentration range from 1/20 000 to 1/40 000 (v/v) exhibited a marked anti-adhesion activity against *H. pylori* with a >70% reduction in adhesion activity ($p < 0.01$). By contrast, colonies of *H. pylori* J99 attached to the entire surface of control AGS cells after 1.5 h incubation and hummingbird phenotype was observed following infection.

It was concluded that O. minutiflorum EO may potentially be effective for phytotherapy in patients with gastric cancer caused by *H. pylori*.

Abstract no.: P18.15

THE RELATIONSHIP OF CD44 AND SURVIVAL RATE IN GASTRIC CANCER

B. I. Jang,* J. S. Park,* S. H. Lee* and C. H. Yang†
*Division of Gastroenterology and Hepatology, Department of Internal Medicine, Yeungnam University Co, Daegu, Korea; †Division of Gastroenterology and Hepatology, Department of Internal Medicine, Dongguk University College of Medicine, Kyungju, Korea

Backgrounds: CD44 play important roles in carcinogenesis, differentiation, lymph node metastasis and prognosis in gastric cancer. CD44 more detected in *H. pylori* positive gastric cancer due to genetically alteration according to acute and chronic infection with *Helicobacter pylori*. Generally, Lower median survival rate and poor response to treatment in patients with CD44 positive gastric cancer were known. The purpose of this study is to identify the relationship of CD44 and survival rate, CD44 and *H. pylori* infection in gastric cancer patients.

Methods: A total of 62 patients who were diagnosed with histologically proven gastric adenocarcinoma and underwent surgery and chemotherapy for the tratment at Yeungnam University hospital in South Korea between January 2005 and December 2007 were retrospectively analyzed. Age, sex, CD44 stain, *H. pylori*, endoscopic finding, lymph node metastasis, stage, mean survival rate, etc were reviewed.

Results: The male-to-female ratio was 2.1:1, and the mean age was 56.8 ± 10.7 years. *H. pylori* positive were seen in 25 patients (40.3%). CD 44 stain positive was observed in 53 patients (85.5%). The median size of specimen was 6.25 ± 2.98 cm. The most frequent location in stomach was body (34cases, 54.8%). The most frequent endoscopic appearance was Borrmann classification III (35cases, 56.5%). The most frequent depth of invasion was detected in 37 (59.7%) subserosa. Regional lymph node metastasis was detected in 49 cases (79.0%). The most frequent stage was IIA (17caseas, 27.4%). *H. pylori* positive gastric cancer patients were CD44 positive in 23 cases (92%, $p = 0.292$). Median survival rate in CD 44 positive and negative patients were 81.2 ± 3.4, 76.9 ± 7.4 months ($p = 0.386$)

Conclusion: This study showed that *H. pylori* positive gastric cancer patients had a higher CD 44 positive rate but it was not statistically significant. Also, there was no significant differentiation of median survival rate between CD 44 positive and negative.

Abstract no.: P18.16

THE POLYMORPHISM OF INTERLEUKIN 8 -251 T/A BUT NOT INNATE IMMUNITY RELATED PROTEIN, INFLUENCES THE SUSCEPTIBILITY OF GASTRIC CANCER WITH *HELICOBACTER PYLORI* INFECTION IN KOREAN POPULATION

Y. W. Chang, J. Y. Jang, J. W. Kim and W. J. Ko
Kyung Hee University Hospital, Seoul, Korea

Background and Aim: The host response to *Helicobacter pylori* has been related to genetic polymorphisms that influence both innate and adaptive immune responses. Toll-like receptor 4 (TLR4), mannose binding lectin 2 (MBL2), and NOD1 are well-characterized pattern recognition receptors, and are major components of the innate immune system. Interleukin-8 (IL-8) is a potent neutrophil-activating chemokine, central to the immunopathogenesis of *H. pylori*-induced gastric mucosal injury. We examined whether genetic polymorphisms of innate immunity related proteins and IL-8 in gastric disease groups.

Material and Methods: Seventy-eight patients with functional dyspepsia (FD), 65 peptic ulcer disease (PUD), and 124 gastric cancer (GC) were included in this study. All subjects were *H. pylori* positive. The polymorphisms of TLR4 Asp299Gly, TLR4 Thr399Ile, MBL2 codon 54 G/A, G796A NOD1, and IL-8 -251 T/A were examined by PCR-RFLP analysis. The concentrations of serum MBL protein and gastric mucosal IL-8 were measured by ELISA.

Results: There were no significant differences in TLR4, MBL2, and NOD1 polymorphisms among the *H. pylori* positive FD, PUD, and GC groups. IL-8 -251 T/A polymorphism was associated with the significantly higher risk of GC compared with FD or PUD. Serum concentration of MBL protein was not different among the gastric disease groups. Gastric mucosal IL-8 concentration was slightly higher in GC than FD and PUD.

Conclusions: The polymorphisms of TLR4, MBL2, and NOD1 did not influence the susceptibility of PUD and GC. IL-8 -251 T/A polymorphism and *H. pylori* infection could be risk factors for the development of GC in Korean population.

Abstract no.: P18.17

GASTRIC MALT LYMPHOMAS AND RESPONSE TO *HELICOBACTER PYLORI* ERADICATION THERAPY

G. Noa Pedroso,* E. Montes de Oca Megías,† B. Gutiérrez Carrillo,‡ J. A. Más Páez,* R. Pérez Menéndez,* H. Mulet Hernández* and C. Domínguez Álvarez*
*Hermanos Ameijeiras Hospital, La Habana, Cuba; †Centro Nacional de Cirugía de Mínimo Acceso, La Habana, Cuba; ‡Instituto Nacional de Oncología y Radiología, La Habana, Cuba

Introduction: Mucosa-associated lymphoid tissue (MALT) lymphoma is the most common lymphoma of the stomach and is directly related to *Helicobacter pylori* infection in most instances. These tumors show regression after eradication therapy and potential effect on survival.
Objetive: To described the clinical-pathological features and the results of management with *H. pylori* eradication therapy in Gastric MALT lymphoma.
Methods: This cohort study was realized in Ameijeiras Hospital, Cuba, during January 2000-September 2010. A total of 24 patients were evaluated. Data were obtained retrospectively by contacting patients or theirs clinical reports. The information was evaluated by SPSS program.
Results: The mean age of the patients was 61.5 ± 16.525 years. Female (54.8%), white people (69%), ulcer syndrome (76.2%) and ulcer lesions (35.7%), majorly in the antrum (33.3%) were most frequent. Most lymphomas were classified as low-grade (79.2%-19 patients) and the prevalence of *H. pylori* infection was 95%. The other 20.8% (five patients) were high grade MALT with 100% of prevalence of *H. pylori*. All patients (100%) received anti-*Helicobacter pylori* eradication therapy, and second treatment was necessary in 7 (29.2%) of them. The lymphoma remission rate was 89.5% of low grade lymphoma with average survival of 5.6 years and the average survival to high grade lymphoma was of 1.5 years.
Conclusions: The strong relation between *H. pylori* infection and gastric MALT lymphoma was demonstrated. The eradication therapy offered a potential remission of this tumor; the best response was reported in low grade MALT lymphoma. The high grade MALT lymphoma showed worse response and survival.

Abstract no.: P18.18

ASSOCIATION BETWEEN SEVERITY OF *HELICOBACTER PYLORI* INFECTION AND CLINICAL-MORPHOLOGICAL FACTORS OF GASTRIC CANCER PROGRESSION

M. Senchukova and A. Stadnicov
Orenburg State Medical Academy, Orenburg, Russian Federation

Objective: To study the relationship between the severity of *H. pylori* infection and clinical-morphological data in patients with gastric cancer (GC).
Method: The samples of gastric mucosa adjacent to tumors from 45 patients with GC were studied. Hematoxylin-eosin and *H. pylori*-specific immunostaining were performed. The previously described features of *H. pylori* infection (Helicobacter, 2012, 17 (4): 113) were assessed by visual analog way: the presence of coccoid forms (CFs), a specific multiple punctate inclusions (MPI) within the epithelial cells (ECs) and mononuclear cells (MCs) of the lamina propria.
Results: In cases of marked contamination of CFs, compared that of mild the rate of diffuse type of GC, tumors with lymph node metastases and tumors with marked stroma was higher (72.2% vs 33.3%, $p = 0.01$; 72.2% vs 33.4%, $p = 0.03$ and 66.7% vs 36.8%, $p = 0.08$, respectively), but the rate of the severe atrophy and dysplasia was below (50% and 74.1%, $p = 0.102$; 44% and 70.4%, $p = 0.08$, respectively). The presence of specific MPI in large number of ECs, compared that in small, was more frequent in tumors with marked stroma and tumors having a poorly differentiated clusters of tumors cells (66.7% vs 36.8%, $p = 0.08$ and 66.7% vs 28.6%, $p = 0.04$, respectively). The presence of specific MPI in large number of MCs, compared that in small, more frequently observed in tumors located in the upper and middle third of the stomach, compared that in lower (73.3% and 31.8%, $p = 0.059$, respectively).
Conclusion: These data suggest that the severity of *H. pylori* infection may be associated with factors of CG progression.

Abstract no.: P18.19

STOMACH MICROBIOTA COMPOSITION GRADUALLY SHIFTS FROM NON-ATROPHIC GASTRITIS TO INTESTINAL METAPLASIA TO GASTRIC CANCER

J. Torres, F. Aviles, R. Medrano and M. Camorlinga
Infectious Diseases Research Unit and Oncology Hospital, Instituto Mexicano del Seguro Social, Mexico, Mexico

Background: Contrary to what it was assumed previously, studies have documented that human stomach is colonized by a complex microbiota often including Proteobacteria, Firmicutes, Actinobacteria and Fusobacterium phyla. Initial attempts to characterize microbiota in gastric cancer samples have reported no significant differences with dyspeptic controls, although authors used methods with reduced sensitivity.
Objective: We aimed to characterize the changes in the microbiota of the gastric mucosa as it progress to intestinal type of cancer.
Methods: Study included five patients of each, non-atrophic gastritis (NAG), intestinal metaplasia (IM) and intestinal gastric cancer IGC). Gastric tissue was obtained and DNA extracted for microbiota analyses using the G3 phylochip assay (second genome, Inc).
Results: Bacterial richness ranged from 8 to 57, and it steadily decreased from NAG to IM to IGC ($p = 0.004$, two-tailed heteroscadastic t-test). Firmicutes, Proteobacteria and Bacteroidetes where the most abundant phyla. A significant microbiome difference was observed between NAG and IGC based on weighted unifrac abundance and unifrac presence/absence metrics of 283 taxa at $p < 0.05$. HC-AN analyses based on presence/absence of 238 taxa revealed that GC and NAG grouped apart, whereas IM overlapped with both GC and NAG groups. A PCoA analyses based on weighted unifrac distance given abundance of 44 taxa showing significance across categories revealed significant microbiome separation between NAG and IGC groups. Lactobacillus and Lachnospiraceae OTUs were significantly more abundant in IGC samples. TM7 phyla was more abundant in NAG group.
Conclusion: A shift in stomach microbiota was observed from NAG to IM to IGC.

