H. pylori-infected gastric biopsies (five gastritis, five ulcers). Tests were performed in a FACSCalibur.

Results: In *H. pylori*-infected AGS, CD277 (control = 16.4 ± 6.6, [HP2 × 10^8] = 35.3 ± 15.1*), and HLA-DR (control = 12.5 ± 4.9, [HP2 × 10^8] = 20.2 ± 7.5*) expression, were increased independently of colonization density and genotype. In biopsies we detected a 2.2-fold increase CD277 values in ulcers compared to gastritis specimens.

Conclusions: *H. pylori* allows gastric epithelial cells to behave as APC, and increases CD277 expression. Due to the inhibitory properties of butyrophilins the host cells could collaborate to chronicity and severity of infection inducting anergy in T cells. CD277 emerges as a new target for interventions to overcome immune evasion and boost immunity in infected patients.

Abstract no.: P1.20

OXIDATIVE STRESS CAUSED BY *H. PYLORI* DECIDES THE MITOCHONDRIAL NETWORK FRAGMENTATION BY PROTEINS TRANSLOCATION (BAX AND DRP-1) TO FISSION SITES

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Objectives: Apoptosis has been associated with *H. pylori* infection. The crucial step in the apoptotic intrinsic pathway is outer mitochondrial membrane permeabilization, being the mitochondrial pores opening (OMP) responsible for it, although are not clearly identified neither the initialization signals nor the molecules involved.

Aims: To determine oxidative stress role and mechanism that drives the OMP in *H. pylori* infection. Involvement of Bcl-2 and fission proteins family.

Methods: AGS cells were *H. pylori*-infected (10^8 CFU/mL, 24 hours), and incubated with or without VitE or V5''Bax-translocation inhibitor'' (10^{-4} mol/L). It was studied:

- OMP (Calcein-AM with $CoCl_2$) by Confocal Microscopy (CM)
- Mitochondrial network phenotype (NAO) by CM
- Bax and Drp1 oligomerization by cross-linked and Western blot assays
- Bax and Drp1 colocalization by CM

Results: Calcein fluorescence in presence of $CoCl_2$, was reduced in mitochondria of coinfected AGS compared to control, showing that OMP has happened. *H. pylori* switched mitochondrial morphology from ''tubular'' (control) to ''punctuate and swollen'' phenotype (co-infected cells). Mitochondrial Bax in AGS-infected was as monomer, dimmer, trimmer, and heteromultimer with Drp1 (Bax-Drp1 and Bax-Bax-Drp1). Bax and Drp1 colocalized in mitochondria forming clusters at fission prospective sites. Vit E and V5 pretreatment avoided these alterations.

Discussion: Oxidative stress observed in *H. pylori*-infected gastric epithelial cells, is able to initiate an alterations cascade that leads cells to autoelimination, being OMP a crucial step. In the OMP are involved Bax and Drp-1 that are translocated to mitochondria to close proximity. Antioxidants and/or Bax translocation inhibitors treatment could prevent the OMP, the apoptosis development, and consequently, reduce the bacterial toxic effect on gastric epithelium.

Abstract no.: P1.21

COMBINED PRESENCE OF THE *HELICOBACTER PYLORI* JHP0562 AND TNPA GENES PREDICTS THE PRESENCE OF DUODENAL ULCER

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Helicobacter pylori infection is now accepted as the main cause of superficial gastritis and is associated with other gastroduodenal disorders. Infection usually occurs during childhood and when left untreated it will become chronic and last for life. During the first years of the infection the presence of *H. pylori* probably only results in asymptomatic chronic gastritis, but prolonged infection can lead to a variety of digestive illnesses, including peptic ulcer disease and gastric cancer. Recently, several putative virulence factors have been identified but discrepant data exist on their association with disease. The aim of this study was to evaluate a putative relationship between the presence of the jhp0562, cagA, sabB, tnpA and tnpB genes and disease. Patients were collected in the Imam Khomeini hospital between the May 2007 and April 2011. *H. pylori* could be isolated from 360/376 patients. The presence of jhp0562, cagA, sabB, tnpA and tnpB genes in these strains was examined by using a specific Real-Time PCR reaction on the purified DNA from these strains. Of 376 included patients (196 males, average age 42.1 years, range: 17–73) 108 were diagnosed with Duodenal Ulcer, 92 with Gastric Ulcer, 65 with Gastric Cancer, and 95 with Gastritis. A significant association (95% CI = 4.24–9.01; OR = 19.5) was observed between the presence of duodenal ulcers and the combined jhp0562+, tnpA+ genotype. The combined jhp0562+ tnpA+ genotype shows a strong correlation with the presence of duodenal ulcers in *H. pylori* infected patients and might serve to predict the induction of duodenal ulcers before they are clinically manifest.

Abstract no.: P1.22

DEVELOPMENT OF A NOVEL *HELICOBACTER PYLORI* BABA2 GENE-SPECIFIC POLYMERASE CHAIN REACTION (PCR) ASSAY

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Background: *Helicobacter pylori* (*H. pylori*) babA2 is the ABO blood group antigen binding adhesin, which has a closely related paralogue babB with unknown function. Some studies showed that babA2 gene-positive *H. pylori* strains are associated with severe clinical outcome in Western populations. The ability to detect babA2, however, depends on the used PCR method. It has been shown recently that available babA2 primers may generate both false-negative and false-positive results due to sequence variation among *H. pylori* strains and cross-reactivity with babB gene.

Objective: To develop and evaluate a novel babA2 PCR in comparison to two widely used PCRs targeting 850-bp (PNAS USA 1999;96:12778–83) and 271-bp (Gut 2003;52:927–32) fragments of babA2.

Material and Methods: BabA2 primers were designed according to the multiple alignment of 94 babA2 and 24 babB sequences available in GenBank. A total of 217 *H. pylori* DNA isolates were consequently tested with the novel assay.

Results: Three forward and one reverse primer were selected to amplify 146-bp fragment of babA2 gene. Using novel PCR, babA2 was detected in 114/217 (52.5%) *H. pylori* isolates. Using 850-bp and 271-bp PCRs, babA2 was found in 74/217 (34.1%) and 174/217 (80.2%) cases, respectively. Sequencing of 146-bp and 850-bp PCR amplicons confirmed the presence of babA2, while it was not possible to distinguish reliably among babA2 and babB sequences in 271-bp amplicons.

Conclusion: Novel assay significantly improves the detection of babA2 gene over existing assays. Further validation of this assay is needed on a geographically more diverse collection of *H. pylori* strains.

Abstract no.: P1.23

THE ULCEROGENIC PROFILE OF *HELICOBACTER PYLORI* PAEDIATRIC STRAINS ASSOCIATED WITH PEPTIC ULCER DISEASE

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Helicobacter pylori infection is the major cause of paediatric peptic ulcer disease (PUD). In children with no other aetiology for the disease, this rare event occurs shortly after infection, presuming a still poorly understood higher susceptibility of the patient and highlighting the virulence of the implicated strain. Recently, we showed that the enhanced virulence of a group of paediatric ulcerogenic-strains result from a synergy between their ability to better adapt to the hostility of their niche and the expression of *cagA, vacAs1, oipA* ''on'' status, *homB* and *jhp562*[1]. Accordingly, these ulcerogenic strains share a particular proteome profile, providing them with better antioxidant defences, a metabolism favouring the biosynthesis of aromatic amino acids and higher motility[1]. Corroborating these findings, our preliminary data on electronic microscopic analyses demonstrated the presence of more abundant flagella in PUD-associated paediatric strains, in contrast to the control strain, a paediatric strain associated with non-ulcer dyspepsia (NUD). Compared with paediatric NUD-associated isolates, ulcerogenic

H. pylori strains present a greater ability to induce a marked decrease in the gastric cells' viability and to cause them severe cytoskeleton damage and mucins' production/secretion impairment[1]. To uncover the underlying molecular mechanisms, we are now characterizing the modifications induced by these strains in the proteome of human gastric cells, during in vitro infection, by two-dimensional gel electrophoresis followed by mass-spectrometry.

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Reference

1. Vitoriano I, Saraiva-Pava KD, Rocha-Goncalves A, Santos A, Lopes AI, Oleastro M, Roxo-Rosa M. *PLoS One* 2011;6:e26265.

Abstract no.: P1.24

RETHINKING VACA: A TRUE MULTIFUCTIONAL TOXIN OR RATHER A NOVEL TYPE OF MONOFUNCTIONAL A-B TOXINS?

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Helicobacter pylori is the paradigm of a bacterium which favors a carcinogenic process. Into the last decade, careful analyses of two of its most important virulence factors, the vacuolating toxin VacA and the cytotoxin-associated gene A product CagA, have led to important breakthroughs for the study of bacterial-host relationships. From the cell biology point of view, VacA is a fascinating protein toxin which, although classified as a pore-forming toxin, apparently exerts pleiotropic effects on mammalian cells and tissues. It has thus been proposed that VacA may be considered a paradigm for toxin multifunctionality. However, an increasing body of evidence now suggests that VacA may rather be the prototype of a new class of monofunctional A-B toxins in which the A subunit exhibits pore-forming instead of enzymatic activity. Thus a peculiar mechanism of action for VacA, which allows it to intoxicate the human stomach, may be depicted. By combining the action of a cell-binding domain, a specific intracellular trafficking pathway and a novel mitochondrion-targeting sequence, the VacA pore-forming domain is selectively delivered to the inner mitochondrial membrane to efficiently kill target epithelial cells. VacA action on the human host could be exploited and controlled by *H. pylori* through a functional relationship with another virulence factor, CagA, to achieve the best interaction between the bacterium and the hostile gastric environment that represents its ecological niche.

Abstract no.: P1.25

PREVALENCE OF BACTERIAL VIRULENCE FACTORS IN *H. PYLORI* STRAINS ISOLATED IN PATIENTS WITH GASTROINTESTINAL DISEASES IN A PROSPECTIVELY ENROLLED COHORT IN MAGDEBURG (EAST GERMANY) 2011 AND 2012

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Background: The development of gastric cancer is dependent from host-related, environmental and bacterial virulence factors. The aim was to study the presence of *H. pylori* CagA and VacA variants in patients with different types of gastritis.

Methods: From all included patients gastric biopsies were obtained and the *H. pylori* status was determined. VacA and CagA variants were identified by PCR from *H. pylori* DNA. Anti-*H. pylori* and anti-CagA IgG was quantified by ELISA.

Results: As shown in table 1, 186 patients with different diseases were included and 1/3 were infected with *H. pylori*. Overall, 86.7% of all *H. pylori* strains contained the *cagA* gene. Interestingly, a remarkable number (n = 30, 63.8%) of those was not associated with an anti-CagA IgG response in the corresponding patients. Pilot investigation concerning CagA variants (number of EPIYA motifs) in 31 patients revealed predominant presence of ABC (65%), followed by ABCCC (16%), ABCC (13%) and AB (6%). In five patients, colonization with multiple strains having at least three CagA variants was detected. Variants of the vacA gene s1m1 and s2m2 were identified in 40% and 35% of the strains, respectively. Due to small numbers of cases at this moment, statistical analysis was not performed.

Conclusions: *H. pylori* strains isolated from patients in Magdeburg (East Germany) demonstrate a high degree of variability in regard to isoforms of CagA and VacA gene.

Table 1 Clinical data of study group

Diagnosis	Number of patients (Number of patients with isolated strains)	CagA Gene	VacAs 1m1	VacAs 2m2
Normal control	61 (2)	2 (100%)	0	1
Antrum predominant gastritis	15 (13)	9 (69.2%)	3	6
Corpus predominant gastritis	7 (6)	5 (83.3%)	1	4
Pangastritis	14 (10)	8 (80%)	5	2
Atrophic gastrits /intestinal metaplasia	63 (20)	20 (100%)	9	5
Peptic ulcer disease	15 (5)	4 (80%)	2	3
Gastric cancer	11 (4)	4 (100%)	4	0
Total number	186 (60)	52 (86.7%)	24	21

Abstract no.: P1.26

PREVALENCE AND CLINICAL RELEVANCE OF *CAGA*, *VACA*, *ICEA* AND *BABA2* GENES IN SLOVENIAN PEDIATRIC POPULATION INFECTED WITH *HELICOBACTER PYLORI*

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Aims: (1) To determine the prevalence and genetic diversity of *H. pylori cagA, vacA, iceA* and *babA2* genes in Slovenian pediatric population, and (2) to analyze the relationship between infections with different strains and the severity of antral inflammation.

Methods: DNA was extracted from 190 *Helicobacter pylori* (*H. pylori*) positive gastric biopsies. *H. pylori cagA, vacA and iceA* status was determined, as described previously (*J Pediatr Gastroenterol Nutr* 2009;49:289–96). *The presence of babA2* gene was determined by using two different primer sets targeting 850-bp (*Proc Natl Acad Sci USA* 1999;96:12778–83) and 271-bp (*Gut* 2003;52:927–32) fragments of *babA2*. Single gene was compared with density, activity and chronicity of *H. pylori* infection according to the Updated Sydney histological Classification.

Results: Multiple *H. pylori* genotypes were found in 20/190 (10.5%) children which were excluded from further analysis The *cagA* gene, and *s1* and *m1* alleles of the *vacA* gene were found in 66.4%, 73.9% and 40.6% of *H. pylori* isolates, respectively. *IceA1* positive strains were identified in 68.8%. Using 850-bp and 271-bp PCR assays, *babA2* gene was found in 52/170 (30.6%) and 137/170 (80.6%) cases, respectively. The severity of antral inflammation was associated with *cagA, vacAs1, vacAm1* and *babA2* (850–bp) positivity.

Conclusion: The results of this study showed that in contrast to *iceA1, cagA, vacAs1* and *vacAm1* are important virulence determinants of *H. pylori* in Slovenian children. The importance of *babA2* gene for clinical outcome is not clear yet as *babA2* status significantly depends on the used primer set.

Abstract no.: P1.27

CLINICAL VALUE OF HOPQI GENOTYPE OF *HELICOBACTER PYLORI*; ASSOCIATION WITH RESISTANCE PHENOMENA

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Eradication of *Helicobacter pylori* infection is accepted as the first-line treatment among patients with digestive diseases. To date, no previous studies have been carried out to investigate the status of *H. pylori* hopQ genotypes and the pattern of antibiotic resistance. The aim of our study was to investigate the association between hopQ types I and II genotypes of *H. pylori* in patients with resistant and