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

**Results:** In H. pylori-infected AGS, CD277 (control = 16.4 ± 6.6, [HP2 x 10^7] = 35.3 ± 15.1), and HLA-DR (control = 12.5 ± 4.9, [HP2 x 10^9] = 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 by diverting energy 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 MITOCOCHONDL NETWORK FRAGMENTATION BY PROTEINS TRANSLATION (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 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 (10^5 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 CoCl₂) 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 CoCl₂ 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 TNP2 GENES PREDICTS THE PRESENCE OF DUODENAL ULCER**

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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.: P1.22**

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

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**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, vacA, sfp, “on” status, babB and jhp0562. 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. 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. 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. Work supported by Research Grant 2011 – Sociedade Portuguesa de Gastroenterologia.

Table 1 Clinical data of study group

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients (Number of patients with isolated strains)</th>
<th>CagA Gene</th>
<th>VacAs 1m1</th>
<th>VacAs 2m2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>61 (2)</td>
<td>2 (100%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Antrum predominant gastritis</td>
<td>15 (13)</td>
<td>9 (69.2%)</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Corpus</td>
<td>7 (6)</td>
<td>5 (83.3%)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>15 (5)</td>
<td>4 (80%)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>11 (4)</td>
<td>4 (100%)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total number</td>
<td>186 (60)</td>
<td>52 (86.7%)</td>
<td>24</td>
<td>21</td>
</tr>
</tbody>
</table>

Abstract no.: P1.24
RETHINKING VACA: A TRUE MULTIFUNCTIONAL TOXIN OR RATHER A NOVEL TYPE OF MONOFUNCTIONAL A-B TOXINS?
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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
C. Langner, T. Wex, M. Varbanova, T. Tammer, W. Habendorf, J. Bornschein, M. Selgrad, D. Kuester, and P. Mallertheiner* 1Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Germany; 2Institute of Pathology, Otto-von-Guericke University, Magdeburg, Germany

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 vacA 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 ABCC (16%), ABCC (13%) and AB (6%). In five patients, colonization with multiple strains having at least two 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.